The Journal of

PHARMACY *and* PHARMACOLOGY

Successor to The Quarterly Journal of Pharmacy and Pharmacology

33 BEDFORD PLACE, LONDON W.C.1

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Telegrams: Pharmakon, Westcent, London

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British Pharmaceutical Conference

EIGHTY-NINTH ANNUAL MEETING, NOTTINGHAM, 1952

REPORT OF PROCEEDINGS

OFFICERS:

President:
W. J. TRISTRAM

Chairman:
H. B. MACKIE, B.Pharm., Brighton

Vice-President:
(*who filled the office of President prior to* 1923)
E. SAVILLE PECK, M.A., Cambridge.

Vice-Chairmen:
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NORMAN EVERS, Ph.D., F.R.I.C., Ware.
A. D. POWELL, F.R.I.C., Nottingham.
H. BERRY, B.Sc., Dip.Bact. (Lond.), F.R.I.C., London.

Honorary Treasurer:
G. R. BOYES, L.M.S.S.A., B.Sc., F.R.I.C., London.

Honorary General Secretaries:
H. TREVES BROWN, B.Sc., London.
H. G. ROLFE, B.Sc., 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*).

*Miss M. A. BURR, Nottingham.
H. DAVIS, B.Sc., Ph.D., F.R.I.C., London.
J. W. HADGRAFT, London.
F. HARTLEY, B.Sc., Ph.D., F.R.I.C., London.
E. F. HERSANT, Ph.D., B.Pharm., Dagenham.
J. P. TODD, Ph.D., F.R.I.C., Glasgow.
*T. REID, Luton.
T. D. WHITTET, B.Sc., London.

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

PROCEEDINGS OF CONFERENCE

NOTTINGHAM, 1952

THE OPENING SESSION

The opening session of the Conference was held in the Great Hall of the University on Monday, September 1, with Mr. W. J. Tristram, President of the Conference (President of the Pharmaceutical Society) in the chair. On the platform were the Chairman of the Conference (Mr. H. B. Mackie), the Mayor of Nottingham (Councillor Leon Willson), the Vice-Chancellor of the University of Nottingham (Mr. B. L. Hallward), the Chairman and Secretary of the Local Conference Committee (Mr. G. Shaw and Miss G. W. Watson), the Honorary General Secretaries (Mr. H. Treves Brown and Mr. H. G. Rolfe), the Honorary Treasurer (Dr. G. R. Boyes), together with members of the Conference Executive.

The PRESIDENT called upon the Mayor to address the meeting. The Mayor extended to the Conference a warm welcome to Nottingham and said he hoped the deliberations would be successful. The President thanked the Mayor on behalf of the Conference for his welcome.

The President then handed over the further conduct of the Conference to the CHAIRMAN (Mr. H. B. Mackie), who delivered his address entitled "An Education for a Pharmacist," which is printed in full in the *Journal of Pharmacy and Pharmacology*, 1952, **4**, pages 673 to 680.

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

THE CIVIC RECEPTION

On the evening of Monday, September 1, there was a Civic Reception in the Council House. The guests were received by the Mayor of Nottingham (Councillor Leon Willson), the Mayoress, and the Sheriff of Nottingham (Councillor Kenyon). A dance was held after the reception.

THE SCIENCE SESSIONS

Meetings were held on Tuesday, Wednesday and Friday, September 2, 3 and 5, the Chairman presiding, in the Great Hall, or in a lecture theatre.

During the sessions the following 29 papers were communicated:—

1. "The Detection and Determination of *iso*Nicotinyl Hydrazide."—By P. G. W. Scott, B.Sc.
2. "The Estimation of *iso*Nicotinyl Hydrazide."—By E. A. Haugas, Mag. Chem.Techn.(Tarta), and B. W. Mitchell, B.A.(Cantab.), B.Sc., A.R.I.C.
3. "Some Observations on the Structural Requirements for Antibiotic Activity in the Chloramphenicol Series, Part II."—By R. J. Collins, B.Sc., B. Ellis, B.Sc., S. B. Hansen, B.Sc., H. S. Mackenzie, B.Sc., R. J. Moualim, Ph.D., A.R.I.C., V. Petrow, Ph.D., D.Sc., F.R.I.C., O. Stephenson, Ph.D., F.R.I.C., B. Sturgeon, Ph.D.
4. "Changes in the Hydrocyanic Acid Content of Chlorodyne on Storage."—By H. A. Glastonbury, B.Sc., A.R.I.C.
5. "The Application of Emission Spectrography to Pharmaceutical Analysis."—By L. Brealey, B.Sc., D. C. Garratt, B.Sc., Ph.D., F.R.I.C., and K. A. Proctor, B.Sc., A.R.I.C.
6. "Ethyl Esters of Hydnocarpus Oil Stabilised with Creosote."—By G. E. Foster, B.Sc., Ph.D., F.R.I.C., E. L. Kendrick, E. Walton, M.Sc., Ph.D., and W. D. Williams, B.Pharm, Ph.C.
7. "The Determination of Ascaridole in Oil of Chenopodium and in Solution of Oil of Chenopodium in Castor Oil."—By A. H. Beckett, B.Sc., Ph.D., Ph.C., F.R.I.C., and M. Dombrow, B.Sc., B.Pharm., Ph.C., A.R.I.C.
8. "The Polarographic Determination of Riboflavine in Compressed Tablets and Ampoule Solutions."—By Sylvia Fowler, Ph.C., and R. C. Kaye, B.Pharm., Ph.D., Ph.C.

9. "The Determination of Thyroxine with Special Reference to Tablets."—By D. C. M. Adamson, F.R.I.C., A. P. Domleo, B.Sc., A.R.C.S., A.R.I.C., J. P. Jefferies, B.Sc., A.R.I.C., and W. H. C. Shaw, Ph.C., F.R.I.C.
10. "The Partition Chromatography of Alkaloids. Part IV. The Assay of Solanaceous Drugs."—By W. C. Evans, B.Pharm., B.Sc., Ph.D., Ph.C., and M. W. Partridge, B.Pharm., B.Sc., Ph.D., Ph.C.
11. "Observations on the Paper Partition Chromatogram as Applied to the Detection of Alkaloids."—By D. N. Gore, Ph.C., F.R.I.C., and J. M. Adshead, B.Sc.
12. "Digitalis Glycosides. The Colorimetric Assay of the Chloroform-Soluble Glycosides of Digitalis."—By C. J. Eastland, Ph.C., F.R.I.C., Miss D. P. Lawday, B.Sc., and E. H. B. Sellwood, P.Pharm., Ph.C.
13. "Studies in the Genus Digitalis. Part I. The Colorimetric Estimation of Digitoxin and of Preparations of *Digitalis purpurea*."—By J. M. Rowson, M.Sc., Ph.D., Ph.C.
14. "Studies in the Genus Digitalis. Part II. A Comparison of the Colorimetric and Biological Methods for the Evaluation of *Digitalis purpurea*."—By J. M. Rowson, M.Sc., Ph.D., Ph.C., and F. J. Dyer, B.Sc., Ph.D., Ph.C., A.R.I.C.
15. "Some Further Studies on Tuberculostatic Compounds."—By E. M. Bavin, B.Sc., F.R.I.C., D. J. Drain, B.A. (Cantab.), M. Seiler, M.D., and D. E. Seymour, F.R.I.C.
16. "Further Aspects of the Pharmacology of *p*-Aminosalicylic Acid."—By E. M. Bavin, B.Sc., F.R.I.C., and Barbara James, B.Sc.
17. "The Analgesic and Antipyretic Properties of Some Derivatives of Salicylamide."—By E. M. Bavin, B.Sc., F.R.I.C., F. June Macrae, B.Sc., D. E. Seymour, F.R.I.C., and Pamela D. Waterhouse, B.Sc.
18. "Neovitamin A and Vitamin A Alcohol in Commercial Fish-Liver Oils and Vitamin A Concentrates."—By E. Hayes, B.Sc., A.R.I.C., M.P.S.I., and Margaret A. Petitpierre, B.Sc.
19. "Some Observations on the Determination of Vitamin A in Cod-Liver Oil."—By R. V. Swann, B.Sc., A.R.I.C.
20. "The Stability of Vitamin A Alcohol in Aqueous and Oily Media."—By C. L. J. Coles, B.Pharm., Ph.C., and D. F. W. Thomas.
21. "The Assay of Penicillin using Penicillinase."—By A. Royce, Ph.C., C. Bowler, and G. Sykes, M.Sc., F.R.I.C.
22. "Preliminary Observations on the Stabilisation of Penicillin Solutions with Hexamine."—By R. J. Hobbs, B.Pharm., Ph.C., J. L. Livingstone, B.Sc. Pharmacy, Ph.C., J. Reece, Ph.C., and W. A. Woodard, M.P.S.
23. "Bacterial Survival in Systems of Low Moisture Content. Part IV. The Effects of Increasing Moisture Content on Heat Resistance, Viability and Growth of Spores of *B. subtilis*."—By K. Bullock, M.Sc., Ph.D., Ph.C., and A. Tallentire, Ph.C.
24. "The Application of Infra-red Heating to Pharmaceutical Products. Part I. Preliminary Investigations."—By H. W. Fowler, B.Pharm., Ph.C.
25. "The Water Retention Coefficient of Surgical Dressings."—By R. M. Savage, M.A., Ph.D., F.R.I.C., D. M. Bryce, B.Sc., B.Pharm. Ph.C., and J. R. Elliott, Ph.C.
26. "The Use of Surface Active Agents in Pharmaceutical Preparations: The Evaluation of Emulsifying Power."—By J. R. Cockton, and J. B. Wynn, B.Pharm., Ph.C.
27. "The Assay of Bacterial Pyrogens."—By Mrs. M. Dawson, Ph.C., and J. P. Todd, Ph.D., Ph.C., F.R.I.C.
28. "A Simple Apparatus for the Determination of Blood Clotting-Times and its Application to the Assay of Heparin."—By D. M. Bryce, B.Sc., B.Pharm., Ph.C.
29. "The Combination of Mercury Diuretics with Dimercaprol (2:3-Dimercaptopropanol); The Effect on Diuretic Activity and Toxicity."—By Eileen I. Short, B.Sc., B.Pharm., Ph.D., Ph.C., A.R.I.C., with a "Note on the Preparation of the Compounds of Mercurial Diuretics with 2:3-Dimercaptopropanol (Dimercaprol)," by Thomas M. Sharp, M.Sc.(Tech.), A.R.I.C.

The papers are printed in full, with reports of discussions, in the *Journal of Pharmacy and Pharmacology*, 1952, **4**, pages 681 to 780 and 803 to 1000.

THE SYMPOSIUM SESSION

A symposium on Recent Developments in the Pharmacy of Antibiotics was held on Friday, September 5. The Chairman presided, and Messrs. W. A. Woodard, W. Trillwood and J. O. Davidson read introductory papers. A report of the meeting is printed in the *Journal of Pharmacy and Pharmacology*, 1952, **4**, pages 1037 to 1046.

MEETING OF BRANCH REPRESENTATIVES

During the Conference the Council of the Society arranged a meeting of Branch Representatives. Sessions were held in the morning and afternoon of Wednesday, September 3, with the President of the Pharmaceutical Society, Mr. W. J. Tristram, in the chair. The Agenda comprised two subjects, (*a*) Draft Revised Statement on Matters of Professional Conduct, (*b*) Report on Assistants in Pharmacy.

A report of the sessions was published in *The Pharmaceutical Journal*, 1952, 169, pages 183 to 184, 189 to 192.

THE CLOSING SESSION

The closing Session of the Conference was held on Friday, September 5, in the Great Hall, the Chairman, Mr. H. B. Mackie, presiding.

Vote of Thanks to the Nottingham Committee

The CHAIRMAN called on Mr. J. C. Hanbury to propose a vote of thanks to the Local Committee. This was seconded by Dr. E. Höst-Madsen. The Chairman then presented to the Nottingham Branch of the Pharmaceutical Society an ivory gavel provided from the Bell and Hills Fund. Mr. G. Shaw (Chairman of the Nottingham Committee) replied to the vote of thanks and acknowledged the gift.

Annual Report

MR. H. TREVES BROWN presented the following Annual Report of the Executive Committee:—

Your Executive have pleasure in presenting the Eighty-ninth Annual Report.

MEMBERSHIP.—In addition to Members, Honorary Members and Student Associates of the Pharmaceutical Society, together with the Members of the Pharmaceutical Society of Northern Ireland, the Conference includes 66 members elected by the Executive.

CONFERENCE RESEARCH PAPERS.—Thirty-three papers were submitted and twenty-nine accepted for presentation to the Conference. The Executive thank the authors for their contributions.

FINANCIAL ASSISTANCE FROM THE SOCIETY.—The Council of the Society again placed at the disposal of the Executive a sum of up to £500 which has been used to assist younger members to attend the Conference. Twenty-seven members took advantage of the offer. The Executive are grateful to the Council for their generous assistance.

JOURNAL OF PHARMACY AND PHARMACOLOGY.—The report of the meeting of the Conference at Harrogate was published in the 3rd Volume of the Journal of Pharmacy and Pharmacology. The Executive have been represented on the Editorial Committee by the Chairman (Mr. H. B. Mackie), Dr. G. R. Boyes, and the Senior Honorary General Secretary.

RELATIONSHIP WITH THE SOCIETY.—Discussions have continued with the Council of the Society in regard to the inclusion of a section in the Society's byelaws dealing with the Conference.

BADGE OF OFFICE FOR THE CHAIRMAN.—Arrangements have been made for the manufacture of the badge from a design prepared by the College of Arms.

BELL AND HILLS FUND.—Your Executive have decided in future to use the income from this fund for the purchase of a suitable memento to be presented to the Branch of the Society entertaining the Conference, which need not be limited to books as hitherto.

FUTURE MEETINGS.—An invitation will be presented at this meeting for the Conference to meet in London in 1953.

Invitations for future meetings of the Conference have been provisionally accepted as follows:—

1954 from the Oxfordshire Branch to meet in Oxford.

1955 from the Aberdeen and North-Eastern Scottish Branch to meet in Aberdeen.

1956 from the Pharmaceutical Society of Ireland to meet in Dublin.

OFFICERS OF THE CONFERENCE.—Your Executive have nominated the following officers for 1952–1953:—

President (ex officio): The President of the Pharmaceutical Society of Great Britain. *Chairman:* G. R. Boyes. *Vice-President:* (who held the office of President prior to 1923) E. Saville Peck. *Vice-Chairmen:* R. R. Bennett, C. H. Hampshire, F. W. Crossley-Holland, H. Deane, H. Humphreys Jones, T. E. Wallis, H. Brindle, B. A. Bull, Norman Evers, A. D. Powell, H. Berry and H. B. Mackie. *Honorary Treasurer:* H. Davis. *Honorary General Secretaries:* H. Treves Brown and H. G. Rolfe. *Other Members of the Executive:* K. Bullock, T. C. Denston, G. E. Foster, J. W. Hadgraft, F. Hartley, J. P. Todd, with the following nominated by the Council of the Pharmaceutical Society of Great Britain: T. Heseltine, G. H. Hughes and F. C. Wilson and 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 Chairman of the Local Committee and the Honorary Local Secretary.

MR. H. CLEMENT SHAW proposed the acceptance of the Report and the election of officers of the Conference for the ensuing year, and congratulated the newly-elected officers, in particular the Chairman-Elect, Dr. G. R. Boyes. Mr. C. J. Eastland seconded, and the Report was adopted.

DR. G. R. BOYES thanked the Conference on behalf of the newly-elected officers.

TREASURER'S REPORT

DR. G. R. BOYES presented the following Report and Statement of Accounts for the year 1951:—

During the year ended December 31st, 1951, the excess of income over expenditure in the Bell and Hills Fund amounted to £28 10*s.* 11*d.* The Accumulated Fund on December 31st, 1951, amounted to £1,838 9*s.* 8*d.*, which is represented by Cash at Bank £80 4*s.* 6*d.*, Post Office Savings Bank Account £308 5*s.* 2*d.*, and Savings Bonds £200, together with the original donation by Alderman Clayton of Consols to the nominal value of £1,250.

Subscriptions from elected members, including a composition feee from the Pharmaceutical Society of Northern Ireland, amounted to £138 15*s.* 0*d.*, and were credited to the account of the Journal of Pharmacy and Pharmacology.

The expenditure of £665 13*s.* 8*d.*, which included £205 13*s.* 9*d.*, the cost of the scheme for assisting young pharmacists and £74 9*s.* 10*d.*, for the entertainment of foreign visitors, paid by the Pharmaceutical Society 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

(Bell and Hills Fund)

INCOME AND EXPENDITURE ACCOUNT, 1951

Expenditure.	£	s.	d.	*Income.*	£	s.	d.
Donation of Books to the Harrogate Branch	6	11	11	Interest on Consols	21	12	10
Balance to Accumulated Fund	28	10	11	Interest on 3 per cent. Savings Bonds	6	0	0
				Interest on Post Office Savings Bank Account;	7	10	0
	£35	2	10		£35	2	10

BALANCE SHEET AT DECEMBER 31ST, 1951

Liabilities.	£	s.	d.	*Assets.*	£	s.	d.
*Accumulated Fund—				Investments:—			
As at 31.12.50	1,809	18	9	(*a*) £1,250 2½ per cent. Consols (Donation by the late Alderman Clayton of Birmingham) (Market value at 31st December, 1950, £768)	1,250	0	0
Add Surplus	28	10	11	(*b*) £200 3 per cent. Savings Bonds, 1960-70 (Market value at 31st December, 1951, £173	200	0	0
				Post Office Savings Bank Account	308	5	2
				Cash at Westminster Bank, 31.12.51	80	4	6
	£1,838	9	8		£1,838	9	8

* Includes £121 2s. 9d. balance on London Conference, 1946.

G. R. BOYES,
Honorary Treasurer

On the proposition of the President, seconded by Dr. K. Bullock, the Report was adopted.

[*Continued on page 7*

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.
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Years	Places of Meeting	Chairmen	Local Secretaries
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1940	LONDON	H. HUMPHREYS JONES, F.R.I.C.	———
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Closing Session (continued).

PLACE OF MEETING FOR 1953

MR. F. G. WELLS, on behalf of the Branches in the Metropolitan area, extended an invitation to hold the Conference in London in 1953.

MR. H. T. THOMAS proposed that the invitation be accepted, and MR. H. W. GAMBLE seconded. The vote was put to the meeting and unanimously carried.

VOTE OF THANKS TO CHAIRMAN

Mr. A. G. FISHBURN proposed a vote of thanks to the Chairman.

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

MR. MACKIE 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-Presidents, 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 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 30*s*. annually, which shall entitle them, on application, to receive the JOURNAL OF PHARMACY AND PHARMACOLOGY, as published. Subscriptions shall become due on 1st January, and membership shall cease if subscriptions are not paid by 1st June.

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REVIEW ARTICLE

APPLICATIONS OF POLAROGRAPHY IN PHARMACEUTICAL ANALYSIS

By J. E. Page, B.Sc., Ph.D., F.R.I.C.

Glaxo Laboratories, Ltd., Greenford, Middlesex

Introduction

The polarographic method of analysis was devised by Professor J. Heyrovský in Prague about 25 years ago, but it is only during the last ten years that polarography has been accepted for pharmaceutical analysis. Nevertheless, it is now agreed that the technique is of unquestionable value and the polarograph is assured of a permanent place among the complex equipment of a modern analytical laboratory.

In the past there has been a tendency among some enthusiasts to be uncritical of polarographic methods and to use a polarographic procedure whether or not some other more satisfactory method exists. Some of the polarographic analyses suggested in the literature need complicated chemical separations and do not compare favourably with simpler colorimetric and volumetric methods. Whenever a new analytical problem is being examined, a polarographic approach should always be considered, but, before any method is adopted for routine use, its merits must be compared with those of methods based on other analytical techniques. For many pharmaceutical problems the polarograph can offer a method that has advantages over all other techniques.

The fundamental principles of polarographic analysis were worked out in Heyrovský's laboratory and are adequately described in Kolthoff and Lingane's[1] and Heyrovský's[2] monographs. These survey polarographic literature up to the beginning of 1941. Stock's[3] and Müller's[4] reviews provide useful introductions to practical polarography and von Stackelberg's[5] book covers the literature until the end of 1949.

Characteristics of Polarography

In polarography the sample to be examined is dissolved in a base solution containing an excess of a base or supporting electrolyte and is placed in a special electrolytic cell, which has a pool of mercury as anode and mercury dropping from a capillary at the rate of one drop every 2 to 4 seconds as cathode. When a gradually increasing voltage is applied to the cell and the corresponding current is measured on a galvanometer, it is possible to determine from the resulting current-voltage curve both the nature and the concentration of the reducible substances in the sample. If the dropping mercury electrode is made the anode, analytical data on oxidisable substances may be obtained. The current-voltage curves, or polarograms, as they are usually called, may be plotted manually or recorded, either photographically or by means of a pen-recorder. A recording instrument is recommended for pharmaceutical investigations.

A typical polarogram obtained with an air-free solution of 0·02 per cent. of cadmium sulphate in 0·1N potassium chloride is shown in Figure 1. Under standard conditions, the diffusion current (i.e., the height of the step or wave) is proportional to the concentration of electroreducible substance present. This relationship is the basis of quantitative polarography. The half-wave potential, which, as its name implies, is the value of the potential of the dropping mercury electrode, measured against a standard external reference electrode, at the point on the current-voltage curve at which the current is one-half of its limiting value, is a special property of the particular electroreducible substance present and is independent of the concentration of the substance and of the characteristics of the dropping mercury electrode used. Qualitative polarography is based on these facts. The saturated calomel electrode is usually accepted as the standard reference electrode and all half-wave potentials mentioned in this review will be referred to it.

The magnitude of the diffusion current is governed by Ilkovič's equation,[6] $i_d = 605nD^{\frac{1}{2}}Cm^{\frac{2}{3}}t^{\frac{1}{6}}$ in which i_d is the diffusion current in microamp., n is the number of electrons involved in the reduction of one molecule of reducible substance, D is the diffusion coefficient of the reducible substance in sq. cm. per sec., C is its concentration in millimoles per l., m is the weight of mercury in mg. flowing out of the capillary per sec. and t is the drop time in sec. Recent work has shown that one of the assumptions made during the mathematical derivation of the Ilkovič equation is of doubtful validity and it is recommended that the equation should be multiplied by the factor $(1 + X.D^{\frac{1}{2}}t^{\frac{1}{6}}m^{-\frac{1}{3}})$. Lingane and Loveridge[7] give a value of 39 for the factor X, while Strehlow and von Stackelberg[8] quote a figure of 17. From the Ilkovič relation, it follows that results obtained with different capillaries can be compared provided that the product, $m^{\frac{2}{3}}t^{\frac{1}{6}}$ is known. Data for m and t should therefore be included in all polarographic reports.

Scope of Polarography

Theoretically every substance that is either electro-reducible or -oxidisable within the potential range of the dropping-mercury electrode (i.e., between +0·6 and −2·6 v.) can be determined polarographically, but for most solutions the range is much smaller. If several electroreducible substances are present together in the same solution, they can all be estimated if their half-wave potentials are more than 0·2 v. apart. Steps that are less than 0·2 v. apart can sometimes be separated by either changing the *p*H value of the base solution or by converting the reactants into complexes from which they deposit at potentials far enough apart for each step to be measured separately.

Under normal conditions, the accuracy of polarographic analysis is about ±2 per cent. in the concentration range 10^{-2} to 10^{-4} molar and ±5 per cent. between 10^{-4} and 10^{-5} molar. By taking special precautions it is sometimes possible to obtain greater accuracy. However, when the small quantity of material needed for an analysis is considered, the degree of accuracy is seen to compare favourably with that of other

micro-analytical techniques. The sample is not destroyed during measurement and can if necessary be recovered for further studies.

Practical Polarography

Current-voltage curves do not always have the ideal shapes shown in Figure 1 and are often surmounted by so-called "maxima." The discharge curve rises sharply, but, instead of gradually developing into a

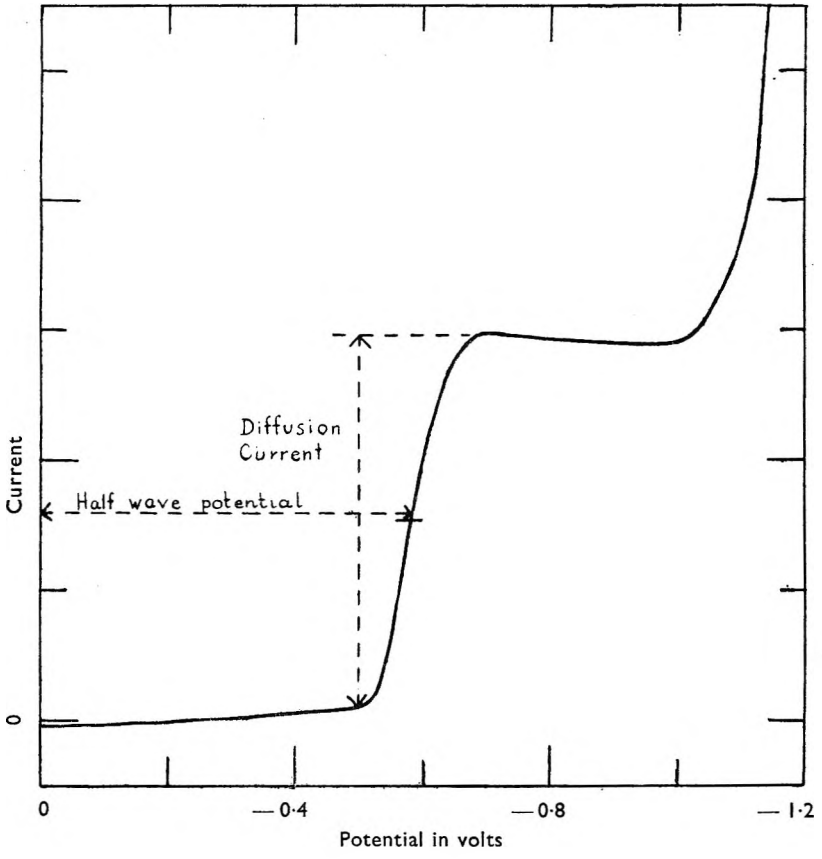

Fig. 1. Typical Polarogram.
Air-free solution of 0·02 per cent. of cadmium sulphate in 0·1N potassium chloride.

limiting current, the current increases abnormally until a critical value is reached and then decreases towards a limiting value corresponding with the normal diffusion current. The more or less sharp maxima shown in polarogram 1 of Figure 2 results. Fortunately maxima of this type can usually be suppressed by adding to the solution small quantities of surface-active substances. Gelatin and methyl red at a concentration of 0·01 to 0·1 per cent. are frequently used for this purpose. Maxima on catalytic waves (*vide infra*) do not normally respond to small amounts of surface-active substances.

Since oxygen is reduced at the dropping mercury electrode and interferes with the polarograms of most substances, it is generally necessary to remove dissolved air from polarographic solutions by bubbling either oxygen-free nitrogen or hydrogen through the cell before electrolysis or, if the solution is neutral or alkaline, adding about 1 per cent. of solid sodium sulphite.

Special care is needed in the selection of base solutions for the examination of organic substances. Since the *p*H value of the medium has considerable effect on half-wave potential, all solutions should be buffered. In unbuffered solutions, *p*H changes at the electrode surface frequently give rise to the development of drawn-out steps and sometimes to the formation of two steps. If the organic compound is insoluble in water, a mixture of water with a miscible organic solvent that will dissolve both buffer mixture and organic compound must be used. All the ingredients of the base solution should be tested polarographically to ensure the absence of impurities that would be reduced at a more positive potential than the substance under examination. Ethanol, *iso*propanol and dioxane need to be freed from aldehydes and peroxides.

Under certain conditions, ions that deposit at more negative potentials than hydrogen and are able to increase greatly the hydrogen over-potential form, in addition to ordinary reduction steps, catalytic waves. These waves can be distinguished from reduction steps by observing the effect of *p*H value on their height. Catalytic waves show big changes in height for small changes in *p*H value and frequently attain a height many hundreds of times greater than that of the corresponding reduction step. The height of a catalytic wave cannot be associated with a normal reduction mechanism.

Special Polarographic Techniques

For certain measurements the dropping mercury electrode may be replaced by either a rotating or a vibrating platinum micro-electrode. So far platinum electrodes have not been used extensively in pharmaceutical analysis, but they are useful for measurements in the positive potential range, in which mercury is oxidised, and for respiration studies on living organisms, when mercury might be toxic.

Amperometric titrations, in which either a dropping mercury electrode or a platinum electrode is employed as indicator electrode, have been developed during the last 15 years. They arise directly from polarography; the alternative name, polarometric titrations, was suggested by Majer to indicate their relationship to polarography. The technique depends on the measurement, at a constant potential, of the change in diffusion current of a reducible or oxidisable substance when the substance is titrated with a suitable reagent. The method is particularly useful for titrating small amounts of metal ions and can frequently be used when reversible indicator electrodes for titration by the classical potentiometric method are not available. Amperometric titrations need relatively simple equipment and sometimes yield more accurate results than ordinary polarography.

Recently, new techniques such as differential, derivative and oscillographic polarography[4] have been introduced. Derivative polarography promises to be of value for determining in the presence of each other substances whose half-wave potentials are close together. Oscillographic polarography should find many applications in the field of reaction kinetics.

Pharmaceutical Applications

The pharmaceutical applications of polarography will be discussed under the following headings:—

(1) Dissolved oxygen and peroxides.
(2) Trace metals and metal-containing drugs.
(3) Antiseptics and insecticides.
(4) Vitamins.
(5) Hormones.
(6) Antibiotics.
(7) Alkaloids.
(8) Miscellaneous pharmaceutical substances.
(9) Blood serum and cancer diagnosis.

Dissolved Oxygen and Peroxides

The determination of dissolved oxygen provides a simple exercise in quantitative polarography. Oxygen dissolved in an electrolyte solution (cf. Fig. 2) is reduced at the dropping mercury electrode and yields two distinct steps. The first step (Kolthoff and Miller[9]), at −0·1 v., is due to the reduction of oxygen to hydrogen peroxide and the second, at −0·9 v., to the reduction of hydrogen peroxide to either water or hydroxyl ion. The second oxygen step coincides with that obtained for the electrolysis of an air-free solution of hydrogen peroxide. These steps have been used for measuring the oxygen content of a wide range of fluids, including whole blood (Weisinger[10]), body fluids (Beecher, Follansbee, Murphy and Craig[11]), fermentation liquors (Hixson and Gaden,[12] Bartholomew, Karow, Sfat and Wilhelm[13] and Wise[14]) and milk (Hartman and Garrett[15]) and for studying the respiration rates of micro-organisms (Winzler[16] and Skerman and Millis[17]).

If the potential applied to the polarograph cell is kept constant at about −0·5 v., the variation of step height (i.e., oxygen level) with time can be recorded. When a suspension of yeast cells in a glucose medium is placed in a special air-tight polarograph cell, the height of the oxygen step falls at a steady rate, proportional to the number of yeast cells in the suspension. As soon as the amount of oxygen in the solution falls below a certain minimum value, respiration ceases and a horizontal trace is obtained. In this way, Petering and Daniels[18] were able to study the respiration of micro-organisms such as green algae, yeast, blood-cells and animal tissues. Winzler[16] examined the effect of cyanide and azide on the respiration of baker's yeast and Skerman and Millis[17] studied oxygen consumption in bacterial culture media. The method is sensitive and compares favourably with the classical manometric methods.

Recently the technique has been used to measure oxygen levels during the submerged culture production of penicillin and streptomycin. During fermentation, oxygen is supplied to the mould by bubbling air or oxygen through the medium. At high rates of penicillin production, the growth of the mould may be limited by the rate at which oxygen dissolves from

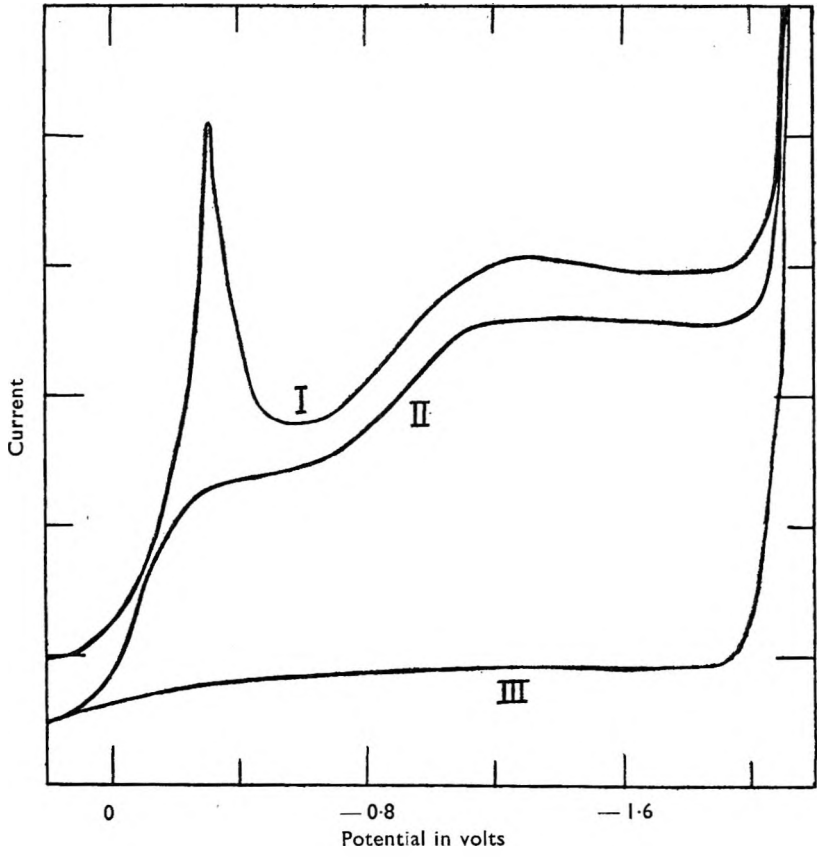

FIG. 2. Polarograms for oxygen.

I. 0·05N potassium chloride solution saturated with air.
II. After addition of a trace of methyl red.
III. After removal of oxygen by nitrogen.

the gas phase. Polarographic measurements on penicillin and streptomycin fermentation liquors have been reported by Hixson and Gaden[12] and by Bartholomew, Karow, Sfat and Wilhelm[13] in America and by Wise[14] in this country.

Peroxides and aldehydes are reduced at the dropping mercury electrode. Reimers[19] has described a method for the determination of peroxides and aldehydes in ether. A mixture of 5·0 ml. of the ether sample, 5·0 ml. of aldehyde-free ethanol and 10 ml. of 0·04N tetramethylammonium hydroxide is polarographed before and after removal of oxygen with

ether-saturated nitrogen. Examination of the first polarogram gives information on the aldehyde content of the sample and of the second on peroxide content. Benzoyl peroxide, lard peroxide, methyl linoleate peroxide andethyl, *iso*propyl and *n*-butyl ether peroxides may be determined in an ethanol-benzene mixture containing lithium chloride as base electrolyte (Lewis, Quackenbush and de Vries[20]); the technique has been used to study changes in lard (Lewis and Quackenbush[21]).

Trace Metals and Metal-containing Drugs

Polarography has been used extensively to determine trace metals in pharmaceutical products and to estimate drugs that contain metals as a constituent. The metals examined include antimony, arsenic, cadmium, copper, iron, lead, magnesium, mercury, vanadium and zinc. Unfortunately the procedures are not always so simple as the corresponding polarographic methods for these metals in metallurgical samples. If the organic matter is reduced at the same or at a lower potential than that of the metal a preliminary chemical separation or ashing procedure must be undertaken. Blood and urine containing traces of arsenic or vanadium need lengthy pre-treatments to remove reducible organic matter before an extract suitable for polarography is obtained. These pre-treatments are probably as complicated as those needed before any other analytical method can be used and it is doubtful whether for these particular analyses anything is to be gained by polarography.

On the other hand, Page and Robinson[22] have shown that the polarograph can offer advantages in special problems such as the estimation of antimony in pharmaceutical preparations and Goodwin and Page[23] have used the technique for studying the metabolism of the antimonial drugs for treatment of bilharzia and kala-azar. It is difficult to distinguish by ordinary chemical methods between ter- and quinquevalent antimony in organic matter. However, tervalent antimony in 1·0N hydrochloric acid forms a good polarographic step with a half-wave potential of −0·15 v., whereas quinquevalent antimony does not. Consequently, tervalent antimony can be determined in the presence of quinquevalent. This technique has been adopted as the British Pharmaceutical Codex (1949) test for tervalent antimony in sodium stibogluconate (sodium antimony$^{(V)}$ gluconate). Gelatin (0·01 per cent.) is included in the polarograph solution as a maximum suppressor.

Since the half-wave potential of tervalent antimony in 1·0N hydrochloric acid is relatively low, −0·15 v., the step appears before those due to most biological substances and tervalent antimony can therefore be determined directly in urine; blood samples need relatively little additional pre-treatment. Quinquevalent antimony can be measured after reduction with sodium sulphite. In this way, the valency state and the quantity of antimony appearing in the organs and body fluids of animals that had received stibophen (sodium antimony$^{(III)}$ biscatechol 3 : 5-disulphonate), sodium stibogluconate and other antimonial drugs could be ascertained.

Methods for determining a wide range of metals in materials of pharmaceutical interest are described in the literature. These include copperin

powdered and liquid milk (Cranston and Thompson[24]), milk fat (Coleman, Thompson and Branum[25]) and lard (Lupton, Mitchell, Oemler and Woolaver[26]), lead in lard[26] and urine (Cholak and Bambach,[27] Reed and Gant,[28] Weber[29] and Baker[30]), arsenic in biological material (Bambach[31]) and iron in pharmaceutical preparations (Bingenheimer and Christian[32]). The three earlier polarographic methods for lead in urine involved dry ashing at high temperatures; one method[27] also included a preliminary electrolytic separation. By using an entrainment technique, Baker[30] has been able to simplify the procedure considerably.

In Bambach's method[31] for arsenic, the arsenic is evolved as arsine and absorbed in mercuric chloride solution; the mercury arsenides are converted to arsenious oxide and mercurous chloride and the mercury is precipitated with hydroxylamine. The residual solution is treated with hydrochloric acid and examined polarographically. The method covers the range, 1 μg. to 1 mg. of arsenic and has an accuracy of 3 to 5 per cent. for quantities above 10 μg. Brdička[32] has used polarography to determine quinquevalent arsenic in arsphenamine, 0·01N lithium chloride being used as base electrolyte.

In Bingenheimer and Christian's method[33] for iron in American pharmaceutical preparations, a solution containing about 0·1 per cent. of ferric iron in 0·5M sodium citrate, containing 0·005 per cent. of gelatin as maximum suppressor, is polarographed. The iron must be dissolved completely in the ferric state.

Antiseptics and Insecticides

Several mercury-containing antiseptics and the new insecticides, gammexane and parathion, can be determined polarographically.

Page and Waller[34] have shown that thiomersalate (sodium ethyl mercurithiosalicylate) in 1·0N hydrochloric acid gives well-defined steps. The steps occur at a relatively low potential, $-0{\cdot}48$ v., and are suitable for the routine determination of thiomersalate in vaccines and other pharmaceutical preparations. Phenylmercuric acetate and nitrate, but not mercurochrome or mercuric chloride, can be determined in this way. Benesch and Benesch[35] have used the method to determine the mercurial diuretics and Osborn[36] has adopted it for the routine determination of phenylmercuric acetate in a gelatin base. Owing to the large amounts of gelatin in Osborn's preparation, it was necessary to carry out the measurments in a special cell maintained at 58° C.

Commercial gammexane, which is the γ-isomer of 1 : 2 : 3 : 4 : 5 : 6-hexachlorobenzene, may occur mixed with at least four other isomers, of which only gammexane itself is both biologically active and polarographically reducible. It is unusual for only one isomer of a substance to be reducible; the reducibility of the γ-isomer is attributed to its polar structure. Ingram and Southern[37] used an aqueous-ethanolic solution containing 1·0 per cent. of potassium chloride as base solution. Dragt[38] preferred a potassium chloride-sodium acetate buffer mixture in aqueous acetone and Schwabe[39] 0·1 tetraethylammonium iodide in 80 per cent. aqueous ethanol. Heptachlorocyclohexane (Monnier, Roesgen and

Monnier[40]) is also reduced polarographically, but it is unstable at *p*H 11·5 while the γ-isomer is stable at *p*H 13·0 so that gammexane may be determined in the presence of heptachlorocyclohexane in aqueous acetone buffered at *p*H 11·5.

Bowen and Edwards[41] have determined parathion (O : O-diethyl-O-*p*-nitrophenyl thiophosphate) with an accuracy of ±1 per cent. in commercial preparations. The procedure depends on the reduction of the nitro group and is carried out in aqueous acetone with 0·05N potassium chloride as base electrolyte.

Vitamins

Most water-soluble and some oil-soluble vitamins are either oxidised or reduced at the dropping mercury electrode and can be estimated in pure solution. Unfortunately most of the assays are unsuitable for foods and natural products. Methods have been reported for the following vitamins:—aneurine, riboflavine, nicotinic acid, pantothenic acid, pyridoxine, folic acid, vitamin B_{12}, ascorbic acid, α-tocopherol and vitamin K.

Lingane and Davis[42] determined riboflavine in the presence of aneurine and nicotinic acid and in pharmaceutical products such as liver extracts. The steps formed by aneurine and nicotinic acid appear at higher potentials than the riboflavine step and are therefore masked by steps due to more easily reducible organic matter. The mechanism of the riboflavine step has been studied by Brdička and Knobloch[43] and that of the nicotinic acid wave by Tompkins and Schmidt.[44] Knobloch[45] has determined nicotinamide in solutions intended for injection; 0·1N potassium chloride was used as base solution.

Wollenberger[46] has shown that under certain *p*H conditions aneurine forms a catalytic wave about 4,000 times higher than the normal aneurine reduction step. This catalytic wave can be used to detect traces of aneurine but is unsuitable for quantitative work. Pyridoxine and pantothenic acid[42] are reduced only at relatively high potentials.

Folic acid in 0·1M lithium borate gives a step at −0·8 v.; xanthopterin, rhizopterin and aporhizopterin give similar steps at the same potential. When Rickes, Trenner, Conn and Keresztesy[47] made the original observation, the structure of vitamin Bc, as folic acid was then called, was unknown, but the similarity in the steps suggested that the four substances had the same pterin structure. For routine estimations of folic acid in tablets, Mader and Frediani[48] recommend 1 per cent. tetramethylammonium chloride (*p*H 9·5) as base electrolyte with methyl red as maximum suppressor. Duncan and Christian[49] prefer a mixture of boric acid, potassium chloride and sodium hydroxide (*p*H 9).

Diehl, Sealock and Morrison[50] have shown that 9×10^{-4}M vitamin B_{12} in acid, neutral and alkaline solution gives a well-defined step at −1·12 v., which in 0·1M potassium cyanide shifts to −1·33 v. owing to the formation of a cyanide complex.[51] In neutral solution vitamin B_{12} also forms a catalytic wave with a half-wave potential of −1·53 v. (Fantes, Page, Parker and Smith[52]). The wave can be used to detect small amounts

($1{\cdot}0 \times 10^{-6}$ molar) of vitamin B_{12}, but is unsuitable for quantitative purposes.

By virtue of its reducing properties ascorbic acid yields an anodic step (cf. Fig. 3), a fact that was first discovered by Kodiček and Wenig.[53]

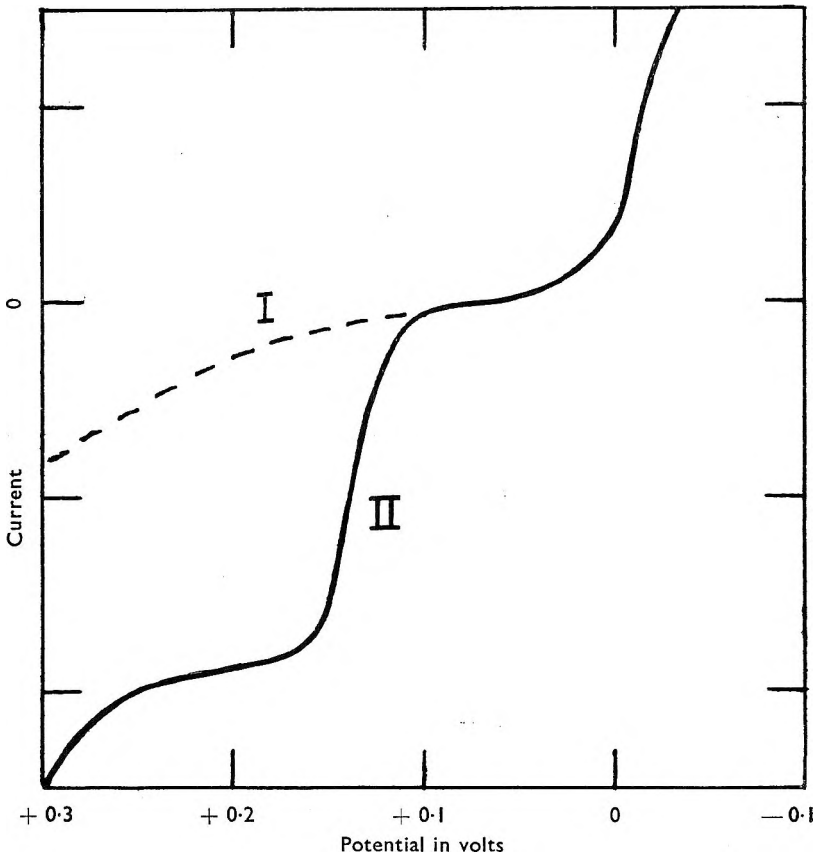

FIG. 3. Polarograms for ascorbic acid.
I. 0·05M phthalate buffer solution (*p*H 4·0).
II. 0·0002M ascorbic acid in 0·05M phthalate buffer solution.

These authors used a phosphate buffer solution (*p*H 7) for estimating ascorbic acid in fruit juices. Gillam[54] obtained better results by using 0·05M potassium hydrogen phthalate (*p*H 4·0) as base electrolyte. If the *p*H value is too low, the anodic step is shifted to such a high positive potential that the limiting current cannot develop. At high *p*H values the vitamin is unstable. Steps that are easier to measure (Page and Waller[55]) may be obtained by expanding the potential range of the instrument so that the voltage range of the potentiometer corresponds to 0 to $-1{\cdot}0$ v. instead of 0 to $-3{\cdot}0$ v. In this way, the steps are spread out and their height can be measured more accurately. Polarography has been used

to estimate ascorbic acid in fruit, vegetables[54] and milk (Perrin and Perrin[56]) and in tablet and ampoule preparations (Mata[57]).

Smith, Spillane and Kolthoff[58] showed that α-tocopherol in an aniline-anilinium perchlorate buffer solution gives an anodic step that can be used within certain limits for the determination of α-tocopherol in the presence of β- and γ-tocopherol. Unfortunately 2·5 to 5·0 per cent. of either sesame oil or fish oil or 0·15 per cent. of cholesterol will interfere with the determination and in the presence of these substances α-tocopherol cannot be determined at concentrations of less than 10^{-3} molar (Beaver and Kaunitz[59]).

McCawley and Gurchot[60] have shown that synthetic vitamin K_1, menaphthone (2-methyl-1 : 4-naphthoquinone), and phthiocol give well-defined polarographic steps in a 60 per cent. ethanolic acetate buffer solution (*p*H 6·56). The polarographic behaviour of vitamin K_1 in 50 per cent. aqueous *iso*propanolic 0·05N potassium chloride and of menaphthone in a 75 per cent. ethanolic acetate buffer solution (*p*H 6·24) has been reported by Hershberg, Wolfe and Fieser[61] and by Page and Robinson[62] respectively. Recently Onrust and Wöstmann[63] have described a polarographic procedure for determining menaphthone in mixtures with ground wheat germ and maize meal; Knobloch[64] has determined vitamin K_5 (4-amino-2-methyl-naphthol) in a phosphate buffer solution (*p*H 7·0).

Hormones

Thyroxine, insulin, adrenaline and several sex hormones give characteristic polarograms, that under certain conditions can be used for their determination.

The polarographic reduction of thyroxine was first reported by Simpson and Traill[65] who showed that thyroxine in 40 per cent. ethanolic 0·5N sodium carbonate containing 1 per cent. of tetramethylammonium bromide gave three steps, the first step having a half-wave potential of −1·24 v. In the same medium, 3 : 5-diiodotyrosine gave a double step with a half-wave potential of −1·72 v., thus making it possible to determine thyroxine in the presence of diiodotyrosine (cf. Fig. 4). Borrows, Hems and Page[66] studied the effect of various substances chemically related to thyroxine and obtained better polarograms by using a base solution containing 20 per cent. of *iso*propanol instead of 40 per cent. of ethanol. The technique has been extended to the measurement of thyroxine in iodinated proteins[66,67] and has been used by Clayton, Free, Page, Somers and Woollett[68] to estimate the chemical purity of thyroxine labelled with radioactive iodine.

The polarographic behaviour of adrenaline has been discussed by Kockelmeyer and Hauss[69] and by Sartori and Cattaneo.[70] Wielle and Gerlich[71] used polarography to determine the purity of an insulin sample; the colorimetric and polarographic methods for measuring the cystine content of insulin have been compared by Sullivan, Hess and Smith.[72]

Eisenbrand and Picher[73] first showed that certain sex hormones can be determined polarographically. 4 : 5-Unsaturated 3-ketosteroids, such as testosterone, progesterone, corticosterone and desoxycorticosterone, are

reducible at the dropping mercury electrode, but unsaturated 17-keto-steroids (e.g., androsterone) are not. However Wolfe, Hershberg and Fieser[74] condensed the latter with Girard T reagent (tetramethylammonium acethydrazide) to give water-soluble hydrazones that are electro-reducible.

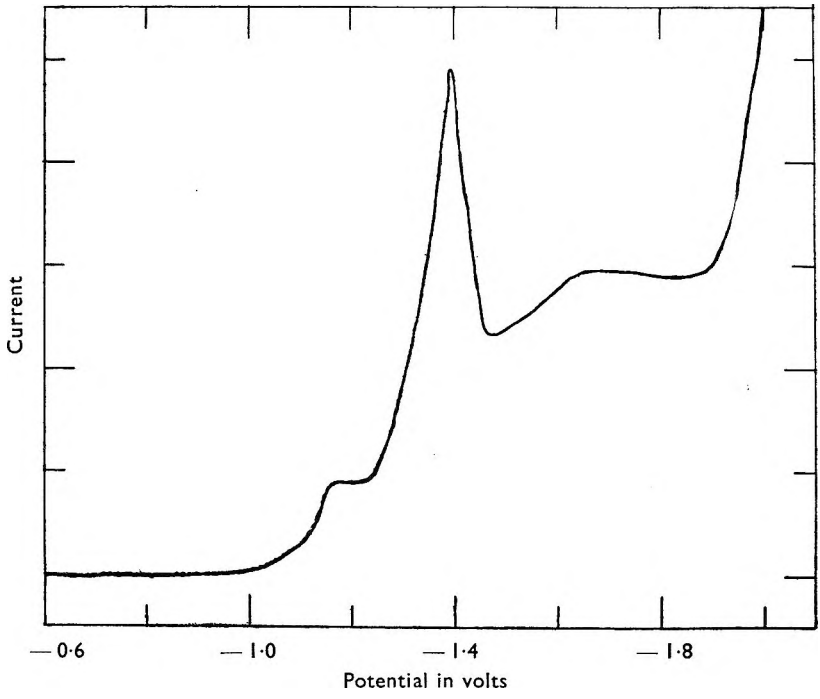

FIG. 4. Polarogram for 0·05 per cent. of thyroxine in 20 per cent. *iso*propanol containing 1·0 per cent. of tetramethyl ammonium bromide and 2·65 per cent. of sodium carbonate.

The condensation is undertaken in the presence of glacial acetic acid and the product examined in a base solution containing sodium hydroxide and chloride.

Girard T derivatives of 4 : 5-unsaturated ketonic steroids also give well-defined steps that can be used for analytical purposes. Certain non-ketonic steroids such as cholesterol can be determined after oxidation with aluminium *tert*-butoxide to the corresponding ketone and subsequent condensation with Girard's reagent (Hershberg, Wolfe and Fieser[75]).

Barnett, Henly and Morris[76] have used polarography to determine total 17-ketosteroids in urine. By employing a special preliminary separation, they[77] were able to differentiate between 3(α)- and 3(β)-hydroxy-17-ketosteroids. Some separation of the steps for individual ketosteroids may be achieved by using Werthessen and Baker's[78] expanded voltage scale technique, but otherwise the steps are too close together for individual ketosteroids to be estimated. A comparison of results for urinary 17-ketosteroids obtained by the polarographic and colorimetric methods

has been reported by Barnett, Henly, Morris and Warren.[79] Sartori and Bianchi[80] used polarography to determine methyltestosterone in pharmaceutical preparations.

Björnson and Ottesen[81] have shown that œstrone forms a polarographically-reducible Girard T derivative and have used the technique to estimate œstrone in pregnant mare urine. The method is suitable for analytical control of the large-scale production of œstrone. Œstrone, œstradiol and œstriol (Heusghem[82]) and the synthetic œstrogens, stilbœstrol, hexœstrol and dienœstrol (Gry[83]) may be determined after conversion to the corresponding nitroso derivatives. It is claimed that 5 to 20 μg. of the œstrogen can be detected.

Bingenheimer and Christian[84] discovered that stilbœstrol suppresses the oxygen maximum in the polarogram for 10 per cent. ethanolic 0·001N potassium chloride and used the property to determine stilbœstrol in tablets. These were extracted with ether, the ether was removed and the residue dissolved in the base solution. The solution was saturated with oxygen and polarographed. The quantity of stilbœstrol in the tablets was obtained by comparing the height of the oxygen maximum with that for a standard stilbœstrol solution.

Antibiotics

Polarographic methods for the determination of antibiotics such as penicillin, streptomycin and chloramphenicol have been described. Although the procedure for penicillin (Page,[85] cf. Clarke, Johnson and Robinson[86]) has been superseded by other methods, the procedure is of some interest as being the first non-biological assay method to be developed for penicillin. The technique depends on the observation that penicillamine (β:β-dimethylcysteine), a hydrolytic product of penicillin, in an ammoniacal cobalt buffer solution yields a catalytic wave similar to that given by cysteine.

Brdička[87] noticed that either cysteine or cystine reduced in buffered cobalt or nickel solution gave a single large catalytic wave (cf. Fig. 5). The height of the catalytic wave formed by cystine was twice that given by the same molar quantity of cysteine, but was 500 times as great as that of the step for the normal reduction of cystine to cysteine. These waves can be used to determine cysteine and penicillamine in the concentration range, 0·025 to 0·125 mg. per 100 ml. At higher concentrations, the catalytic wave height becomes independent of further increases in cysteine concentration. For analytical work on penicillin, the sample is inactivated with 0·1N sodium hydroxide, hydrolysed by warming with N hydrochloric acid, dissolved in the ammoniacal cobalt buffer mixture and polarographed immediately. The method can only be used for relatively pure penicillin samples and is unsuitable for metabolism solutions.

Levy, Schwed and Sackett[88] found that streptomycin in 3·0 per cent. tetramethylammonium hydroxide solution gives at $-1{\cdot}45$ v. well-defined steps due to reduction of the maltol group and used the procedure to estimate streptomycin in simple solutions. The technique cannot

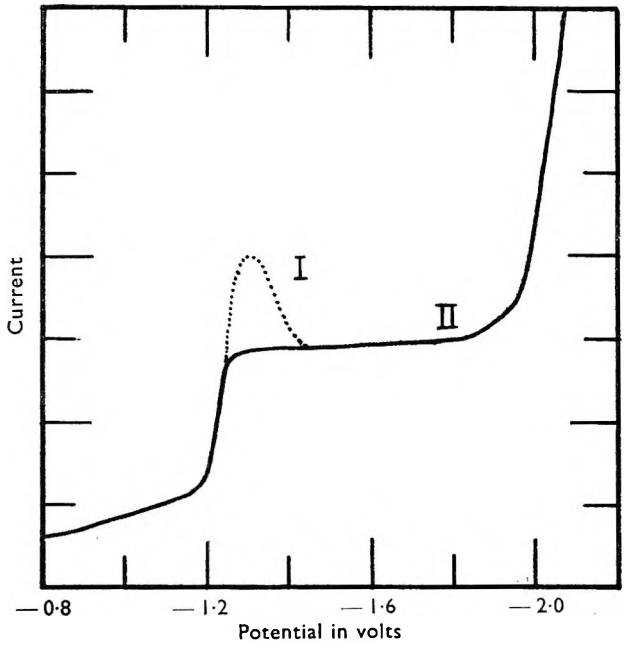

FIG. 5A.

I. Ammoniacal cobalt buffer solution.
II. Ammoniacal cobalt buffer solution containing 0·01 per cent. of gelatin.

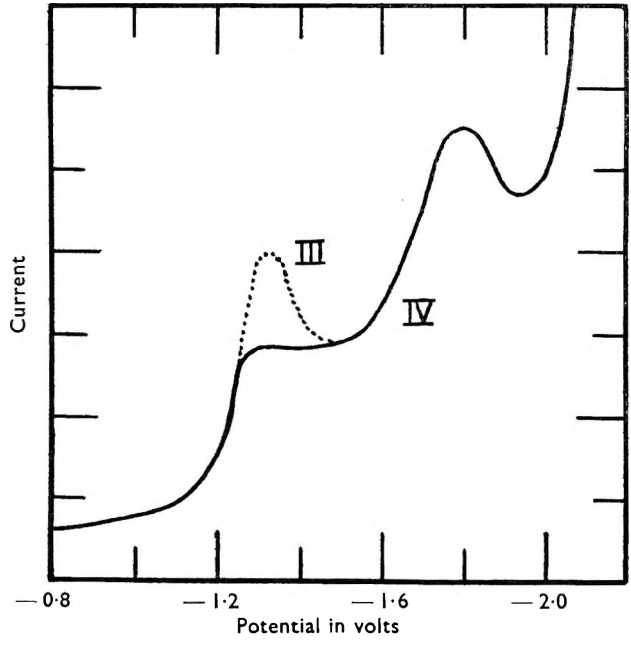

FIG. 5B.

III. Ammoniacal cobalt buffer solution containing 0·00005 per cent. of cystine.
IV. Ammoniacal cobalt buffer solution containing 0·00005 per cent. of cystine and 0·01 per cent. of gelatin.

differentiate between streptomycin and mannosidostreptomycin (Bricker and Vail[89]) and has been largely replaced by colorimetric methods.

Hess[90] has described an interesting polarographic method for chloramphenicol. The method, which depends on the reduction of the nitro group to hydroxylamine, is suitable for the routine analysis of broths. A potassium hydrogen phthalate buffer solution is used as base electrolyte.

Alkaloids

Most alkaloids yield catalytic waves and a few give reduction steps. Pech[91] in a preliminary survey of the quinoline and *iso*quinoline alkaloids found that quinine and quinidine in ammonium chloride solution yield identical catalytic waves, whose heights are proportional to the concentration of the alkaloids. The waves could be used to detect quinine and quinidine at concentrations as low as 10^{-7} molar. Cinchonine and cinchonidine behaved qualitatively in the same way as quinine, but the *iso*quinoline alkaloids, codeine, morphine, narcotine and hydrastine, gave much smaller catalytic waves than quinine. Reimers[92] observed that strychnine in 0·1N sodium sulphite gives a wave suitable for the rough determination of strychnine in the presence of quinine.

The only systematic polarographic investigation of the alkaloids has been conducted by Kirkpatrick.[93] In a study of over 30 alkaloids, he noticed that the catalytic waves formed by most alkaloids are in their height, formation, types of maxima and variation with *p*H value characteristic of the individual alkaloid and, provided the examination is conducted under standard conditions, can help in the identification and estimation of an unknown alkaloid. Preliminary purification is as necessary for the polarographic method as for the application of chemical tests, but polarography has the advantage that the alkaloid may be recovered unchanged.

The effect of *p*H value on the catalytic wave formed by morphine is shown in Figure 6. The wave at *p*H 8·0 has been selected for quantitative work, the relationship between diffusion current and concentration being linear over the range, $0{\cdot}3 \times 10^{-4}$ to $1{\cdot}3 \times 10^{-4}$ molar.

Kirkpatrick recommends polarography for the routine determination of morphine, diamorphine, emetine, strychnine and atropine in injection solutions. Single alkaloids may be extracted with chloroform from tablets and dissolved, after solvent removal, in 0·001N hydrochloric acid, mixed with the appropriate buffer solution and examined polarographically. Strychnine may be determined in mixtures with brucine and in nux vomica preparations. Mixtures containing 0·05 to 1·75 mg. of strychnine gave results that agreed within 3 per cent. of those obtained by the British Pharmacopœia 1932 procedure.

The polarographic behaviour of morphine has also been studied by Rasmussen, Hahn and Ilver.[94] If the nitroso derivative is formed, morphine can be determined in the presence of narcotine, papaverine and codeine. The technique has been extended to the determination of morphine in poppy capsules.[95] Further observations on the polarography of thujaplicin, berberine, hydrastine and cotarnine have been reported by

Šantavý,[96] who has also described a method for determining colchicine in seeds and in tincture of colchicum.[97] A polarographic procedure for lobeline in lobelia was published by Nyman and Reimers[98] but it gives lower results than those obtained by the Swiss Pharmacopœia method.

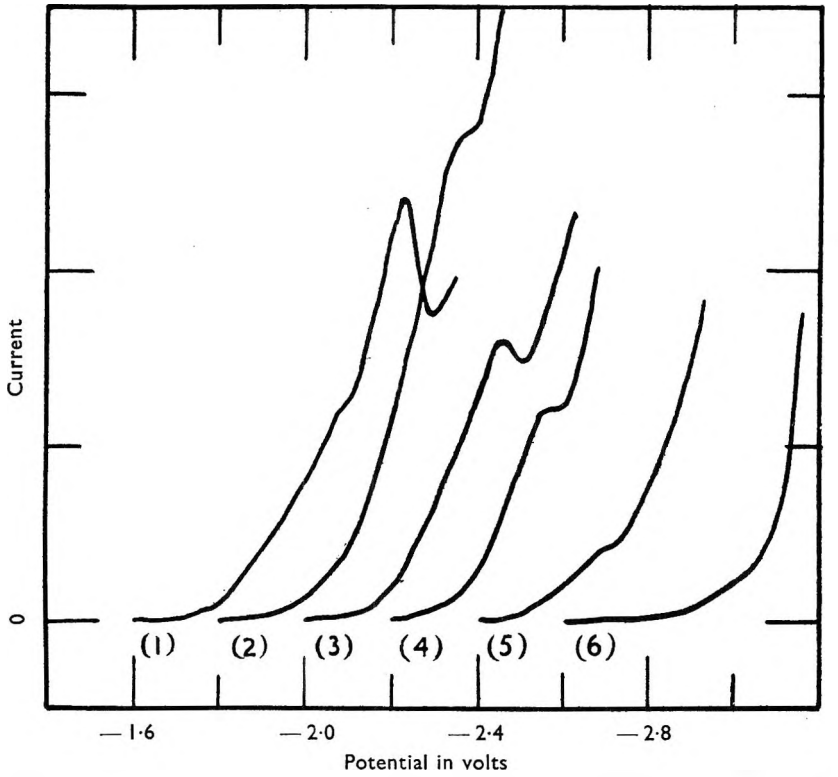

FIG. 6. 1·00M × 10^{-4} morphine in buffer solutions of *p*H value (1) 5·0, (2) 6·0, (3) 7·0, (4) 8·0, (5) 9·0, (6) 10·0. Each polarogram starts at −1·6 v.

MISCELLANEOUS PHARMACEUTICAL SUBSTANCES

Many miscellaneous substances of pharmaceutical interest, such as sugars, saccharin and the iodine-containing contrast agents, can be determined polarographically. Aldoses, such as glucose, rhamnose arabinose, mannose, galactose and lyxose, and disaccharides, such as maltose, lactose and sucrose, give small steps that are attributed to the aldehydo form of the sugar in the equilibrated solutions. Cantor and Peniston[99] used polarography to determine the amount of the aldehydo form present in sugar solutions under different *p*H, concentration and temperature conditions. The sugar solutions were buffered and 5-hydroxymethylfurfural, an aldehyde with approximately the same molecular weight as the sugar, was used as standard (cf., however, Weisner[100]). Ketoses, such as fructose and sorbose, in 0·02N lithium chloride give larger steps than were used by Heyrovský and Smöler[101] to determine

fructose in honey and in urine containing sucrose and glucose. Williams, McComb and Potter[102] recommend calcium chloride as base electrolyte and gelatin as maximum suppressor for the determination of fructose in fruit. Saccharin yields well-defined steps in acid, neutral and alkaline media; the steps have been used by Pech[103] to determine saccharin in tablets and by Momose[104] to determine saccharin in the presence of sugar. A base solution consisting of 0·05N hydrochloric acid and 0·05N potassium chloride is used in quantitative measurements.

The iodine-containing contrast agents that are used for the X-ray examination of various body organs yield characteristic polarograms (Page[105]), which can be used to identify and determine the contrast agents in simple solution. Diodone (diethanolamine salt of 3 : 5-di-iodo-4-pyridone-*N*-acetic acid) and iodoxyl (*N*-methyl-3 : 5-di-iodo-4-pyridone-2 : 6-di-carboxylic acid) in either acid or alkaline base solution given two well-defined steps. The first diodone step is surmounted by a maximum which may be suppressed by including a trace of gelatin in the base solution. Pheniodol (α-phenyl-β-(4-hydroxy-3 : 5-di-iodophenyl)-propionic acid) in sodium carbonate solution forms a double step that was utilised by Free, Page and Woollett[106] to determine the chemical purity of pheniodol labelled with radioactive iodine: a mixture of 1 per cent. of tetramethylammonium bromide and 2·65 per cent. of sodium carbonate in 20 per cent. *iso*propanol was the base solution.

Other applications of polarography include the measurement of formaldehyde in pharmaceutical products (Portillo and Mosquera[107]), digitoxin and gitoxin in tincture of digitalis (Hilton[108]), aloin in aloes, soap, gums, podophyllin and belladonna (Stone[109]) and santonin in *Flores cinae* and in tablets (Šantavý[110]). Knobloch and Schraufstätter[111] have listed the half-wave potentials of many pharmaceutical substances, including barbituric acid, chloramine, 8-hydroxyquinoline and phenolphthalein.

Blood Serum and Cancer Diagnosis

Polarography is of some importance in the study of pathological sera. In 1936, Brdička[112] found that blood serum from cancer patients contained fewer sulphydryl groups than that from normal persons. Direct comparison of untreated serum showed little difference, but significant differences between the two types of serum were observed when the serum was partially hydrolysed with dilute alkali to unmask disulphide groups inside the protein molecule. Still greater differences were noticed if the alkali-treated serum was precipitated with sulphosalicylic acid and the filtrate polarographed in an ammoniacal cobalt buffer solution. Under these conditions the difference was reversed and the cancer serum showed higher waves. Further work has shown that a high filtrate wave is not specific for cancer serum. Most foreign proteins, whether from the break-down of cancer tumour tissue or from centres of infection or inflammation, cause large filtrate waves when they are introduced into the blood stream. Since 1939, over 15,000 serum samples have been examined in Prague by these methods. Brdička's[113] general conclusions are that there

is a 20 per cent. overlap between the upper limit for normal serum and the lower limit for early cancer, but that there is a statistical correlation between wave height and the degree of advancement of the cancer. New growths are indicated by a gradual increase in wave size: after successful treatment of the cancer, whether by surgery or X-rays, the wave reverts to normal size. Robinson,[114] working in London, has largely confirmed the Czech work and has shown that the wave heights of serum filtrates from patients with prostatic cancer decreased when the patients were treated with synthetic œstrogens. The decrease in wave-height could be correlated with an improvement in the patient's condition.

The term "protein index" was introduced by Müller and Davis[115] to characterise and compare results obtained from the polarographic examination of blood serum. The protein index is a function of the ratio of the wave-heights obtained by the digest and filtrate tests. Both tests are conducted under standard conditions on different portions of the same blood sample. The technique is particularly suitable for routine analysis, since it is independent of small temperature changes and is unaffected by relatively large changes in mercury drop-time and drop-size. The protein index for normal persons is fairly constant,[116] but the values for patients with different diseases differ sufficiently to make the test useful in clinical diagnosis.[117]

Conclusion

Many of the applications described in this review are of limited scope, but they are all of some significance in their own particular field and contribute to the general importance of polarography in modern pharmaceutical analysis. This is a rapidly developing subject and we can anticipate that many new applications of polarography will be found in the near future.

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RESEARCH PAPERS

THE SYNTHESIS AND LOCAL ANÆSTHETIC PROPERTIES OF ARYLOXYPROPANOLAMINES

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It is well known that alkamines which contain a sufficiently hydrophobic radical (e.g., an aryl group or a long-chain alkyl group) possess local anæsthetic properties. Quinine is the classical example but MacIntosh and Work[1] described a series of alkamines of the general type $RR'COH{\cdot}CH_2NR''_2$, where R contained an aryl nucleus (or in one compound was *n*-undecyl), R′ was usually H and NR''_2 usually NC_5H_{10}; most of these compounds had about the same order of activity as procaine in the guinea-pig weal test, but were much more active than procaine on the guinea-pig cornea. Fourneau and Samdahl[2] had previously prepared a series of dialkamines, derivatives of piperazine of the type

$$ROCH_2CHOH{\cdot}CH_2N{<}C_4H_8{>}NCH_2CHOH{\cdot}CH_2OR,$$

and had found that high local anæsthetic activity appeared when R was *n*-hexyl or *n*-heptyl. Although alkamines of the general types mentioned are unsuitable for clinical use, because they usually cause irritation at the site of injection, it seemed of interest to study another series in the hope that light would be thrown on the structural features which govern high local anæsthetic activity in this type of compound. We have prepared a series of aryloxypropanolamines of the general type: $ArOCH_2CHOH{\cdot}CH_2NR_2$, where NR_2 was piperidine (NC_5H_{10}) and Ar was an unsubstituted or substituted aryl nucleus; we have also prepared a short series of the same type in which Ar was α-naphthyl and R was H, C_2H_5, *n*-C_3H_7, *iso*-C_3H_7 and *n*-C_4H_9.

Local Anæsthetic Activity

Method. The local anæsthetic activities of our compounds were compared with that of procaine by the guinea-pig weal method of Bulbring and Wajda.[3] Two modifications of their method were introduced.

(1) Bulbring and Wajda divided the animal's back into 4 areas and gave each animal 4 injections, 2 concentrations of the standard drug and 2 of the compound of unknown potency. Since we wished to test the linearity of the log dose-response regression lines we used a device introduced by Somers and Edge[4] whereby 3 concentrations of a compound were compared with 3 concentrations of the standard in each of 6 animals, the locus of injection of each dose being changed from animal to animal. The use of 6 injections per animal probably sacrificed some accuracy in the method, since the area of maximum sensitivity on the animal's back is limited, but it was felt that this device was likely to be more satisfactory

for obtaining three-point regression lines than the use of different animals, of unknown variability, for a third point.

(2) Bulbring and Wajda used doses which gave a linear relation between log dose and response. The complete curve over the whole range of effective doses would of course be sigmoid and it is not always easy to arrange the dosage so that linearity is obtained. Somers and Edge used a probit scale, which improves the linearity but is based on the assumption that the responses are distributed normally. We used the method of Angular Transformation, whereby the percentage response is expressed as an angle from 0° to 90°. This method merely transforms the results from one arbitrary scale into another without involving any assumption about the normal distribution of responses. In practice we found that plotting the results on a degree scale gave better linearity than plotting them on a probit scale.

Results

The compounds tested and their molar potencies in terms of procaine are listed in Table I. The potencies were estimated graphically from the

TABLE I

Molar activities in the guinea-pig weal test in terms of procaine = 100

$ArOCH_2CHOH{\cdot}CH_2NR_2$

Ar	NR_2	Salt	Molar potency, procaine = 100
phenyl	piperidino	HCl	105
m-tolyl	,,	,,	130
p-tolyl	,,	,,	95
o-chlorophenyl	,,	,,	(90)
m-chlorophenyl	,,	,,	(95)
p-chlorophenyl	,,	,,	110
p-nitrophenyl	,,	free base	(70)
p-aminophenyl	,,	di-HCl	0
o-diphenyl	,,	free base	185 (approx.)
p-diphenyl	,,	HCl	140
α-naphthyl	,,	,,	(95)
β-naphthyl	,,	,,	170
α-naphthyl	NH_2	,,	56
,,	$N(C_2H_5)_2$	,,	140
,,	$N(n\text{-}C_3H_7)_2$	,,	200
,,	$N(iso\text{-}C_3H_7)_2$	,,	200

regression lines, the log dose corresponding to the ordinate of 45° being taken as the "median effective dose." A few compounds gave regression lines which were not parallel to that for procaine; for these compounds the molar potencies are recorded in parentheses in Table I, since compounds which do not bear a constant log dose-effect relationship with procaine cannot strictly be compared with it. Departure of the regression lines from parallelism with that of procaine was always associated with lesser slope, a result which suggests that the compounds had some vasodilator action, but this possibility was not investigated further. Aryloxyethylamine derivatives are well-known to have vasodilator actions, and our compounds might be expected to share this property.

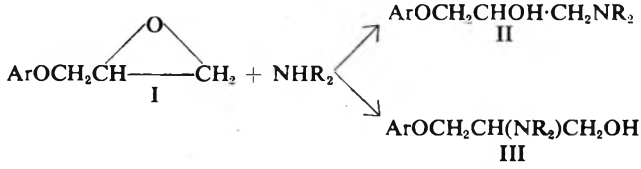
The potencies vary over a relatively narrow range from a half to twice that of procaine, except for *p*-aminophenoxypropanolpiperidine, which

was inactive. The *p*-amino group in this compound is, however, more strongly basic than that in procaine, as is shown by the fact that the *p*-aminophenoxy compound was isolated as its dihydrochloride. Apart from this, no clear-cut correlation between structure and action emerges other than the expected increase in potency in the α-naphthoxy series as the size of the N-alkyl group increases.

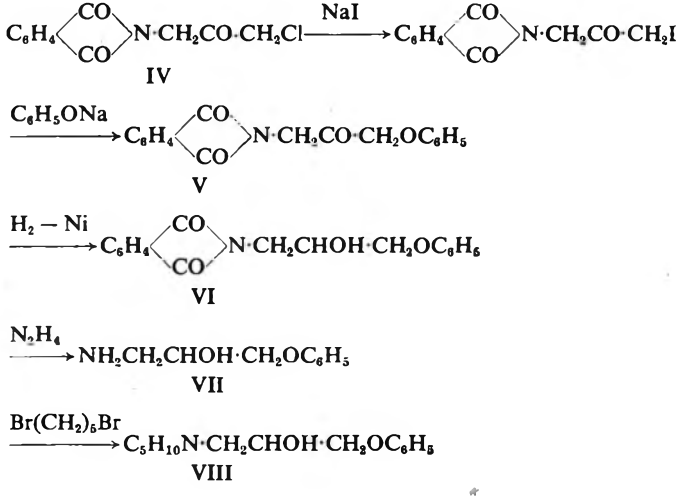
None of the compounds produced noticeable irritation at the site of injection except *o*-phenylphenoxypropanolpiperidine and α-naphthoxypropanolamine.

Chemical Section

The aryloxypropanolamines were all prepared by the condensation of the appropriate aryloxypropylene epoxide (I) with a secondary amine. Theoretically this reaction could give two products, (II) and (III),



of which (II) is the more likely. However, 3-phenoxy-2-hydroxypropylpiperidine was synthesised by an alternative route, the stages of which are outlined below:



The hydrochloride of the product (VIII) was identical with that of the base formed from phenoxypropylene epoxide and piperidine and consequently there seems to be no doubt that the general method (reaction of and aryloxypropylene epoxide with a secondary amine) gives products of the general structure (II).

Also 3-phenoxy-2-hydroxypropylphthalimide (VI) was prepared by three methods: from phenoxyphthalimido-acetone (V) as shown above,

from phenoxypropylene epoxide and phthalimide, and from 3-phenoxy-2-hydroxypropyl chloride and potassium phthalimide. Each method gave the same product, which after treatment with hydrazine (Ing and Manske)[5] gave the same phenoxypropanolamine hydrochloride (HCl salt of VII). This salt displayed a curious "double melting point": it melted at 135° to 136° C. to an opaque liquid which only became clear at 228° C. The hydrobromide behaved similarly, melting at 141° to 142° C. to an opaque liquid which became clear at 162° C.

Although epichlorhydrin reacts with phthalimide at 140° to 150° C. to give phthalimidopropylchlorhydrin, the structure of which is settled by its oxidation to phthalimido-chloro-acetone (IV) (Gabriel and Ohle[6]), phenoxypropylene epoxide can be heated with phthalimide at 180° C. for several hours without condensation occurring. If, however, a trace of potassium phthalimide is added to the mixture, condensation occurs rapidly and exothermically at 180° C., a result which suggests that it is the phthalimide anion which attacks the epoxide ring.

The aryloxypropanolamine salts prepared are listed in Table II, together with their melting-points and analyses. It will be noticed that few of them gave sharp melting-points although the analytical figures show them to have been substantially pure compounds. Fourneau,[7] who prepared a number of aryloxypropanoldimethylamines, found that these bases did not give crystalline hydrochlorides and we found it impossible to isolate a pure hydrochloride of α-naphthoxypropanoldimethylamine, although it gave a pure methiodide (Table II).

TABLE II

ARYLOXYPROPANOLAMINE SALTS

$ArOCH_2CHOH{\cdot}CH_2NR_2$

Ar	NR_2	Salt	M.pt. °C.	Formula	Found C per cent.	Found H per cent.	Theory C per cent.	Theory H per cent.
phenyl	NH_2	HCl	228	$C_9H_{14}O_2NCl$	53·0	7·0	53·1	6·9
		HBr	162	$C_9H_{14}O_2NBr$	43·5	5·6	43·6	5·6
phenyl	NC_5H_{10}	HCl (*a*)	150 to 152	$C_{14}H_{22}O_2NCl$	62·0	8·2		
		,, (*b*)	151 to 153	,,	61·7	8·0	62·0	8·2
m-tolyl	,,	,,	126 to 128	$C_{15}H_{24}O_2NCl$	63·1	8·3	63·2	8·3
p-tolyl	,,	,,	171 to 173	$C_{15}H_{24}O_2NCl$	62·8	8·0	63·2	8·3
o-chlorophenyl	,,	,,	148 to 150		54·8	6·8		
m-chlorophenyl	,,	,,	155 to 156	$C_{14}H_{21}O_2NCl_2$	55·4	7·0	54·9	6·9
p-chlorophenyl	,,	,,	156 to 158		54·6	6·8		
p-nitrophenyl	,,	free base	79 to 80	$C_{12}H_{20}O_4N_2$[1]	60·0	6·8	60·0	7·1
p-aminophenyl	,,	di HCl	262 decomp.	$C_{14}H_{24}O_2N_2Cl_2$	52·0	7·4	52·0	7·4
o-diphenyl	,,	free base	b.p. 240 to 250°/10 to 15 min.	$C_{20}H_{25}O_2N$	75·5	8·0	77·2	8·0
p-diphenyl	,,	HCl	197 to 199	$C_{20}H_{26}O_2NCl$	69·4	7·4	69·2	7·5
β-naphthyl	NC_5H_{10}	,,	175 to 176	$C_{18}H_{24}O_2NCl$	67·2	7·3	67·3	7·5
α-naphthyl	NH_2	,,	decomp. 200	$C_{13}H_{16}O_2NCl$	61·7	6·3	61·7	6·3
,,	NMe_2	methiodide	172 to 173	$C_{16}H_{22}O_2NI$	50·0	5·7	49·4	5·7
,,	NEt_2	HCl	127 to 130	$C_{17}H_{24}O_2NCl$	65·1	7·8	65·1	7·8
,,	NC_5H_{10}	,, (*a*)	183 to 186	$C_{18}H_{24}O_2NCl$	67·7	7·0	67·3	7·5
,,	,,	,, (*b*)	185 to 186		66·8	7·5		
,,	NPr^{α}_2	,,	155 to 156	$C_{19}H_{28}O_2NCl$	67·5	8·4	67·6	8·3
,,	NPr^{β}_2	,,	173	$C_{19}H_{28}O_2NCl$	67·6	8·3	67·6	8·3
,,	NBu^{α}_2	,,	95 to 98	$C_{21}H_{32}O_2NCl$	68·4	8·7	69·0	8·8

1. Found: N, 9·9. Required N, 10·0 per cent.

EXPERIMENTAL

(Analyses by Weiler and Strauss. Melting-points uncorrected.)

The aryloxypropylene epoxides were prepared by the method of Boyd and Marle.[8] The crude oily product was warmed on the water-bath with 50 per cent. aqueous potash in order to convert any chlorohydrin present into the epoxide and the latter was usually isolated by fractional distillation *in vacuo*, and converted into the alkamine without further purification. Three of the epoxides used proved to be crystalline solids, viz., *p*-nitrophenoxypropylene epoxide, crystallised from ether, m.p. 66° to 68° C. (not analysed); *p*-phenylphenoxypropylene epoxide, crystallised from light petroleum, m.p. 80° to 83° C. Found: C, 79·7; H, 6·3, $C_{15}H_{15}O_2$ requires C, 79·7; H, 6·2 per cent.; and β-naphthoxypropylene epoxide, crystallised from light petroleum, m.p. 62° to 64° C. Found: C, 78·0; H, 6·1. $C_{13}H_{12}O_2$ requires C, 78·0; H, 6·0 per cent.

The aryloxypropanolamines were prepared by heating equimolar amounts of epoxide and secondary amine for a short time on the water-bath. The reaction is exothermic so that completion of the reaction was readily ascertained by observation of the temperature; no solvent was necessary unless the amine was very volatile; e.g., dimethylamine was used in ethanol in a pressure bottle. The product was dissolved in acetone and acidified with concentrated hydrochloric acid. The alkamine hydrochloride usually crystallised, or could be induced to do so by the addition of ether. The hydrochlorides were recrystallised at least twice from ethanol-ether mixtures. Their melting-points and analyses are given in Table II.

The two primary amines listed in Table II, viz., phenoxy- and α-naphthoxy-propanolamine, were prepared from the corresponding phthalimides by Ing and Manske's method.[5] The phthalimides were prepared by heating equimolar amounts of epoxide and phthalimide with a trace of potassium phthalimide at 180° C. 3-Phenoxy-2-hydroxypropylphthalimide crystallised from ethanol or benzene-light petroleum, m.p. 116° to 117° C. Found: C, 68·7; H, 5·2. $C_{17}H_{15}O_4N$ requires C, 68·7; H, 5·1 per cent. 3-α-Naphthoxy-2-hydroxypropylphthalimide crystallised from ethanol, m.p. 152° to 153° C.

Alternative synthesis of 3-*phenoxy*-2-*hydroxypropylpiperidine*. 3-Chloro-1-phthalimido-acetone (Gabriel and Ohle[6]), dissolved in acetone, was treated with excess of sodium iodide in acetone. 3-Iodo-1-phthalimido-acetone crystallised almost at once; it was recrystallised from benzene, m.p. 180° to 183° C., yield 63 per cent. Found: C, 40·6; H, 2·5. $C_{11}H_8O_3NI$ requires C, 40·1; H, 2·4 per cent. 3-Phenoxy-1-phthalimido-acetone was prepared by treating the iodo-compound with a benzene suspension of sodium phenate (prepared from phenol and powdered sodium in benzene) for 12 hr. at room temperature. The reaction mixture was carefully acidified and steam-distilled to remove excess of phenol and benzene. The non-volatile residue was extracted with boiling xylene; the extract, after treatment with charcoal, deposited 3-phenoxy-1-phthalimido-acetone; m.p. 163° to 164·5° C., after recrystallisation from ethanol.

Found: C, 69·4; H, 4·6. $C_{17}H_{13}O_4N$ requires C, 69·2; H, 4·4 per cent. The same product was obtained by oxidising 3-phenoxy-2-hydroxypropylphthalimide (prepared from phenoxypropylene epoxide) with chromium trioxide in acetic acid.

3-Phenoxy-1-phthalimido-acetone, suspended in ethanol, was reduced over Raney nickel with hydrogen at room temperature and pressure (reaction time, 24 hours). The ready solubility of the product in ethanol made its isolation easy; it was crystallised from benzene light petroleum, m.pt. 112° to 114° C. Found: C, 69·5; H, 5·1. $C_{17}H_{15}O_4N$ requires C, 68·7; H, 5·1 per cent. Clearly the product was not so pure as the specimen prepared from phenoxypropylene epoxide and phthalimide, but removal of the phthalyl group with hydrazine gave 3-phenoxy-2-hydroxypropylamine hydrochloride identical with that produced through the epoxide, i.e., melting to an opaque liquid at 135° to 136° C. and to a clear liquid at 228° C. The free base, liberated from the hydrochloride, was heated on the water-bath with pentamethylene dibromide for 1 hour; alcoholic potassium hydroxide (1 mol.) was now added and heating continued; finally excess of amine was fixed by warming the mixture in aqueous alkali with toluene sulphonyl chloride. The mixture was acidified, extracted with ether, then basified with ammonia and the free base converted into its hydrochloride, which, after crystallisation from ethanol-ether, proved to be identical with 3-phenoxy-2-hydroxypropylpiperidine hydrochloride prepared from phenoxypropylene epoxide and piperidine, m.pt. 185° to 186° C., mixed m.pt. unchanged.

Summary

Sixteen aryloxypropanolamines of the general formula

$$ArOCH_2CHOH{\cdot}CH_2NR_2$$

were prepared and tested for local anæsthetic activity by the guinea-pig weal method. All but one were local anæsthetics, varying in activity from about a half to twice that of procaine.

We are indebted to the Medical Research Council for a grant to one of us (W.E.O.) which enabled us to undertake this work.

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THE MICROBIOLOGICAL ASSAY OF VITAMIN B_{12} IN CRUDE LIVER EXTRACTS

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The first indication that vitamin B_{12} was a growth factor for micro-organisms was provided by the work of Shorb,[1] who showed that it was essential for the growth of *Lactobacillus lactis* Dorner and that the amount of growth was proportional to the amount of vitamin B_{12} present. Shorb developed a turbidimetric method of assay, but this has since been found to give somewhat unsatisfactory results; in the first place the organism is rather unstable,[2] secondly, it will apparently grow without vitamin B_{12} under anaerobic or reducing conditions,[3,4] and, thirdly, thymidine and other deoxyribosides also stimulate the growth of the organism, making it essential to separate these from vitamin B_{12} prior to carrying out the assay.

Cuthbertson, Pegler and Lloyd[5] obtained more satisfactory results with *L. lactis* Dorner by using the cup-plate method of Bacharach and Cuthbertson.[6] In this method petri dishes containing a solid medium seeded with the test organism are used, holes are cut into the agar and filled with dilutions of the solution to be tested and the plates are then incubated. The organism forms zones of stimulation around the holes containing the vitamin B_{12} solution and the diameters of these zones are proportional to the concentration of the vitamin. Using this method Cuthbertson *et al.*[5] obtained satisfactory results with fiducial limits of ± 15 per cent. ($P = 0{\cdot}05$)—sufficiently narrow for routine purposes. Thymidine and other deoxyribosides when present form zones of stimulation somewhat fainter and more diffuse than those formed by vitamin B_{12} and these may interfere with the accurate measurement of the latter. Interference due to deoxyribosides can be eliminated by paper chromatography as described by Winsten and Eigen[7] and by Smith and Cuthbertson.[8]

Vitamin B_{12} is also necessary for the growth of *Lactobacillus leichmannii*; on the whole this has given more satisfactory results than has *L. lactis* in the assay of the vitamin turbidimetrically.[9,10,11] Like *L. lactis*, however, *L. leichmannii* is also stimulated by thymidine and other deoxyribosides.

We have used *L. leichmannii* for the assay of vitamin B_{12} by the cup-plate method and obtained satisfactory results with relatively pure solutions of the vitamin. With certain crude liver extracts, however, difficulties were encountered that have apparently not been previously reported.

In the turbidimetric assay of vitamin B_{12} by means of *L. leichmannii*, Hoffmann *et al.*[10] recommend that a second assay of the vitamin B_{12} solution should be carried out after digestion with alkali, e.g., by boiling the solution for 30 minutes after adjustment to *p*H 10. The difference

between the value thus obtained and that obtained on the untreated solution is said to be proportional to the amount of vitamin B_{12} present, since this is completely destroyed by alkali under the conditions used whereas thymidine and other deoxyribosides are unaffected.

In assaying crude liver extracts by the cup-plate method 3, or even 4, zones could sometimes be seen. The largest of these was easily distinguished from the others by reason of the less dense growth and was identical in appearance with the zone produced by thymidine, a specimen of which was kindly supplied to us by Professor A. R. Todd. This zone may occasionally interfere with the measurement of the other zones, but normally the cup-plate method enables vitamin B_{12} to be assayed in presence of thymidine without difficulty. In order to determine which of the other zones was due to vitamin B_{12} the solution was re-tested after digestion with alkali; it was found that the innermost zone generally disappeared and this zone was therefore assumed to be due to vitamin B_{12}. The results were accordingly calculated from the diameter of this zone. Sometimes, however, there appeared to be little difference in the appearance of the plates before and after alkaline hydrolysis, whilst occasionally one of the intermediate zones disappeared, and in such instances the results calculated from the diameter of the innermost zone were unsatisfactory for, when known amounts of vitamin B_{12} were added to the test solution and the assay repeated, the results obtained did not agree with the amount of vitamin B_{12} known to be present.

Winsten and Eigen[7] showed that vitamin B_{12} could be separated from thymidine and other deoxyribosides by paper chromatography. When the developed paper chromatogram was applied to agar plates seeded with *L. leichmannii* at least 6 zones of stimulation were observed; a similar result was obtained by Cuthbertson and Smith[8] with *L. lactis*. We applied this method to crude liver extracts and obtained several zones of stimulation. We had no difficulty in identifying the zones due to vitamin B_{12} and vitamin B_{12a}. However when the extracts were digested with alkali and the hydrolysates chromatographed on filter paper strips we were surprised to find that the chromatograms were apparently unchanged, a zone of stimulation being still present at the site normally occupied by the different forms of vitamin B_{12}. This indicated that in the crude liver extracts under examination there was present an alkali-stable growth factor for *L. leichmannii* with an R_F value close to that of vitamin B_{12} itself. In the turbidimetric assay of such crude extracts the method of Hoffmann *et al.* for correcting for the presence of alkali-stable substances would presumably give satisfactory results, but merely removing the deoxyribosides by paper chromatography would still give too high a result for the vitamin B_{12} content.

Attempts have been made to separate the alkali-stable factor from vitamin B_{12} in order to obtain more consistent assay results. Crude liver extracts were applied to columns of various ion exchange resins and some evidence of separation was obtained with Zeokarb 215. Better separation was obtained, however, by extraction with phenol which removed the vitamin B_{12}, leaving most of the alkali-stable factor in the aqueous phase.

The most satisfactory method proved to be that of Peeler and Norris,[12] in which the extract acidified to *p*H 2 was extracted repeatedly with a mixture of phenol and butanol equilibrated with water. Using this method the phenol-butanol extract gave a zone of stimulation that completely disappeared on alkaline hydrolysis, whereas the zone of stimulation given by the aqueous phase was unaffected by treatment with alkali.

In general it would appear that the results obtained in the microbiological assay of vitamin B_{12} in crude extracts by means of *L. leichmannii* should be interpreted with great caution. One of the great advantages of the cup-plate method over the turbidimetric method is that the former will discriminate between different growth factors (and inhibitors) present, whereas with the latter the amount of growth is a measure of the combined effect of each growth factor and growth inhibitor present. We believe that many of the results reported in the literature for the vitamin B_{12} contents of various natural products and crude extracts may be too high owing to the presence of other growth factors for which no allowance has been made.

It has been reported[13,14] that a mutant of *Escherichia coli* responds to methionine and vitamin B_{12} but not to thymidine. We have investigated the assay of crude liver extracts with this organism using the cup-plate method, and compared the results obtained with those given by *L. leichmannii*. In general the *E. coli* mutant gave results in good agreement with those given by *L. leichmannii*, although with some crude liver extracts, *E. coli* sometimes gave lower results. Such extracts also gave complex zones of stimulation with *E. coli*, although it has been stated that this organism responds only to methionine and to vitamin B_{12}. One of the zones of stimulation appeared to be due to the same alkali-stable factor as that which stimulated the growth of *L. leichmannii*. However, whereas *L. leichmannii* gave fairly satisfactory recoveries when known amounts of vitamin B_{12} were added to a solution of the alkali-stable factor freed from vitamin B_{12}, the results obtained with *E. coli* were much lower than would have been expected from the amount of vitamin B_{12} known to be present. This suggests that the solution contains in addition to the alkali-stable substance that stimulates the growth of both *L. leichmannii* and *E. coli* another substance that interferes with the utilisation of vitamin B_{12} by *E. coli*. This presumably explains the low results sometimes observed in the assay of the cruder types of liver extract by means of the *E. coli* mutant.

It has been suggested that the multiple zones of stimulation observed with the *E. coli* mutant might have been due to the formation of a mixture of variants in our particular culture, but precisely similar results have been obtained with a purified strain kindly sent to us by Dr. W. F. J. Cuthbertson.

Experimental

Assays with L. leichmannii. The medium now used as a routine has the composition given in Table I.

The *p*H of the medium can be varied between 5·0 and 6·5 without

affecting the diameters of the zones of stimulation; normally a *p*H of 5·5 is used. The test solutions are made up in phosphate buffer solution of *p*H 5·2.

Stock cultures of the organism are maintained on a soya bean medium and up to the present no indication has been obtained that the response of the organism to vitamin B_{12} has varied since the work was commenced. Cultures for assay are prepared by transferring a loopful of the stock culture to 10 ml. of the basal medium from which salt and agar have been omitted and to which has been added 2 per cent. of crude liver extract ("Hepolon"). The tube is incubated for 18 hours and then centrifuged for 10 minutes. The supernatant liquid is withdrawn and replaced by 10 ml. of 0·86 per cent. saline. The tube is then well shaken and the turbidity measured in an Eel nephelometer, and the result compared with that of an arbitrary standard. Each culture is diluted if necessary in order to obtain approximately the same density for each test. Too light an inoculum tends to give large zones with sparse growth.

An adequate amount of the basal medium is sterilised, cooled to 50° C. and the inoculum added with stirring. The requisite number of sterile 9 cm. petri dishes are put on to a level surface, and 25 ml. of the medium poured on to each plate, giving a depth of medium that in our hands has given the most satisfactory results. When the agar has set, 6 holes are cut in each plate with a sterile cork borer 6 mm. in diameter, any liquid that has collected in the holes is then withdrawn by suction, and 5 of the holes are then filled with the solutions to be tested, and the sixth hole with a standard solution (0·2 μg./ml.) prepared from crystalline vitamin B_{12} e.g., "Anacobin"). The plates are incubated at 37° C. for 18 hours. With each set of plates at least 2 plates are included containing standard solutions only, at concentrations of 0·03, 0·05, 0·1, 0·2, 0·4 and 0·6 μg./ml.

TABLE I

COMPOSITION OF *L. leichmannii* ASSAY MEDIUM

Glucose	10 g.
Sodium acetate	10 g.
Sodium citrate	10 g.
Acid-hydrolysed casein	5 g.
Dipotassium hydrogen phosphate	3 g.
Potassium dihydrogen phosphate	3 g.
Magnesium sulphate ($7H_2O$)	2·8 g.
Manganese sulphate	0·6 g.
Ferrous sulphate ($7H_2O$)	0·17 g.
Tween 80	1·0 g.
Sodium chloride	20 g.
L-Cystine	0·2 g.
Asparagine	0·1 g.
DL-Tryptophane	0·1 g.
Thioglycollic acid	1 ml.
Xanthine	10 mg.
Adenine	10 mg.
Guanine	10 mg.
Uracil	10 mg.
p-Aminobenzoic acid	2·5 mg.
Nicotinic acid	1·0 mg.
Riboflavine	0·8 mg.
Pyridoxine	0·2 mg.
Calcium pantothenate	0·2 mg.
Aneurine	0·2 mg.
Biotin	0·002 mg.
Pteroylglutamic acid	0·002 mg.
Agar	18 g.
Distilled water	1·0 l.

The diameters of the zones of stimulation obtained with these standard solutions are plotted against the logarithms of the concentrations of vitamin B_{12} and this gives the day's standard curve, from which the vitamin B_{12} contents of the unknown solutions are calculated. The inclusion of a standard solution on each plate enables any variation from plate to plate to be detected. A typical standard curve is shown in Figure 1A.

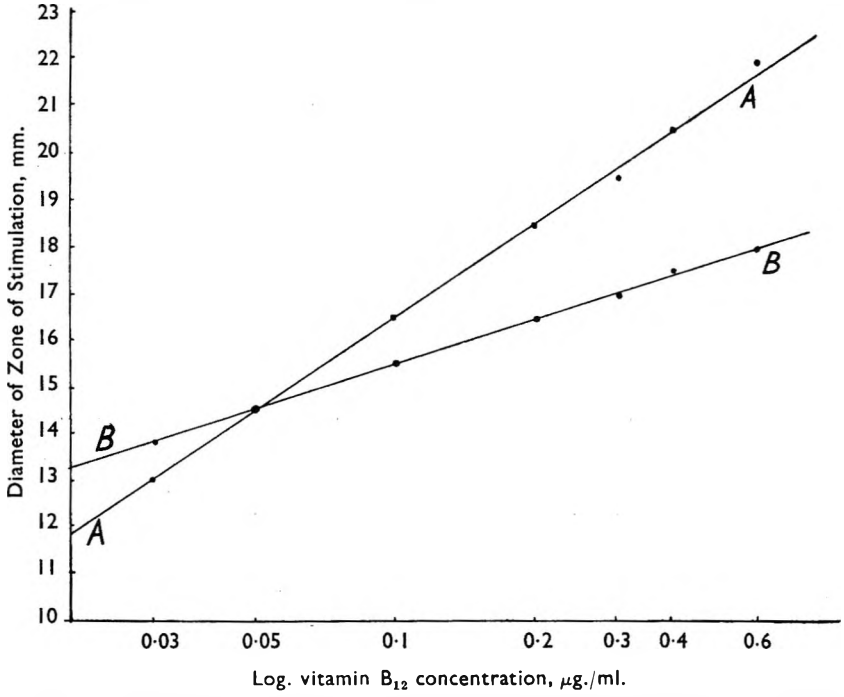

FIG. 1. Standard curves obtained by plotting the diameters of the zones of stimulation against the logarithms of the concentrations of vitamin B_{12} solution using (A) *L. leichmannii* and (B) the *E. coli* mutant, as test organisms.

Assays with E. coli. The medium used was that described by Davis and Mingioli,[13] and the organism was maintained in accordance with the recommendations of Bessell *et al.*[14] The experimental procedure was the same as with *L. leichmannii*, except that the medium was adjusted to *p*H 7·2 and the test solutions were diluted with a phosphate buffer solution of *p*H 7·0 to 7·2. A typical standard curve is shown in Figure 1B.

Results. Assays were carried out on a number of liver extracts made in Great Britain. The results, recorded in Table II, indicate that with most extracts *L. leichmannii* and *E. coli* give results that are in reasonably close agreement. Several of the crude liver extracts, however, gave lower results with *E. coli* and gave more than one zone with both organisms; one or two of the extracts gave at least 3 zones. The results are calculated on the assumption that the innermost zone is due to vitamin B_{12}.

Recovery experiments. Table III illustrates the results obtained when known amounts of vitamin B_{12} were added to liver extracts that gave only

TABLE II

POTENCIES OF LIVER EXTRACTS ASSAYED BY *L. leichmannii* AND *E. coli*

Extract and type	Stated potency	Potency found (μg./ml.)		Remarks
		L. leichmannii	*E. coli*	
A.1 Crude	—	1·7	1·0	Small zone after hydrolysis
A.2 Concentrated	—	4·5	4·3	Faint zone after hydrolysis
C. Crude	2 U.S.P. units	1·5	1·3	Faint zone after hydrolysis
E. Concentrated	12 μg./ml.	10·5	11·5	Faint zone after hydrolysis
H.1 Crude	—	0·5	0·1	Three zones, only one disappeared on hydrolysis
H.2 Concentrated	—	7·0	7·5	Faint zone after hydrolysis
H.3 Crude	2 μg./ml.	0·6	0·6	Faint zone after hydrolysis
L. Crude	—	1·2	0·8	Two zones, one remained after hydrolysis
N. Concentrated	12 μg./ml.	9·0	9·5	Faint zone after hydrolysis
P.1 Crude	1·5 μg./ml.	1·6	1·2	Two zones, haze after hydrolysis
P.2 Concentrated	20 μg./ml.	22·5	24	One zone, disappeared on hydrolysis
P.3 Crude	4 μg./ml.	4·5	4·0	Three zones; large zone left after hydrolysis

TABLE III

RECOVERY OF VITAMIN B_{12} ADDED TO LIVER EXTRACTS

Extract	Vitamin B_{12} content (μg./ml.)			
	L. Leichmannii assays		*E. coli* assays	
	Calculated	Found	Calculated	Found
A.2 + 1 μg. B_{12}	0·38	0·35	0·38	0·36
C. + 1 μg. B_{12}	0·32	0·28	0·31	0·28
E. + 1 μg. B_{12}	0·40	0·44	0·44	0·36
H.2 + 1 μg. B_{12}	0·52	0·44	0·52	0·50
H.3 + 1 μg. B_{12}	0·29	0·33	0·29	0·28
L. + 1 μg. B_{12}	0·40	0·35	0·34	0·28
N. + 1 μg. B_{12}	0·38	0·44	0·38	0·36
P.4 + 2 μg. B_{12}	0·5	0·5	0·8	0·8
P.4 + 6 μg. B_{12}	0·9	1·0	1·2	0·8
P.4 + 10 μg. B_{12}	1·3	1·6	1·6	1·2
P.5 + 1 μg. B_{12}	0·35	0·30	0·30	0·15
P.5 + 2 μg. B_{12}	0·55	0·40	0·50	0·40

2 zones with *L. leichmannii*, one due to vitamin B_{12} and the other to thymidine, and one zone with *E. coli*. The values thus obtained are in fair agreement with the values calculated from the amounts of vitamin B_{12} added, and indicate that the presence of thymidine in such extracts does not seriously interfere with measurement of the vitamin B_{12} zone. Table IV records similar results with an extract that gave a more complex system of zones and shows that unsatisfactory results are obtained whichever of the two inner zones is used as the basis of calculation.

Paper Chromatography. In view of the unsatisfactory results obtained with several of the cruder liver extracts, the method of Winsten and Eigen[7] was investigated. A 10-μl. spot of the test solution was applied to a strip of filter paper which was then dried and developed from the top

TABLE IV

RECOVERY OF VITAMIN B_{12} ADDED TO CRUDE LIVER EXTRACTS

Extract	Result calculated from	Vitamin B_{12} content (μg./ml.)			
		L. leichmannii assays		*E. coli* assays	
		Calculated	Found	Calculated	Found
H.3	Zone 1	—	0·2	—	0·25
	Zone 2	—	2·6	—	5·1
H.3 + 0·1 μg. B_{12}	Zone 1	0·15	0·1	0·2	0·1
	Zone 2	1·35	3·0	2·6	2·2
H.3 + 0·4 μg. B_{12}	Zone 1	0·3	0·15	0·3	0·1
	Zone 2	1·5	3·0	2·7	3·9
H.3 + 1·0 μg. B_{12}	Zone 1	0·6	0·1	0·6	0·2
	Zone 2	1·8	2·1	3·1	0·5

downwards with *n*-butanol saturated with water in the usual way. The paper strip was then dried and transferred to an agar plate seeded with *L. leichmannii*. The paper was either removed after a few minutes and the plate then incubated or the plate was incubated with the paper still in position. The second procedure gave the better defined zones, although both gave substantially the same results. The position of the vitamin B_{12} zone and the amount of vitamin B_{12} were determined by comparison with the chromatogram obtained when a 10-μl. spot of a standard solution of vitamin B_{12} was treated in the same way. Generally this standard solution gave a single zone, but occasionally a smaller second zone appeared slightly below it; this is presumably due to the formation of vitamin B_{12a} during chromatography.[15] Both these zones were absent after alkaline hydrolysis, as was the top zone of the chromatograms of all the concentrated liver extracts examined. With some crude liver extracts, however, the appearance of the chromatogram after alkaline hydrolysis was almost the same as before hydrolysis. In Table V are recorded some of the results obtained with different liver extracts when assayed directly and after paper chromatography. There is not very good agreement between the two sets of results, and it is by no means certain which method gives the more reliable estimate.

TABLE V

COMPARISON OF VITAMIN B_{12} CONTENT OF LIVER EXTRACTS, AS ESTIMATED BY DIRECT ASSAY AND BY ASSAY AFTER CHROMATOGRAPHY

Extract and type	Vitamin B_{12} content (μg./ml.)	
	Direct assay	Assay after chromatography
H.4 Crude	0·4	1·4
H.5 Crude	0·9	1·5
H.6 Concentrated ..	3·4	2·8
H.7 Concentrated ..	5·3	4·3
H.8 Crude	0·9	1·5
H.9 Crude	1·6	2·8
H.10 Crude	1·0	2·2
H.11 Crude	1·0	2·2
H.12 Crude	0·5	0·7
W. Concentrated ..	4·0	5·0, 6·0
A.3 Concentrated ..	1·7, 2·0	1·2

Effect of Cyanide, Reducing Agents, etc. When vitamin B_{12} is assayed turbidimetrically with *L. leichmannii* the depth of the medium in the assay tubes affects the response,[11,16] because the vitamin B_{12} requirement of the organism depends on the E_H of the medium. Hoffmann *et al.*[10] reported that thioglycollic acid increased the response of *L. leichmannii* to vitamin B_{12}. The addition of thioglycollic acid to crude liver extracts, however, did not affect the response under the conditions of assay used by us and did not minimise the interference caused by the alkali-stable factor. Aeration and treatment with mild oxidising agents likewise failed to affect the assay. It has been shown[17] that vitamin B_{12a} can be converted into vitamin B_{12} by the addition of cyanide to the aqueous solution, whilst it has also been suggested[18] that in liver extracts some or all of the vitamin B_{12} may be present in the form of a complex which is broken down by the addition of cyanide. With the possibility in mind that the alkali-stable substance present in crude liver extracts might be another form of vitamin B_{12} or might be a vitamin B_{12} conjugate, the effect of adding different concentrations of cyanide was investigated; no obvious effect could be detected.

Separation of Vitamin B_{12} from the alkali-stable factor. The first attempts to separate vitamin B_{12} from the alkali-stable factor were made with columns of ion-exchange resin. Columns of Amberlite IRC50, buffered at *p*H 5, 6, 7 and 8, were developed with 0·1N sodium hydroxide, but both factors ran quickly through the columns without any evident separation of one from the other. Similar results were obtained with columns of Amberlite IR400 and Amberlite IR100 buffered at *p*H 3 and 4 and with columns of Zeokarb 215 and 216 developed with 0·1N hydrochloric acid, although the eluates from the Zeokarb 215 columns gave larger vitamin B_{12} zones than did the original extracts.

Next, the extracts were acidified to *p*H 2 and extracted twice with an equal volume of 88 per cent. phenol. The phenol extracts were shaken with water and ether and the aqueous solutions tested. Again, larger vitamin B_{12} zones were obtained than were given by the original extracts, although some alkali-stable factor was probably still present.

Finally a method suggested to us by Peeler and Norris[12] was tried and was found to separate vitamin B_{12} completely from the alkali-stable factor. A 30 per cent. solution of phenol in *n*-butanol was shaken with a 2 per cent. solution of glacial acetic acid adjusted to *p*H 2·0 with hydrochloric acid and the two phases were allowed to separate. 5 ml. of the liver extract was diluted to 100 ml. with the aqueous phase of the equilibrium mixture and the *p*H maintained at 2·0. The solution was then shaken with an equal volume of the alcohol phase of the equilibrium mixture, and the resulting aqueous phase was separated and extracted another seven times with the alcohol-phenol mixture. The residual aqueous phase was shaken with ether to remove phenol and butanol, and then autoclaved at *p*H 1 to destroy thymidine. When assayed by means of *L. leichmannii* or *E. coli* it was found to contain no detectable amount of vitamin B_{12} but with both organisms it gave a large zone of stimulation that was still present after the solution had been boiled at *p*H 10 for 30 minutes. The

combined alcohol-phenol extracts were extracted with water in presence of ether and the aqueous phase evaporated to small volume. The resulting concentrate when assayed gave a zone of stimulation frequently greater than that produced by the original extract, and the zone disappeared completely on alkaline hydrolysis.

Recommended Procedure

In assaying crude liver extracts for vitamin B_{12} by the *L. leichmannii* or *E. coli* cup-plate methods, it is essential to repeat the assay after the extract has been boiled at *p*H 10 for 30 minutes. If a zone of stimulation similar to that given by vitamin B_{12} is still present, then it is recommended that an extraction with phenol-butanol be carried out as described above, and the concentrated extract assayed in the usual way.

Discussion

Although, so far as we are aware, this is the first publication in which attention has been called to the presence in liver extracts of an alkali-stable factor (other than deoxyribosides) that interferes with the microbiological assay of vitamin B_{12}, and in which a method of separating it from vitamin B_{12} has been suggested, there have been a number of references to such substances in earlier publications.

Thus Combs *et al.*[19] reported the existence in a liver concentrate of factors that promoted early growth in chicks and stimulated the growth of *L. leichmannii*. It was shown subsequently[20] that one or other of these factors could replace vitamin B_{12} in the metabolism of *L. leichmannii* and could be differentiated from vitamin B_{12} either by paper chromatography or by treatment with alkali. The new factor stimulated *L. leichmannii* in the early stages of the assay but prolonged incubation eliminated the need for the factor.[21] An alkali-stable factor was also found to be present in alfalfa, an observation confirmed by Bickoff *et al.*[22] Cronheim and Dannenburg[23] have also reported that the "vitamin B_{12}" activity of liver extracts as determined by *L. leichmannii* assays is not completely destroyed by treatment with alkali.

The interfering factor now reported by us appears to be different from that described by Peeler and Norris.[21] Whereas our factor appears to be present only in crude extracts and not in refined extracts Peeler and Norris isolated their factor from 15 unit U.S.P. liver extracts. Again, our factor appears to stimulate the growth of *L. leichmannii* independently of the presence of vitamin B_{12}, whereas Peeler and Norris had to use a dilute inoculum and a short period of incubation to differentiate their factor from vitamin B_{12}; indeed the factor appeared to be synthesised by *L. leichmannii* in the later stages of incubation.

Summary

1. Cup-plate methods for the microbiological assay of vitamin B_{12} with *Lactobacillus leichmannii* and a mutant of *Escherichia coli* are described. The results obtained by the two methods were in reasonably good agreement when applied to most commercial liver extracts.

2. Some crude liver extracts produced several zones of stimulation with both test organisms. One of these zones appeared to be caused by an alkali-stable substance which formed a zone near the vitamin B_{12} zone on paper chromatography. A method for separating this factor from vitamin B_{12} is described.

3. Some evidence was obtained that certain crude liver extracts also contain a substance that interferes with the response of the *E. coli* mutant to vitamin B_{12}.

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THE EFFECTS OF CORTISONE AND ADRENOCORTICOTROPHIC HORMONE ON POLIOMYELITIS AND ON OTHER VIRUS INFECTIONS

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There is growing evidence that under certain circumstances cortisone reduces the resistance of animals to bacterial infections; thus the infection becomes more intense after cortisone in experimental tuberculosis, syphilis, and pneumococcal septicæmia. There is also evidence that cortisone has a similar action in some virus infections. Schwartzmann[1] found that cortisone had an accelerating action on poliomyelitis in mice when the virus was inoculated intracerebrally. In addition, golden hamsters, *Mesocricetus auratus*, which normally are highly resistant to intracerebral inoculation with Lansing types of poliomyelitis virus, were found to become highly susceptible to this route of infection when MEF1 poliomyelitis virus was injected intracerebrally.

Experiments on Poliomyelitis in Mice

Experiment 1. 30 mice were inoculated intracerebrally with 0·03 ml. of a suspension containing approximately 100 LD50 doses of MEF1 poliomyelitis virus. 10 mice were left as controls: a second group of 10 mice was given 5 mg. of cortisone acetate 2 hours, and again 19 hours, after the intracerebral injection of virus: a third group of 10 mice received 5 mg. of ACTH intramuscularly 2 hours before being injected with the virus; 5 mg. of cortisone acetate was then injected intramuscularly 2 hours after the injection of the virus and the same dose was repeated 19 hours after the injection of virus. The average period from inoculation of virus to death is shown in Table I.

TABLE I

Number of mice	Treatment	Average period from inoculation to death days	Percentage mortality
10	Control	12·3 ± 3·2	90
10	ACTH and Cortisone	6·4 ± 2·6	100
10	Cortisone	7·5 ± 2·2	100

Experiment 2. A similar experiment was carried out with 30 mice, 15 mice being used as control and 15 receiving cortisone alone in the dosage as before. The results were similar: the controls died in an average of 14·2 days while the cortisone-treated mice died in an average of 6·2 days. One curious fact was that whereas the cortisone was invariably injected intramuscularly into the hind leg, paralysis almost always occurred in the front legs, thus showing that the paralysis was not due to the trauma occasioned by the cortisone injection.

Experiment 3. In this experiment ACTH alone was used. 40 mice were inoculated intracerebrally with 0·03 ml. of approximately 50 LD50 doses of MEF1 poliomyelitis virus in a suspension of mouse cord in saline solution. 20 mice were given 5 mg. of ACTH intramuscularly 2 hours before being injected with the poliomyelitis virus and again with the same

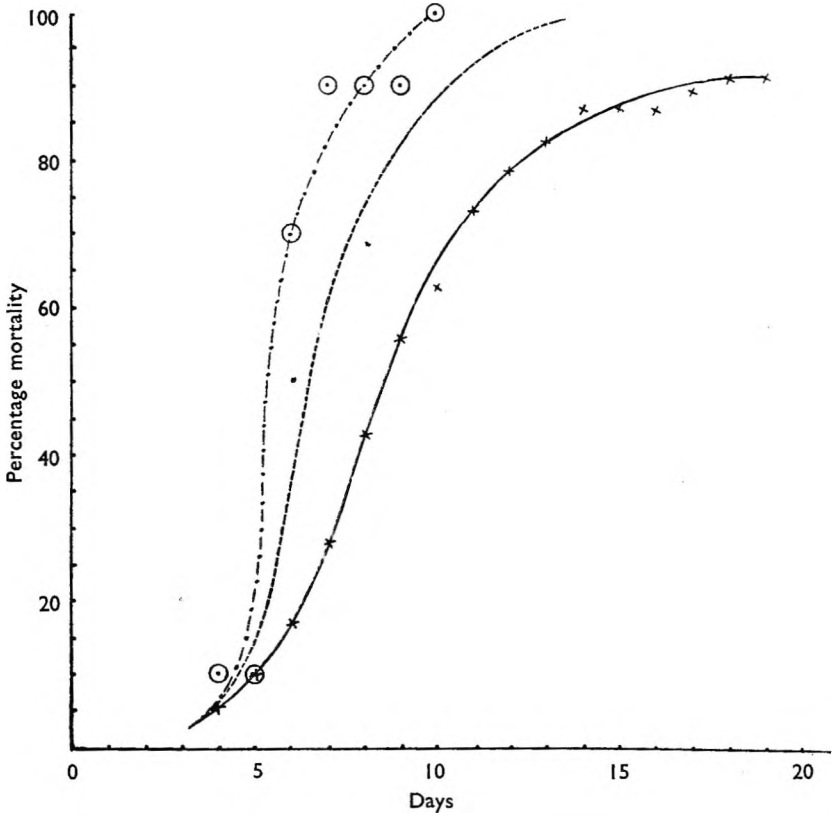

FIG. 1. Percentage mortality in days after inoculation of MEF1 poliomyelitis virus to:

——————— normal mice.
– – – – – – mice treated with cortisone.
– · – · – · – mice treated with cortisone and ACTH.

dose 22 hours after injection of the virus. The average period from inoculation to death was 10·2 days in the control mice and 9·8 days in the ACTH-treated mice. Of the ACTH-treated mice half were dead by the seventh day after infection, whereas of the control mice half were dead only by the tenth day after infection. Thereafter the ACTH-treated mice showed a slight retardation and 2 mice survived the observation period of 25 days. The effect of ACTH on the poliomyelitis infection was not therefore very noticeable.

Experiment 4. The experimental results described suggested that cortisone in some way reduced the resistance of the central nervous system

to poliomyelitis virus once it had gained entrance to the central nervous system. This reduced resistance might be due to a change in the nervous system whereby paralysis and death occurred in the presence of a smaller amount of virus than was required to produce paralysis in the normal mouse or to the more rapid multiplication of virus in the spinal cord.

In order to throw light on this point 6 mice were each injected intramuscularly in the right leg with 5·0 mg. of cortisone in 0·5 ml. of saline solution: 6 control mice were similarly given an intramuscular injection of 0·5 ml. of saline solution. 2 hours later the 6 cortisone-treated and the 6 control mice were injected with 0·03 ml. of 50 MLD50 of MEF1 poliomyelitis virus in the form of a saline suspension of mouse cord. 18 hours later on the following day the cortisone-treated group were again injected with 5·0 mg. of cortisone in 0·5 ml. of saline solution and the control mice received 0·5 ml. of saline solution. 24 hours after the original injection of cortisone 3 mice from the cortisone-infected and 3 from the saline-treated mice were killed. Their cervical and lumbar cords were dissected out. The 3 cervical cords were weighed together and ground up to form a 1 in 10 suspension in physiological saline solution: the 3 lumbar cords were similarly treated. Dilutions of the 1 in 10 suspensions in saline solution were diluted so as to provide suspensions of 1 in 100, 1 in 200, and 1 in 400. Batches of 4 mice were then inoculated intracerebrally with 0·03 ml. of each dilution of cervical cord and of each dilution of lumbar cord. 48 hours after the original injection of cortisone the remaining 3 mice in each batch were killed and suspensions of their cervical and lumbar cords were prepared as before, batches of mice receiving 0·03 ml. of each dilution as before. The period from intracerebral inoculation to death is shown in Table II: mice were observed for 25 days; S signifies survival for this period.

TABLE II

PERIOD OF SURVIVAL OF MICE IN DAYS AFTER INTRACEREBRAL INOCULATION OF CORDS FROM CORTISONE-TREATED AND FROM NORMAL MICE INFECTED WITH POLIOMYELITIS (MEF1 STRAIN)

Dilution of cord suspension	Cords from cortisone-treated mice killed after		Cords from saline-treated mice killed after	
	24 hours	48 hours	24 hours	48 hours
Cervical cord:				
1 in 10 ..	9, 14, 15, 16	9, 10, 10, 16	S, S, S, S	22, S, S, S
1 in 100 ..	4, 9, 13, 14	6, 10, 11, 20	S, S, S, S	S, S, S, S
1 in 200 ..	16, 20, 20, S	9, 11, 11, 13	S, S, S, S	S, S, S, S
1 in 400 ..	13, 17, S, S	11, 11, 13, S	S, S, S, S	S, S, S, S
Lumbar cord:				
1 in 10 ..	22, S, S, S	6, 15, 13, S	S, S, S, S	S, S, S, S
1 in 100 ..	S, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S
1 in 200 ..	S, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S
1 in 400 ..	S, S, S, S	S, S, S, S	S, S, S, S	S, S, S, S

S = Survival.

The evidence thus shows that poliomyelitis virus was present in cervical cords even 24 hours after intracerebral inoculation of suspensions of cervical cord from mice inoculated with MEF1 poliomyelitis and treated with cortisone whereas no virus was present in the cervical cords of mice similarly infected with MEF1 virus but injected intramuscularly with

saline solution only. This difference between cortisone-treated and saline-treated mice is even more marked when the cervical and lumbar cords were removed from mice 48 hours after injection of MEF1 virus.

The evidence thus suggests that cortisone increases the rate of multiplication of poliomyelitis virus in the spinal cord of mice. Cultures from the heart blood and brains of cortisone-treated mice showed no evidence of bacterial infection.

Experiments in mice using 5-pregnene-3-β-ol-20-one, in the same dosage as with cortisone, showed no effect in accelerating the development of poliomyelitis.

Experiments on Poliomyelitis in Golden Hamsters

Even more striking than the effect of cortisone on MEF1 poliomyelitis virus infection in mice were the results obtained by Schwartzman[1] in the golden hamster (*Mesocricetus auratus*). The experiments here recorded were carried out with the Lansing strain of poliomyelitis. This strain in mice is normally rather less active than the MEF1 virus and the intervals between inoculation and death of a batch of mice show a more irregular distribution. From time to time batches of golden hamsters have been inoculated intracerebrally with the Lansing strain of poliomyelitis virus: no symptoms have been seen and the animals have survived.

10 half-grown golden hamsters were inoculated intracerebrally with 0·05 ml. of a 1 in 20 suspension in saline solution of mouse cord infected with the Lansing strain of poliomyelitis virus. 5 of the hamsters were injected intramuscularly in the hind leg with 5 mg. of cortisone acetate in 0·5 ml. of physiological saline solution: the same dose of cortisone was injected intramuscularly 3 hours after the intracerebral injection of the poliomyelitis virus. 5 control hamsters were given intramuscular injections of physiological saline solution. Of the control hamsters 1 died within 3 hours as a result of the shock of inoculation. Of the cortisone-treated hamsters, 1 became paralysed after 3 days, 1 after 8 days, 1 after 11 days and 1 after 28 days. The fifth hamster survived. The control hamsters were observed for 35 days without symptoms. Just as was described by Schwartzman, the cortisone-treated hamsters at the same time as they developed paralysis were seen to have hunched backs, ruffled fur and conjunctivitis. As in the case of mice, paralysis first appeared in the front legs.

Cultures of heart blood from the paralysed hamsters were bacteriologically sterile. In order to be quite certain that an encephalo-myocarditis virus was not being carried over together with the Lansing poliomyelitis virus suspensions 1 in 5, 1 in 50 and 1 in 200 of the spinal cords from paralysed mice were mixed with normal rabbit serum and with rabbit serum containing immune bodies against Columbia SK virus. Mice were then injected intracerebrally with 0·03 ml. of the various suspensions. There was no difference in the time at which the two series of mice developed paralysis and died: in other words the Columbia SK immune serum had no action on the virus present in the cord suspensions which was therefore not a virus of the encephalo-myocarditis group.

One point of considerable interest was in the histological appearances of the cords from hamsters which had developed paralysis as a result of cortisone treatment. The anterior horn cells in the cervical cord especially showed widespread necrosis but there was remarkably little round cell or neuroglial reaction in the neighbourhood of the degenerate neurones. As no material was available for comparison from normal hamsters inoculated with the Lansing virus 6 young hamsters were injected intracerebrally with 0·05 ml. of a 1 in 10 suspension in saline of spinal cord from mice paralysed by the MEF1 virus. 2 of these hamsters became paralysed in 18 and 24 days after inoculation: histological examination of their spinal cords showed a much more intense reaction round the anterior horn cells.

Experiments with Other Viruses

A small number of experiments were carried out with other viruses to determine the effects of cortisone.

Rift Valley Fever Virus. The viscerotropic strain of this virus when inoculated intraperitoneally into mice causes diffuse necrosis of the liver. 2 experiments were carried out. 20 mice were injected intramuscularly with 5 mg. of cortisone acetate: 2 hours later these 20 mice and 24 controls were inoculated intraperitoneally with 0·1 ml. of dilutions of mouse blood infected with Rift Valley fever virus. The cortisone-treated mice were given a further intramuscular injection of 5 mg. of cortisone 3 hours after the intraperitoneal injection of the virus. The period from inoculation to death is shown in Table III.

TABLE III

MICE INOCULATED INTRAPERITONEALLY WITH RIFT VALLEY FEVER VIRUS

Dilution of blood	Time in days from infection to death	
	Control mice	Cortisone-treated mice
10^{-1}	2, 2, 2, 2	2, 2, 2, 2
10^{-4}	3, 3, 4, 5	2, 2, 2, 2
10^{-5}	3, 3, 4, 5	2, 2. 2, 2
10^{-6}	3, 4, 4, 5	2, 2. 2, 2
10^{-7}	S, S, S, S	3, 3. 3, 3
10^{-8}	S, S, S, S	5, 5. 6, S

S = Survival.

It will be seen that the infection in the cortisone-treated mice was much more rapid than in the control mice. Histological examination of the livers of the cortisone-treated mice, dying in 48 hours after an injection of 0·1 ml. of blood diluted 10^{-6} showed a diffuse liver necrosis with margination of the nuclear chromatin and acidophilic intranuclear inclusions entirely comparable to that seen in mice inoculated with infected blood diluted 10^{-1}.

Coxsackie Virus. Evidence has been obtained that young adult mice which show only a very slight multiplication of Coxsackie Virus in the muscles, when injected with cortisone exhibit a much more intense production of virus.

Encephalo-myocarditis Viruses. The Columbia SK Virus when inoculated intraperitoneally into mice gives rise to viræmia followed by the

production of encephalitis and myocarditis. When cortisone-treated and control mice are inoculated intraperitoneally with dilutions of Columbia SK virus it is again found that the cortisone-treated mice exhibit a rapid infection after inoculation with dilutions of infected mouse brain which kill normal mice only after a delay of 4 to 5 days.

Discussion

The evidence obtained with poliomyelitis viruses of the Lansing type, with Rift Valley fever virus, with Coxsackie viruses, and with encephalomyocarditis virus (Columbia SK and Senger Viruses) all points to the fact that cortisone and ACTH permit a more rapid multiplication of these viruses. How exactly this increased virus multiplication occurs is not known although there is a suggestion that the tissue reaction of the host to infection is in some way inhibited. This effect on the tissue reactivity is not specific to any one tissue; Kass *et al.*[2] found that in mice infected with influenza virus (a dilution of 10^{-5} intranasally) and treated with cortisone, 10 of 10 mice died in an average of 6·5 days: only 6 of 10 control mice died in an average of 8·5 days. In the survivors after 12 days the lung lesions were not extensive. Kligman *et al.*[3] similarly found that when 5 mg. of cortisone was injected daily into guinea-pigs for 2 days and vaccinia virus was then inoculated intradermally the lesions were more intense in the cortisone-treated animals than in normal guinea-pigs.

Conclusions

1. Treatment of mice infected with poliomyelitis virus by cortisone early in the infection results in a more rapid onset of paralysis and death than in control mice untreated by cortisone.

2. The evidence suggests that there is more rapid multiplication of virus in the cervical cord of cortisone-treated mice.

3. Injections of ACTH early in infection produce early paralysis and death in some mice but the effect is less marked than with cortisone.

4. Golden hamsters infected with Lansing poliomyelitis are highly resistant but if cortisone is injected at the time of infection the majority develop paralysis and die. This infection is due to poliomyelitis virus and not to an intercurrent infection.

5. There is evidence that cortisone increases the rate of multiplication and hence the rapidity and severity of symptoms in mice infected with the viruses of Rift Valley fever, encephalomyocarditis (Columbia SK) and Coxsackie (type A).

The cortisone used in this work was provided from a generous gift made jointly to the Medical Research Council and the Nuffield Foundation by Merck & Co., Inc. We wish to express our gratitude for this gift. One of us (G.M.F.) is in receipt of an expenses grant from the Medical Research Council.

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THE BIOLOGICAL ACTIVITY OF *trans*-4-CARBOXYVITAMIN-A ACID

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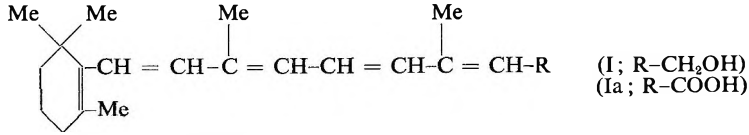
Although the biological activity characteristic of vitamin A (I) is shown by a number of simpler or derived products, the structural features associated with this type of activity have yet to be strictly defined. Marked structural alterations of (I) are known to lead to a pronounced drop in biological potency.[1,2] Replacement of the terminal $-CH_2OH$ group of (I) by carboxyl (Ia), however, gives a compound with biological activity equal to that of the parent vitamin.[3] (Ia) is, in fact, the most effective compound of this type yet to be synthesised. It was, therefore, of interest to determine the effect, if any, on biological activity exerted by a carboxyl group in position 4. To this end we have synthesised compounds A to D by methods described in detail elsewhere,[4] and now report the biological assay of these compounds employing the growth response of young, vitamin A-depleted, rats as a criterion of potency.

Compound A: *trans*-4-carboxyvitamin-A acid (II).

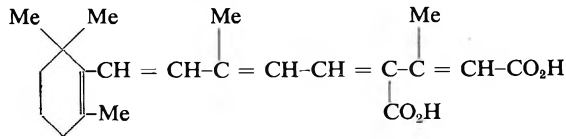
Compound B: Lithium aluminium hydride reduction product of Compound A.

Compound C: *cis*-4-Carboxy-vitamin-A-acid anhydride (III).

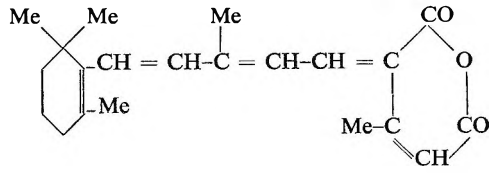
Compound D: The product $C_{22}H_{30}O_7$ obtained by condensing β-ionylidene acetaldehyde with α-carboxy-β-methylglutaconic acid triethyl-ester in the presence of methanolic potash. Two preparations of this compound (D1 and D2) were examined. [The formulation (IV) assigned to compound D from its mode of formation is not supported by light absorption data. Its structure must be considered unproven (cf. ref. 4).]



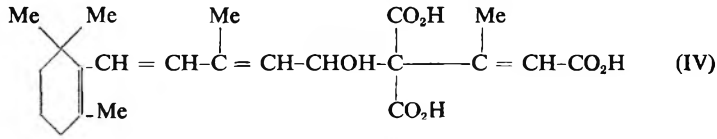
(I; R–CH_2OH)
(Ia; R–COOH)



(II)



(III)



(IV)

EXPERIMENTAL

The vitamin A-deficient diet used had the following composition:—

Fat-free casein	20 per cent.
Rice starch	57 „ „
Salts	5 „ „
Hardened vegetable oil ..	9·9 „ „
Dried yeast	8 „ „
Radiostol solution	0·1 „ „

The rats were placed on the diet at weaning or when they had reached a weight of 40 to 50 g. The animals were of either sex, but litter-mates were always taken in pairs of the same sex. After 3 to 4 weeks on the vitamin A-deficient diet, when depleted of vitamin A, the rats were given the test oils. In each experiment a certain number of animals were kept on the diet with no supplementary dose of vitamin A, as negative controls. In all experiments, groups of animals were given 2 or 3 different doses of vitamin A in the form of either the International standard for vitamin A (β-carotene in 1948-49), or a standardised solution of vitamin A acetate, the potency of which had been previously determined in terms of the International standard.

In experiment No. 3, compound D1, the International standard β-carotene solution, 200 I.U./g., was given in doses of 0·02 g. once or twice

TABLE I

GROWTH RESPONSES

Experiment	Dose per week I.U. or g.		Number of rats	Average growth per week g.
1	Vitamin A acetate (I.U.)	6 3 1·5	10 6 3	3·6 1·8 – 1
	Compound A (g.)	0·004	3	1
	Compound C (g.)	0·004	1	– 8 (5 died at 3 weeks)
2	Vitamin A acetate (I.U.)	4 2 1	14 12 7	2·7 1·1 0·7
	Compound B (g.)	0·004	21	3·3
3	β-carotene (I.U.)	8 4	5 4	6·8 4·9
	Compound D1 (g.)	0·004	11	4·6
4	Vitamin A acetate (I.U.)	4 2 1	14 10 7	2·7 1·2 0·8
	Compound D2 (g.)	0·004	28	4·1

weekly by pipette, i.e., 4 and 8 units. The animals receiving the dose once a week were given alternate doses of arachis oil.

In the other experiments the standardised solution of vitamin A acetate was diluted 1 : 70 with arachis oil and the potency checked by the Carr-Price blue value. Sub-dilutions were then made for 3 dosage groups: the doses were given in 0·02 g. by pipette. All the compounds were diluted 1 : 10 by weight with arachis oil. Compounds B, D and D2 were first dissolved in ether before the arachis oil was added, the ether then being evaporated off at 37° C. Compound A remained insoluble in ether and so was given as a 1 in 10 suspension in arachis oil. The growth responses are given in Table I. The experimental period was 3 weeks.

The approximate potencies calculated from the figures given in Table I are, therefore, as shown in Table II.

TABLE II

APPROXIMATE POTENCIES

Compound	Estimated potency I.U./g.
A	Approximately 500
B	Approximately 2,000
C	No activity
D1	Approximately 1,000
D2	Approximately 2,000

SUMMARY

The introduction of a carboxyl-group into position 4 of vitamin A and vitamin A acid leads to compounds with only slight biological activity.

The authors thank the Directors of The British Drug Houses, Ltd., for permission to publish these results.

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A PHOTOELECTRIC COLORIMETRIC METHOD FOR THE ESTIMATION OF ASCORBIC ACID

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Chemically, the outstanding property of ascorbic acid is its ease of oxidation. The oxidation-reduction properties of ascorbic acid are widely used as the fundamental reaction in the measurement of vitamin C. In such methods, acid extracts of the materials being assayed are prepared and the reducing capacity of the extract is measured by treatment with a suitable oxidising agent such as 2 : 6-dichlorophenolindophenol, iodine, ferricyanide, methylene blue, etc. Of these, oxidation with the dye 2 : 6-dichlorophenolindophenol[1,2,3] has found extensive use as the basis of techniques for determining ascorbic acid and has generally been found to be the most satisfactory.

In some materials, the value of the 2 : 6-dichlorophenolindophenol reagent for measuring ascorbic acid is limited by the presence of other reducing substances such as reductones, reductic acid and ferrous iron.[4,5] Ferrous ion reduces the dye in the presence of metaphosphoric acid, so that pharmaceutical preparations containing reduced iron should be titrated in 8 per cent. acetic acid solution, free from metaphosphoric acid. On the other hand, ferric ion interferes with the end-point in the absence of metaphosphoric acid, so that the metaphosphoric-acetic acid mixture should be employed as the titration medium when testing pharmaceutical preparations containing oxidised iron.[6]

Reaction of derivatives of ascorbic acid with 2 : 4-dinitrophenylhydrazine permits the determination of ascorbic acid by methods not based upon oxidation-reduction properties. Dehydroascorbic acid couples with 2 : 4-dinitrophenylhydrazine to produce a compound which, when treated with strong sulphuric acid, yields a red colour. These reactions have been employed[7,8] for the direct determination of dehydroascorbic acid, as well as for the measurement of the reduced form of the vitamin after oxidation by treatment with activated charcoal.

A serious objection to this method is the fact that diketogulonic acid, a biologically inactive oxidation product of vitamin C, reacts like the vitamin with dinitrophenylhydrazine.[9,10]

The Photoelectric Colorimetric Method

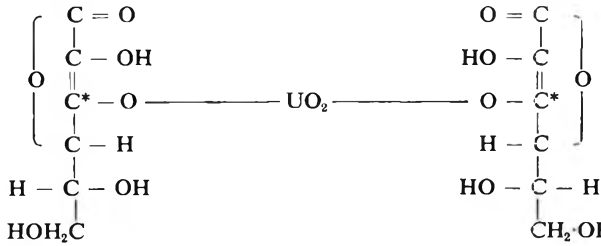
A new, simple and rapid method for the determination of ascorbic acid in pure solutions and in pharmaceutical products, which overcomes the disadvantages previously mentioned is advanced. This method is based on the colour reaction of aqueous 30 per cent. uranium nitrate solution with ascorbic acid (red in concentrated solutions and orange-red in dilute solutions). The colour is immediately obtained by mixing ascorbic acid

solution with the reagent at room temperature and the intensity is directly proportional to the vitamin content. The colour is stable in neutral solutions as well as in slightly acid and alkaline media, and is not affected by the action of heat. In a strongly acid medium the colour is discharged and in strongly alkaline solution a deep brown colour is obtained (on the gradual addition of the alkali) followed by precipitation of sodium uranate.[11,12] Since ascorbic acid is unstable in solution, especially in the presence of air or traces of metals such as copper and iron, and in light,[13,14] the estimation should not be delayed more than half an hour after mixing the ascorbic acid solution with the reagent.

Nature of the Colour Test. In solution, vitamin C exhibits acidic properties, the dissociation constants being $pK_1 = 4{\cdot}17$ and $pK_2 = 11{\cdot}57$. Ascorbic acid has the empirical formula $C_6H_8O_6$. It is a monobasic acid, giving well-defined salts of the type $C_6H_7O_6M$ (M = monovalent metal). The acidic properties of the vitamin are due to the hydroxy (enolic) group attached to the third carbon atom (asterisk in the formula given).[15,16,17,18,19]

A number of salts, for example, the sodium, copper, manganese and iron salts of ascorbic acid have already been isolated and found to possess antiscorbutic activity.[20]

Accordingly it is believed that this colour test is due to the formation of the corresponding metallic derivative, uranium ascorbate.



Dehydroascorbic acid and diketogulonic acid give no colour with the reagent.

This colour test is not based on the strong reducing ability of ascorbic acid, since it is not produced when a current of hydrogen is passed into 30 per cent. aqueous uranium nitrate.

A similar orange-red colour is given by the reagent with phenolic compounds such as an alcoholic solution of 2 : 4-dihydroxybenzaldehyde or salicylic acid and an aqueous solution of sodium salicylate or resorcinol.

On the other hand, a much paler colour is obtained with an alcoholic solution of phenol, and no colour is developed with an alcoholic solution of benzoic acid or an aqueous solution of sodium benzoate. This observation supports the idea that the enolic group of ascorbic acid is responsible for the development of the colour. Salicylates, if present in pharmaceutical preparations, can be eliminated in the form of salicylic acid by rendering the solution just acid.

This colour test may be applied for the estimation of ascorbic acid in food materials since the following substances which might interfere have

no influence on the colour; amino-acids such as glycine, alanine, valine, arginine and isoleucine; thiamine hydrochloride and riboflavine, carbohydrates such as glucose, lactose, sucrose and starch; diketogulonic acid, urea and uric acid, acetone, ethyl acetate and aceto-acetic ester. This colour test is not affected by the presence of other reducing substances such as ferrous salts, tartaric acid, oxalates, tartrates and citrates, formaldehyde, sodium sulphite, bisulphite and thiosulphate, thio-urea, hydrogen sulphide and pyridinium compounds. The presence of ferric salts does not interfere with the test; inorganic and organic ferrous and ferric compounds also interfere with the determination by the dye 2 : 6-dichlorophenolindophenol.[21]

This method avoids interference by reducing substances that may be present in association with ascorbic acid in pharmaceutical products and food materials. It includes only the determination of ascorbic acid, not of dehydroascorbic acid; the latter can be determined after reduction with hydrogen sulphide.[22]

The sensitivity of the test is 1 in 17,000.

Colour Tests for Ascorbic Acid. The following colour tests are based on the strong reducing property of ascorbic acid:

(1) To a solution of 10 mg. of ascorbic acid in 1 ml. of distilled water add 2 ml. of 8 per cent. aqueous ammonium molybdate solution; a yellow, green and then a deep blue colour, "molybdenum blue," develops in the cold.

(2) (*a*) When a solution of 10 mg. of ascorbic acid in 1 ml. of distilled water is treated with 2 ml. of sodium tungstate solution (10 per cent.) and the mixture warmed for 1 minute and treated with 3 drops of 20 per cent. sulphuric acid, a blue colour, "tungstic blue," is obtained.

(*b*) To 10 mg. of ascorbic acid in 1 ml. of distilled water, add 2 ml. of 10 per cent. sodium tungstate solution and 3 drops of 20 per cent. sulphuric acid in the cold; an orange-red colour develops which darkens on standing.

(3) To 10 mg. of ascorbic acid in 1 ml. of distilled water, add 2 ml. of potassium ferricyanide solution (5 per cent.) and heat for 1 minute; a blue colour develops which turns green on standing and deposits a blue precipitate.

(4) To 10 mg. of ascorbic acid in 1 ml. of distilled water, add 2 ml. of potassium chromate solution (10 per cent.); a green colour develops in the cold which turns to wine-red on adding 3 drops of 20 per cent. sulphuric acid.

(5) To 20 mg. of ascorbic acid in 1 ml. of distilled water, add 1 ml. of 10 per cent. platinic chloride solution and heat for 1 minute; a deep red colour is obtained.

Experimental

Preparation of the Metallic Derivative. To a solution of 1 g. of pure ascorbic acid (2 mol.) in 5 ml. of ethanol in a small crucible, 1·43 g. of uranium nitrate (1 mol.) in 5 ml. ethanol is added; on mixing well an

intensely dark red solution is obtained. This solution is concentrated on the electric plate to about 3 ml. and allowed to stand at room temperature, after which reddish-brown crystals separate out; these are filtered off and dried; m.pt. 178° to 180° C. with decomposition. The salt dissolves in distilled water giving a red colour, which is discharged on adding strong mineral acids; the colour becomes deep brown on adding 30 per cent. sodium hydroxide solution with the final precipitation of sodium uranate. The reddish, crystalline solid salt decomposes at room temperature when exposed to the atmosphere, being changed gradually to a yellow, somewhat elastic, non-crystalline mass.

Equipment and Reagents. (1) Lumetron photoelectric colorimeter using a yellow-green filter, 530 against water as the blank, set at 100 per cent. transmission.

(2) Two micro-pipettes (5 ml.), one of which is graduated.

(3) Uranium nitrate reagent, prepared by dissolving 30 g. of uranium nitrate in 100 ml. of distilled water and filtering if necessary.

(4) Standard stock solution of ascorbic acid prepared by dissolving 0·4 g. of pure ascorbic acid (previously dried over concentrated sulphuric acid in a desiccator to constant weight) in distilled water and completed to 200 ml. in a standard flask.

Procedure. Into a 25-ml. volumetric flask x ml. of the standard stock solution of ascorbic acid (1 ml. represents 2 mg. of ascorbic acid) is introduced. The volume is then completed to 25 ml. with distilled water, well mixed, and left to stand for about 5 minutes. Then 5 ml. of this solution is transferred to a dry colorimeter tube and 3 ml. of the uranium nitrate reagent added. The mixture is well mixed and its percentage transmission is read after 3 minutes in a Lumetron photoelectric colorimeter, Model 400–A using a yellow green filter, 530 against water as the blank, set at 100 per cent. transmission. $x = 25$ ml. of the standard stock solution of ascorbic acid (0·4 g. per 200 ml. w/v), representing a concentration of 10 mg. of ascorbic acid per 5 ml. of solution or 22·5, 20, 17·5, 15, 12·5, 10, 7·5, 5, 2·5 and 1·25 ml. of the standard stock solution of ascorbic acid representing concentrations of 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0·5 mg. of ascorbic acid per 5 ml. w/v respectively.

From the results obtained it was found that:—(*a*) transmission readings should be spread out sufficiently to allow a determination of ascorbic acid to be made within concentrations ranging from 0·5 to 10 mg. per 5 ml. w/v of ascorbic acid; (*b*) since the graph shows that within these concentrations there is a slight deviation from Beer's Law, a calibration table can replace the graph and give more accurate results, provided that the estimations are carried out at the same (room) temperature and under the same conditions.

Calibration Table. From the standard stock solution of ascorbic acid (0·4 g. per 200 ml.) standard solutions are prepared, so that 5 ml. of each dilution contains an amount of ascorbic acid ranging from 1 mg. to 10 mg., increasing in the order of 1 mg.; 5 ml. of each dilution is accurately measured in a dry, colorimeter tube; 3 ml. of uranium nitrate reagent is

added, well mixed and left to stand for about 3 minutes. The percentage transmission of the solution is then read. The results obtained at room temperature (28° C.) are shown in Table I.

TABLE I

CALIBRATION TABLE

Ascorbic acid mg.	Percentage transmission	Ascorbic acid mg.	Percentage transmission
10	22	5	45
9	25	4	52
8	28	3	61
7	32	2	72
6	38	1	84
		½	94

METHOD OF ASSAY

(1) *Test Solutions.* A dilution of the test solution is made so that 5 ml. contains an amount of ascorbic acid ranging between 0·5 and 10 mg. 5 ml. of this dilution is accurately measured into a dry colorimeter tube, 3 ml. of the uranium nitrate reagent is added, mixed well and left to stand for about 3 minutes. The percentage transmission of the solution is measured and the amount of ascorbic acid corresponding to this transmission is obtained from the calibration table, and the amount in the original test solution calculated. On applying this method to accurately weighed amounts of pure ascorbic acid the results obtained did not differ by more than ± 1 per cent.

(2) *Injections.* For the estimation of ascorbic acid in solutions prepared for injection the following method is recommended.

A known volume of the solution is diluted with distilled water in a standard flask so that 5 ml. contains 0·5 to 10 mg. of ascorbic acid. 5 ml. of this dilution is accurately measured and introduced into a dry colorimeter tube; 3 ml. of the uranium nitrate reagent is added and mixed well. The percentage transmission of the solution is measured and the concentration read from the calibration table. The figure obtained is the amount in mg. contained in 5 ml. of the diluted solution.

This method has been successfully applied to several kinds of injections obtainable in Egypt.

(3) *Tablets.* 10 tablets are weighed and powdered and an accurately weighed quantity of the powder, equivalent to 50 mg. of ascorbic acid, is introduced into a 50-ml. standard flask. Successive small quantities of distilled water (10 ml.) are added with continuous and vigorous shaking and the volume made up to the mark with distilled water. The mixture is well shaken for 15 minutes and filtered. The assay is carried out using 5 ml. of the filtrate, as described for injections. The result obtained multiplied by 10, gives the amount of ascorbic acid in the original weight taken. This method has been applied to two kinds of tablets common in Egypt giving very satisfactory results. The assay can be carried out even on one tablet giving reproducible results.

SUMMARY

1. A new colour test for ascorbic acid is described. The colour is shown to be due to the coloured uranium derivative.

2. A photoelectric colorimetric method for the assay of ascorbic acid is described. This method is recommended for the assay of pharmaceutical preparations such as injections and tablets. The assay is carried out within the limits of 0·5 to 10 mg. of ascorbic acid. Work is proceeding on the application of this method for citrus fruits.

3. New colour tests based on the strong reducing property of ascorbic acid are mentioned.

The authors wish to express their thanks to the Memphis Chemical Co. for the help extended to them in carrying out this work and for the supply of ascorbic acid preparations and instruments required.

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THE ASSAY OF LEPTAZOL

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Until recent years the determination of pentamethylenetetrazole presented great difficulties due to the absence of typical "functional groups"; furthermore it was found[1] that the estimation of the nitrogen content by the usual Kjeldahl process gave results up to 60 per cent. too low. Ultraviolet absorption measurements, suitable in the case of some tetrazoles, were also useless for leptazol as solutions were transparent in all utilisable parts of the spectrum.[2]

The compound is now determined by taking advantage of the sparingly-soluble nature of complexes formed with salts of the heavy metals, especially mercuric chloride[3] and cuprous chloride and that formed with the latter was thoroughly investigated by Dister.[4] The precipitate formed upon adding a solution of cuprous chloride to a solution of leptazol, under certain conditions, he found to have the composition $8C_6H_{10}N_4$, $7Cu_2Cl_2$, and to be practically insoluble in 1 per cent. acetic acid, which was therefore used for washing the complex. Dister assayed the complex for cuprous copper by dissolving it in warm ferric alum solution and then estimating the ferrous iron thereby produced by titrating with potassium permanganate solution. Good results were obtained.

The present author has found that the complex soon turns blue in the air, due to oxidation, and hence the assay must be completed quickly; to obviate this the method has been modified, the total copper in the filtrate being estimated after oxidising with hydrogen peroxide. By using a pipetted quantity of cuprous chloride solution for the assay and performing a "blank" the amount of copper removed as complex can readily be calculated. As total copper is estimated in each case, two or three precipitations and filtrations can be performed together and the filtrates assayed at leisure, as air oxidation will not vitiate the result once the complex has been removed.

At first great difficulty was experienced in obtaining consistent figures both by Dister's method, and by the modified method described; the fault was eventually traced to the cuprous chloride used in preparing the reagent. Not only must the reagent be freshly prepared but the cuprous chloride itself must be of good quality and should assay well for cuprous chloride by the ferric alum/permanganate method described below. Old samples of cuprous chloride are likely to consist largely of cupric compounds and although the reagent contains sodium metabisulphite (which would be expected to reduce all the copper to the cuprous state) poor results are obtained. The reason for this is not clear for although reagent made with an old sample was more acidic (pH 1·6) than one made with freshly prepared cuprous chloride (pH 2·7) the hydrogen ion concentration

of the reagent does not appreciably affect the results. This was readily ascertained by reversing the *p*H values of the two reagents by adding a sufficient quantity of N sodium hydroxide to the "old" reagent to raise its *p*H from 1·6 to 2·7, and N hydrochloric acid to the other to lower its *p*H from 2·7 to 1·6 and then repeating the assay with both of them. (See Table I.)

TABLE I

RESULTS OBTAINED WITH DIFFERENT SAMPLES OF CUPROUS CHLORIDE REAGENT

Reagent	pH	$C_6H_{10}N_4$ per cent.
(1) Prepared with fresh Cu_2Cl_2	2·7	99·8
(2) Prepared with old Cu_2Cl_2	1·6	77·5 84·2 68·0
(3) Prepared with fresh Cu_2Cl_2 but *p*H adjusted with HCl ..	1·6	97·5
(4) Prepared with old Cu_2Cl_2 but *p*H adjusted with NaOH	2·7	84·2

EXPERIMENTAL

Assay of Leptazol. Dissolve about 0·1 g. of sample, accurately weighed, in 25 ml. of water, and add slowly with stirring, 25 ml. of solution of cuprous chloride. Stopper the flask and allow to stand for 3 hours with occasional shaking. Filter through a sintered-glass crucible (No. 3) and wash the precipitate and flask with 30 ml. of water containing 1 per cent. w/w of glacial acetic acid. To the mixed filtrate and washings add 10 ml. of solution of hydrogen peroxide, and if necessary, a few drops of dilute sulphuric acid to clarify the solution. After effervescence has ceased, bring gently to the boil and continue boiling for 10 minutes to decompose the excess of hydrogen peroxide. Cool to room temperature add solution of ammonia drop by drop to obtain an opalescence and clear by addition, drop by drop, of acetic acid. Add 5 g. of potassium iodide, titrate with 0·1N sodium thiosulphate until the liquid is pale brown, add mucilage of starch and 3 g. of ammonium thiocyanate and continue the titration until the colour is discharged. Note the number of ml. required for the whole titration. Repeat the determination with 25 ml. of solution of cuprous chloride commencing with the words ". . . add 10 ml. of solution of hydrogen peroxide. . . ." The difference between the two titrations is equivalent to the amount of copper present in the precipitate. Each ml. of 0·1N sodium thiosulphate is equivalent to 0·007891 g. of $C_6H_{10}N_4$.

Cuprous chloride. This should be freshly prepared[5] or of reagent purity.

Assay. Dissolve by warming about 0·3 g., accurately weighed, in 20 ml. of a solution of ferric ammonium sulphate (5 per cent.) in sulphuric acid (20 per cent. w/w). Cool to room temperature and titrate with 0·1N potassium permanganate. Each ml. of 0·1N potassium permanganate is equivalent to 0·009903 g. of Cu_2Cl_2.

Solution of Cuprous Chloride (Dister's formula[4]). Dissolve 1·25 g. of cuprous chloride in 100 ml. of solution of ammonium chloride containing

1 g. of sodium metabisulphite. When solution is complete, filter if necessary. The solution should be freshly prepared.

Results. 0·1205 g. of sample gave (*a*) 100·1, (*b*) 99·5, (*c*) 99·8, (*d*) 99·8 per cent. of $C_6H_{10}N_4$.

Summary

1. The assay of leptazol introduced by Dister has been modified, the filtrate being titrated for total copper instead of the complex being assayed for cuprous copper. It is claimed that the method is quicker and more convenient, potassium permanganate being replaced by the more stable sodium thiosulphate as volumetric reagent.

2. It has been shown that the cuprous chloride used for preparing the precipitant must be either freshly prepared or of reagent purity.

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CHEMOTHERAPEUTIC STUDIES IN BACTERIOSTASIS

PART II. TERTIARY AMINES AND QUATERNARY AMMONIUM SALTS CONTAINING THE SKELETON OF *p*-TOLUIDINE

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From the Pharmaceutical Chemistry Research Laboratories of the School of Pharmacy, University of London

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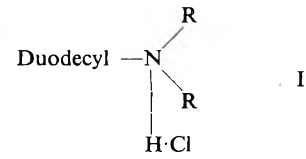
AMONG the chemical substances having antiseptic action, the surface active cations (such as long chain amino and quaternary salts) represent a well-defined class. The antibacterial action of these substances was reported by Hartmann and Kegi.[1] Domagk[2] first gave a detailed description of the bacteriological properties of some of the potent members of this group. Since then an ever-increasing number of cations with different types of substituents have been prepared and studied chemotherapeutically.

Stanley and his colleagues[3] who investigated a series of cyclohexyl-substituted amines of the formula I showed that the molecule of 16 to 18 carbon atoms possessed the highest antibacterial action.

$$C_6H_{11}\cdot(CH_2)_n\cdot N(Et)_2HCl \qquad \text{I}$$

Leffler and Volwiler[4] who investigated various duodecyl amines of the type II reported that the bacterial toxicity was greater when R was alkyl than when it was hydrogen.

Volko and Dubois[5] studied the effect of the side chain on the bactericidal power of several amines and quaternary salts, and made some important observations. (*a*) In a series of primary and tertiary amine hydrochlorides the activity reaches its maximum with the duodecyl chain; (*b*) In a series of higher aliphatic dimethylbenzylammonium chlorides the maximum potency is achieved with 12 to 14 carbon atoms; (*c*) in homologous series of alkyl dimethyl alkyl ammonium bromides, the antibacterial activity reached its peak at a chain length of 14 to 16 carbon atoms. It appears from these results that so long as the general molecular structure and the polar groups are unchanged, the length of the alkyl chain has a regular effect.



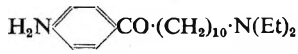
II

The mechanism by which amines and quaternary salts kill micro-organisms has not yet been clearly demonstrated but it bears no obvious relation to their effectiveness as emulsifiers, wetting agents or foaming agents, nor does it appear to be *entirely* connected with their ability as surface tension depressants. The bacterial action, like surface activity, reaches its peak and then declines. This probably means that as with phenols the initial action of these compounds depends on surface activity, when surface activity is present and is sufficient to pierce the plasma

membrane and render it permeable to the cation the difference in activity of the various cations may be a reflection of their efficiency as enzyme poisons.

In consideration of these facts, it appeared to be a matter of interest to introduce a skeleton of *p*-toluidine (*p*-aminobenzoyl and *p*-aminobenzyl group) into such compounds, possibly in the hydrocarbon chain. Such compounds, while retaining their surface active properties, might exhibit, at the same time, an increased enzyme activity associated with the metabolite group.

The synthesis of the following compounds was therefore undertaken.



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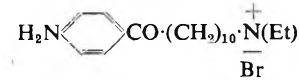

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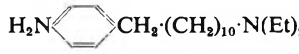

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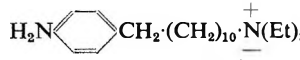

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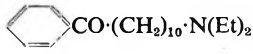

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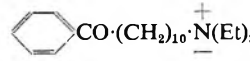

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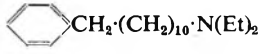

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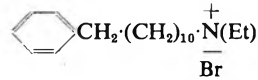
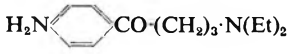


X

Unfortunately, the synthesis of the compounds III, IV, V and VI could not be accomplished because of certain difficulties which are mentioned in the experimental part. The synthesis of the last 4 has been accomplished.

During the investigation of the different methods for the synthesis of the first 4 compounds using easily available intermediates such as *β*-diethylaminoethanol, the following compounds were prepared:—



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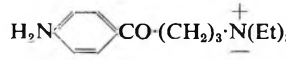

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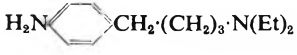

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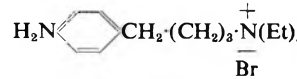


XIV

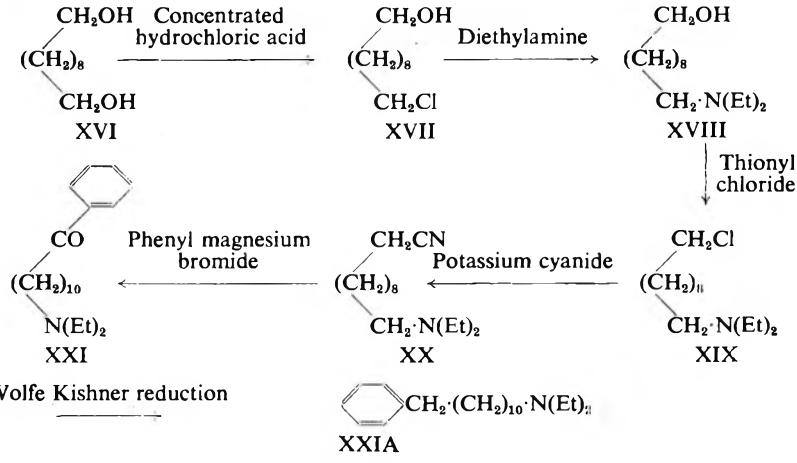
The therapeutic properties of these compounds are of interest since Woods[6] has reported that diethylaminoethyl-*p*-aminobenzoate (XV),

XV

a local anæsthetic, is as active as *p*-aminobenzoic acid in antagonising sulphonamide action. It appears likely that this molecule is hydrolysed to *p*-aminobenzoic acid in the body. The above substances which are similar in structure to this ester are incapable of hydrolysis and may, therefore, act as enzyme inhibitors.

The methods hitherto used for the synthesis of such compounds, consist of either reacting a diethylaminoalkyl cyanide with the appropriate Grignard reagent (phenyl magnesium bromide or *p*-aminophenyl lithium)[7] or condensing a long chain ω-halogeno-acid chloride with benzene or acetanilide by Friedel and Craft's reaction and reacting the resulting aroyl alkyl halide with diethylamine.[8]

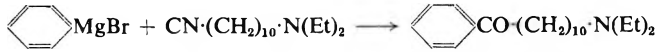
The application of the first method required the compound ω-diethylaminodecyl cyanide as an intermediate. This was prepared by chlorinating decamethylene glycol with hydrochloric acid, treating the chlorohydrin formed with diethylamine, chlorinating the resulting alcohol with thionyl chloride and replacing the chlorine atom by the cyano group with potassium cyanide. This method, although very lengthy, is comparatively easy to carry out.



The second step of this reaction series, viz., the synthesis of diethylamine decanol (XVIII) from the chlorohydrin, has been the subject of a patent.[9] It consists of refluxing chlorohydrin with large excess of diethylamine in anhydrous conditions for several hours. This procedure gave only about a 25 per cent. yield of compound XVIII. Increasing the quantity of diethylamine caused a drop in the refluxing temperature and a consequent drop in the yield. On the other hand, if an equimolecular quantity of diethylamine is used, the reaction can be carried out at a much higher temperature and the yield is quantitative.

The synthesis of the proposed ketones from the cyano compound XX by the Grignard reaction proved to be a difficult problem. Although compound XX reacted easily with phenyl magnesium bromide in ether to give 1-benzoyl-10-diethylaminodecane (XXI) in 50 per cent. yield it

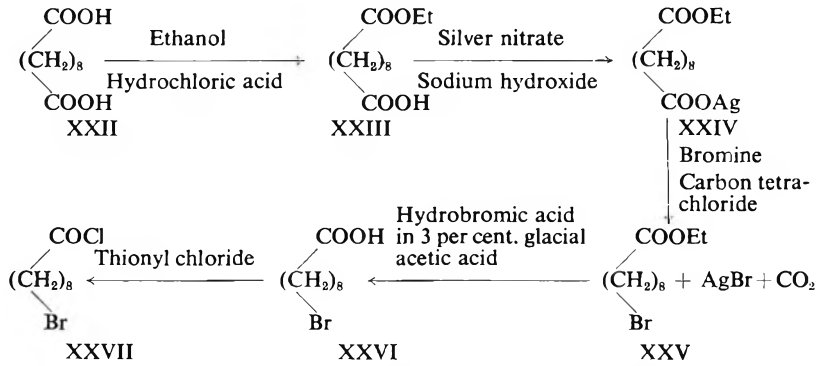
would not react with *p*-aminophenyl lithium in the same manner (even after refluxing for 12 hours).



XXI

At this stage resort was made to the second method—the Friedel and Craft reaction between ω-halogeno acid chloride and acetanilide.

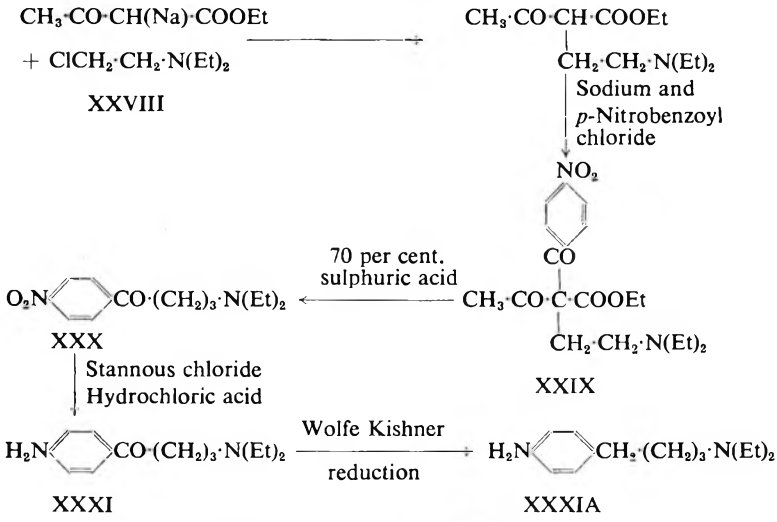
Sebacic acid provided a convenient starting point for this process. This acid was half esterified according to the process of Swan and Oehler[10]; the resulting acid ester XXIII was converted into its silver salt XXIV and brominated in carbon tetrachloride. The decarboxylation and bromination took place simultaneously and ethyl-ω-bromo octoate (XXV) was produced in excellent yields. This was hydrolysed with 3 per cent. hydrobromic acid in glacial acetic acid and the acid (XXVI) produced was converted to acid chloride (XXVII) with thionyl chloride.



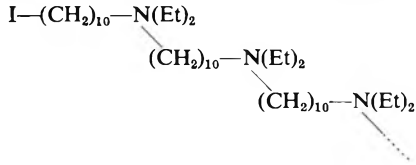
The condensation of XXVII with acetanilide was attempted in carbon disulphide using aluminium chloride catalyst according to the directions of Kunkell,[8] but unfortunately the activity of the long chain acid chloride proved to be insufficient to cause the acylation. The use of nitrobenzene as solvent also proved ineffective.

A third attack on the synthesis of the long chain amino ketones was developed using acetoacetic ester. Owing to the difficulty of obtaining the required diethylaminodecyl chloride, the method had to be explored with diethylaminoethyl chloride which was easily available by treating the corresponding alcohol with thionyl chloride. The reaction of this chloride (XXVIII) with sodioacetoacetic ester followed by replacement of the second hydrogen atom with *p*-nitrobenzoyl chloride gave the compound XXIX. This on treatment with 70 per cent. v/v sulphuric acid at 100° C. gave compound XXX, the decarboxylation and hydrolysis of the acetyl group taking place simultaneously.

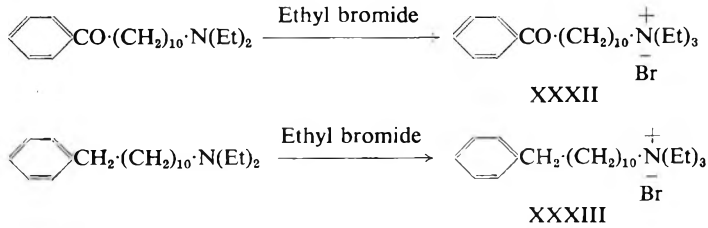
Application of the above method to the synthesis of long chain ketones proved to be unsuccessful. Diethylaminodecyl chloride, unlike compound XXVIII, failed to react with acetoacetic ester even after 48 hours; the activity of the terminal chlorine atom of the former compound appeared



to be insufficient. Replacement of chlorine by iodine was attempted by refluxing the compound with sodium iodide in acetone, but this gave rise to polymerisation probably on the lines shown below.



The reduction of the ketones (XXI and XXXI) to the corresponding alkyl derivatives (XXIA and XXXIA) was achieved in good yields by using the Haung-Minlon modification[11] of the Wolfe Kishner process. The compounds were isolated from the reaction mixture by dilution with water and extraction with ether.



The quaternary ammonium salts of all the 4 tertiary amines (XXI, XXIA, XXXI and XXXIA) were prepared by treating them with ethyl bromide. The compounds XXI and XXIA were easily quaternised by refluxing with ethyl bromide for 2 hours on a water-bath. Evaporation of excess of ethyl bromide followed by crystallisation from absolute ethanol gave colourless very hygroscopic crystals of XXXII and XXXIII.

The quaternisation of the amines XXXI and XXXIA presented some

difficulty. As they contain an aromatic primary amino group which is easily attacked by the alkyl halide, the above simple procedure could not be used, and an alternative method had to be developed. Successful results were obtained by the following procedure. The amines were acetylated in benzene solution with a similar solution of acetic anhydride, the acetyl derivatives were quaternised with ethyl bromide and the acetyl group was removed by heating with hydrobromic acid. Both compounds XII and XIV crystallised from boiling anhydrous methanol in pale yellow crystals changing to brown on standing. These were moderately readily soluble in hot methanol, practically insoluble in absolute ethanol and were extremely hygroscopic.

Bacteriological tests are presented at the end of the paper.

Experimental

Decamethylene glycol (*XVI*). This was obtained by the reduction of ethyl sebacate with sodium and ethanol, according to the method of Menske.[12] Yield 82 per cent., m.pt. 71° C.

Decamethylene chlorohydrin (*XVII*). This was synthesised by refluxing the glycol with concentrated hydrochloric acid sp. gr. (1·18) for 4 hours according to the directions of Alberti and Smiecinszewski.[13] Yield 60 per cent.; b.pt. 164° to 166° C./24 mm.

1-*Hydroxy*-10-*diethylaminodecane* (*XVIII*). Compound XVII 30 g. and diethylamine (11 g.) were refluxed on an oil bath for 24 hours. Crystals gradually separated. The mixture was made strongly alkaline with dilute sodium hydroxide solution and extracted with ether. The ether and excess diethylamine were removed on a water-bath, the higher boiling residue was mixed with ether and extracted with 2N hydrochloric acid. The acid phase, on being made alkaline, liberated the base which was extracted with ether dried over anhydrous magnesium sulphate and distilled. Pale yellow oil; b.pt. 178 to 183° C./16 mm.; $n_D^{18°C.}$, 1·4581. Yield 90 per cent.

1-*Chloro*-10-*diethylaminodecane* (*XIX*). This was prepared according to a method described in a patent.[9] A colourless oil, b.pt. 173° to 175° C./20 mm. Yield 6 g.

1-*Cyano*-10-*diethylaminodecane* (*XX*). In a 50-ml. flask equipped with a mercury-sealed stirrer, a reflux condenser and a dropping funnel, was placed a solution of potassium cyanide, 95 per cent. (2·3 g.) in water (5 ml.). The flask was heated on a sand bath and, while refluxing, a solution of XIX (8·5 g.) in ethanol (15 ml.) was added drop by drop with stirring. The mixture was kept refluxing and stirring for 12 hours, cooled, saturated with sodium carbonate and extracted with ether. The ether phase was dried over anhydrous sodium sulphate. The ether was removed and the residual oil subjected to fractional distillation.

1-*Cyano*-10-*diethylaminodecane* (*XX*) distilled at 125° to 130° C./6 mm. as a colourless liquid with a slightly fishy odour. $n_D^{17°C.}$, 1·4518. Yield 5·1 g. Found: C, 74·8; H, 12·8; N, 12·02; $C_{15}H_{30}N_2$ requires C, 75·63; H, 12·6; N, 11·75 per cent.

1-*Benzoyl*-10-*diethylaminodecane* (*XXI*). In a 100 ml. round-bottomed

flask equipped with a mercury-sealed stirrer, a reflux condenser protected by a calcium chloride tube and a dropping funnel (also protected by a calcium chloride tube) were placed clean, dry magnesium turnings (0·3 g.) ether (5 ml.) and a crystal of iodine. In the funnel was placed a solution of bromobenzene (1·9 g.) in ether (5 ml.). The addition of 1 ml. of this solution and gentle heating started a prompt reaction; with stirring the remainder of the solution of bromobenzene was added at a rate which maintained vigorous refluxing. The mixture was stirred and refluxed for 30 minutes and then a solution of compound XX (3 g.) in ether (60 ml.) was added drop by drop over a period of 30 minutes. Stirring and refluxing were continued for a further 6 hours. The cooled mixture was then decomposed by the slow addition of aqueous ammonium chloride (10 ml.). The ether was removed on a water-bath. After heating for 1 hour to insure hydrolysis of ketimines the product was extracted with ether, dried over anhydrous sodium sulphate and fractionally distilled. The first fraction, boiling at 162° to 175° C./6 mm., was discarded. 1-Benzoyl-10-diethylaminodecane came over at 214° to 216° C./6 mm. as a pale yellow oil; $n_D^{20°C.}$, 1·4954. Yield 1·8 g. (50 per cent.). *Picrate*, pale yellow needles from ethanol, m.pt. 58° to 59° C. Found: C, 59·21; H, 6·87; N, 9·95; $C_{27}H_{38}N_4O_8$ requires C, 59·33; H, 6·96; N, 10·25 per cent.

1-*Benzyl*-10-*diethylaminodecane* (*XXIA*). The ketone XXI was added to ethylene glycol (35 ml.) containing hydrazine hydrate 85 per cent. (3 ml.) and sodium hydroxide (2·5 g.). The mixture was refluxed over a free flame for 1 hour. The condenser was then removed and the thermometer fixed so that the bulb was placed in the liquid, the refluxing was continued thus until the thermometer recorded 195° C. approx., when the condenser was replaced, and the refluxing continued for 3 more hours. After cooling, the contents of the flask were diluted with water (100 ml.) and extracted with ether; the ether extract was dried over anhydrous sodium sulphate and distilled. 1-Benzyl-10-diethylaminodecane distilled at 160° C./0·2 mm. as a colourless oil, soluble in all the usual organic solvents and in dilute mineral acids. Yield 2·2 g. Found: C, 83·36; H, 12·04; N, 4·30; $C_{21}H_{37}N_1$ requires C, 83·44; H, 11·92; N, 4·63 per cent.

Attempted Synthesis of *p*-Aminobenzoyl-alkyl-diethylamine by Friedel and Crafts's Reaction

Ethyl hydrogen sebacate (*XXIII*). This was prepared from sebacic acid according to the method described in "Organic Synthesis."[10] B.pt. 183° to 187° C./6 mm., m.pt. 34° to 36° C. Yield 68 per cent.

Silver salt of ethyl hydrogen sebacate. Ethyl hydrogen sebacate (130 g.) was dissolved in N potassium hydroxide, and silver nitrate (92 g.) was added. The solution on heating formed a white bulky precipitate of the silver salt which was filtered, washed free from silver nitrate and dried at 60° to 70° C., powdered and dried again. Yield 152 g.

1-*Carbethoxy*-8-*bromo-octane* (*XXV*). The silver salt 130 g. was suspended in anhydrous carbon tetrachloride (260 g.) and bromine (62 g.)

was added drop by drop with occasional stirring and cooling over a period of 30 minutes. The mixture was then heated on a water-bath for 15 minutes, cooled and filtered; the filtrate was shaken with anhydrous potassium carbonate to remove any unchanged acid, the solvent distilled off and the residual oil distilled under reduced pressure. 1-Carbethoxy-8-bromoctane was obtained as a colourless oil b.pt. 180° C./30 mm. $n_D^{20°C.}$, 1·4610. Yield 65 per cent. (with reference to the silver salt).

8-*Bromo-octane*-1-*carboxylic acid* (*XXVI*). The ester XXV was dissolved in a solution of hydrobromic acid 3 per cent. in glacial acetic acid 120 ml. and water 10 ml. and refluxed on a free flame for 10 hours. The acetic acid was removed under slight vacuum. The residual acid solidified on cooling. It was purified by dissolving in dilute sodium hydroxide, extracting the impurities with ether and acidifying the aqueous solution with hydrochloric acid. The acid separated as an oil and on cooling solidified to crystals of m.pt. 25° to 30° C. Recrystallisation from aqueous methanol raised the m.pt. to 36° C. Yield 11 g. (45 per cent.).

Evaporation of the ether phase gave 7 g. of the unchanged ester.

8-*Bromo-octane*-1-*carboxylic acid chloride* (*XXVII*). The acid (XXVI) (9 g.) and thionyl chloride (13·2 g.) were refluxed together for 2 hours. The excess of thionyl chloride was removed under reduced pressure, using a water-pump, and the residue distilled *in vacuo* using an oil pump. 8-Bromo-octane-1-carboxylic acid chloride came over as a colourless oil b.pt. 153° to 155° C./12 mm. Yield 87 per cent.

This substance gave a test for ionisable chlorine, and on boiling with aqueous sodium hydroxide and acidification gave the corresponding acid, thus proving its constitution.

The attempted condensation of compound XXVII with acetanilide in the presence of anhydrous aluminium chloride failed to give the desired ketone. The reaction mixture on distillation gave back all the acetanilide and some 8-bromo-octane-1-carboxylic acid.

Attempts to Synthesise *p*-Aminobenzoyl-alkyl-diethylamine Using Acetoacetic Ester

3-*Carbethoxy*-1-*diethylaminopentan*-4-*one*. This was prepared from ω-diethylaminoethyl chloride according to the directions of Breslow, Yost *et al.*[14] The product was purified by vacuum distillation. 3-Carbethoxy-1-diethylaminopentan-4-one was obtained as a colourless oil, b.pt. 144° to 148° C./12 mm. Yield 82 per cent.

1-(4′-*Nitrobenzoyl*)-3-*diethylaminopropane* (*XXX*). Sodium (4·8 g.) was finely divided in toluene, washed with dry benzene and suspended in dry benzene (260 ml.) in a flask provided with a mercury-sealed stirrer, a reflux condenser and a dropping funnel and 3-carbethoxy-1-diethylaminopentan-4-one (50 g.) was added at room temperature. The mixture was heated on a steam bath with stirring until all the sodium had dissolved (3 hours). The solution was cooled to about 40° C. and a solution of *p*-nitrobenzoyl chloride (37 g.) in benzene (140 ml.) was added drop by drop with stirring. The reaction mixture was heated on a steam bath with stirring for 2 hours; kept at room temperature overnight and refluxed for

2 more hours next day. The separated solid, which consisted of *p*-nitrobenzoic anhydride and sodium chloride, was filtered off, and the benzene removed under slight vacuum. The residue was diluted with anhydrous ether and some more *p*-nitrobenzoic anhydride which separated, was again filtered off. The ether solution was shaken twice with aqueous sodium carbonate, dried over anhydrous sodium sulphate and evaporated to remove the ether.

The viscous residue (3-carbethoxy-1-diethylamino-3-(4′-nitrobenzoyl)-pentan-4-one) was warmed on a steam bath for 20 minutes with 70 per cent. v/v sulphuric acid (280 ml.), stirring by hand in a vessel large enough to allow frothing. Some more *p*-nitrobenzoic acid separated; this was filtered off quickly through sintered glass; the filtrate was cooled and neutralised cautiously with solid sodium carbonate. The liberated base was extracted with ether, dried over anhydrous sodium sulphate; the ether was removed and the residue fractionally distilled under vacuum. The 1(4′-nitrobenzoyl)-3-diethylaminopropane distilled at 180° to 190° C./0·1 mm. as a dark red oil, soluble in ether, in benzene, in ethanol and in dilute mineral acids, insoluble in light petroleum. Yield 25 g. (43 per cent.).

The following derivatives were prepared:—*Picrate*, long yellow needles from acetic acid m.pt. 169° C. Found: C, 48·8; H, 4·8; N, 14·17; $C_{20}H_{23}N_5O_{10}$ requires C, 48·67; H, 4·6; N, 14·2. 2 : 4-*Dinitrophenyl hydrazone*; saffron coloured plates from nitrobenzene, m.pt. 231° to 232° C. *Semicarbazone*, small greenish cubes from hot water, m.pt. 198° C. *Ethiodide*; hygroscopic white prisms from absolute ethanol changing to light brown on standing.

1-(4′-*Aminobenzoyl*)-3-*diethylaminopropane* (*XXXI*). The nitro derivative (XXX) (5 g.) was dissolved in concentrated hydrochloric acid (100 ml.) and stannous chloride (20 g.) was added slowly with stirring. After the addition, the mixture was heated on a water-bath for 1 hour, cooled, made strongly alkaline with sodium hydroxide and extracted with ether. The ether extract after drying over anhydrous sodium sulphate on evaporation gave a residue which solidified on cooling. It was recrystallised from light petroleum (b.pt. 100° to 120° C.) in long pale yellow needles, m.pt. 68° C. Yield 3·1 g. (68 per cent.). Found: C, 51·78, H, 5·42; N, 14·6; $C_{14}H_{22}N_2O$ requires C, 51·83; H, 5·4; N, 15·4 per cent. 2 : 4 *Dinitrophenylhydrazone*, orange coloured plates from ethanol m.pt. 165° to 167° C. (frothing).

1-(4′-*Aminobenzyl*)-3-*diethylaminopropane* (*XXXIA*). The ketone (XXXI) (3 g.) was reduced to 1(4′-aminobenzyl)-3-diethylaminopropane (XXXII) by the Wolfe Kishner process in exactly the same way as described for compound XXIA. It distilled at 140° to 142° C./6 mm. as a colourless oil, soluble in benzene, ether and chloroform, but insoluble in light petroleum. Yield 2·3 g. *Chloroplatinate complex* from dilute hydrochloric acid solution as yellow silky needles; m.pt. 215° C. (decomp.). Found: C, 27·2; H, 4·19; N, 4·61; Pt, 31·0; $C_{14}H_{24}N_2PtCl_6$ requires C, 26·8; H, 4·13; N, 4·44; Pt, 31·6 per cent.

1-*Benzoyl decyltriethyl ammonium bromide* (*XXXII*). The tertiary

amine (XXI) 1 g. was mixed with ethyl bromide (4 ml.) in a dry test-tube provided with a calcium chloride tube and heated on an oil bath at 100° C. for half an hour. The solution on cooling solidified to a crystalline mass which on recrystallisation from absolute ethanol gave colourless cubes (0·8 g.) insoluble in benzene and light petroleum, but soluble in water. Found: C, 65·1; H, 9·16; N, 3·23. $C_{23}H_{40}N_1OBr$ requires C, 64·7; H, 9·39; N, 3·28 per cent. Eq. wt. 435; required 426.

1-*Benzyldecyltriethylammonium bromide* (*XXXIII*). The amine XXIA was treated with ethyl bromide in exactly the same way as described above. Colourless cubes from absolute methanol, changing to brown. Very hygroscopic. Eq. wt. 421; required, 412.

1-(4′-*Aminobenzoyl*)-*propyltriethylammonium bromide*. 1(4′-Amino-benzoyl)-3-diethylaminopropane (1 g.) in benzene solution was refluxed with a similar solution of acetic anhydride (1 g.) for 2 hours and the residue left after the removal of the solvent was treated with sodium hydroxide solution. Chloroform then extracted a solid, which, after the evaporation of the chloroform, was boiled with ethyl bromide. The precipitated salt of 1-(4′-acetylaminobenzoyl)-propyltriethylammonium bromide was filtered off, washed with hot benzene, dried by suction, dissolved in aqueous hydrobromic acid and evaporated to dryness on a water-bath. The semi-solid residues on trituration with absolute ethanol gave 1-(4′-aminobenzoyl) propyltriethylammonium bromide which crystallised from boiling anhydrous methanol to give colourless prisms (0·7 g.). It was moderately soluble in hot methanol, practically insoluble in ethanol and very hygroscopic. Found: C, 55·8; H, 7·67; N, 8·5; $C_{16}H_{27}N_2BrO$ requires C, 56·0; H, 7·87; N, 8·18. Eq. wt. 340; required, 343.

1-(4′-*Aminobenzyl*)-*propyltriethylammonium bromide*. This was prepared from 1-(4′-aminobenzyl)-3-diethylaminopropane (1 g.) in exactly the same way as described above. Colourless crystals from absolute methanol changing to brown on standing. Yield 0·64 g. Found: C, 57·7; H, 9·3; N, 8·6; $C_{16}H_{29}N_2Br$ requires C, 58·2; H, 8·8; N, 8·43 per cent. Eq. wt. 325; required, 329.

Bacteriological Tests

Bacteriological tests were performed in a nutrient broth medium using *Staphylococcus aureus* and *Pseudomonas pyocyanea* as test organisms. The tests were also performed simultaneously using a nutrient broth containing the sodium salt of *p*-aminobenzoic acid at a concentration of 1 in 1,000. The addition of 1 : 1,000 of sodium salt of *p*-amino-benzoic acid did not alter the results, which are shown in Table I.

Conclusions

In general it can be said that the introduction of a metabolite structure into the molecule of the antibacterial agents chosen does not improve their chemotherapeutic properties. Both the amino and the ketone groupings appear to be distherapeutic. The fact that the activity of these compounds is not affected by the presence of *p*-aminobenzoic acid probably indicates that the mode of action of these substances is different

from that of the sulphonamide type compounds. Indeed, these results seem to militate against the popular concept that the resemblance of sulphonamides to *p*-aminobenzoic acid is the basis of their activity.

In the long chain aliphatic series the activity of compounds VI and VIII is very significant. In general the reduction of the ketone to the corresponding alkyl derivative and quaternisation of the tertiary amines increase the activity considerably. The total inactivity of all the compounds against Gram-negative organisms is in accordance with the general behaviour of the cationic detergents.

In the 8-hydroxyquinoline series the small activity shown by compounds I, II and IV may be due to the oxine portion rather than the metabolite structure. The higher antibacterial activity of compound III than of compound IV fits in well with the general theory of chelation, discussed by Albert, who has suggested that the presence of an electron-attracting structure such as benzoyl group in the ring containing the –OH group in the *ortho* or *para* position, should cause an increased ionisation of the –OH group resulting in the formation of more stable metal complexes.

TABLE I

Compound	*Staph. aureus* ("Oxford strain")		*Ps. pyocyanea* ("C.N. 200")	
	Inhibition	Growth	Inhibition	Growth
(1) 5(4′-Aminobenzoyl)-8-hydroxyquinoline	1 : 4000	1 : 8000	1 : 2000	1 : 4000
(2) 5(4′-Aminobenzyl)-8-hydroxyquinoline	1 : 34000	1 : 64000	No inhibition	
(3) 5(Benzoyl)-8-hydroxyquinoline	1 : 1024000	1 : 2048000	1 : 2000	1 : 4000
(4) 5(Benzyl)-8-hydroxyquinoline	1 : 34000	1 : 64000	No inhibition	
(5) 1-Benzoyl-10-diethylaminodecane HBr	1 : 64000	1 : 128000	1 : 1000	1 : 2000
(6) 1-Benzyl-10-diethylaminodecane HBr	1 : 1024000	1 : 2048000	1 : 1000	1 : 2000
(7) Benzoyldecyltriethylammonium bromide	1 : 256000	1 : 51200	No inhibition	
(8) Benzyldecyltriethylammonium bromide	1 : 2048000	1 : 4096000	,,	
(9) 1-(4′-Aminobenzoyl)-10-diethylaminopropane HBr	1 : 1000	1 : 2000	,,	
(10) 1-(4′-Aminobenzyl)-10-diethylaminopropane HBr	1 : 2000	1 : 4000	,,	
(11) 4′-Aminobenzoylpropyltriethylammonium bromide	1 : 4000	1 : 8000	,,	
(12) 4′-Aminobenzylpropyltriethylammonium bromide	1 : 128000	1 : 256000	,,	

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloids, Paper Chromatography of. J. E. Carless and H. B. Woodhead. (*Nature, Lond.*, 1951, **168**, 203.) Buffered filter paper was used for the separation of the strongly basic solanaceous alkaloids, and the feebly basic water-insoluble alkaloics of ergot. In the case of solanaceous alkaloids, separation of atropine and hyoscine occurs, and buffers of *p*H 5 to 9 were used, a weak solution of iodine in potassium iodide and water being used to detect the alkaloid on the paper. Graphical representations of separations obtained at various *p*H values of a mixture of apoatropine, methyl nitroatropine, atropine and hyoscine are given. Similar separations can be made of brucine and strychnine; morphine, narcotine, cocaine and the quinine alkaloids give spots of varying R_F, using butanol and buffered paper. By the use of buffered filter papers over the range *p*H 2·2 to 6, varying degrees of separation of the water-insoluble alkaloids of ergot have been carried out, the most satisfactory solvent being diethyl ether saturated with water. Quantitative estimations by elution of the alkaloid were not satisfactory, recoveries varying from 75 to 88 per cent. using 15 to 40 μg. of ergotoxine. No resolution of ergotoxine into ergocristine, ergocornine or ergokryptine was observed in the systems used. By the use of variations in *p*H and different solvents, very complex mixtures of alkaloids should be separable provided that partition coefficients and/or the *p*H's differ. R. E. S.

Brucine, a New Reduction Product of. S. P. Findlay. (*J. Amer. chem. Soc.*, 1951, **73**, 3008.) In the hope of obtaining brucidine, brucine was treated with lithium aluminium hydride. A new product, m.pt. 188° to 189° C., containing two hydrogen atoms fewer than brucidine was obtained and named dehydrobrucidine. Brucine apparently behaves peculiarly in this reaction, because strychnine, α-colubrine, β-colubrine and dihydrobrucine are reduced in the expected manner by lithium aluminium hydride. The identity of the ring structure of the colubrines with that of strychnine and brucine is established because, like strychnidine and brucidine, α- and β-colubridine are oxidised by chromic acid to diketonucidine. A. H. B.

Cinchona Alkaloids, Optical Isomers of. P. Baranger and R. Maréchal. (*C.R. Acad. Sci. Paris*, 1951, **233**, 550.) Quinine and quinidine can be separated into optical isomers by chromatography, cinchonine and cinchonidine cannot. The alkaloids were purified in the normal way by hot recrystallisation of the sulphates or bases, and these are called A in the table below. Aqueous solutions were made by adding a concentrated alcoholic solution to a large volume of water, double-distilled in glass. The concentration was about 0·04 per cent. The *p*H was adjusted to 11 with ammonia, and the chromatographic material used was resublimed and powdered paradichlorbenzene. The adsorbed base was washed out by N hydrochloric acid, whence it was reprecipitated by ammonia; this is called B. The non-adsorbed base was extracted with trichlorethylene, the base taken up by N hydrochloric acid and reprecipitated by ammonia; this is called C. B and C were obtained in equal proportions from quinine and quinidine, and had the following properties:

	M.p.	α $^{15°\,C.}_{D}$	Solubility in water at 20° C.
Quinine A	172° C.	−284·3	0·061
Quinine B	166	−310	0·050
Quinine C	205	−258	0·035
Quinidine A ..	171	+334	0·056
Quinidine B ..	169	+310	0·042
Quinidine C ..	166	+354	0·022

Under the same conditions cinchonine and cinchonidine give 15 parts of B to 7 parts of C, but the rotation and m.pts. are the same as those of A. H. D.

Scopadonnine, a New Scopolamine Alkaloid. W. Kussner and H. W. Voightlander. (*Arch. Pharm. Berl.*, 1951, **284**, 197.) The new alkaloid is obtained by dimerisation of aposcopolamine by heating for several hours. It is thus comparable with the product (belladonnine) which has previously been obtained by dimerisation of apoatropine. The product has lost the unsaturated character of aposcopolamine, and is difficult to saponify. G. M.

ANALYTICAL

Acetylsalicylic Acid, Phenacetin, and Caffeine, and Combinations with Codeine or Thenylpyramine, Infra-red Analysis of. T. V. Parke, A. M. Ribley, E. E. Kennedy and W. W. Hilty. (*Anal. Chem.*, 1951, **23**, 953.) A method is described for the simultaneous determination, by infra-red spectrophotometry, of acetylsalicylic acid, phenacetin and caffeine in pharmaceutical products, even in the presence of codeine phosphate or thenylpyramine hydrochloride, and without separation. The method applies infra-red absorption in the 5 to 7 μ region to a solution made directly in chloroform, and relative freedom from mutual interference permits direct calculation of results. Diluents present in the tablets and powders analysed were shown to exhibit no interference in the 5 to 7 μ region. Codeine is determined by infra-red absorption at 10·62 μ following extraction into carbon disulphide. Thenylpyramine is determined by ultra-violet absorption. In mixtures of acetylsalicylic acid, phenacetin, caffeine and thenylpyramine hydrochloride, the average deviation of analyses of weighed samples was less than 2 per cent. for all components. About 1 hour per sample was required for the complete analysis of the above mixture, including all weighings, preparation of samples, and calculation of results. The method is suitable for routine control analysis of these mixtures. A. H. B.

Aspirin, Phenacetin, and Caffeine in Mixtures, Spectrophotometric Determination of. M. Jones and R. L. Thatcher. (*Anal. Chem.*, 1951, **23**, 957.) A simple, reliable control procedure for the assay of aspirin, phenacetin and caffeine in pharmaceutical products, based on the ultra-violet absorption of the three substances, is presented. Caffeine and phenacetin are determined together in chloroform solution after the removal of aspirin with sodium bicarbonate. The aspirin-sodium bicarbonate solution is acidified, and the aspirin extracted and determined spectrophotometrically in chloroform. The entire procedure can be completed in about 2 hours, and the precision is ± 2 per cent. for aspirin and phenacetin and ± 5 per cent. for caffeine. A. H. B.

Camphor in Spirit of Camphor, Determination of. A. Özger. (*Pharm. Acta Helvet.*, 1951, **26**, 177.) The method of the British Pharmacopœia for the determination of camphor gives results which are too low, owing to decomposition of the dinitrophenylhydrazone by heat on the evaporation of the alcohol.

In the U.S.P. method this heating is avoided and the results are more accurate. In the volumetric determination with hydroxylamine hydrochloride, choice of indicators is important, and the author recommends methyl orange for the first neutralisation, with phenol red or thymolphthalein for the titration. G. M.

Castor Oils, Determination of Acetyl Values of. J. P. Riley. (*Analyst*, 1951, **76**, 40.) In the present work a comparison has been made between the British Standards Institution (B.S.I. No. 684, 1950) and the Association of Official Agricultural Chemists methods for the determination of acetyl values, both of which depend on the determination of the saponification values of the oil and the acetylated oil. It has been found that both methods give very similar results, but it is suggested that where results of high accuracy are required the saponification values both of the oil and its acetyl compound should be the mean of at least 5 determinations. The accuracy of the B.S.I. method has been tested by using pure specimens of methyl ricinoleate and methyl 12-hydroxystearate; and it is concluded that with care results having an accuracy of ± 1 per cent. can be obtained. The acetyl value method, when applied to castor oils, does not give a true estimate of the amount of ricinoleo-glycerides present, since small amounts (about 0·6 per cent.) of 9 : 10-dihydroxystearo-glycerides are also estimated. The dihydroxystearic acid may be determined with sufficient accuracy by crystallisation of the mixed fatty acids of the oil, after removal of unsaponifiable matter, from ethyl acetate at 0° C.; the precipitated dihydroxystearic acid, which has a low solubility is weighed after filtration and washing, and to this weight is added a correction for its slight solubility in the ethyl acetate used as solvent and for washing. From the acetyl value and the percentage of dihydroxystearic acid, the percentage of ricinoleic acid in the oil can be calculated. The other principal constituent of castor oils is linoleic acid, to the extent of about 4 to 6 per cent., which may be determined by spectrophotometric examination of the mixed acids (after removal of unsaponifiable matter), after alkali isomerisation at 180° C. for 60 minutes under the conditions of Hilditch *et al.* (*Analyst*, 1945, **70**, 67). Oleic and saturated acids, which occur in small amounts in the oil, are determined by iodine value and by difference respectively. Examples are given of results obtained in the complete analysis of castor oils. R. E. S.

Karl Fischer Reagent, Review of the Use of. A. G. Jones. (*Analyst*, 1951, **76**, 5.) The theory of the use of the Karl Fischer Reagent is discussed and the various methods are reviewed. The visual end-point method by direct and back titration can be used if approximate results only are required in non-coloured solutions; the electrometric end-point can also be used with both forms of titration. The "dead-stop" electrometric end-point with either a direct or a back titration was most commonly used and gave accurate results. Examples of types of apparatus and electrometric titration circuits are given. A number of uses are briefly reviewed including the determination of the degree of hydration of salts and the method of following the course of various organic reactions in which water is liberated or consumed. R. E. S.

Methoxy and Ethoxy Groups in Admixture, Micro-detection of. C. J. de Wolff, A. L. O. M. Smithuis and A. F. C. Sterk. (*Pharm. Weekbl.*, 1951, **86**, 429.) About 0·1 mg. of material is heated with 10 ml. of 50 per cent. sulphuric acid to 200° C. in a sealed apparatus as described previously (*Pharm. Weekbl.*, 1951, **86**, 273). The top is cut off and the reaction mixture is distilled after the addition of 20 ml. of 50 per cent. potassium hydroxide. The distillate is redistilled with 4N sulphuric acid to remove bases, and oxidised with a red

hot copper wire. If a methoxy group was present, a violet red colour is given on the addition of Schiff's reagent, turning blue on the addition of an equal volume of 70 per cent. sulphuric acid. For the detection of the ethoxy group, the first distillate is heated in a sealed tube with solid potassium dichromate and a little concentrated sulphuric acid to 150° C., and then distilled. Acetic acid may be detected in the distillate by the lanthanum nitrate reaction. For the detection of methyl and ethyl esters, the original hydrolysis is carried out with 20 ml. of 4N sodium hydroxide at 150° C. G. M.

Morphine in Poppy Capsules, Estimation of. M. Valente. (*Boll. chim. farm.*, 1951, **90**, 223.) The following method based on that of Wuest and Frey is recommended. After freeing the capsules from seeds and drying at 100° C. they are ground in a mortar. 100 g. of coarse powder is placed in a percolator and covered with 300 ml. of ethanol (70 per cent.) containing 5 per cent. of hydrochloric acid and left for 2 days. Then the ethanol is drained off and replaced with 300 ml. of hot water. After 2 days this is drained off, and, after recovery of the ethanol, the two percolates are evaporated on a water-bath. To the residue 2 g. of calcium hydroxide and 20 ml. of water are added, shaken for a long time and filtered, sodium carbonate is then added to the filtrate, and it is shaken and filtered again. The morphine is extracted from the filtrate with 60 ml. of a mixture of equal parts of butanol and benzene, and 20 ml. of dilute sulphuric acid is added to the butanol-benzene solution. After 24 hours the aqueous liquid, in which the morphine is found, is treated with sodium carbonate in slight excess in the presence of ethyl ether, into which the morphine passes. The ethereal solution is evaporated to dryness in a tared dish and weighed. Poppy heads from Brindisi and Taranto yielded 0·0776 and 0·0921 per cent. respectively and a sample from Bari 0·1012 per cent. H. D.

Sugars, Colorimetric Determination of. M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith. (*Nature, Lond.*, 1951, **168**. 168.) A simple, rapid method for the colorimetric determination of ketoses and aldoses and their methyl derivatives on a sub-micro scale is reported. The orange-yellow colour, produced by adding sulphuric acid (5 ml.) to the sugar solution (2 ml.) containing phenol, is permanent; its optical density (measured at 490 mμ for hexoses and hexuronic acid and their derivatives and at 475 mμ for pentoses and their derivatives) when referred to a standard curve gives the concentration of the sugar. This method, which is applicable to all carbohydrates with either a free or potential reducing group, is particularly useful for determining sugars which have been separated by partition chromatography using phenol-water as the solvent. The sugars can be extracted from the strips of paper cut from the chromatogram by simply immersing in water at room temperature, and thus there is no danger of decomposing the sugars.

A. H. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Esterases, Spectrophotometric Determinations of. B. H. J. Hofstee. (*Science*, 1951, **114**, 128.) Salicylic acid strongly absorbs ultraviolet light of wavelength 290 mμ to 300 mμ, whereas acetylsalicylic acid and other fatty acid esters of salicylic acid do not. This fact allows a spectrophotometric determination of esterases in general to be made, because hydrolysis of as little as 0·01 μM of salicylic esters can be detected. The reference cell contained buffer, substrate and water to a final volume of 3 ml. The control cell contained

buffer substrate, water, and enzyme to a final volume of 3 ml. The other cells contained, in addition to the elements of the control cell, substances the influence of which upon the enzyme reaction were to be observed. Thus the influence of spontaneous hydrolysis on the observed increase of absorption was cancelled. Spontaneous hydrolysis was considerable in many cases. The esterolytic activity of normal blood serum with acetylsalicylic acid as substrate was investigated. The observed activities are apparently due entirely to choline esterase (*s*-type) because there is practically complete inhibition by eserine concentrations less than 10^{-5}M, and parathione and tri-*o*-cresylphosphate cause inactivation and Ca^{++}, Mn^{++}, and Mg^{++} cause activation. It was found that steapsin liberates only traces of salicylic acid from acetylsalicylic acid, but causes a significant rate of hydrolysis of longer chain fatty acid esters of salicylic acid. In the case of the hydrolysis of butyrylsalicylic acid, steapsin is not inactivated by eserine and this enzyme seems to act in the absence of Ca^{++}. Evidence is submitted which suggests that the observed hydrolysis of the salicylic acid esters is effected by the same enzyme that hydrolyses glycerides such as monobutyrin and tributyrin. A. H. B.

***Strophanthus Intermedius* Pax, Glycosides of Seeds of.** M. R. Salmon, E. Smith and W. G. Bywater. (*J. Amer. chem. Soc.*, 1951, **73**, 3824.) The seeds of *Strophanthus intermedius* were extracted according to the general method of Katz (*Helv. Chim. Acta*, 1948, **31**, 993), and the resulting aqueous solution of the total glycosides was then extracted successively with ether and chloroform. From the ether extract were obtained a crystalline material which agreed in properties with Reichstein's substance 761, and an approximately equal amount of oily glycosides which did not yield crystalline material after chromatography. The chloroform extract yielded a crystalline material from which were obtained, after chromatography, Reichstein's Substance 761 and substance 762. The total yield of ether- and chloroform-soluble components from *Strophanthus intermedius* seeds was 4·5 per cent. of which 2·2 per cent. was crystalline glycosides. Substance 761 gave sarverogenin upon hydrolysis with dilute acid.

A. H. B.

INORGANIC CHEMISTRY

Mercuric Chloride, Volatility when Evaporating Aqueous Solutions. I. Bellucci and A. Casini. (*Ann. Chim.*, 1951, **41**, 374.) Mercuric chloride is very volatile in dilute aqueous solutions, for instance a solution containing 1 mg. in 100 ml. exposed to air at ordinary temperatures will lose 24 per cent. in 48 hours. When evaporated on a water-bath to half its volume 38·7 per cent. is lost. Sodium chloride will reduce, and hydrochloric acid, chlorine or albumen will prevent, this loss, but the use of the latter substances is not always convenient. The authors found that the addition of an excess of potassium iodide will prevent it and this can be conveniently combined with Rupp's method of estimation. To 10 ml. of 0·1N mercuric chloride (0·1358 g. of the salt) add 1·5 g. of potassium iodide in 15 ml. of water, 5 ml. of 10 per cent. sodium hydroxide solution and, with agitation, 2 ml. of formaldehyde solution diluted with 10 ml. of water, shake thoroughly for 2 minutes, acidify with acetic acid and add 20 ml. of 0·1N iodine, shaking again to dissolve the precipitated mercury and titrate with 0·1N thiosulphate. This also works satisfactorily with 0·01N solutions, using the same amounts of the other reagents, and no loss occurred if the solution of mercury was evaporated to dryness on the water-bath after adding the potassium iodide. H. D.

ORGANIC CHEMISTRY

Antihistamines, Geometrical Isomers of. D. W. Adamson, P. A. Barrett, J. W. Billinghurst, A. F. Green and T. S. G. Jones. (*Nature, Lond.*, 1951, **168**, 204.) Further study of 1-*p*-chlorophenyl-1-α-pyridyl-3-pyrrolidinoprop-1-ene (405C49) has revealed the presence of an isomeric base (496C50) in the product of dehydration. The isomers were separated by fractional crystallisation of their oxalates (405C49 base, m.pt. 61° to 62° C.; oxalate, m.pt. 184° C. (decomp.); 496C50 base, oil; oxalate, m.pt. 156° to 157° C. (decomp.)). The bases were apparently geometrical isomers each giving a high yield of the same ketone (*p*-chlorophenyl α-pyridyl ketone, m.pt. 63° C.) on oxidation with chromic acid, and the same propylamine ($n_D^{19°C}$, 1·570; oxalate, m.pt. 147° C.), when catalytically hydrogenated. The ultra-violet absorption spectra are given and have been interpreted as indicating that in 405C49, as in α-pyridyl-ethylene, the α-pyridyl and ethylenic groups are approximately co-planar, on the generally accepted assumption that this configuration is required for maximal electronic interaction of the two groups to which the characteristic light absorption is related. By the same reasoning, the *p*-chlorophenyl group of 496C50, rather than the α-pyridyl group, is in the same plane as the ethylenic group. Outstanding differences in chemical behaviour of the isomers were observed. The dehydration of many related α-pyridyl carbinols has been re-investigated. In each case the results were similar to the example quoted above: two isomers were formed, one being chemically stable and exhibiting the α-pyridylethylene type of spectrum, the other being relatively unstable and having a spectrum similar to the corresponding substituted phenylethylene. Separation of the isomers was usually effected by fractional crystallisation of the oxalates or by chromatography, the progress of the separation being followed by measurements of the ultra-violet absorption spectra. The pharmacological properties of the isomers differed in an interesting manner, high and specific antihistamine activity being shown only by the isomers having the α-pyridylethylene type of structure, the other isomers of each pair invariably being considerably less active in this respect. In contrast, other pharmacological activities were approximately of the same low order in both isomers. R. E. S.

Nitrogen Mustards. E. Wilson and M. Tishler. (*J. Amer. chem. Soc.*, 1951, **73**, 3635.) The methods of preparation and other chemical data relating to many compounds belonging to the general class of nitrogen mustards, which were synthesised for testing as chemotherapeutic agents against neoplastic diseases, are reported. The compounds and structural variants of the effective nitrogen mustard N-methyl-β : β′-dichlorodiethylamine, and have the general formula $R - N(CH_2CH(X)Y)_2$. The variations consisted of: (1) a change in the nature of the R-group; (2) the introduction of an additional β : β′-dichlorodiethylamino group; (3) substitution of the β-chloro-*n*-propyl group (X = Cl, Y = CH_3) for the usual β-chloroethyl group (X = Cl, Y = H); (4) introduction of an additional β : β′-dichlorodipropylamino group into the molecule; (5). substitution of bromine for chlorine; (6) substitution of fluorine for chlorine. Some of the compounds combined some of the above variations. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline and Noradrenaline; Effects of Stimulation of Rat Adrenals. A. S. Outschoorn. (*Nature*, 1951, **167**, 722.) Three drugs, insulin 1 unit, morphine hydrochloride 2 mg., and tetrahydro-β-naphthylamine carbonate 7·5

mg. per 100 g. of rat, known to cause a release of adrenal medullary hormone were administered subcutaneously to groups of 2 to 4 rats. The rats were killed at varying times after injection simultaneously with equal numbers of uninjected controls. Extracts were made of the adrenals and the amounts of adrenaline and noradrenaline in each individual's glands estimated. The mean concentrations of the amines, in terms of bodyweight per rat in the injected groups, were calculated as percentages of the mean concentrations in the control groups killed in each experiment simultaneously with the injected. The results obtained show that with prolonged activity of the glands utilisation of adrenaline increasingly outpaces synthesis; all three drugs caused a depletion. The amounts of noradrenaline, however, did not follow the trend of the methylated amine; neither morphine nor tetrahydronaphthylamine produced any lowering of the content of noradrenaline below the range of the controls, and insulin produced no more than a transient and statistically nonsignificant depletion. These results suggest that under certain conditions of adrenal medullary activity there is either no output of noradrenaline, or an output with which synthesis can keep pace. There does in fact seem to be a tendency for the earliest response of the glands to be an increased production of noradrenaline even while the demand for adrenaline can still be met; although this increase was too small to be significant it occurred following all three of the agents used. S. L. W.

Cortisone, Detection in Body Fluids. C. L. Cope. (*Brit. med. J.*, 1951, **1**, 271.) A 24- or 48-hour collection of urine is acidified to about *p*H 1 with sulphuric acid, allowed to stand at room temperature overnight, and extracted by shaking in separating funnels with 4 successive quantities of chloroform, each equal to 15 per cent. of the total urine. The chloroform emulsions are then centrifuged and the clear chloroform phase evaporated to a volume of about 30 ml. The concentrated chloroform extract is transferred quantitatively to a small separating funnel and washed 3 times with 0·1N sodium hydroxide (6 ml.) and 3 times with water (6 ml.), each washing being extracted with chloroform, which is added to the extract. The washed chloroform solution is then evaporated to dryness. The most suitable solvent for injection is propylene glycol, 0·2 ml. of which is added to the dried extract. After dissolving by gently warming the tube to 45° C. normal saline solution 0·8 ml. is added and the tube shaken to give a homogeneous mixture. Blood is drawn from the tail vein of adrenalectomised mice to the 0·5 mark on a white-cell pipette, diluted to the top mark with Randolph's diluting fluid and eosinophil cells counted in duplicate in the Ruchs-Rosenthal chamber. The diluting fluid consists of 0·1 per cent. of phloxine and 0·1 per cent. of methylene blue, each dissolved separately in 50 per cent. propylene glycol; the solutions are mixed in the proportions of 2 of phloxine to 1 of methylene blue immediately before use. The equivalent of 12 hours' urine extract in 20 per cent. propylene glycol is injected subcutaneously and the animals returned to their cages. Further eosinophil counts are made at 4 and at 6 hours after injection. The significant response is the maximum drop in eosinophil cells expressed as a percentage of the initial value; this drop may be greatest at either 4 or 6 hours. The method is shown to be useful and practical for detecting cortisone-like activity in urine extracts; its applicability to blood is uncertain. S. L. W.

Vitamin B_{12}, Formation of a Competitive Antagonist of, by Oxidation. J. M. Beiler, J. N. Moss and G. J. Martin. (*Science*, 1951, **114**, 122.) Treatment of vitamin B_{12} in strong acid solution with hydrogen peroxide caused decolorisation, and this solution then exhibited a competitive antagonism to

vitamin B_{12}. The activity of this solution was assayed directly on *Lactobacillus leichmanii* 4797, and an inhibitory effect on the growth of this organism, counteracted by vitamin B_{12}, was exhibited. Only at the highest level of inhibitor tested was there incomplete counteraction. The solution was also tested on *Staph. aureus*, *S. typhosa*, and *Ps. aeruginosa*, three organisms which do not require preformed vitamin B_{12} as a growth factor, and no inhibitory effect was observed. It appears that the substance produced by the above treatment of vitamin B_{12} has a specific antagonistic effect to vitamin B_{12}, since it is counteracted by the vitamin and has no inhibitory effect where the vitamin is not an essential factor. A. H. B.

BIOCHEMICAL ANALYSIS

Amino-acids, A New Paper Chromatography Solvent for. F. Bryant and B. T. Overell. (*Nature, Lond.*, 1951, **168**, 167.) Mesityl oxide, which has previously been mentioned as a possible solvent for the paper partition chromatography of organic acids (Lugg and Overell, *Aust. J. Sci. Res.*, 1948, A.**1**, 98), has now been used as a solvent for the separation of amino-acids. Before use, the mesityl oxide is re-distilled and fractionated to prevent condensation in the presence of formic acid. The fractionated solvent alone does not give any movement of amino-acids; but the acid spots move when formic acid is added to the solvent system. The method of obtaining the most desirable mobile phase is to shake one volume of mesityl oxide with 1 volume of formic acid (85 per cent.) and 2 volumes of water. The paper is allowed to become equilibrated thoroughly in the vapours of the stationary phase before introducing the mobile phase. Small volumes of the mobile phase are made just prior to use and discarded after two days. A table of R_F values is recorded. A. H. B.

Methionine, Colorimetric Determination of. M. N. Rudra and L. M. Chouhbury. (*Analyst*, 1951, **76**, 432.) A method for the determination of methionine, involving modifications of previously reported methods, is described. 1 g. of air-dried powdered material was hydrolysed with 5 ml. of 20 per cent. hydrochloric acid for 24 hours. The basic amino acids were precipitated with 100 per cent. phosphotungstic acid, the filtrate was made up to a known volume (usually 8 to 10 ml.) and filtered through a dry filter. 4 ml. of the filtrate was taken, 2 ml. of 5N sodium hydroxide and 1 ml. of 1 per cent. sodium nitroprusside solution were added, the solution was warmed for 8 minutes in a water-bath at 40° C. and cooled in an ice-bath for 5 minutes, and then 2 ml. of concentrated hydrochloric acid was added to develop the red colour. A "Lumetron" photo-electric colorimeter with filter No. 530 was used for preparing the standard calibration curve for colour comparisons. A. H. B.

Nicotinamide, Fluorimetric Determination by Use of Synthetic Ion Exchange Resins. M. Kato and H. Shimizu. (*Science*, 1951, **114**, 12.) The fluorimetric determination of nicotinamide by treatment with cyanogen bromide solution under specific conditions, after the removal of other fluorescent substances according to the method of Chaudhuri and Kodíček (*Biochem. J.*, 1949, **44**, 343), failed in the case of the attempted determination of the nicotinamide content of silkworm because of the fluorescences caused by kynurenine, 3-hydroxykynurenine and other unknown substances. These contaminating fluorescences were completely eliminated by the use of KH–4B–Na (a synthetic cation exchange resin) and Amberlite IRA–400–OH (a synthetic anion exchange resin) and the estimation of nicotinamide was then performed without difficulty. The apparatus used and the details of the method are described. A. H. B.

CHEMOTHERAPY

2 : 4-Diaminopyrimidines; A New Series of Antimalarials. E. A. Falco, L. G. Goodwin, G. H. Hitchings, I. M. Rollo and P. B. Russell. (*Brit. J. Pharmacol.*, 1951, **6**, 185.) A series of derivatives of 2 : 4-diaminopyrimidine was prepared and tested for antimalarial activity. High activity was shown by many members against *P. gallinaceum* in chicks and *P. berghei* in mice, substances with a 5-phenyl substituent being the most active, 5-benzyl and 5-phenoxy derivatives being somewhat less active. Substitution of halogen or nitro groups in the *para* position of the benzene nucleus of the 5-substituent was found to enhance activity. Substitution of an alkyl group in the 6-position enhanced activity, and, in the 5-phenyl derivatives, a peak of activity was reached with the 6-ethyl compound. 2 : 4-Diamino-5-*p*-chlorophenyl-6-ethylpyrimidine was found to be 60 times as active as proguanil against *P. gallinaceum* and 200 times as active against *P. berghei*. Longer chain alkyl derivatives were less active. The drugs were also found to be active against the blood-forms of *P. cynomolgi* in monkeys but to have no pronounced action on the exoerythrocytic stages. It is hoped that these drugs will prove of value in the suppression and treatment of human malaria, and especially in the treatment of proguanil-resistant strains. S. L. W.

1 : 2 : 3 : 4-Tetrahydrocarbazolylcarboxylic Acid Esters with Local Anæsthetic Activity. H. W. Murphy. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 373.) The hydrochlorides of β-diethylaminoethyl, 3-(2-methylpiperidino) propyl, 3-di-*n*-butylaminopropyl, 3-morpholinopropyl, and 1-methyl-3-diethylaminopropyl esters of 1 : 2 : 3 : 4-tetrahydro-6-carbazolylcarboxylic acid were prepared, characterised and studied for local anæsthetic activity, toxicity and irritation. All are potent local anæsthetics when applied topically, but concentrated solutions are irritating. They have anæsthetic activity comparable to that of dibucaine but are more stable in solution and have lower toxicities. The 3-di-*n*-butylaminopropyl ester salt appeared to be the best local anæsthetic of the compounds prepared. They do not interfere with the action of sulpha drugs and they have a germicidal action. The local anæsthetic activity of the amino-ester hydrochlorides of the isomeric 8-carboxylic acid was lacking or of a very low order, but the compounds from either acid were capable of relaxing smooth muscle. The irritant effects, toxicity and limited solubility in the presence of salts, might limit the utility of even the highly active anæsthetic compounds prepared. A. H. B.

Thiocyanates, Antifungal Activity of a Series of. L. Landis, D. Kley and N. Ercoli. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 321.) The substances tested comprised 8 derivatives of 2-phenoxyethyl thiocyanate, 6 of 3-phenoxypropyl thiocyanate and 6 of 4-phenoxybutyl thiocyanate. They were examined for fungicidal, fungistatic and sporostatic activity against *Tricophyton mentagrophytes*, *T. purpureum*, *Epidermophyton floccosum*, *Cryptococcus neoformans*, *Microsporum felineum* and *Aspergillus fumigatus*, and simultaneously compared with undecylenic acid, propionic acid, 8-hydroxyquinoline, and similar substances. The fungistatic activity indicated that the most effective of all the substances tested was 3-(*p*-bromo)phenoxypropyl thiocyanate. The highest dilutions showing fungistasis were: 3-(*p*-bromo)phenoxypropyl thiocyanate, 1 in 90,000; undecylenic acid, 1 in 25,000; propionic acid, 1 in 10,000; and 8-hydroxyquinoline benzoate, 1 in 35,000. In the presence of 10 per cent. of rabbit

serum, the results were 3-(*p*-bromo)phenoxypropyl thiocyanate, 1 in 40,000; undecylenic acid, 1 in 5,000, propionic acid, 1 in 1,000; and 8-hydroxyquinoline benzoate, 1 in 9,000. G. R. K.

PHARMACY

NOTES AND FORMULÆ

Cortisone Acetate (Cortone Acetate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **146**, 1422.) Cortisone acetate, $C_{23}H_{30}O_6$, is 11-dehydro-17-hydroxycorticosterone 21-acetate. It is a white, odourless powder, melting at 242° to 248° C. with decomposition, practically insoluble in water, slightly soluble in ether and alcohol, and freely soluble in chloroform. When dissolved in alcohol and treated with a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N hydrochloric acid, it yields an orange-coloured precipitate which melts at 235° to 242° C. with decomposition, after recrystallisation from ethyl acetate. A 0·2 per cent. solution in acetone has $[\alpha]_D^{25°C}$, 177° to 185°; a 0·001 per cent. solution in alcohol exhibits an ultraviolet absorption maximum at about 2,380 Å ($E_{1\,cm}^{1\ per\ cent.}$, about 390). When dried over phosphorus pentoxide *in vacuo*, it loses not more than 3·0 per cent. of its weight; sulphated ash, not more than 0·1 per cent. It is assayed by determining the optical density of a 0·001 per cent. solution in alcohol at 2,380 Å and multiplying by 39 to obtain the concentration in mg./ml.; it contains 95·0 to 105·0 per cent. of cortisone acetate. G. R. K.

PHARMACOGNOSY

Peroxidase Content of Drugs, as Measure of Age. L. Hörhammer and R. Hänsel. (*Arch. Pharm. Berl.*, 1951, **284**, 110.) For determination of the peroxidase value of a drug, a portion (equivalent to about 1 g. of fresh drug) is rubbed down with 1 g. of sand and extracted for 3 hours with 50 ml. of water. The peroxidase determination is carried out by the method of Diemair and Häusser (*Z. analyt. Chem.*, 1941, **122**, 12) using 0·2 to 2 ml. of the extract and the leuco compound of 2 : 6-dichlorphenolindophenol with hydrogen peroxide. After 30 sec. the reaction is stopped by the addition of 50 ml. of acidified ether, and the colour is shaken out into the ether. Tests with a variety of drugs (including belladonna leaf and root, caraway fruit, male fern, colchicum seed, strophanthus seed, showed that in all cases old drugs had lost activity, which was often reduced to zero after several years storage. G. M.

Jalap Resins, Nomenclature of. P. Duquenois and G. E. Trease. (*Ann. pharm. franc.*, 1951, **9**, 114.) There is some confusion in the nomenclature of the resins of jalap. Names which have been given are: for the ether-soluble fraction, jalapine, scammonine and orizabine: and for the ether-insoluble fraction, jalappine, convolvuline. rhodeoretine and jalapurgine. In English practice scammonin is the soluble resin, convolvulin or jalapin the insoluble one; while on the Continent jalapine is the soluble resin and convolvuline the insoluble one. The confusion is due to W. Mayer, who changed jalapin into convolvulin and then called the resin of *Ipomoea orizabensis* (orizabin) jalapin. It is recommended that the name jalapine should not be used, but that the ether-soluble resin should be called scammonin and the insoluble resin convolvulin. This is justified by the fact that scammonin is in practice prepared from scammony. G. M.

Opium Poppy of Macedonia. B. Akačić, D. Marković and J. Petričič. (*Acta Pharm. Jug.*, 1951, **1**, 3.) The pure white variety of poppy (*Papaver somniferum* L. var. *album* D.C.) is seldom used in Macedonia for the production of opium; instead, a cross between this and a violet-grey form is used. The capsule is similar morphologically to those of other varieties but the latex vessels are always closely associated with the vascular strands. The alkaloids are practically all contained in the latex though traces occur in the epidermal cells. The upper part of the stem is also rich in alkaloid and contains 30 to 50 per cent. of the morphine content of the capsule. Where the soil composition was optimal the highest morphine content was found in the ripe unincised capsule; where not optimal the highest content was in the unripe capsule. Stabilisation by alcohol vapour immediately after collection had no apparent effect on the conservation of morphine during subsequent storage for 7 to 8 months; on the other hand samples which became mouldy lost much morphine. The morphine contents were determined by a method based on those of van Arkel (*Pharm. Weekbl.*, 1935, **72**, 366) and Wieland and Kappelmeyer (*Leibigs Ann.*, 1911, 382, 319). J. W. F.

PHARMACOLOGY AND THERAPEUTICS

Acenaphthene Derivatives, Fungistatic Properties of. J. E. McDavid and T. C. Daniels. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 325.) Seven 5-substituted derivatives of acenaphthene (chloro, bromo, nitro, amino, sulpho (sodium salt), sulphonamido and carboxy) and two 5-substituted derivatives of acenaphthenequinone (chloro and bromo) were examined by the cup-plate method, using 7-day-old cultures of *Tricophyton mentagrophytes*. All the compounds displayed some fungistatic activity but in none was the effect more than moderate. G. R. K.

Adrenocortical Hormones, Assays on Small Laboratory Animals. M. Vogt. (*Analyst*, 1951, **76**, 478.) The three biological assays of cortical hormone on rodents of which enough is known to justify their use as routine or screening tests are discussed and practical details given. These assays are (1) the survival test in low environmental temperature, originated in 1938 by Selye and Schenker ("Cold test"); (2) the test using deposition of liver glycogen in fasting mice given glucose parenterally described by Venning, Kazmin and Bell in 1946; (3) the test using the fall in circulating eosinophils in the mouse described by Speirs and Meyer in 1949. A. H. B.

Adrenocorticotrophic Hormone Activity, Assessment of. M. Reiss, I. D. K. Halkerston, F. E. Badrick and J. M. M. Halkerston. (*Analyst*, 1951, **76**, 461.) The attempts to assess adrenocorticotrophic hormone activity biologically, such as those based on change of adrenal weight and those utilising histological changes within the adrenal cortex, are reviewed. The use of phosphorus–32 as an index of adrenal function is described. Indirect assay methods, based on changes due to adrenal hormones mobilised by the adrenocorticotrophic hormone, are also discussed. Results obtained using a number of the methods are reported. Because of the shortcomings of the biological assay it is pointed out that it would be a great advantage if the assay animal could be dispensed with and the influence of the hormone on adrenal cortex tissue *in vitro* investigated. A. H. B.

Adrenocorticotrophic Hormone, Adrenal Ascorbic Acid Depletion and Adrena, Repair Methods for the Bio-assay of. C. J. O. R. Morris. (*Analyst*, 1951-**76**, 470.) The technique of the adrenal repair method for the assay on the hypol physectomised rat is described and the methods that use histological evidence of repair as the criterion of activity are discussed. After a description and discussion of the adrenal ascorbic acid depletion method of assay, modifications are mentioned. A. H. B.

Adrenocorticotrophic Hormone, Ascorbic Acid Depletion Method for the Bio-Assay of, and the Use of Inhibition of Tissue Repair. B. E. Clayton and E. T. E. Prunty. (*Analyst*, 1951, **76**, 474.) The adrenal ascorbic acid depletion method for the assay of adrenocorticotrophic hormone is described, along with modifications and the results obtained. The hormone inhibits the formation of granulation tissue in response to trauma and this fact was used as the basis of its assay on mice. The details of the method are reported. A. H. B.

Antihistamine Drugs; Method of Assessment of Activity in Man. A. A. C. Ross. (*Lancet*, 1951, **261**, 62.) After preliminary cleaning with ether, 4 areas of skin on the dorsal surface of the forearm (or the outer surface of the thigh) are exposed to ultra-violet radiation from a water-cooled mercury-vapour lamp by holding the quartz window of the lamp in contact with the skin for 15, 30, 45 and 60 sec. Small lead diaphragms are used, one for each length of exposure, so cut that the area exposed is constant (12·5 sq. cm.) for all exposures, but the outline of each differing to avoid confusion when recording results. Twenty-four hours after exposure the reactions are recorded as "—," negative "+" (faint erythema) or "++" (definite or intense erythema). Exposures are made first as controls and again a week or more later during treatment with antihistaminic drugs. The drugs are given in a dosage of 50 mg. by mouth at 10 p.m. on the day before irradiation, 50 mg. at 8 a.m., 3 p.m. and 10 p.m. on the day of irradiation and 50 mg. at 8 a.m. the next day. Irradiation is done in the early afternoon and the results assessed 24 hours later. A positive result is regarded as one where the erythema threshold is raised during the treatment with the antihistamine drug compared with the control. The paper records the comparative results of the test on 10 young adults using three antihistaminic drugs. S. L. W.

Dextran Sulphate as an Anticoagulant. K. Walton. (*Proc. Roy. Soc. Med.*, 1951, **44**, 563.) Dextran sulphate has a heparin-like anticoagulant action. When prepared from dextran of molecular weight 35,000 and over, it is unsuitable for therapeutic use, causing precipitation of fibrinogen, agglutination of platelets and deposition of granular material in reticulo-endothelial cells. In contrast, when prepared from dextran of molecular weight 20,000 and under, it has no effect on platelets and causes no deposits in reticulo-endothelial cells in experimental animals. One typical batch of this material, when assayed against International Standard Heparin, had an activity of 17 to 20 I.U./mg. It was found to be effective by parenteral injection only. When given intravenously to rabbits, a dose of 5 mg./kg. of body weight reduced the clotting time from 19 to 3 minutes in an hour and a half, and a dose of 10 mg./kg. body weight reduced it from 54 to 3 minutes in 3 hours. Doses up to 0·5 g./kg. body weight produced no toxic effects in the mouse, rat, rabbit, dog, or monkey. It seems probable that the anticoagulant action is due to the strong electro-negative charge upon the acidic groups, since it is opposed by strongly basic substances such as protamine. The presence of a co-factor in the serum or plasma is essential, and in its

presence the action is that of an antithrombin. If further chemical trials are satisfactory, dextran sulphate may serve as a cheap synthetic analogue of heparin.
G. R. K.

Laburnum Poisoning in Children. R. G. Mitchell. (*Lancet*, 1951, **261**, 57.) 10 recent cases of poisoning due to eating the pods or seeds of laburnum are reported. The ages of the children ranged from 3 to 10 years, with an average of 5½ years. There were no deaths. The symptoms usually commenced about 30 minutes after ingestion. The common features were nausea and vomiting, pallor and drowsiness, with dizziness and incoordination in the severest cases. There was no diarrhœa, and no record of headache or other pain, and none of the children had a temperature of over 98·8° F. The children usually looked pale, with tachycardia and dilated pupils. With gastric lavage, supplemented by stimulants such as nikethamide in the more severe cases, all the children completely recovered within 24 hours. The active principle of laburnum is an alkaloid, cytisine, which resembles nicotine in its actions. The dose of laburnum lethal to man has not been determined. 2 or 3 seeds may be sufficient to produce symptons, but a boy of 4 recovered after eating at least 18 seeds. The possibility of a fatal issue, however, renders immediate treatment imperative. Cytisine is rapidly excreted by the kidneys so an adequate fluid intake must be assured. S. L. W.

Morphine Derivatives. Pharmacological Properties of. R. Giudicelli, P. Chabrier and K. Kristensson. (*Therapie.*, 1951, **6**, 146.) The pharmacological properties of morphine alkaloids were compared with those of their hydrogenation products and with quaternary ammonium derivatives. The study of morpholylethylmorphine confirmed the view of Sumwalt, that ether-oxides of morphine all have a depressive action on the cough centre similar to that of codeine, and independent of the nature of the radical used. Among the various ether-oxides of morphine, morpholylethylmorphine is characterised by its low toxicity: which is 1/4 that of codeine, intravenously, and 2/15 subcutaneously. This compound also gives a dibromomethylate which has a curarising power 12 times that of the monobromomethylates of morphine and codeine and 1/35 that of curare, the toxicity being 1/50 that of curare. G. M.

Pituitary, Antidiuretic Hormone of the Posterior Lobe. C. Cavallero and M. Zanchi. (*J. Path. Bact.*, 1951, **63**, 249.) The antidiuretic activity of human posterior lobes was estimated by Burn's method. The glands were removed 10 to 25 hours after death from post-mortem material which included instances of disease in which disturbances of the water metabolism are known to occur. When expressed as antidiuretic potency per mg. of fresh neurohypophysial tissue, marked increases of hormone content were observed in cases of liver cirrhosis with ascites, arterial hypertension, cardiac œdema, diabetes mellitus, eclampsia and Addison's disease. A pronounced reduction of antidiuretic potency was found in single cases of diabetes insipidus and hæmochromatosis. S. L. W.

BOOK REVIEWS

THE MODE OF ACTION OF ANÆSTHETICS, by T. A. B. Harris. Pp. xii + 768, including Index. E. and S. Livingstone, Ltd., Edinburgh. 1951. 42s.

This book is another "classic" addition to Messrs. Livingstone's imposing list of medical publications and it should rapidly become a standard textbook for all who are specialising in this branch of medicine. It is not just another book dealing with the controversial theories of how anæsthetics act. In contrast, it is an extremely comprehensive treatise on the chemical, pharmacological and therapeutic properties of the typical anæsthetics in common use. Throughout, the author has endeavoured to correlate the clinical effects observed with the pharmacological and physiological mechanisms involved. While the information it contains is really extensive, it is readily understood, due to the clear and concise manner in which it is presented.

The book is divided into four parts, each consisting of a number of chapters, followed by a very complete list of references. Part 1 deals with the chemical and physico-chemical properties of narcotics and their mode of action on living cells. Part 2 describes their absorption and selective action, together with a review of the biological responses observed in clinical practice. Part 3 correlates the level of anæsthesia with the activity of the motor nerves and the autonomic nervous system. Two excellent chapters adequately describe the chemical transmission of the nervous impulse, while another explains very clearly the properties and uses of D-tubocurarine chloride. It is perhaps unfortunate that the newer neuromuscular blocking drugs, "Flaxedil" and decamethonium, could not have been included. Part 4 underlines the scope of the book, no less than 190 pages being devoted to the side actions of anæsthetics.

G. F. SOMERS.

PHARMACOGNOSY, by Robertson Pratt and Heber W. Youngken, Jr. Pp. xi + 644 (including 4 plates in colour and 63 other illustrations). J. B. Lippincott Company, London. 1951. 70s.

This book presents the subject of pharmacognosy from an angle quite different from that usually found in textbooks. The main emphasis is laid on the constituents of crude drugs rather than on the crude drugs themselves, and the authors point out that this shift of emphasis really stems from the work of Pelletier, Caventou and others who were the first to isolate active principles from crude drugs. A distinction is made between the therapeutically active constituents and those chiefly of pharmaceutical importance, e.g., acacia, starch. This novel presentation of the subject effects the plan of the book. The early chapters give a general account of the constituents as follows: their formation in the living cell, their function in the plant or animal in which they are formed and their pharmacological activities. The crude drugs themselves are then studied and are classified on a pharmacological basis, the chief emphasis being on the chemistry of their constituents and their therapeutic and pharmaceutical uses. The later chapters deal with the production of crude drugs (including a discussion of plant breeding and weed control), their evaluation and the pests which cause deterioration during storage. This book should be considered as complementary to, rather than a substitute for, the normal textbooks of pharmacognosy. The crude drugs themselves are not the real object of study so that the information on their morphological, anatomical or even botanical characters is scanty, and the few drawings illustrating these

features lack the precision we expect in pharmacognosy. Thus the drawing of the trichomes in Figure 37 gives little real information as to the structure of nux vomica trichomes and in Figure 34 a rare type of glandular trichome of digitalis is drawn, whereas the much more common type is omitted. However, the book does not claim to cover these "highly specialised phases of the subject" and the student or practising pharmacist who has already had some basic training in pharmacognosy will find this book stimulating, interesting and informative. The authors are to be congratulated for this refreshingly different study of vegetable and animal materia medica. J. W. FAIRBAIRN.

QUALITATIVE ARZNEIMITTEL-ANALYSE, by Hans Mühlemann and Adolf Bürgin. Pp. 278 + Index. Ernst Reinhardt, Basle. 1951. Cardboard cover, 10 Swiss francs. Linen cover, 12.50 Swiss francs.

This book is intended primarily for pharmacists and students of pharmacy as an aid to the ready identification of common medicinal substances. The opening chapters give a brief description of the more commonly employed physical and chemical techniques, including distillation, fractionation, crystallisation and sublimation, which are available for the isolation of pure substances. There follows an explanation of the way in which these methods are applicable to the examination of various types of dispensed preparation. Aqueous, alcoholic and other non-oily substances are classified together and preliminary tests are suggested for (*a*) reaction to litmus, (*b*) miscibility, (*c*) residue on evaporation, (*d*) alkaloids and bases, (*e*) carbohydrates, (*f*) ammonium salts, and (*g*) substances reacting with ferric chloride. Powders, tablets, pills and dragees are treated as a group and are examined for water- and alcohol-soluble substances. Special methods are described for the examination of preparations containing oils and fats, such as emulsions and suppositories. The remainder of the work is devoted to a series of short monographs describing the physical and chemical tests for the identification of a number of simple chemical and medicinal substances. These are arranged in groups of compounds, chemically related, and include alcohols, aldehydes, ketones, carbohydrates, phenols, acids, esters, ethers, organo sulphur compounds, cyano compounds, organo arsenicals, halogen derivatives, organic bases, alkaloids, sulpha drugs and a group of miscellaneous natural products. The book does not provide a truly systematic approach to the problem of identifying organic medicinal substances, though it does fill a need for guidance in the use of the methods which are available for the isolation and identification of the active ingredients of dispensed medicines.

JOHN B. STENLAKE.

BOOKS RECEIVED

ENZYMATISCHE ANALYSE by Herman Stetter. Pp. 196 and Index. Verlag Chemie, GMBH., Weinheim/Bergstr. 1951. DM. 17.50.

PAPIERCHROMATOGRAPHIE by Friedrich Cramer. Pp. 81 with 47 illustrations. Verlag Chemie, GMBH,, Weinheim/Bergstr. 1952. DM. 9.80.

PHYSICAL BIOCHEMISTRY (2nd Ed.) by H. B. Bull. Pp. viii + 334 and Index. Chapman and Hall, London, 1951, 46s.

THE VITAMIN B COMPLEX by F. A. Robinson. Pp. xi + 629 and Index. Chapman and Hall, London, 1951, 60s.