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Successor to The Quarterly Journal of Pharmacy and Pharmacology

INCORPORATING THE YEAR-BOOK OF PHARMACY

VOLUME II, 1950

WITH THE

TRANSACTIONS

OF THE

BRITISH PHARMACEUTICAL

CONFERENCE

HELD AT

GLASGOW

SEPTEMBER 4th to 8th, 1950

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British Pharmaceutical Conference

EIGHTY-SEVENTH ANNUAL MEETING, GLASGOW, 1950

REPORT OF PROCEEDINGS

OFFICERS :

President :

A. A. MELDRUM.

Chairman :

A. D. POWELL, F.R.I.C. Nottingham.

Vice-Presidents :

(*who have filled the office of President prior to* 1923)

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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 PRESIDENT of the Pharmaceutical Society of Ireland (*ex officio*).
The PRESIDENT of the Pharmaceutical Society of Northern Ireland (*ex officio*).
The CHAIRMAN of the Executive of the Scottish Department of the Pharmaceutical Society of Great Britain (*ex officio*).

K. BULLOCK, Ph.D., M.Sc., Manchester.
* MISS M. A. BURR, Nottingham.
H. DAVIS, Ph.D., F.R.I.C., London.
A. G. FISHBURN, F.R.I.C., D.P.A., Manchester.
E. F. HERSANT, Ph.D., B.Pharm., Dagenham.
* J. F. MCNEAL, Amersham.
T. MARNS, London.
T. D. WHITTET, London.

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

PROCEEDINGS OF CONFERENCE GLASGOW, 1950

THE OPENING SESSION

The opening session of the Conference was held in the St. Andrew's Halls on the afternoon of Monday, 4th September, the President of the Conference, Mr. A. A. Meldrum (President of the Pharmaceutical Society of Great Britain), in the Chair. On the platform were the Chairman of the Conference (Mr. A. D. Powell), the Right Honourable the Lord Provost of Glasgow, the Chairman of the Local Conference Committee (Mr. S. Hughan) the Honorary General Secretaries (Mr. H. Treves Brown and Mr. H. G. Rolfe), the Treasurer (Dr. G. R. Boyes), and the following Members of the Executive: Professor H. Brindle, Mr. B. A. Bull, Dr. K. Bullock. Miss M. A. Burr, Dr. H. Davis, Mr. H. Deane, Dr. Norman Evers, Mr. A. G, Fishburn, Dr C,. H. Hampshire, Dr. E. F. Hersant, Mr. H. Humphreys Jones, Mr. J. F. McNeal, Mr. A. Officer, Mr. T. C. Scott (President of the Pharmaceutical Society of Ireland), Mr. F. W. R. Skinner (President of the Pharmaceutical Society of Northern Ireland), Dr. T. E. Wallis, Mr. T. D. Whittet.

THE PRESIDENT called upon the Lord Provost to address the meeting.

The Lord Provost expressed pleasure in welcoming the Conference to Glasgow. He pointed out that Glasgow had a very important Medical School and the Glasgow hospitals had long enjoyed a high reputation. He hoped the Conference would have a most successful week.

The President thanked the Lord Provost, on behalf of the Conference, for his welcome.

THE CHAIRMAN'S ADDRESS

THE CHAIRMAN (Mr. A. D. Powell, F.R.I.C.) then delivered his Address, entitled " Fifty Years of Pharmaceutical Progress," which is printed in full in the *Journal of Pharmacy and Pharmacology*, 1950, **2**, No. 10, pages 609 to 618.

On the proposition of Dr. Norman Evers, seconded by Professor J. P. Todd, the Conference accorded a hearty vote of thanks to the Chairman for his address.

THE CIVIC RECEPTION

A civic reception, at the invitation of the Corporation of Glasgow, was held at the City Chambers on Monday evening, 4th September. The guests were received by the Right Honourable the Lord Provost of Glasgow (Mr. Victor D. Warren, M.B.E., T.D.). The reception was followed by dancing and music.

THE SCIENCE SESSIONS

Meetings were held on Tuesday, Wednesday and Friday, the 5th, 6th and 8th September, the Chairman presiding. The meetings took place in the St. Andrew's Halls.

The following twenty-one papers were communicated :—

1. "The Preparation of Compressed Tablets. Part III.—A study of the value of Potato Starch and Alginic Acid as Disintegrating Agents."—By H. Berry, B.Sc., Ph.C., F.R.I.C., Dip. Bact. (Lond.), and C. W. Ridout, Ph.C.
2. "Disintegration of Compressed Tablets. The Effect of Age and certain Associated Factors."—By H. Burlinson, Ph.C., and C. Pickering, Ph.C.
3. "The Removal of Bacteria from Oils by Filtration."—By G. Sykes, M.Sc., F.R.I.C., and A. Royce, Ph.C.
4. "Some Pharmaceutical Aspects of Vitamin B_{12}."—By F. Hartley, Ph.D., B.Sc., F.R.I.C., Ph.C., P. Stross, B.Sc., and R. E. Stuckey, Ph.D., B.Sc. F.R.I.C., Ph.C.
5. "Bacterial Survival in Systems of Low Moisture Content. Part II.—The Bactericidal Effects of Certain Substances during the Spray-Drying Process." —By K. Bullock, Ph.D., M.Sc., Ph.C., and E. A. Rawlins, Ph.C.

6. "Antagonism between Non-Ionic Detergents and Antiseptics."—By A. Mirimanoff, D. ès Sc. and Mlle. A. Bolle, D. ès Sc.
7. "The Stability of Injection of Morphine Sulphate."—By G. E. Foster, Ph.D., B.Sc., F.R.I.C., Miss J. M. Macdonald, B.Sc., A.R.C.S., and T. D. Whittet, Ph.C., D.B.A.
8. "The Tryptic Activity of Pancreatin: A Critical Study of Some Assay Processes and Standards."—By K. Bullock, Ph.D., M.Sc., Ph.C., and Jitendra Kumar Sen, B.Sc., M.Sc. (Calcutta).
9. "A Note on the B.P. 1948 Assay Process for Trypsin in Pancreatin."—By G. E. Foster, Ph.D., B.Sc., F.R.I.C., and W. Smith, B.Sc., F.R.I.C.
10. "The Arsenic Limit Tests of the British Pharmacopœia."—By W. Mitchell-Ph.D., B.Sc., F.R.I.C., Miss H. M. Perry, M.Sc., F.R.I.C., and L. A. Shearing, B.Sc., F.R.I.C.
11. "The Behaviour of some Thiosemicarbazones towards Silver Nitrate and a Gravimetric Estimation of 4-Acetamidobenzaldehyde Thiosemicarbazone."—By E. A. Haugas and B. W. Mitchell, B.A. (Cantab.), B.Sc., A.R.I.C.
12. "The Tuberculostatic Activity of some Thiosemicarbazones."—By E. M. Bavin, B.Sc., F.R.I.C., R. J. W. Rees, B.Sc., M.B., M.R.C.S., L.R.C.P., J. M. Robson, F.R.S.E., D.Sc., M.D., M. Seiler, M.D., D. E. Seymour, F.R.I.C., and D. Suddaby, B.Sc., F.R.I.C.
13. "Studies in the Chromatography of Senna and Related Compounds."—By G. H. MacMorran, Ph.D., Ph.C.
14. "The Partition Chromatography of Alkaloids. Part III.—The Alkaloids of Punica Granatum."—By J. Chilton, M.Pharm., Ph.C., and M. W. Partridge, Ph.D., B.Sc., B.Pharm., Ph.C.
15. "A Comparative Study of Agars from various Geographical Sources."—By J. L. Forsdike, B.Pharm., Ph.C., A.R.I.C.
16. "Vegetable Purgatives containing Anthracene Derivatives. Part III.—The Evaluation of Senna Pod and its Preparations."—By J. W. Fairbairn, Ph.D., B.Sc., Ph.C., and I. Michaels, Ph.D., B.Sc., Ph.C., F.R.I.C.
17. "Vegetable Purgatives containing Anthracene Derivatives. Part III.—Galenical Preparations of Senna Pod."—By J. W. Fairbairn, Ph.D., B.Sc., Ph.C., and I. Michaels, Ph.D., B.Sc., Ph.C., F.R.I.C.
18. "A Polarographic Investigation of the Redox Characters of the Amino, Acridines, considered in relation to Antibacterial Action."—By R. C. Kaye, Ph.D., B.Pharm., Ph.C.
19. "The Biological and Chemical Assay of Tinctures of Digitalis."—By H. Brindle, M.Sc., F.R.I.C., and G. Rigby, B.Sc., Ph.C.
20. "The Preparation of Liquid Human Plasma by the Kaolin Process."—By G. R. Milne, Ph.C., and G. M. Todd, Ph.C., D.B.A.
21. "The Use of Sulphated Whole Blood in the Assay of Heparin."—By S. S. Adams, B.Pharm., Ph.C., with an interpretation of the data by K. L. Smith.

The papers, with a report of the discussions, are printed in the *Journal of Pharmacy and Pharmacology*, 1950, **2**, No. 10, pages 619 to 715; No. 11, pages 747 to 846; No. 12, pages 880 to 918.

THE SYMPOSIUM SESSION

A symposium on Formularies and Formulation was held on Friday, the 8th September. The Chairman presided and Professor J. P. Todd, Mr. H. S. Grainger, Miss M. A. Burr and Dr. A. Wilson were the opening speakers. A report of the meeting is printed in the *Journal of Pharmacy and Pharmacology*, 1950, **2**, No. 12, pages 919 to 936.

MEETING OF BRANCH REPRESENTATIVES

Two sessions were held in the morning and afternoon of Wednesday, the 6th September, the President, Mr. A. A. Meldrum, in the Chair. The Agenda comprised two subjects—consideration of (*a*) the Draft Supplementary Charter and Bye-Laws Consequent thereon, and (*b*) the Council's report on the man

power situation. A report of the meeting was published in *The Pharmaceutical Journal*, 1950, **165**, pages 190 to 193 ; 229 to 232 ; 240 and 241.

THE CLOSING SESSION

The Closing Session of the Conference was held on Friday, 8th September, in the St. Andrew's Halls, the Chairman, Mr. A. D. Powell, presiding. The Lord Provost was represented by Bailie John McAslan.

Vote of Thanks to the Glasgow Committee and Presentation of Books

The Chairman called on Dr. C. H. Hampshire to propose a vote of thanks to the Local Committee. This was seconded by Mr. C. J. Eastland. The Chairman then presented to the Glasgow Branch of the Pharmaceutical Society the books provided from the Bell and Hills Fund. Mr. S. Hughan (Chairman of the Glasgow Committee) replied to the vote of thanks and acknowledged the gift of books.

Annual Report

Mr. H. Treves Brown presented the following Annual Report of the Executive Committee :—

Your Executive have pleasure in presenting the Eighty-seventh 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 70 members elected by the Executive.

Obituary.—The Executive record with deep regret the death of Mr. A. R. Melhuish, who was Chairman of the Conference at the London meeting in 1941.

Conference Research Papers.—Twenty-six papers were submitted and twenty-one 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. Forty 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 Blackpool was published in the 1st Volume of the *Journal of Pharmacy and Pharmacology*. The Executive has been represented on the Editorial Committee by the Chairman (Mr. A. D. Powell), Dr. G. R. Boyes, and the Senior Honorary General Secretary.

Relationship with Society.—The Council of the Society have recently submitted a draft of the sections of the new bye-laws of the Society dealing with the Conference and these will be carefully considered by your Executive.

It is understood that the suggestions regarding the organisation of pharmaceutical research made by your Executive to the Council of the Society are still under consideration by the Council.

Arrangement for Future Meetings.—An invitation will be presented at this meeting on behalf of the Harrogate and District Branch for the Conference to meet in Harrogate in 1951. The Executive are grateful for this offer of hospitality and are confident that it will be much appreciated by members of the Conference.

Officers of the Conference.—Your Executive have nominated the following officers for 1950-1951 :—

President (ex-officio): The President of the Pharmaceutical Society of Great Britain. *Chairman:* H. Berry. *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 and A. D. Powell. *Honorary Treasurer:* G. R. Boyes. *Honorary General Secretaries:* H. Treves Brown and H. G. Rolfe. *Other Members of the Executive:* K. Bullock, A. G. Fishburn, J. W. Hadgraft, E. F. Hersant, J. P. Todd, T. D. Whittet, with the following nominated by the Council of the Pharmaceutical Society of Great Britain : Miss M. A. Burr, F. C. Wilson and T. Reid, and the following *ex-officio:* The President of the

Pharmaceutical Society of Ireland, the President of the Pharmaceutical Society of Northern Ireland, the Chairman of the Executive of the Scottish Department, the Chairman of the Local Committee and the Honorary Local Secretary.

MR. E. W. MANN proposed the acceptance of the Report and the election of officers of the Conference for the ensuing year. He had particular pleasure in proposing the election of Professor H. Berry as Chairman for 1951. Dr. K. R. Capper seconded and the Report was adopted.

PROFESSOR H. BERRY 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 1949.

In the Bell and Hills Fund the excess of income over expenditure during the year ended 31st December, 1949, was £25 10*s.* 6*d.* With this addition, the Accumulated Fund now amounts to £1,794 0*s.* 4*d.*, consisting of Cash £344 0*s.* 4*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 fee from the Pharmaceutical Society of Northern Ireland, amounted to £114 16*s.* 0*d.*, and were credited to the account of the *Journal of Pharmacy and Pharmacology*.

The expenditure of £641 8*s.* 5*d.*, which included £290 9*s.* 10*d.*, the cost of the scheme for assisting young pharmacists, and £75 19*s.* 0*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 Hill's Fund.)

INCOME AND EXPENDITURE ACCOUNT, 1949.

Expenditure.	£	*s.*	*d.*	*Income.*	£	*s.*	*d.*
Donation of Book to the Blackpool Branch	2	12	6	Interest on Consols	22	3	0
Balance to Accumulated Fund ...	25	10	6	Interest on 3 per cent. Savings Bonds	6	0	0
	£28	3	0		£28	3	0

BALANCE SHEET AT 31ST DECEMBER, 1949.

Liabilities.	£	*s.*	*d.*	*Assets.*	£	*s.*	*d.*
Accumulated Fund :—				Investments :—			
As at 31/12/48	1,768	9	10	(*a*) £1,250 2½ per cent. Consols (Donation by the late Alderman Clayton of Birmingham) (Market value at 31st December, 1949, £879 13*s.* 9*d.*) ...	1,250	0	0
Add: Surplus	25	10	6	(*b*) £200 3 per cent. Savings Bonds, 1960-70 (Market value at 31st December, 1949, £193 10*s.* 0*d.*) ...	200	0	0
				Cash at Westminster Bank, 31/12/49	344	0	4
	£1,794	0	4		£1,794	0	4

G. R. BOYES,
Honorary Treasurer

On the proposition of THE PRESIDENT, seconded by DR. E. F. HERSANT, the Report was adopted.

PLACE OF MEETING FOR 1951

MR. R. C. WILBERFORCE, on behalf of the Harrogate Branch, extended an invitation to hold the Conference in Harrogate during the week beginning 10th September, 1951. He said Harrogate was situated centrally, so that no choice could be made with less prejudice to any particular area of the country. The Municipality would spare no effort for the comfort and convenience of members of the Conference.

MISS D. MURGATROYD proposed that the invitation be accepted and MR. J. W. HADGRAFT seconded. The vote was put to the meeting and carried with acclamation.

(*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.
1889	Newcastle-on-Tyne.	C. UMNEY, F.I.C., F.C.S.	T. M. CLAGUE.
1890	Leeds	C. UMNEY, F.I.C., F.C.S.	F. W. BRANSON, F.C.S.
1891	Cardiff ...	W. MARTINDALE, F.C.S.	ALFRED COLEMAN.
1892	Edinburgh ...	E. C. C. STANFORD, F.C.S.	PETER BOA.
1893	Nottingham ...	OCTAVIUS CORDER.	C. A. BOLTON.
1894	Oxford ...	N. H. MARTIN, F.L.S., F.R.M.S.	H. MATTHEWS.
1895	Bournemouth	N. H. MARTIN, F.L.S., F.R.M.S.	STEWART HARDWICK.
1896	Liverpool ...	W. MARTINDALE, F.C.S.	T. H. WARDLEWORTH. H. O. DUTTON (Birkenhead)
1897	Glasgow ...	Dr. C. SYMES.	J. A. RUSSELL.
1898	Belfast ...	Dr. C. SYMES.	R. W. McKNIGHT. W. J. RANKIN.
1899	Plymouth ...	J. C. C. PAYNE, J.P.	J. DAVY TURNEY.
1900	London ...	E. M. HOLMES, F.L.S.	W. WARREN. HERBERT CRACKNELL.
1901	Dublin ...	G. C. DRUCE, M.A., F.L.S.	J. I. BERNARD.
1902	Dundee ...	G. C. DRUCE, M.A., F.L.S.	W. CUMMINGS.
1903	Bristol ...	T. H. W. IDRIS, M.P., F.C.S.	H. E. BOORNE.
1904	Sheffield ...	T. H. W. IDRIS, M.P., F.C.S.	H. ANTCLIFFE.
1905	Brighton ...	W. A. H. NAYLOR, F.I.C., F.C.S.	W. W. SAVAGE. C. G. YATES.
1906	Birmingham ...	W. A. H. NAYLOR, F.I.C., F.C.S.	C. THOMPSON.
1907	Manchester ...	THOS. TYRER, F.I.C., F.C.S.	W. KIRKBY.
1908	Aberdeen ...	ROBT. WRIGHT, F.C.S.	W. F. HAY.
1909	Newcastle ...	J. F. TOCHER, B.Sc., F.R.I.C.	T. M. CLAGUE. H. W. NOBLE.
1910	Cambridge ...	FRANCIS RANSOM, F.C.S.	A. A. DECK. T. J. MALLETT.
1911	Portsmouth ...	W. F. WELLS.	T. O. BARLOW. T. POSTLETHWAIT.
1912	Edinburgh ...	Sir EDWARD EVANS, J.P.	THOS. STEPHENSON.
1913	London ...	JOHN C. UMNEY, F.C.S.	W. J. UGLOW WOOLCOCK.
1914	Chester ...	E. H. FARR, F.C.S.	R. CECIL OWEN, B.Sc.
1915	London ...	E. SAVILLE PECK, M.A.	———
1916	London ...	DAVID HOOPER, LL.D., F.R.I.C.	———
1917	London ...	CHAS. ALEX. HILL, B.Sc., F.R.I.C.	———
1918	London ...	CHAS. ALEX. HILL, B.Sc., F.R.I.C.	———
1919	London ...	W. KIRKBY, M.Sc., F.C.S.	———
1920	Liverpool ...	CHAS. ALEX. HILL, B.Sc., F.R.I.C.	H. HUMPHREYS JONES, F.R.I.C.
1921	Scarborough	E. SAVILLE PECK, M.A.	E. R. CROSS.
1922	Nottingham ...	Prof. H. G. GREENISH, D ès Sc., F.I.C.	E. C. CARR.
1923	London ...	F. W. GAMBLE.	W. J. U. WOOLCOCK, C.B.E.
1924	Bath	EDMUND WHITE, B.Sc., F.I.C.	P. J. THOMPSON. W. H. HALLETT.
1925	Glasgow ...	EDMUND WHITE, B.Sc., F.I.C.	P. M. DUFF.
1926	Leicester ...	D. LLOYD HOWARD, J.P.	J. BARKER.
1927	Brighton ...	D. LLOYD HOWARD, J.P.	F. W. BURGESS.
1928	Cheltenham ...	R. R. BENNETT, B.Sc., F.R.I.C.	P. JAMES.
1929	Dublin ...	R. R. BENNETT, B.Sc., F.R.I.C.	V. E. HANNA.
1930	Cardiff ...	J. T. HUMPHREY.	J. MURRAY.

Years	*Places of Meeting*	*Chairmen*	*Local Secretaries*
1931	MANCHESTER ...	J. H. FRANKLIN.	R. G. EDWARDS.
1932	ABERDEEN ...	H. SKINNER.	H. M. DUGAN.
1933	LONDON ...	C. H. HAMPSHIRE, M.B., B.S., B.Sc., F.R.I.C.	H. N. LINSTEAD.
1934	LEEDS	C. H. HAMPSHIRE, M.B., B.S., B.Sc., F.R.I.C.	G. C. CRUMMACK. J. F. SIMON.
1935	BELFAST ...	F. W. CROSSLEY-HOLLAND, L.M.S.S.A.	D. L. KIRKPATRICK.
1936	BOURNEMOUTH	HAROLD DEANE, B.Sc., F.R.I.C.	V. J. SCAMPTON.
1937	LIVERPOOL ...	T. EDWARD LESCHER, O.B.E.	W. E. HUMPHREYS.
1938	EDINBURGH ...	J. RUTHERFORD HILL, O.B.E.	C. G. DRUMMOND.
1939	BIRMINGHAM ...	J. RUTHERFORD HILL, O.B.E.	D. J. RUSHTON.
1940	LONDON ...	H. HUMPHREYS JONES, F.R.I.C.	———
1941	LONDON ...	A. R. MELHUISH.	———
1942	LONDON ...	T. E. WALLIS, D.Sc., F.R.I.C., F.L.S.	———
1943	LONDON ...	T. E. WALLIS, D.Sc., F.R.I.C., F.L.S.	———
1944	LONDON ...	H. BRINDLE, B.Sc., F.R.I.C.	———
1945	LONDON ...	H. BRINDLE, B.Sc., F.R.I.C.	———
1946	LONDON ...	B. A. BULL, A.R.I.C.	———
1947	TORQUAY ...	B. A. BULL, A.R.I.C.	T. D. EVANS.
1948	BRIGHTON ...	NORMAN EVERS, PH.D., F.R.I.C.	A. WILSON.
1949	BLACKPOOL ...	NORMAN EVERS, PH.D., F.R.I.C.	P. VARLEY. T. A. DURKIN.
1950	GLASGOW	A. D. POWELL, F.R.I.C.	A. OFFICER

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1944 to , H. TREVES BROWN, B.Sc.
1947 to , H. G. ROLFE, B.Sc., F.R.I.C.

PROCEEDINGS OF THE CONFERENCE (*Continued from page 5*)

VOTE OF THANKS TO CHAIRMAN

PROFESSOR H. BRINDLE proposed a vote of thanks to the Chairman who, he thought, had presided over the business of the Conference in a charming manner. His criticism was constructive ; his address, his work and his style had been at all times powerful and kindly, and, above all, he possessed a keen sense of humour.

DR. J. M. ROWSON seconded : the vote was put to the meeting by the President and unanimously carried.

MR. A. D. POWELL said that he had appreciated the honour of being elected Chairman and thanked Professor Brindle and Dr. Rowson for proposing and seconding the vote of thanks. He also thanked Mr. S. Hughan and Mr. A. Officer for their endeavours on his behalf.

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 25*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.

REVIEW ARTICLE

LES ANTI-EPILEPTIQUES

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Pharmacien de l'Hôtel-Dieu
Membre de l'Academie de Médicine

JEAN CHEYMOL,
Dr. en Méd., Dr. ès Sc.,
Dr. en Pharm.
Professeur agrégé à la Faculté de Médecine de Paris
Pharmacien de l'Hôpital Tenon

L'ÉPILEPSIE ne recevra un traitement vraiment rationnel que le jour où ses causes profondes nous auront été enfin dévoilées et nous n'en sommes pas encore à ce degré de la connaissance. Cependant des progrès notables ont été récemment accomplis touchant le mécanisme de ses manifestations les plus frappantes et sa thérapeutique au moins symptomatique.

Ces progrès sont dus essentiellement à la lumière que l'épilepsie expérimentale a projetée sur l'épilepsie-maladie. La reproduction chez l'animal des manifestations les plus caractéristiques de l'épilepsie permet en outre l'étude systématique des médicaments les plus capables de la faire disparaître chez l'Homme. Enfin la comparaison des formules des anti-épileptiques synthétiques récemment obtenus mène à la connaissance des groupements chimiques les plus actifs et ouvre la voie à de nouvelles recherches.

Ces divers points feront l'objet de cette brève étude.

I) EPILEPSIE-MALADIE

a) *Symptômes.* - La forme qui nous intéressera surtout ici est la forme classique ou épilepsie-coma, inconsciente et généralisée. C'est la plus répandue chez l'Homme et la plus étudiée expérimentalement. Nous rappellerons seulement que d'autres formes peuvent se rencontrer: épilepsie bravais-jacksonienne, consciente et localisée; épilepsie réflexe de Brown-Séquard (insensible au phénobarbital); épilepsies réflexes sensorielles, etc. . . .

Résumons brièvement la symptomatologie de cette épilepsie classique. Dans le grand mal, la manifestation la plus frappante est la crise tonique et clonique. Précédée ou non de l'aura, de la chute du sujet, elle est suivie par la stupeur et le sommeil; c'est cette forme dont l'expérimentation peut le mieux reproduire les traits essentiels.

Dans le petit mal, on observe la perte transitoire de conscience (pikno-épilepsie ou piknolepsie), des secousses musculaires (myoclonies), localisées, quelquefois généralisées, et la perte brusque du tonus de certains muscles (crises akinétiques). On note aussi les crises psychomotrices ou équivalents psychiques avec leurs actes parfois violents mais toujours inconscients, leurs épisodes ambulatoires et ces curieuses " absences " surtout fréquentes chez l'Enfant.

Grand mal et petit mal peuvent souvent mêler leurs symptômes. Dans la complication la plus grave, il peut s'établir progressivement un état de mal permanent, souvent mortel. Même dans ses formes les moins graves l'épilepsie se caractérise par la variabilité du rythme des crises, parfois

interrompues par des rémissions spontanées, parfois remplacées par de simples formes frustes.

b) *Mécanisme.* – Malgré les recherches les plus récentes des neuro-chirurgiens et des neuro-psychiâtres, les causes profondes de l'épilepsie nous sont encore inconnues. Au moins les tracés électroencéphalographiques ont-ils pu mettre en évidence: dans le grand mal des arythmies cérébrales, variables suivant le type de crises; dans le petit mal une succession lente d'ondes en pointes et d'ondes en dômes.

L'étude des convulsions produites chez l'animal par l'électrochoc et le pentétrazol nous ont appris aussi qu'il n'existe pas un centre épileptogène unique. L'excitation des centres sous-corticaux peut à elle seule engendrer les crises caractéristiques.

Les convulsions provoquées par le pentétrazol montrent qu'il existe un centre tonique bulbaire, un centre clonique protubérantiel et que certains mouvements coordonnés dépendraient du mésencéphale; l'écorce n'est pas ici indispensable[1].

Mais le facteur déterminant des crises reste toujours à élucider. La chimie biologique grâce à ses méthodes d'investigation les plus fines. a pu montrer qu'il s'opérait de curieuses modifications chimiques dans l'intimité même des zones épileptogènes au moment des crises expérimentales. Les constatations les plus frappantes concernent la tendance à l'alcalose sanguine (qui justifie la prescription d'un régime acidosant chez les épileptiques) et le changement au niveau des neurones centraux des rapports normaux entre les ions: abaissement du potassium et du calcium, augmentation du sodium, dont les taux reviennent à la normale après la crise; hydratation cellulaire qui fait comprendre l'abaissement du seuil convulsivant par privation d'électrolytes. Notons aussi la libération d'acétylcholine, la privation d'oxygène entraînant glycolyse et hyperglycémie, la formation de guanidine, d'histamine, etc.

L'avenir seul pourra nous fixer sur la valeur de ces constatations.

2) Epilepsie Expérimentale

De nombreuses techniques permettent de déclancher chez l'animal des crises convulsives épileptiformes plus ou moins étroitement apparentées aux crises de l'épilepsie humaine.

En plus de l'excitation directe de l'encéphale par lésions traumatiques. trois groupes de méthodes principales s'avèrent utilisables:

a) excitation électrique; b) excitation chimique; c) réactions psychomotrices.

a) *Excitation électrique.* – Réalisée pour la première fois par Batelli, étudiée par Cerletti, l'excitation par passage transcérébral du courant électrique est couramment utilisée depuis qu'elle a fait l'objet des travaux de Putnam et Merrit sur le Chat[2]. D'autres animaux peuvent être utilisés: l'essentiel est d'opérer dans des conditions aussi semblables que possible si l'on veut apprécier avec certitude les modifications apportées par l'anti-épileptique étudié.

Les électrodes sont appliquées en des points convenablement choisis pour assurer le meilleur passage du courant: base du crâne et bouche; tempes ou conduits auditifs externes; globes oculaires. On réalise le

passage du courant dans des conditions de voltage, d'intensité et de durée très précises.

Chez le Lapin, que nous avons surtout utilisé, on obtient par une application sur les tempes pendant une 1/2 seconde d'un courant de 50 périodes-seconde sous une différence de potentiel de 35 volts la succession des phénomènes suivants: 1) phase tonique (15 secondes); 2) phase clonique (30 secondes); 3) phase de stupeur avec grincement des dents (15 secondes); 4) phase de relâchement musculaire (90 secondes); au total environ 2 minutes 30 secondes.

Goodman et ses collaborateurs[3] réalisent des crises électriques dites maximales au cours desquelles la durée de l'extension tonique des membres postérieurs est prise comme critérium de l'action convulsivante. Ils ont pu d'autre part abaisser le seuil des crises convulsives tant électriques que chimiques par hydratation du tissu cérébral. La dilution ou la perte des électrolytes par absorption d'eau ou de sérum glucosé isotonique ou la rétention d'eau dans l'organisme sous l'action des extraits post-hypophysaires rend les crises plus nombreuses, ou plus faciles à produire chez l'animal. Ces différents tests de Goodman permettent une étude plus fine des anti-épileptiques[4].

b) *Excitation chimique.* – De nombreuses substances peuvent produire des convulsions épileptiformes. Plusieurs d'entre elles sont d'un emploi exceptionnel: camphre et bromure de camphre, picrotoxine, strychnine, thuyone (de l'essence d'absinthe). Le produit le plus couramment utilisé est le *pentétrazol.* Chez le Lapin, son injection intraveineuse à la dose de 0,025 g. par kg. provoque la succession des phénomènes que l'on peut résumer ainsi: 1) une phase de convulsions toniques avec opistothonos qui dure environ 20 secondes; 2) une phase de convulsions cloniques (40 secondes); 3) une décontraction progressive (60 secondes) avec grincement des dents et morsures; 4) le relâchement musculaire avec asthénie (environ 180 secondes); au total de 5 à 6 minutes. Chez le Rat et la Souris on peut obtenir la même suite de phénomènes avec quelques différences dans l'attitude des animaux notamment au cours de la phase tonique.

c) *Réactions psychiques et psychomotrices.* – Les crises nerveuses que l'on peut provoquer chez l'animal par la perturbation apportée aux réflexes conditionnés s'écartent sensiblement de l'épilepsie proprement dite.

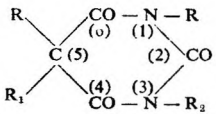
Il n'en est pas de même pour la crise audiogène (Mayer, Morgan et coll.) produite chez le Rat par une excitation auditive assez vive et notamment par différents bruits (sonnerie, sifflet). En suivant la technique préconisée par Morin[5,6,7], on peut ainsi provoquer chez certains animaux après une fuite éperdue, la succession des crises toniques et cloniques et de la dépression avec stupeur dont le déroulement rappelle à l'intensité près les phases successives déjà décrites pour l'épilepsie-maladie et l'épilepsie expérimentale.

La crise audiogène peut être empêchée par les différents anti-épileptiques actuellement utilisés, à des doses qui vont en augmentant du phénobarbital (4 mg.) à la phénytoïne (25 mg.) et la tridione (300 mg.).

Bien qu'il soit, on le conçoit, de réalisation plus difficile, le petit mal –

PRINCIPAUX ANTI-ÉPILEPTIQUES ORGANIQUES

SCHEMAS DE CONSTITUTION



Barbituriques

- R = $-CH_3$ } *rutonal*
- R_1 = $-C_5H_8$ } H.M.

- R = $-C_2H_5$ } phénobarbital
- R_1 = $-C_6H_5$ } *gardénal* *luminal* H.M.

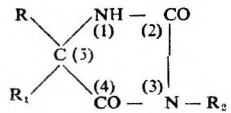
- R = $-C_2H_5$ } *prominal*
- R_1 = $-C_6H_5$ } *isonal*
- R_2 = $-CH_3$ } *mébaral* H.M.

Action sur :

H.M. = haut mal

P.M. = petit mal

Ps.M. = crise psychomotrice



Hydantoïnes

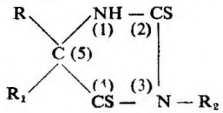
- R = $-C_2H_5$ } *nirvanol* très toxique
- R_1 = $-C_6H_5$ } H.M.(?)

- R = $-C_2H_5$
- R_1 = $-C_6H_5$ } *mésantoïne* *sédantoïnal*
- R_2 = $-CH_3$ } H.M., Ps.M.

- R = $-C_6H_5$ } phénytoïne
- R_1 = $-C_6H_5$ } *dihydan*

dilantin } sel de Na
epanutin } sel de Na
solantyl } sel de Na
H.M., Ps.M.

- R = $-C_6H_5$ } *thiantoïn*
- R_1 = thiényl }

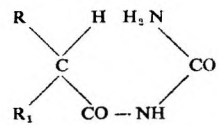


Dithiohydantoïnes

- R = $-CH_3$ } *diméthyl dithio- hydantoïne*
- R_1 = $-CH_3$ } H.M.

- R = $-C_2H_5$ } *éthylphényl dithio- hydantoïne*
- R_1 = $-C_6H_5$ } très actif mais très toxique

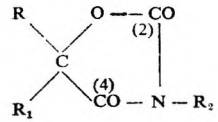
H.M.



Acylurée

- R = $-C_6H_5$ } phénacétylurée
- R_1 = $-H$ } *phénurone*

H.M., P.M., Ps.M.



oxazolidines 2 : 4 dione

- R = $-CH_3$ } *propazone*
- R_1 = $-CH_3$ }

- R = $-CH_3$ } *tridione*
- R_1 = $-CH_3$ } *épidione*
- R_2 = $-CH_3$ } *triméthadione*

P.M.

- R = $-C_2H_5$ } *paradione*
- R_1 = $-CH_3$ }
- R_2 = $-CH_3$ }

P.M.

- R = $-C_6H_5$ } *épidione*
- R_1 = $-C_6H_5$ }

H.M.

caractérisé expérimentalement par les modifications apportées à l'électroencéphalogramme – peut être provoqué, lui aussi, chez l'animal soit par des moyens électriques (excitation portée au niveau du thalamus[8]) soit par des moyens chimiques: injection de fluoroacétate ou de méthyl-fluoroacétate[9], ou de pentétrazol à dose non convulsivante[10,11].

3) LES MEDICAMENTS ANTI-EPILEPTIQUES

Il nous semble inutile d'insister sur les vieilles médications qui ont d'ailleurs fait leurs preuves et rendent encore des services: bromures et dérivés du bore.

Les *bromures,* qui ne sont efficaces chez l'animal qu'à dose toxique, luttent bien chez l'Homme contre la crise d'épilepsie proprement dite. S'ils empêchent la crise convulsive elle-même, ils agissent moins bien dans l'épilepsie psychomotrice; ils font parfois empirer le petit mal. Enfin, on sait qu'ils provoquent les divers accidents décrits sous le nom de bromisme.

Le tartrate *borico-potassique* est assez actif mais il perd rapidement son efficacité.

Barbituriques. – Utilisés depuis 1912, les dérivés de l'acide barbiturique ou malonylurée sont restés les plus utilisés jusqu'à l'apparition des hydantoïnes et des oxazolidines. Ce sont des anti-épilept ques majeurs. Le plus utilisé d'entre eux, le *phénobarbital,* combat efficacement chez l'animal les crises provoquées par le choc électrique et le pentétrazol.

Chez l'Homme il supprime le grand mal; il agit peu sur les crises psychomotrices, et dans le petit mal il fait disparaître les vertiges mais non les absences.

Sa posologie, qu'il faut adapter aux cas individuels, varie de 0,10 g. à 0,30 g. par jour, en prises fractionnées. Le traitement doit être continué sans interruption et même la diminution des doses peut faire apparaître l'état de mal. Ses inconvénients, à commencer par son action hypnotique ici gênante, sont multiples: dans 15 p. 100 des cas environ on peut trouver des symptômes connus sous le nom de petit gardénalisme: somnolence, torpeur, troubles psychiques et digestifs. Dans 5 p. 100 des cas apparaît, après une ou deux semaines, ou plusieurs mois, le grand gardénalisme: éruptions diverses, oedèmes, arthralgies. La fièvre qu'il provoque parfois peut commander l'interruption du traitement et l'administration d'un autre anti-épileptique.

Le rutonal agit comme le phénobarbital mais il exige des doses doubles.

L'isonal, qui porte un radical-CH_3 sur l'azote, est moins hypnotique que les précédents; sa posologie est également double de celle du phénobarbital.

Les barbituriques peuvent être associés à de nombreux produits: soit pour compléter leur action anti-épileptique (phénytoïne, tridione), soit pour corriger leurs effets dépressifs (caféine, amphétamine, etc. . . .).

Dérivés de l'hydantoïne.

a) *Phénytoïne.* – La diphénylhydantoïne ou phénytoïne était connue depuis 1908 (Biltz et Angeli). Insoluble dans l'eau, elle donne un dérivé sodique soluble qui a servi aux expériences de Merrit et Putnam[12,13] qui établissaient en 1938 sa supériorité sur de nombreuses substances de

ce groupe; comme on emploie assez indifféremment la phénytoïne et son sel de sodium nous ne parlerons ici que de la phénytoïne elle-même.

Chez l'animal, elle jugule les crises provoquées par le courant électrique; elle exagère les convulsions pentétrazoliques. Elle provoque à dose élevée des troubles cardiovasculaires graves.

Chez l'Homme, elle se montre spécialement active contre le grand mal; elle le combat plus énergiquement que les bromures et autant que les barbituriques, dont elle n'a pas l'action dépressive sur les fonctions cérébrales. Elle n'agit qu'après une administration répétée pendant quelques jours.

Elle est cependant rapidement détruite dans l'organisme, ou éliminée. On l'administres à la dose de 0,30 g. à 0,50 g. par jour, généralement au moment des repas. On l'associe souvent aux barbituriques surtout dans le traitement des grandes crises.

La tolérance des malades à son égard est très variable. Elle peut provoquer, en plus de quelques troubles digestifs, d'assez nombreux incidents. Dans 15 p. 100 des cas ceux-ci sont légers: phénomènes d'alarme, titubation, diplopie, tremblement, troubles de la mémoire; parfois même anxiété, céphalée, nausées, fièvre, incoordination motrice. On peut à la suite d'un traitement prolongé observer de l'hyperplasie buccale et de la gingivite; ces accidents commandent la suspension du traitement, laquelle peut ici être brusque sans inconvénients.

Dans 5 p. 100 des cas l'intolérance à la phénytoïne peut être beaucoup plus grave: purpura, gastrite hémorragique, etc. . . . On a même enregistré quelques cas de mort.

b) *Autres dérivés de l'hydantoïne.* – Par substitution sur le $-CH_2-$ on obtient la phénylméthylhydantoïne et la phényléthylhydantoïne (nirvanol), très toxiques; et la phénylthiénylhydantoïne, encore en cours d'études. Sur le $-CH_2-$ et un -NH-, on obtient l'éthylphényl-N-méthylhydantoïne ou mésantoïne.

c) *Diméthyldithiohydantoïne.* – Parmi les substitutions diverses que nous avons nous-mêmes réalisées sur le noyau formateur de l'hydantoïne les plus intéressantes ont été obtenues par l'entré de 2 atomes de soufre dans le noyau. La substance la plus digne d'être retenue parmi la quarantaine de corps préparés à partir de la dithiohydantoïne est la diméthyldithiohydantoïne[14,15]. Insoluble comme la diphénylhydantoïne, ce dérivé soufré peut donner des sels solubles et notamment celui de calcium. Chez le Lapin, la diméthyldithiohydantoïne se montre active contre les convulsions provoquées par le pentétrazol, et non pas contre la crise électrique comme la phénytoïne. Elle se sépare aussi de celle-ci par l'absence d'action cardiovasculaire dépressive, comme on peut l'observer chez le Chien: son sel de calcium est même cardiotonique.

Chez l'Homme, les premiers essais ont montré qu'elle se comportait comme un anti-épileptique majeur. Particulièrement active contre le grand mal, elle a même donné quelques succès dans le petit mal.

On l'administre à la dose de 0,80 g. par jour donnée par fractions. Elle est bien tolérée même à doses plus élevées que la phénytoïne; elle n'est pas hypnotique comme le phénobarbital.

Dans le grand mal, elle peut être associée à la dose de 0,60 g. à une petite quantité (0,10 g.) de phénobarbital.

Le seul incident, provoqué par la présence dans sa molécule du groupement $-N=C=S$ (que l'on trouve dans les antithyroïdiens de synthèse), peut être l'hypertrophie de la thyroïde, sans modification très marquée de l'aspect histologique de la glande. Cette action, dont nous avons eu quelques exemples chez des sujets jeunes en état d'instabilité glandulaire, disparaît aisément sous l'influence d'un traitement thyroïdien associé.

Dérivés de l'oxazolidine. – La triméthyloxazolidine ou tridione, produit de substitution de l'oxazolidine-2 : 4-dione, a été étudiée chez l'animal par Everett et Richards[16,17,18] en 1944 et proposée comme anti-épileptique chez l'Homme par Richards et Perlstein en 1945.

Expérimentalement, la tridione est sans grande efficacité contre le choc électrique ou pentétrazolique, sauf à dose élevée; elle est au contraire efficace (Goodman-1949) contre les manifestations électriques caractéristiques du petit mal provoqué par les doses sous-convulsivantes de pentétrazol.

Chez l'Homme, la tridione se montre de même inactive sur le grand mal dont elle pourrait même, d'après certains auteurs, aggraver les symptômes surtout quand il est associé au petit mal. Elle est par contre, surtout chez l'Enfant, très active à l'égard du petit mal et les attaques psychomotrices. Elle n'est hypnotique qu'à doses très élevées.

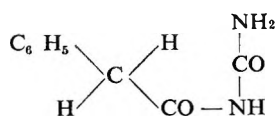
On la prescrit à la dose quotidienne de 0,90 g. à 1,20 g. par jour et d'environ 0,15 g. par année d'age chez l'Enfant.

Si elle est peu toxique et sans effet nocif sur le cœur et la respiration, la tridione peut provoquer divers incidents (somnolence, fièvre, troubles digestifs et cutanés) et des accidents. Ces derniers, observés dans 75 p. 100 des cas environ, sont d'abord d'ordre visuel: sensibilité à la lumière, éblouissement, troubles de la vision colorée qu'accompagnent des douleurs au niveau du globe oculaire. Tous ces troubles s'amendent par cessation du traitement qui, ici encore, peut être brusque.

Les accidents d'ordre hématologique sont plus rares mais graves; on a signalé des cas de mort par anémie aplastique et agranulocytose.

Il convient donc de surveiller la formule sanguine des malades en cours de traitement.

Phénacétylurée:



L'action anti-épileptique de ce produit n'est connue que depuis 1948. Son étude expérimentale a été entreprise seulement en 1949 par Everett, et par Goodman et ses collaborateurs[19,20].

Chez l'animal il se comporte comme un anti-épileptique puissant actif contre les épilepsies diversement provoquées.

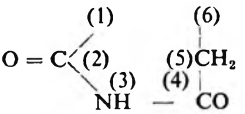
L'étude clinique faite par Gibbs et ses collaborateurs[21] ainsi que par Davidson et Lennox[22] montre des résultats thérapeutiques favorables même chez certains malades réfractaires aux autres anti-épileptiques.

Elle est prescrite à la dose moyenne de 2,5 g., réparties en 5 prises de 0,5 g., au cours de la journée.

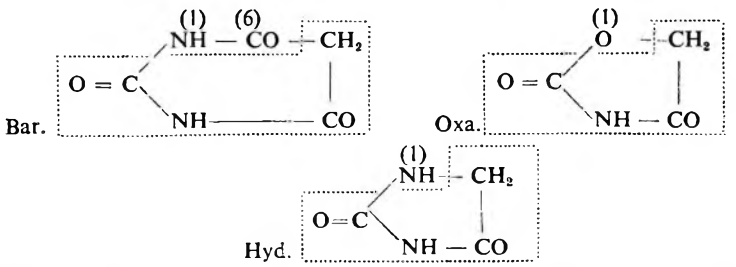
Les auteurs signalent des effets secondaires variés, nombreux et gênants (troubles cutanés, gastriques, psychiques, parfois lésions hépatiques) nécessitant un contrôle médical sérieux.

4) RAPPORT ENTRE ACTION ET CONSTITUTION

Si l'on compare la configuration des *noyaux formateurs* des antiépileptiques de synthèse actuellement connus, on est frappé par la présence d'un groupement commun à tous ces noyaux, que l'on peut pour la commodité de l'exposition représenter de la sorte:

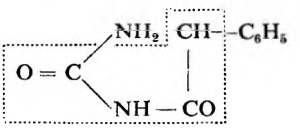


Dans les barbituriques (*Bar.*) le carbone 2 est uni au carbone 5 par la chaine - NH - CO -,



dans les hydantoïnes (*Hyd.*) par le groupement - NH -, dans les oxazolidines (*Oxa.*) par un atome d'oxygène.

La phénacétylurée trouve sa place ici, puisqu'on peut l'écrire de la sorte:



formule qui permet de la considérer comme une hydantoïne ouverte avec l'activité que confère dans bien des cas déjà connus l'ouverture de la molécule.

Les dithiohydantoïnes ont bien, elles aussi, ce groupement commun, l'oxygène étant ici remplacé par le soufre. L'entrée de ce métalloïde transforme l'activité de la molécule et fait qu'elle est mieux tolérée par l'organisme.

Remarquons enfin, en ce qui concerne la constitution du noyau, la présence de 2N dans les barbituriques et les hydantoïnes actives contre le grand mal et d'un seul N dans les oxazolidines actives contre le petit mal.

L'effet des *substitutions* faites sur le *carbone 5* est considérable. On en connaissait déjà l'importance en ce qui concernait les barbituriques. Dans leur cas, le radical $-C_6H_5$ tient sous sa dépendance l'action sédative mais il doit être unique: la diphénylmalonylurée est en effet convul-

sivante. Le – C_6H_5 est unique aussi dans la phénacétylurée. Dans le cas des hydantoïnes au contraire c'est la présence de 2 – C_6H_5 qui donne les meilleurs résultats. Remarquons que la réunion d'un – C_6H_5 et d'un – C_2H_5 favorable dans le cas des barbituriques, ne l'est pas dans le cas des hydantoïnes: la phényléthylhydantoïne est moins active et plus toxique que la diphénylhydantoïne. Dans les dithiohydantoïnes, c'est la substitution par 2-CH_3 qui est la plus avantageuse.

La méthylation portant sur l'*azote* en 3 diminue généralement les actions hypnotique et anti-convulsivante: cas du prominal dans les barbituriques, de la mésantoïne dans les hydantoïnes, de la tridione dans les oxazolidines.

L'étude des rapports qui lient l'action physiologique d'une molécule organique à sa constitution physico-chimique doit porter, nous le savons maintenant, sur des séries homogènes. On peut espérer que la constatation que l'on peut faire dans la série des anti-épileptiques au noyau formateur commun permettra d'orienter des recherches nouvelles.

Au terme de cette rapide revue[23], on peut conclure que l'application des méthodes expérimentales au problème de l'épilepsie a, en quelques années, mené à des acquisitions importantes sur le plan théorique et pathogénique et surtout sur le plan pratique.

Nous possédons dès à présent une gamme de produits dont quelques-uns permettent à l'épileptique de mener une existence à peu près normale. Cette thérapeutique reste cependant encore symptomatique et bien des progrès restent à accomplir.

Peu de questions autant que celle-ci montrent la prééminence actuelle de la pharmacologie, science expérimentale par excellence, mais dont les découvertes contribuent si largement au développement de la thérapeutique.

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RESEARCH PAPERS

THE IDENTIFICATION OF THE SYNTHETIC STILBENE ŒSTROGENS

By J. W. G. Smith and G. E. Turfitt

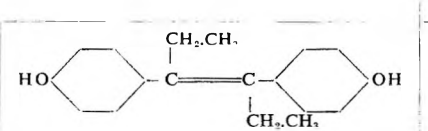
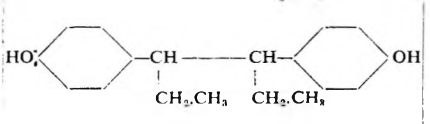
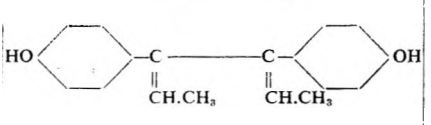
From the Metropolitan Police Laboratory

Received August 20, 1949

Following the demonstration by Dodds[1] of the œstrogenic activity of certain synthetic stilbene derivatives, various compounds of this type have been introduced into œstrogen therapy. Of these, the structurally similar stilbœstrol, hexœstrol and dienœstrol, and the dipropionate of stilbœstrol (Table I) are commercially available in this country, either in tablet form, in oil solution for injection purposes, or as ointments.

TABLE I

Synthetic stilbene œstrogens

	Structure	M.pt. °C.	Solubilities
Stilbœstrol B.P.		169	Very slightly soluble water Slightly soluble benzene Soluble ethyl alcohol, ether, acetone, chloroform, sodium hydroxide
Hexœstrol B.P.		186	Insoluble water Slightly soluble chloroform, benzene Soluble ethyl alcohol, ether, acetone, sodium hydroxide
Dienœstrol B.P.		233	Insoluble water Slightly soluble chloroform, benzene Soluble ethyl alcohol, ether, acetone, sodium hydroxide
Stilbœstrol dipropionate B.P.C.		105	Slightly soluble water Soluble ethyl alcohol, ether, acetone, chloroform, benzene

The toxicity of these drugs is of the same order as that of the natural œstrogens, and toxic symptoms during clinical usage may develop as a result of excessive doses or prolonged administration. Although various colorimetric procedures are available for the quantitative determination of these synthetic products (Dingemanse[2]; Tubis and Bloom[3]; Huf and Widmann[4]; Malpress[5]), their qualitative identification and differentiation appear to have received but little attention. The interesting colour reactions of Cocking[6,7] whilst clearly differentiating hexœstrol from stilbœstrol and dienœstrol, permit of no distinction between the two latter compounds themselves, and are entirely negative in the case of the ester.

In the present publication a series of five simple colour reactions is described, from the results of which the three parent œstrogens and stilbœstrol dipropionate may be readily identified and differentiated.

EXPERIMENTAL

In various instances the tests may be performed directly on tablets, oil solutions or ointments with entirely satisfactory results, but, in general, it is desirable for the drug to be isolated in the pure state, prior to the examination.

Colour reactions. The complete series of five tests described below requires, for any particular compound, a quantity of material of not more than 0·5 mg.; the resulting colours (Table II) are distinctive and free from ambiguity.

TABLE II

COLOUR REACTIONS OF SYNTHETIC STILBENE ŒSTROGENS

Test	Stilbœstrol	Hexœstrol	Dienœstrol	Stilbœstrol Dipropionate
Sulphuric acid	Bright red yellow	Colourless	Yellow	Orange-yellow
Heated	Orange	Colourless	Green	Orange
Sulphuric acid-sodium nitrite	Intense red	Intense purple	Intense red	Intense red
Sulphuric acid-vanillin ...	Orange-red ; purple margin	Yellow	Greenish-brown ; purple margin	Orange-reddish-brown ; purple streaks
Addition of ethyl alcohol ...	Violet	Colourless	Pink	Violet
Phosphoric acid-vanillin ...	Yellow-purple-carmine	Yellow-reddish-brown-purple; solution turbid	Yellow-deep blue	Yellow-purple
Hydrochloric acid-vanillin ...	Colourless	Colourless	Blue	Colourless
Addition of bleaching powder	Green or blue	Colourless	—	Green

(1) *Sulphuric acid test.* A quantity of material of the order of 0·01 to 0·1 mg. in a white porcelain dish is treated with one drop of concentrated sulphuric acid, and heated for 1 minute on a boiling water-bath.

(2) *Sulphuric acid-sodium nitrite test.* 0·1 mg. of sodium nitrite is treated with one drop of concentrated sulphuric acid in a white porcelain dish, which is then heated for 10 seconds on a boiling water-bath. 0·01 mg. of the test material is sprinkled over the surface of the still hot acid mixture.

(3) *Sulphuric acid-vanillin test.* 0·1 mg. each of test material and vanillin is placed in a white porcelain dish, and treated with one drop of concentrated sulphuric acid. The dish is warmed for one minute on a boiling water-bath with occasional gentle rocking and cooled, and the acid mixture is treated with 1 to 2 ml. of alcohol.

(4) *Phosphoric acid-vanillin test.* 0·01 to 0·1 mg. each of test material and vanillin is treated in a small (2″ × ¼″) test tube with one drop of syrupy phosphoric acid, and the liquid heated to boiling for 10 to 15 seconds.

(5) *Hydrochloric acid-vanillin test.* 0·1 mg. each of test material and vanillin in a 3″ × $\frac{3}{8}$″ test tube is treated with one drop of glacial acetic acid, solution being affected by gentle warming. 2 to 3 drops of concentrated hydrochloric acid are added, and the liquid gently boiled for 10 to 15 seconds. If no colour develops, approximately 0·1 mg. of bleaching powder is added, and the liquid again boiled.

Although the results of this series of tests provide conclusive identification within the group, the conclusion should be checked whenever practicable by a micro mixed melting-point determination.

Summary

1. A series of five simple colour reactions is described whereby the synthetic stilbene œstrogens, stilbœstrol, hexœstrol, dienœstrol and stilbœstrol dipropionate may be identified and distinguished.

2. The tests involve the use of no unusual reagents and may be completed within 5 to 10 minutes, using a total amount of only 0·5 mg. of test material.

The authors wish to express their thanks to Messrs. Boots Pure Drug Co. for their kind co-operation in supplying samples of pure œstrogens, and to Dr. H. S. Holden, Director of the Metropolitan Police Laboratory, for his permission to publish the results of this investigation.

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A NOTE ON RUPE'S REARRANGEMENT

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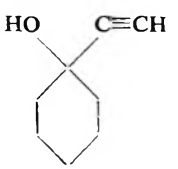
RUPE, *et al.*[1] described a rearrangement in which 1-ethynyl*cyclo*hexanol-1 (I) was heated with 85 per cent. formic acid. They asserted that the compound obtained was *cyclo*hexylidene acetaldehyde (II) on the evidence of a positive colour test with Schiff's reagent, but the physical constants of its hydrogenated product were not quite those expected. On the other hand, ozonolysis of the compound gave not *cyclo*hexanone but adipic acid. It was later shown by Fischer and Loewenberg[2] that the compound was actually 1-acetyl-*cyclo*hexene-1(III). Further, it was established by Hurd and Jones[3] that, in an analogous manner, 1-ethynyl-*cyclo*hexanol-1 was converted by thionyl chloride in presence of pyridine into 1-α-chlorovinyl-*cyclo*hexene-1 (IV).



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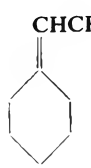

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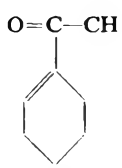

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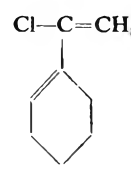


IV.

Hurd and Christ[4] repeated Rupe's rearrangement and the product, boiling at 60° to 64°C./4 mm., was identical in every respect with the 1-acetyl*cyclo*hexene-1 prepared by Darzen's method from acetyl chloride and *cyclo*hexene. Both gave a semicarbazone melting at 220° to 221°C. and no depression was observed on determining a mixed melting-point.

On attempted dehydration of 1-ethynyl*cyclo*hexanol-1 with anhydrous oxalic acid, Levina and Vinogradova[5] obtained the same 1-acetyl-*cyclo*hexene-1, but Bergmann and Bergmann[6] reported *cyclo*hexanone and *cyclo*hexenylacetic acid as the product.

In this laboratory, experiments were carried out under similar conditions. On heating 1-ethynyl*cyclo*hexanol-1 with anhydrous oxalic acid at 120°C. a vigorous reaction soon set in, after the subsidence of which the mixture was heated for another $1\frac{1}{2}$ hours and the product isolated by extraction with benzene and fractionation. Invariably, a yield of about 40 per cent. of 1-acetyl*cyclo*hexene-1 was obtained together with a little of a low-boiling fraction, the residue in the flask being polymerised tarry matter. The main fraction showed the following constants b.pt. 63° to 64°C./6mm., $n_D^{23°C.}$ 1·4903, $n_D^{16°C.}$ 1·4928, $d_4^{20°C.}$ 0·9678, EM_D 0·79, and gave no colour with Schiff's reagent after standing overnight, although it reduced Fehling's solution on shaking in a boiling water-bath. It gave a

semicarbazone (from acetone/chloroform), melting at 210°C. and a 2:4-dinitrophenylhydrazone (from acetone/chloroform), melting at 204° to 205°C. Its ultra-violet absorption spectrum was taken and is shown in Figure 1; λ max. 234 mμ, $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 957, log. ε 4·075 (in absolute alcohol). Additional direct proof of the methyl ketone structure was obtained by a positive iodoform test.

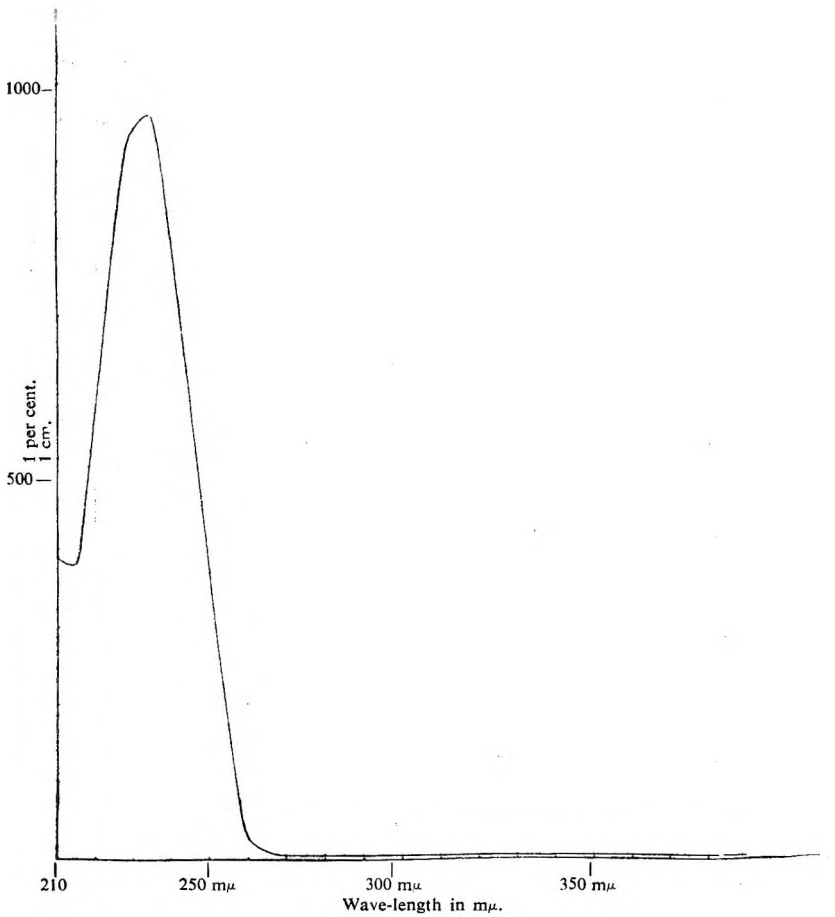
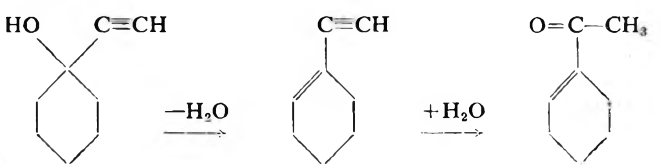


FIG. 1.

An attempt was then made to explain the mechanism of this reaction. As both 85 per cent. formic acid and anhydrous oxalic acid are dehydrating agents, it was postulated that the reaction proceeded by a primary dehydration and a subsequent hydration:



This was substantiated by subjecting 1-ethynyl*cyclo*hexene-1 to similar treatment with 85 per cent. formic acid, and with annydrous oxalic acid plus a little water. In the former case a 52 per cent. yield and in the latter a 29·5 per cent. yield, of the 1-acetyl*cyclo*hexene-1 were obtained. The product, on analysis and determination of the physical constants, gave the required figures and no depression of the melting-point was observed when the semicarbazone and the 2:4-dinitrophenylhydrazone obtained from it were mixed with those obtained as described above. Absorption maximum 234μ, and $E^{1\ per\ cent.}_{1\ cm.}$ were 958 and 954 respectively (in absolute alcohol).

Experimental

1. *Rearrangement of 1-ethynyl*cyclo*hexanol-1 by heating with anhydrous oxalic acid.*—1-ethynyl-*cyclo*hexanol-1 (13·0 g.) was mixed with anhydrous oxalic acid (9·0 g.) and the mixture was heated under a reflux condenser in an oil bath at 120°C. A vigorous reaction soon set in and the heating was interrupted by lifting the flask out of the oil bath. When the reaction had subsided the blackish mixture was heated at 120°C. for another 1½ hours. After cooling, the product was extracted with benzene and the benzene extract was washed and dried. On fractionation, the fraction distilling at 60° to 70°C./6 to 10 mm. was collected, which on redistillation, mainly distilled at 63° to 64°C./6mm. Hg. pressure. Yield: 5 g. (38 per cent.). Found: C, 77·75; H, 9·87 per cent.; $C_8H_{12}O$ requires C, 77·40; H, 9·75 per cent. $n_D^{16°C.}$ 1·4928, $n_D^{23°C.}$ 1·4903, $d_4^{20°C.}$ 0·9678; molecular refraction, according to 1-acetyl-cyclohexene-1: found 37·28; calculated 36·49, EM_D 0·79. It gave no precipitate with ammoniacal silver nitrate, gave no active hydrogen (Zerewitinoff); did not restore Schiff's reagent on standing overnight, but reduced Fehling's solution on heating in a boiling water-bath with shaking. To a few drops of the product in a test tube, 5 ml. of a solution of iodine in potassium iodide and 2 ml. of 20 per cent. sodium hydroxide solution were added, a yellow crystalline precipitate formed was identified as iodoform. The semicarbazone, recrystallised from a mixture of chloroform and acetone melted at 210°C. Found: C, 59·86; H, 8·47; N, 22·60 per cent. $C_9H_{15}ON_3$ requires C, 59·64; H, 8·34; N, 23·19 per cent. The 2:4-dinitrophenylhydrazone, recrystallised from a mixture of chloroform and acetone, melted at 204° to 205°C. Found: C, 55·26; H, 5·44; N, 18·1 per cent. $C_{14}H_{16}O_4N_4$ requires C, 55·26; H, 5·30; N, 18·42 per cent. Absorption spectrum: max. 234 mμ, $E^{1\ per\ cent.}_{1\ cm.}$ 957, log ε 4·075 (in absolute alcohol).

2. *Addition of water on to 1-ethynyl*cyclo*hexene-1 in presence of oxalic acid.*—1-ethynyl*cyclo*hexene-1 (5 g.) was heated with anhydrous oxalic acid (3·5 g.) and water (0·5 g.) in exactly the same manner as before. On fractionation of the benzene extract of the product, the fraction 55 to 60°C./7 mm. Hg pressure was collected, which on redistillation gave a main distillate at 58°C./5 mm. Hg. pressure. Yield, 1·7 g. (29.5 per cent.) $n_D^{18°C.}$ 1·4923, $n_D^{23°C.}$ 1·4900. Reactions were found

to be the same as before. Found: C, 77·23; H, 9·74 per cent. $C_8H_{12}O$ requires C, 77·40, H, 9·75 per cent. The semicarbazone and 2:4-dinitrophenylhydrazone showed the same melting-point, and mixed melting-points showed no depressions when mixed with the corresponding derivatives obtained from experiment 1. Absorption maximum 234 mμ, $E_{1\ cm.}^{1\ per\ cent.}$ 954 (in absolute alcohol).

3. *Addition of water to 1-ethyl*cyclo*hexene-1 in presence of 58 per cent. formic acid.*—The reaction was carried out according to the conditions given by Rupe[1] for his rearrangement of 1-ethynyl*cyclo*hexanol-1. 1-Ethynyl*cyclo*hexene-1 (5 g.) was refluxed with 85 per cent. formic acid (40 ml.) in an oil bath at 120°C. A vigorous but smooth reaction started and the mixture turned dark coloured. After further heating for 2½ hours, the acid was neutralised with sodium carbonate and the product was extracted with benzene. The benzene extract was then washed and dried. On fractionation a distillate was collected between 50 and 58°C./7 mm. Hg pressure which on redistillation distilled mainly at 58°C./5 mm. Hg pressure. Yield: 3 g. (52 per cent.) $n_D^{18°C.}$ 1·4925; $n_D^{23°C.}$ 1·4901. The reactions were found to be identical with those previously recorded above. Found: C, 77·13; H, 9·60 per cent. $C_8H_{12}O$ requires C, 77·40; H, 9·75 per cent. The semicarbazone and 2:4-dinitrophenylhydrazone gave the same melting-points as those from experiments 1 and 2 and the mixed melting-points showed no depression.

Absorption maximum: 234 mμ, $E_{1\ cm.}^{1\ per\ cent.}$ 958 (in absolute alcohol).

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THE COLCHICINE CONTENT AND TOXICITY OF COLCHICUM SEEDS AND CORMS COLLECTED AT DIFFERENT HEIGHTS

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Since 1944 systematic studies have been carried out in this Institute (Mascherpa[1]), in order to discover the relation between mountain climate and the activity of certain drugs. Marangoni [2] and Dordi[3] have studied valerian, frangula and aconite; similar researches are now being made on *Digitalis lutea*, and on some vitamin-containing fruits. Many authors have already investigated this subject, which is interesting from both the scientific and practical points of view; for references, besides the cited authors, the reviews in Meier's[4] and Bänninger's[5] works may be consulted. No research has yet been made, as far as I know, on the relation between the toxicity and colchicine content of colchicum, and mountain climate.

Material and Technique

The material used were the seeds and corms of *Colchicum autumnale* L., collected in the months of July and August on the Alps (valleys of Aosta and Fiemme), on the Apennines and in the plain (province of Pavia), at the heights of 50, 100, 1000, 1200, 1400 and 2200 metres above sea level. At the heights for which it was possible, the material was collected in different places, in the attempt to exclude other factors than altitude, such as soil, sunlight, winds, rains, eventual fertilisation, etc. Plants growing wild on untilled ground and showing ripe seeds in dried capsules were collected. The seeds and corms, after being dried first at room temperature, then in an oven at 45°C. until of constant weight, were powdered and all stored under the same conditions.

For the chemical determination of colchicine, from the numerous methods suggested the method of Self and Corfield[6] adopted in the British Pharmacopœia was selected because of its greater quickness and accuracy. For the toxicity tests tinctures were prepared by percolation (10 per cent.) according to the Pharmacopœia Helvetica V (Italian edition). These tests were made, for each sample gathered, on 3 groups of albino mice (average weight 25 g.), which received intraperitoneally doses of 0·0010, 0·0015 and 0·0020 g. of drug per g. of weight. The mortality was observed after 96 hours.

Experimental Results

Chemical assays.—The results of the chemical assays are reported in Table I; the percentages of colchicine are referred to the dry drug.

These results show that the colchicine generally decreases as the altitude increases; this diminution is far more definite in the seeds than in the corms.

TABLE I

CHEMICAL ASSAYS

Metres above sea level	Average weight of 100 seeds g.	Colchicine in seeds per cent.	Average weight of one corm g.	Colchicine in corms per cent.
50	0·541	0·45	0·646	0·150
100	0·299	0·55	0·750	0·098
1000	0·218	0·35	5·066	0·077
1200	0·341	0·37	1·415	0·125
1400	0·462	0·22	2·042	0·132
2200	0·337	0·24	4·617	0·085

Toxicity Tests.—The results of the toxicity tests for seeds and corms are shown in Table II.

TABLE II

TOXICITY TESTS

Metres above sea level	Mortality per cent. to the doses					
	0·0010 g.		0·0015 g.		0·0020 g.	
	Seeds	Corms	Seeds	Corms	Seeds	Corms
50	80	70	100	60	100	90
100	90	30	90	30	100	70
1000	60	40	60	80	60	30
1200	50	60	60	50	70	100
1400	30	20	50	50	60	60
2200	30	30	40	80	60	50

The results of the determination of colchicine obtained are generally confirmed: in seeds a diminution of toxicity is observed as the altitude increases; in corms a relation between toxicity variations and altitude cannot be detected.

SUMMARY AND CONCLUSIONS

1. The content of colchicine, and toxicity, were studied in relation to altitude in the seeds and corms of *Colchicum autumnale* L., gathered on the Alps, Apennines and the plain, at the heights ranging from 50 metres to 2200 metres above sea level; the results obtained permit only general conclusions to be drawn.

2. In general, as altitude increases, there is a diminution in the colchicine content and the toxicity of seeds; the colchicine content and the toxicity of corms do not seem to be in evident relation to altitude.

3. With regard to these results, the greater influence of climatic variations on overground than on underground organs, and the fact that the latter, being storage organs, take generally a less active part in the metabolic changes caused by climatic factors on plants may be considered.

4. The advantage of using seeds rather than corms is confirmed. The necessity for a strict chemical and pharmacological control of colchicum to be used for therapeutic purposes is also confirmed. From the chemical point of view, colchicum gathered as far as 1000 metres above sea level is to be preferred.

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ANTIBACTERIAL COLLOIDAL ELECTROLYTES : THE POTENTIATION OF THE ACTIVITIES OF MERCURIC-, PHENYLMERCURIC- AND SILVER IONS BY A COLLOIDAL SULPHONIC ANION

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The impairment of the antibacterial activity of inorganic mercuric salts by blood serum, pus and tissue debris has lead in recent years to the introduction of salts of phenylmercuric hydroxide, notably the acetate, nitrate and borate[1,2,3,4,5,6]. Armangue and Maestres[7] and later Jensen[8] concluded from work with these organomercuric salts that while the acid radical influences solubility and other physical properties the antibacterial activity is an independent function of the phenylmercuric cation and this activity is not significantly effected by the presence of serum.

Evidence was presented by Süpfle and Miller[9] to show that mercuric ions are adsorbed upon the cortical layer of the bacterial cells thereby poisoning essential enzyme systems ; the union, however, is loose and treated cells can be revived by shaking with a stronger absorbent such as blood charcoal. The superiority of phenylmercuric salts may be associated with the presence of the benzene nucleus which, by conferring lipophilic properties upon the active cation, renders the union of the organo-mercurial with the bacterial envelope less easily reversible.

The enhancement of the antibacterial activity of inorganic mercuric salts by surface active agents was first reported by Hamilton[10] who observed that the activity of potassium mercuric iodide against *Staphylococcus aureus* was increased threefold by the presence of sodium salts of long chain aliphatic carboxylic acids. Later, Hampil[11] showed that certain concentrations of sodium oleate greatly augment the activity of mercuric chloride against this organism. Tobie and Orr[12] found that sodium dioctyl sulphosuccinate strongly potentiates the bactericidal activity of phenylmercuric nitrate and suggested that this was due to the formation of phenylmercuric dioctyl sulphosuccinate although they failed to isolate the compound. The enhancement of activity by surface active agents is not confined to mercurials. Ordal, Wilson and Borg[13] reported that both sodium dioctyl sulphosuccinate and sodium lauryl sulphate increase the activity of 2 : 4-dichlorophenol and ascribed the action to the greater penetrative power of the antibacterial reagent when associated with surface active compounds ; the same effect has been observed[13,14,15] with phenol in the presence of various soaps. In work with cetylpyridinium chloride and sodium tetradecyl sulphate Dyar and Ordal[16] showed that there is much greater adsorption of the former than of the latter upon all bacteria studied ; this accords with the established

fact that cationic soaps are much more lethal to bacteria than the anionic soaps.

Ionic surface active agents are essentially colloidal electrolytes which possess lipophile-hydrophile balance. Colloidal electrolytes possess two properties by virtue of which they influence biological activity, viz., their strong tendency to adsorb at interfaces and their power to form charged hydrated colloidal aggregates (micelles). Micelles, owing to their structure, adsorb ions carrying a charge opposite to that upon the micelle and in addition are able by the process of molecular adsorption to solubilise many organic substances which are normally insoluble in water. Micellar solubilisation is known to facilitate transport of sparingly soluble substances at a rate which may be enormously greater than the normal process of solution and diffusion. These properties of surface active compounds led to an interest in colloidal electrolytes containing, as the constituent cation, an ion of known toxicity to bacteria, viz., the mercuric, phenylmercuric and silver ions. Provided the resulting colloidal electrolyte possessed the requisite physical characteristics it was expected that the colloidal anion would potentiate the lethal action of the metallic or organometallic cation by promoting adsorption of the latter upon the bacterial envelope and that inactivation of the cation by formation of a coagulum with serum electrolytes would be suppressed by the process of micellar solubilisation of the coagulum.

The colloidal acid selected for the present work was dinaphthylmethane disulphonic acid obtained by a Lederer-Manasse condensation of naphthalene-2-sulphonic acid with formaldehyde. The salts of this acid are strongly ionised in aqueous solution ; they behave as typical colloidal electrolytes and exhibit the phenomenon of hydrotropy. The standardised solutions used contained 1.0 g. of mercuric-, silver- or phenylmercuric-dinaphthylmethane disulphonates and 19 g. of potassium dinaphthylmethane disulphonate per litre. Comparison has been made between the bacteriostatic activities of these solutions with those of mercuric chloride, silver nitrate and phenylmercuric acetate, these being crystalloid (non-colloidal) electrolytes containing the corresponding antibacterial cations. Three Gram-positive and three Gram-negative test organisms were used, *Staphylococcus aureus* NC7361, *Streptococcus pyogenes* NC2432, *B. subtilis* NC3610, *E. coli* NC86, *Ps. pyocyaneus* NC1999 and *Proteus vulgaris* NC5887. The media were Lab-Lemco broth (B) and broth containing 10 per cent., 50 per cent. and 80 per cent. of normal horse serum (10 per cent., S 50 per cent. S and 80 per cent. S respectively[7]) Tables I and II show the experimentally determined maximum dilutions which completely inhibit visible growth of the organism after 48 hours incubation at 37°C. *p*-Chloro-*m*-xylenol and phenol are included for the purpose of comparison.

In Table II, relating to the silver compounds, are included the experimentally found maximum bacteriostatic dilutions of silver proteinate, silver vitellin and " colloidal silver." It was of interest to include these well known protein silver—silver oxide—silver hydroxide dispersions

since these were specifically introduced as silver compounds which, by virtue of their low content of *ionised* silver, avoid in varying degrees coagulation and consequent inactivation by serum ampholytes.

TABLE I

MAXIMUM BACTERIOSTATIC DILUTIONS IN MULTIPLES OF 1,000
(48 hours incubation)

Compound	*Staph. aureus*	*Strep. pyogenes*	*E. coli*	*B. subtilis*	*Ps. pyocyaneus*	*B. Proteus vulgaris*
Mercuric chloride; Hg, 74 per cent. :—						
B	20	20	14	10	10	14
10 per cent. S...	20	20	14	14	10	14
50 per cent. S...	10	10	8	8	8	8
80 per cent. S...	8	8	5	4	2	4
Mercury dinaphthylmethane disulphonate, Hg, 32 per cent.:—						
B	12	12	8	8	6	8
10 per cent. S...	18	18	8	10	8	10
50 per cent. S...	18	18	10	10	8	10
80 per cent. S...	18	18	10	10	8	10
Phenylmercuric acetate; C_6H_6 Hg, 82·5 per cent. :—						
B	400	80	20	20	20	20
10 per cent. S...	500	200	40	30	30	30
50 per cent. S...	200	200	30	10	20	20
80 per cent. S...	100	100	20	8	15	15
Phenylmercuric dinaphthylmethane disulphonate; C_6H_6Hg, 56·5 per cent. :—						
B	250	64	16	10	16	16
10 per cent. S...	350	100	25	20	20	20
50 per cent. S...	400	120	30	25	30	25
80 per cent. S...	400	120	30	25	30	25
p-Chloro-*m*-xylenol :—						
B	7	7	3·5	3	3·5	3·5
10 per cent. S...	4	4	2	1	2	2
50 per cent. S...	4	4	2	1	2	2
80 per cent. S...	4	4	2	1	2	2

It can be seen that the activity of mercury dinaphthylmethane disulphonate in pure broth slightly exceeds that of mercuric chloride per unit of mercuric ion content. While the activity of mercuric chloride falls rapidly as the serum content of the medium rises, that of mercury dinaphthylmethane disulphonate is increased by the presence of all the concentrations of serum employed. In 80 per cent. serum the activity of mercury dinaphthylmethane disulphonate is approximately 5 times as high as that of mercuric chloride per unit of mercuric ion content.

The activity of phenylmercuric dinaphthylmethane disulphonate in broth is of the same order as that of phenylmercuric acetate per unit of phenylmercury content and with both compounds the presence of 10 per cent. serum augments activity. With phenylmercuric acetate, however, as the serum content rises through 50 per cent. to 80 per cent. there is a significant fall in activity; in the case of phenylmercuric dinaphthylmethane disulphonate the initial increase in activity is maintained as the serum content rises to 80 per cent. and at this serum concentration the activity of the compound is of the order of 3 times that of phenylmercuric acetate per unit of phenylmercuric ion content.

TABLE II

MAXIMUM BACTERIOSTATIC DILUTIONS IN MULTIPLES OF 1,000
(48 hours incubation)

Compound	*Staph. aureus*	*Strep. pyogenes*	*E. coli*	*B. subtilis*	*Ps. pyocyaneus*	*Proteus vulgaris*
Silver nitrate; Ag, 60 per cent. completely ionisable :—						
B	20	24	16	12	18	16
10 per cent. S...	10	10	6	6	8	6
50 per cent. S...	6	6	4	4	4	4
80 per cent. S...	4	4	2	2	2	2
Silver dinaphthylmethane disulphonate; Ag, 31 per cent. completely ionisable :—						
B	16	20	12	10	12	12
10 per cent. S...	20	22	18	12	18	18
50 per cent. S...	20	22	18	12	18	18
80 per cent. S...	20	22	18	12	18	18
Silver proteinate; Ag, 8 per cent. partly ionisable :—						
B	10	10	8	6	6	8
10 per cent. S...	5	5	4	2	4	4
50 per cent. S...	4	4	4	2	4	4
80 per cent. S...	4	4	4	2	2	4
Silver vitellin; Ag, 20 per cent. very little ionisable :—						
B	8	8	6	4	4	6
10 per cent. S...	6	6	4	2	4	4
50 per cent. S...	4	4	4	2	4	4
80 per cent. S...	4	4	4	2	2	4
Colloidal silver; Ag, 70 per cent. none ionisable :—						
B	8	6	6	3	3	3
10 per cent. S...	8	8	6	3	3	3
50 per cent. S...	8	8	6	3	3	3
80 per cent. S...	6	6	4	2	2	2
Phenol :—						
B	0·2*	0·2	0·15	0·1	0·15	0·15
10 per cent. S...	0·1	0·1	0·1	0·05	0·1	0·1

* i.e. a dilution of 0·2 × 1000 or 1 : 200.

In pure broth the activity of silver dinaphthylmethane disulphonate is *ca.* twice that of silver nitrate per unit of silver ion content. Whereas, however, the activity of silver nitrate falls very rapidly as serum content rises that of silver dinaphthylmethane disulphonate is increased by the presence of serum up to concentrations of the latter of 80 per cent. in the medium. In 80 per cent. serum silver dinaphthylmethane disulphonate has approximately thirteen times the activity of silver nitrate per unit of silver ion content.

A further point of interest presents itself from Table II with reference to the silver protein derivatives. There is a progressive fall in activity in pure broth from silver proteinate, through silver vitellin to " colloidal silver " ; per unit of silver content this progressive fall is exceedingly high. The loss in activity effected by serum also decreases from silver proteinate, through silver vitellin, to " colloidal silver " ; although this is the order of increasing total silver content it is the order of decreasing ionisable silver content. By comparison with silver nitrate it is evident that the loss in activity of the silver protein compounds and also the

inhibition of the deactivating effect of serum is due to the replacement of ionisable by non-ionisable silver. In the case of the colloidal electrolyte, silver dinaphthylmethane disulphonate, the high activity must be associated with the ionic nature of the whole of the silver content and the maintenance of this activity in the presence of serum due to the action of the polyvalent hydrated anionic micelle.

The *IN VITRO* Reaction of Mercuric-, Phenylmercuric- and Silver Dinaphthylmethane Disulphonates with Serum Ampholytes

The loss in activity of mercuric chloride, silver nitrate and phenylmercuric acetate effected by serum and the maintenance of the activity of mercuric-, silver- and phenylmercuric dinaphthylmethane disulphonates in serum can be correlated with the precipitin reactions of the compounds with serum ampholytes. The reagent in 1 : 1,000 dilution was added to a 1 per cent. aqueous solution of the ampholyte, followed by an excess of the reagent in order to determine whether the precipitate (if formed) was redissolved. The results are recorded in Table III.

TABLE III

Compound	*Gelatin*	*Albumen*	*Casein (Sodium)*	*Horse Serum*
Mercuric chloride...	O/i	nil	nil	P/i
Mercury dinaphthylmethane disulphonate ...	nil	nil	nil	nil
Phenylmercuric acetate	P/i	P/i	P/i	P/i
Phenylmercuric dinaphthylmethane disulphonate	O/s	nil	nil	nil
Silver nitrate	O/i	P/s	P/i	P/i
Silver dinaphthylmethane disulphonate ...	P/s	O/s	O/s	O/s

nil=no precipitate or opalescence ; O=no precipitate but definite opalescence ; P=precipitate ; /s=soluble in excess of reagent ; /i=insoluble in excess of reagent.

Toxicity

The oral toxicities of the compounds were determined in order to ascertain whether the colloidal anion possessed any potentiating effect upon the lethal action of the mercuric, phenylmercuric and silver cations. The compounds were given in aqueous solution to 20 g. mice by stomach tube under light ether—oxygen—carbon dioxide anæsthesia in closely spaced doses. Groups of 4 mice were used for each dose, approximately 40 mice being used for the determination of each toxicity range ; the animals were observed for 14 days after the dose.

It is evident that mercuric-, phenylmercuric- and silver- dinaphthylmethane disulphonate are slightly more toxic per unit of active cation content than the corresponding crystalloid electrolytes : this is to be expected from the enhanced adsorption capacities of the compounds. It is of passing interest, however, that the oral toxicities of phenylmercuric acetate and phenylmercuric dinaphthylmethane disulphonate are little more than that of " colloidal silver 70 per cent." and less than that of

silver nitrate both of which compounds are widely used in clinical practice. It would accordingly appear that as far as these two phenylmercuric compounds are concerned the general prejudice against organic mercurials is not founded upon fact.

TABLE IV

ORAL TOXICITIES

Compound	*LD0*	*LD50*	*LD100*
Mercuric chloride	7 mg./kg.	10 mg./kg.	12·5 mg./kg.
Mercuric dinaphthylmethane disulphonate	20	30	40
Phenylmercuric acetate	50	70	80
Phenylmercuric dinaphthylmethane disulphonate	50	70	80
Silver nitrate	30	50	65
Silver dinaphthylmethane disulphonate	25	50	75
Colloidal silver (70 per cent. Ag)	50	100	150
Potassium dinaphthylmethane disulphonate	950	1250	1450

SUMMARY

1. The potentiating effect of surface active agents upon the antibacterial activity of mercurial compounds has led to an interest in colloidal electrolytes which contain, as the constituent cation, an ion of known high antibacterial activity.

2. The colloidal acid selected was dinaphthylmethane disulphonic acid. The mercuric-, phenylmercuric- and silver-salts of this acid have been prepared and their activities against 3 Gram-positive and 3 Gram-negative organisms compared with those of corresponding crystalloid (non-colloid) salts containing the corresponding cations, viz. mercuric chloride, phenylmercuric acetate and silver nitrate. It was expected that the colloidal anion would potentiate the lethal action of the metallic or organometallic cation by promoting adsorption of the latter upon the bacterial envelope and that inactivation of the cation by formation of a coagulum with serum electrolytes would be suppressed by the process of micellar solubilisation of the coagulum.

3. It has been shown that in pure broth the colloidal anion effects a definite, although small, increase in activity of mercuric and silver ions ; the bacteriostatic activity of the lipophile phenylmercuric ion is not altered. In broth containing serum concentrations approaching 80 per cent., however, the activities of mercuric-, phenylmercuric- and silver

dinaphthylmethane disulphonates are of the order of 5, 3 and 13 times as high as those of mercuric chloride, phenylmercuric acetate and silver nitrate per unit of active cation content. These results have been correlated with the precipitin reactions of the compounds with horse serum and other protein ampholytes.

4. The acute oral toxicities of the compounds have been recorded.

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THE EVALUATION OF THE BACTERICIDAL ACTIVITY OF ETHYLENE GLYCOL AND SOME OF ITS MONOALKYL ETHERS AGAINST *BACTERIUM COLI*

PART VIII

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So far, this work has shown that the course of disinfection of *Bact. coli* by ethylene glycol and its monoalkyl ethers follows that of an asymmetrical sigmoid curve; the actual appearance of the curve could be made to vary by alteration of the environmental conditions[1,2]. The regression obtained by plotting the percentage survivors as probits against log. survivor time could be assumed linear over a limited (but useful) range without causing serious error[3,4]; further, when probit-log. time regressions were taken as linear, it could be shown[5] that parallelism existed between the regressions of different concentrations as well as the same concentration of a compound, thereby enabling a characteristic coefficient to be assigned to each disinfectant-organism reaction. The values of the coefficient varied with the temperature of the experiment[6,7].

Sufficient experimental data have now been accumulated to enable the usefulness and accuracy of a chosen level of mortality for assessing the bactericidal activity of the disinfectant compounds to be decided with some confidence.

SELECTION OF AN INTERMEDIATE MORTALITY LEVEL FOR THE COMPARISON OF BACTERICIDAL ACTIVITY

The inaccuracies of an end-point technique for estimating bacterial death times have led to the selection of intermediate mortality levels, determinable with greater precision, for comparing germicidal activities. Adoption of this principle involves the counting of viable organisms during the course of the disinfection process. Until a sound statistical analysis of the results could be developed and thereby afford a means of calculating the limits of error of the experimental technique, it was not possible to compare, on a mathematical basis, the merits of the different mortality levels proposed by different authors.

The Fallacies of Using Reaction Velocities as a Means of Comparison. Counting techniques enable the reaction velocity to be determined at different stages during the disinfection process. Since it was at first believed that the overall death rate for a particular concentration of a disinfectant was constant, the mean of intermediate death rates was used as a criterion of comparison of different disinfectant solutions. Furthermore, the overall reaction velocity was taken as representative of the efficiency of the disinfection process and was recommended by Phelps[8] as the basis for the evaluation of bactericidal activity. However, when it is realised that a constant death rate is often fortuitous

and that the death rate of the disinfection process does indeed vary along its course, comparison of overall death rates must be criticised as being misleading and uninformative.

The mortality levels chosen by previous workers. In bactericidal problems, the comparison of activity by the times taken for mortalities of less than 100 per cent. appears to have been first suggested by Levine, Buchanan and Lease[9], who recommended the use of the time for a 99·9 per cent. kill. Myers[10] based his comparison on a 99 per cent. mortality since he believed that this could be determined with greater accuracy than higher levels; Weber and Levine[11] also used this degree of mortality, whereas Baker and McClung[12] calculated the time for the death of 99·99 per cent. of the initial inoculum. Hobbs and Wilson[13] expressed doubt as to the accuracy of the computation of the times for a 99·9 per cent. mortality and employed reaction velocities to compare the bactericidal activities of the disinfectants used by them.

The choice of a 50 per cent. mortality level. (*a*) *In pharmacological assay problems.* In pharmacological problems the comparison of the potencies of therapeutically active substances has long been made by utilising the dose affecting 50 per cent. of the test animals. Trevan[14] had shown that the slope of the mortality-dose curve was steepest in the neighbourhood of the dose causing 50 per cent. mortality and this was also shown to be true when the logarithm of the dose was used (Gaddum[15]). Trevan coined the expression "LD50" (the dose which caused 50 per cent. mortality); because of the normal characteristics of the mortality curves, statistical methods could be used to determine the LD50 and the error of its estimation with great precision. Technical faults were thereby detectable, which when rectified enabled the accuracy of the biological assay to be improved enormously.

(b) *In bacteriological and insecticidal assay problems.* In microbiological problems of this nature, Henderson Smith[16] had used the time to kill 50 per cent. of the initial inoculum as a means of comparison in determining the temperature coefficient of hot water against *Botrytis* spores. Withell[17] conceived the idea of an "LT50" (i.e., the time to kill 50 per cent. of the initial inoculum) as the basis of comparison of germicidal activity. By means of the statistical techniques developed by Gaddum[15] and Bliss[18,19,20,21,22] he was able to demonstrate an approximate rectilinear relationship between the probit (a function of the percentage mortality) and the logarithm of the time, thereby facilitating the accurate estimation of LT50.

The conditions necessary for the selection of a convenient arbitrary mortality level. When the mortality curve can be transformed to a straight line along its complete course, the level chosen for comparison of activity is of little importance, for in these circumstances any percentage mortality can be computed from the regression with equal facility. Although the greatest accuracy might be obtained at the 50 per cent. mortality, Bliss[20] preferred to use a 97.725 per cent. mortality (corresponding to probit 7) for comparison of insecticides, as he asserted that

this conveyed more useful information to the entomologist than comparisons made on a 50 per cent. kill. Moore and Bliss[23] used a 95 per cent. mortality for a similar work.

The "virtual sterilisation time." Jordan and Jacobs[24,25] used arguments similar to those of Bliss, when comparisons of bactericides were to be made; they used the exceedingly high mortality level of 99·999999 per cent., which they called the "virtual sterilization time" (v.s.t.). This they were able to determine with very little extrapolation of the experimental data, by using a large initial inoculum (approximately 330 million organisms per ml.) and an extremely specialised experimental technique. They believed that for a proper conception of disinfection potentialities, the comparison of activity should be made at the stage nearest to complete disinfection concomitant with accurate determination, since in practice it was the absolute extinction of the organisms which was sought after.

The mortality level adopted for the experiments. One of the objects of this work was to develop the statistical technique of examining disinfection data. So long as a rectilinear regression could be established over a reasonable range, all the refinements of modern statistical methods could be applied usefully. The use of the LT50 as a basis of comparison of the activity of disinfectant solutions greatly simplifies the calculations and has been exploited for this purpose. Provided that a rectilinear probit-log. time regression may be assumed, the mathematical treatment of the disinfection data will be exactly similar for any desired level of mortality within the probit range under investigation. Comparisons based on levels outside this range must be rejustified before they are used. The results in this thesis have shown that the

TABLE I

CALCULATION OF THE SUMS OF SQUARES FOR DEVIATIONS OF LT50 OR LOG.LT50 FOR CONCENTRATIONS OF ETHYLENE GLYCOL MONOMETHYL ETHER AT 20°C.

	Concentrations of ethylene glycol monomethyl ether									
	42·5 per cent.		45·0 per cent.		47·5 per cent.		50·0 per cent.		52·5 per cent.	
	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit	Expt. No.	Mean Probit
	208a	2·7895	208d	2·3895	209c	2·2562	209d	1·4185	210g	0·3712
	209f	2·9373	209e	2·7922	211e	2·2340	210f	2·4706	211g	0·6497
	210c	2·5255	210d	2·1268	213c	1·8588	211f	1·3843	212e	1·0784
	211c	2·6837	211d	2·1033	214c	1·6837	212d	1·8706	213d	0·9229
$S(LT\,50)$		10·9360		9·4118		8·0327		7·1438		3·0222
No. of expts.		4		4		4		4		4
$LT\,50$		2·7340		2·3529		2·0082		1·7859		0·7556
$S(LT\,50)^2$		29·989437		22·453240		16·371178		13·530870		2·574591
$\frac{S^2(LT\,50)}{n}$		29·899024		22·145495		16·131067		12·758470		2·283423
$S(LT\,50-\overline{LT\,50})^2 =$ $S(LT\,50)^2-\frac{S^2(LT\,50)}{n}$ $=SS$		0·090413		0·307745		0·240111		0·772400		0·291168

probit-log. time relationship is not strictly linear but may be assumed to be so between probits 4 and 6 without incurring any serious error.

Calculation of Results from Experiments at 20°C.

1. *Calculation of log. LT50.* Since the probit-log. time regression between probits 4 and 6 may be assumed linear, the function takes the form of

$$y = \bar{y} + b(x - \bar{x}) \qquad (1)$$

from which the value of x (the log. time) may be calculated for any value of y (the probit). By assigning y the value of 5 (the probit corresponding to a 50 per cent. response) x may be computed, since y, b and $\bar{x}$ are all known. For example, in Experiment 164b (the disinfection of *Bact. coli* by 75 per cent. ethylene glycol at 20°C.), the following data were obtained:

Log.time (x)	Probit (y)
1·301	4·102
1·699	4·447
2·255	4·874
2·477	5·418
2·631	5·431
$\bar{x}$=2·073	$\bar{y}$=4·854

The mean slope (b) for 75 per cent. ethylene glycol at 20°C. is 1·2025 (Table X, Part V[5]). Equation (1) may be transposed to

$$x = \frac{y - \bar{y} + b(\bar{x})}{b} \qquad (2)$$

Substituting in equation (2)

$$x = \frac{5 - 4{\cdot}854 + 1{\cdot}2025\ (2{\cdot}037)}{1{\cdot}2025}$$

$$= 2{\cdot}1945 = \text{log. LT50.}$$

The calculations of the log. LT50's for all the individual tests are too numerous to publish (321 separate equations are involved). However, since the mean LT50's for each concentration will be needed to calculate the empirical variance, these have been set out in Table II.

2. *Calculation of the standard errors of the LT50's.* This calculation is essentially the same as that used in the computation of the standard errors of the probit-log. time regressions (Part VIII[8]). The sum of squares for the deviations of each log. LT50 from its mean log. LT50 (the mean value of the mean LT50 for all the tests at a particular concentration) is computed for every concentration of all the compounds (for experiments performed at 20°C.). Table I shows the calculations for the monomethyl ether; the calculations for the other compounds are precisely the same. The sums of squares for the deviations of LT50 for the other compounds are included in Table II.

The next stage is to calculate the mean squares; this is achieved by dividing each sum of squares by the appropriate number of degrees of freedom. In the experiments with 42·5 per cent. monomethyl, for example, the mean square will be $\frac{0{\cdot}090413}{3} = 0{\cdot}030138$. The total sum of squares of the deviations for all the experiments at 20°C. is seen to be 6·243986 (Table II) which for 165 degrees of freedom has a mean square of 0·037842.

TABLE II

THE EMPIRICAL VARIANCE OF THE INDIVIDUAL MEANS FROM THEIR MEAN LT50'S OF EXPERIMENTS WITH CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 20°C.

Compound	Concentration	SS	N	Mean square	$V_{\log.LT50} = V_T$	$r = \sqrt{V_T}$	Mean LT50 = t min.	$S_t = t.s_T$ min.
	per cent.							
Ethylene glycol	72·5	0·160464	11	0·014583	0·003154	±0·05616	173	±9·72
	75·0	0·735303	31	0·023719	0·001183	0·03438	128	4·40
	77·5	0·219221	14	0·015659	0·002523	0·05023	65	3·26
	80·0	0·143436	9	0·015937	0·003784	0·06152	35	2·15
	82·5	0·447628	8	0·055953	0·004205	0·06485	10	0·65
	85·0	0·074278	9	0·008253	0·003784	0·06152	24	1·48
	90·0	1·111313	9	0·123479	0·003784	0·06152	6·21	0·38
Monomethyl ether	42·5	0·090413	3	0·030138	0·009461	0·09721	542	42·93
	45·0	0·307745	3	0·102582	0·009461	0·09721	225	17·82
	47·5	0·240111	3	0·080037	0·009461	0·09721	102	8·08
	50·0	0·772400	3	0·257467	0·009461	0·09721	60	4·83
	52·5	0·291168	3	0·097056	0·009461	0·09721	57	4·51
Monoethyl ether	25·0	0·013826	2	0·005913	0·012614	0·11230	311	34·93
	27·5	0·005202	1	0·005202	0·018921	0·13760	106	14·59
	30·0	0·072759	3	0·024253	0·009461	0·09721	55	5·35
	32·5	0·274433	4	0·068858	0·007568	0·08700	17	1·48
	35·0	0·171692	3	0·057231	0·009461	0·09721	9·5	0·92
Monopropyl ether	7·8	0·038138	3	0·012711	0·009461	0·09721	126	12·25
	9·0	0·049307	3	0·016436	0·009461	0·09721	55	5·35
	10·0	0·071354	3	0·023785	0·009461	0·09721	27	2·62
	11·0	0·049113	3	0·016371	0·009461	0·09721	14	1·36
	12·0	0·056922	3	0·018974	0·009461	0·09721	8	0·77
Monobutyl ether	3·50	0·036101	3	0·012034	0·009461	0·09721	133	12·93
	3·75	0·164006	3	0·054669	0·009461	0·09721	70	6·80
	4·00	0·029107	3	0·009702	0·009461	0·09721	30	2·92
	4·25	0·006001	3	0·002000	0·009461	0·09721	24	2·33
	4·50	0·021083	3	0·007028	0·009461	0·09721	10	0·97
Monohexyl ether	0·400	0·219795	4	0·054949	0·007568	0·08700	247	21·49
	0·425	0·171177	4	0·042794	0·007568	0·08700	85	7·40
	0·450	0·001404	1	0·001404	0·018921	0·13760	45	3·92
	0·475	0·089286	3	0·029762	0·009461	0·09721	37	3·22
	0·500	0·109805	4	0·027451	0·007568	0·08700	25	2·18
Total		6·243986	165	0·037842*				

*$\frac{6{\cdot}243986}{165} = 0{\cdot}037842$

The variance of log. LT50 (V_T) at a particular concentration is obtained by dividing the average mean square (0·037842) by the number of experiments performed at that concentration; in the instance cited it will be $\frac{0{\cdot}037842}{4} = 0{\cdot}009461$. Hence the greater the number of tests performed at a particular concentration the smaller will be the value of V_T. The standard error of log. LT50, (S_T) equals $\sqrt{V_T}$. The standard error

of LT50, (S_T), is given from the relationship $S_t = t.S_T$.* The standard errors of the mean values of the LT50's at each concentration have been calculated and included with their mean LT50's in Table II.

3. *Construction of limits of error curves for the estimation of LT50.* The limits of error for one estimation will depend on the mean value of LT50, determined from a large number of experiments, and on the probability level at which it is desired to work. The following examples illustrate the method of calculating the limits at three probability levels for two widely separated values of LT50.

(a) *Calculations.*

(i) *When the mean LT50 is* 100 *minutes.* The average mean square for the deviations of log. LT50 is given in Table II as 0·037842; its standard error (S_T) will be $\sqrt{0{\cdot}037842} = \pm 0{\cdot}1945$. This is the standard error for one experiment, which in terms of arithmetic time will be $100 \times \pm 0{\cdot}1945 = \pm 19{\cdot}45$ minutes (from $S_t = t.S_T$). When the mean of n experiments is taken,

$$s_t = \pm 100 \sqrt{\frac{0{\cdot}037842}{n}} = \pm \frac{0{\cdot}1945}{\sqrt{n}} \qquad (3)$$

The limits of the estimation are $\pm cs_t$ (where c = normal deviate). Hence, when one experiment is performed, the limits will be as follows:

at $P = 0{\cdot}01$, $\pm 2{\cdot}576 \times 19{\cdot}45 = \pm 50{\cdot}09$ minutes
at $P = 0{\cdot}05$, $\pm 1{\cdot}96 \times 19{\cdot}45 = \pm 38{\cdot}13$ minutes
at $P = 0{\cdot}325$, $\pm 1{\cdot}00 \times 19{\cdot}45 \pm = 19{\cdot}45$ minutes

This means that when the result from only one estimation is taken and the correct value should be 100 minutes, at $P = 0{\cdot}01$, the LT50 in one instance out of every 100, should fall outside the limits $100 \pm 50{\cdot}09$ minutes; at $P = 0{\cdot}5$, the limits will be $\pm 38{\cdot}13$ minutes, i.e., only 5 results out of every 100 fall outside the range $100 \pm 38{\cdot}13$ minutes, whereas at $P = 0{\cdot}325$, one result out of every three should fall outside the limits $100 \pm 19{\cdot}45$ minutes.

When the mean of several tests is taken, the limits of error will be proportionally smaller. The limits at the three probability levels up to 40 experiments have been calculated from equation (3) and set out in Table III(a).

---

* Let t = LT50 and T = log. LT50, then T = log. t

$$V_T = V_t \frac{(dT)^2}{(dt)}$$

$$\text{hence } s_T = s_t \frac{dT}{dt} \text{ (since } S = \sqrt{V})$$

$$\text{But } \frac{dT}{dt} = \frac{1}{t}$$

$$\text{therefore } s_T = \frac{S_t}{t} \text{ or } s_t = t.\, s_T.$$

TABLE III

RELATION BETWEEN THE LIMITS OF ERROR OF ESTIMATION OF LT50 AT DIFFERENT PROBABILITY LEVELS AND THE NUMBER OF REPLICATE TESTS PERFORMED

(*a*) WHEN LT50=100 MINUTES

	Number of replicate tests (n)												
	1	2	3	4	5	9	10	16	20	25	30	36	40
Limits at $P=0{\cdot}01$	50·09	35·41	28·89	22·05	22·40	16·69	15·82	12·53	11·19	10·02	9·14	8·35	7·92
Limits at $P=0{\cdot}05$	38·13	26·91	22·10	19·07	17·02	12·69	12·11	9·54	8·51	7·61	6·94	6·35	6·02
Limits at $P=0{\cdot}325$	19·45	13·76	11·22	9·73	8·71	6·48	6·15	4·87	4·35	3·89	3·55	3·24	3·08

(*b*) WHEN LT50=20 MINUTES

	Number of replicate tests (n)												
	1	2	3	4	5	9	10	16	20	25	30	36	40
Limits at $P=0{\cdot}01$	10·02	7·09	5·79	5·01	4·48	3·34	3·17	2·51	2·24	2·01	1·83	1·67	1·59
Limits at $P=0{\cdot}05$	7·63	5·39	4·40	3·81	3·41	2·54	2·41	1·91	1·70	1·55	1·39	1·27	1·21
Limits at $P=0{\cdot}325$	3·89	2·75	2·24	1·95	1·74	1·30	1·26	0·97	0·87	0·78	0·71	0·65	0·62

(ii) *When the mean LT50 is* 20 *minutes.*

$$\text{Here } s_t = \pm 20\sqrt{\frac{0{\cdot}037842}{n}} = \pm \frac{3{\cdot}98}{\sqrt{n}} \qquad (4)$$

Limits of the estimation. When one experiment is performed, the limits will be as follows:

at $P = 0{\cdot}01$, $\pm 2{\cdot}576 \times 3{\cdot}89 = \pm 10{\cdot}02$ minutes
at $P = 0{\cdot}05$, $\pm 1{\cdot}96 \times 3{\cdot}89 = \pm 7{\cdot}63$ minutes
at $P = 0{\cdot}325$, $\pm 1{\cdot}00 \times 3{\cdot}89 = \pm 3{\cdot}89$ minutes

The limits at the three probability levels up to 40 experiments have been calculated from equation (4) and set out in Table III (b).

From the sets of results in Table III curves have been constructed (Figure I) to illustrate how the limits of error of the estimation diminish as the number of tests from which the mean LT50 is calculated, is increased.

(b) *The use of the limits of error curve.* When a number of limits of error curves have been constructed to cover the range of LT50's expected in a series of experiments, the error of the estimations at the different probability levels can be deduced rapidly.

For most of the mean LT50's for different concentrations of the compounds investigated in this thesis, four estimations have been used. In some instances, e.g., ethylene glycol at 20°C., many more tests were performed at each concentration. However, it is seen from Figure 1 that if an LT50 of 100 minutes is expected, then from the mean of four experiments the experimental times may be expected to fall outside the limits of 81 and 119 minutes 5 times out of every 100 estimations at $P = 0{\cdot}05$). These limits become narrower as the number of tests is increased; in fact to halve the deviation (i.e., to double the accuracy) requires quadruple the number of tests. For example, when the mean of 20 experiments is taken, the limits are 91·5 and 108·5 minutes; yet for 30 experiments they are 93 and 107 minutes, and for 40 experiments only 94 and 106 minutes. It is necessary to decide on the limits of error required in an assay and then to perform the required number of tests to procure this accuracy; although greater precision is obtainable by carrying out a larger number of experiments from which to compute the mean, it may be decided that the benefits of the smaller additional accuracy so obtained is not in keeping with the nature of the assay.

Calculation of Results from Experiments at 30°C.

Calculation of the log. LT50's and the standard errors of LT50's. The log. LT50's for each experiment and the mean figure at each concentration of a substance was calculated as before from the probit-log. time regression equation.

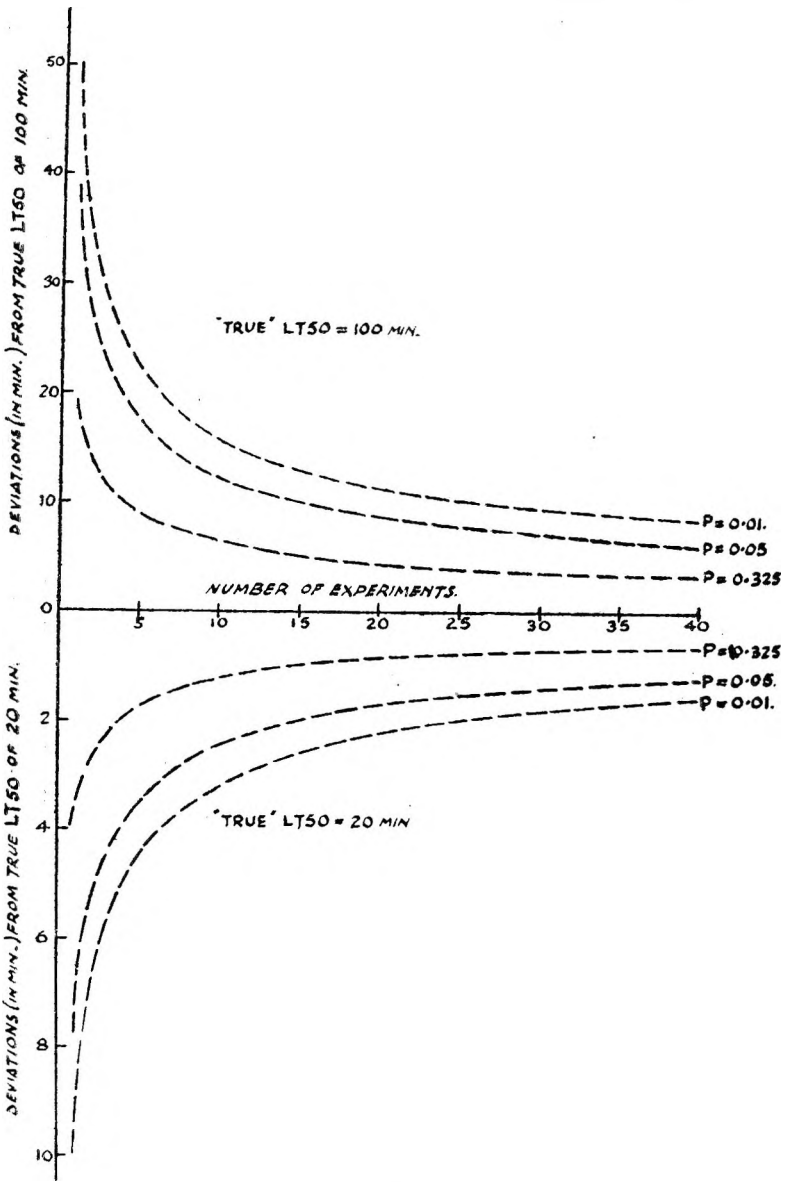

FIG. 1.—Relation between the limits of error of the estimation of LT50 at different probability levels and the number of replicate tests performed.

The sum of squares for the deviations of each log. LT50 from its log. LT50 (the mean value of LT50 for all the tests at a particular concentration) was computed for every concentration of all the compounds. These have been set out in Table IV together with their mean squares. The total sum of squares of the deviations of all the experiments is 3·184845, which for 74 degrees of freedom has a mean square of 0·043038.

The variance of log. LT50 (i.e., V_T) at a particular concentration is obtained by dividing the average mean square (0·043038) by the number of experiments performed at that concentration. Table IV presents a

TABLE IV

THE EMPIRICAL VARIANCE OF THE INDIVIDUAL MEANS FROM THEIR MEAN LT50'S OF EXPERIMENTS WITH CONCENTRATIONS OF ETHYLENE GLYCOL AND ITS MONOALKYL ETHERS AT 30°C.

Compound	Concentration	*SS*	*N*	Mean Square	$V_{\log.\ LT50} = V_T$	$s_T = \sqrt{V_T}$	Mean LT50= *t* min.	$S_t = t.s_T$ min.
	per cent.							
Ethylene glycol	62·5	0·021650	2	0·010825	0·014346	0·1098	±101	±11·09
	65·0	0·425643	3	0·141881	0·010759	0·1038	113	11·73
	67·5	0·779288	5	0·155858	0·007173	0·0847	28	2·37
	70·0	0·021981	3	0·007327	0·010759	0·1038	16	1·66
Monomethyl ether	35·0	0·099914	3	0·033305	0·010759	0·1038	90	9·34
	37·5	0·050939	3	0·016980	0·010759	0·1038	54	5·61
	40·0	0·056315	3	0·018772	0·010759	0·1038	25	2·60
	42·5	0·051599	3	0·017200	0·010759	0·1038	13	1·35
Monoethyl ether	12·5	0·103907	3	0·034636	0·010759	0·1038	156	16·19
	15·0	0·142569	3	0·047523	0·010759	0·1038	87	9·03
	17·5	0·241659	3	0·080556	0·010759	0·1038	37	3·84
	20·0	0·305846	3	0·101949	0·010759	0·1038	8	0·83
Monopropyl ether	3·0	0·028459	3	0·009486	0·010759	0·1038	159	16·50
	4·0	0·034941	3	0·011647	0·010759	0·1038	91	9·45
	5·0	0·021521	3	0·007174	0·010759	0·1038	55	5·71
	6·0	0·008830	3	0·002943	0·010759	0·1038	27	2·80
Monobutyl ether	1·5	0·405014	3	0·135005	0·010759	0·1038	294	30·52
	2·0	0·086614	3	0·028871	0·010759	0·1038	50	5·19
	2·5	0·026305	3	0·008768	0·010759	0·1038	22	2·28
	3·0	0·079045	3	0·026348	0·010759	0·1038	8	0·83
Monohexyl ether	0·325	0·021501	3	0·007167	0·010759	0·1038	98	10·17
	0·350	0·008527	3	0·002842	0·010759	0·1038	64	6·64
	0·375	0·028385	3	0·009462	0·010759	0·1038	41	4·26
	0·400	0·122318	3	0·061159	0·010759	0·1038	18	1·98
	0·425	0·012075	2	0·006038	0·014346	0·1038	9	0·99
Total		3·184845	74	0·043038*				

*$\frac{3 \cdot 184845}{74} = 0 \cdot 043038$

summary of the bactericidal activities at 30°C. of all the concentrations of the different compounds, together with the standard errors of the mean values of the LT50's at each concentration.

It is seen that the mean square at 30°C. (0·043038) and at 20°C. (0·037842) are of the same order; this indicates that the technique is constant and sound.

THE EFFECT OF VARIATION IN THE INITIAL NUMBER OF ORGANISMS ON THE VALUE OF LT50

Experimental part. The experiments in Part VI[6], designed to show the effect of variation in the initial number of organisms on the value of the slope of the regression, are also suitable to demonstrate the effect on the value of the LT50.

Results and calculations. Table V sets out the LT50's obtained for the experiments, which were carried out with 75 per cent. ethylene glycol at 20°C. Log. LT50 was calculated from the equation $y = y + b(x - \bar{x})$

where $y = 5$, y = the mean value of y for a test, b = the mean slope of the regression (1·2025, Table X Part V[5]), x = log. LT50 and $\bar{x}$ = the mean value of x for a test.

TABLE V

VALUES OF LT50 FROM THE DISINFECTION OF *BACT. COLI* BY 75 PER CENT. ETHYLENE GLYCOL AT 20°C. FROM EXPERIMENTS USING DIFFERENT INITIAL NUMBERS OF ORGANISMS

Group	Expt. No.	Initial inoculum. No. of organisms per ml.	Log. LT50	LT50 minutes
A	186f	114,000	2·126	134
	186d	221,000	2·039	109
	186b	379,000	2·005	101
B	199e	156,600	2·109	129
	199g	1·491 millions	2·035	108
	199j	12·83 ,,	2·118	131
	199b	13·30 ,,	2·062	115
C	184b	1·247 ,,	1·984	96
	184d	2·484 ,,	1·933	86
	184f	5·295 ,,	1·985	97
	184h	12·64 ,,	2·000	100
D	168b	9·44 ,,	1·903	80
	167b	16·54 ,,	2·151	142
	169b	28·10 ,,	1·963	92
E	202d	15·25 ,,	2·018	104
	202f	81·27 ,,	2·182	152
	202f	143·1 ,,	2·140	138
	202k	299·6 ,,	2·387	244
F	200b	462·9 ,,	2·290	195
	200c	462·9 ,,	2·375	237
	200d	462·9 ,,	2·324	211
	200e	462·9 ,,	2·490	309

CONCLUSION

There was no correlation betwen the LT50 and the initial number of organisms over a very large range (114,000 organisms per ml. to 143·1 millions per ml.); the results from experiments with still heavier initial inocula, however, gave larger LT50's. The experimental technique was not sufficiently sensitive to detect differences in the values of LT50 over a certain range, and it would appear that there is considerable latitude in the numbers of organisms which should be added to disinfectant solutions when comparing their bactericidal activities under the standardised conditions.

SUMMARY

1. The advantages of using intermediate mortality levels instead of end-points and reaction velocities for the comparison of bactericidal activity have been discussed.

2. The time to kill 50 per cent. of the initial inoculum (LT50) has been employed and its logarithm computed mathematically from the probit-log. time regression equation of the disinfection data between *Bact. coli* and ethylene glycol and its monoalkyl ethers, for experiments at 20°C. and at 30°C.

3. The standard errors of the LT50's for experiments at 20°C. and 30°C. have been computed; from the former, the limits of the estimations at three probability levels (at $P=0{\cdot}01$, $0{\cdot}05$ and $0{\cdot}325$) have been calculated and limits of error curves constructed.

4. The experimental technique was not sufficiently sensitive to detect differences in the values of LT50's over a large range of different initial numbers of organisms under standardised conditions. After a certain stage had been reached, however, heavier initial inocula resulted in larger LT50's.

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A VOLUMETRIC DETERMINATION OF BARBITURIC ACID DERIVATIVES

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Several volumetric methods of determination of barbituric acid derivatives have been described in the literature, most of them depending upon the acidity of the imido hydrogen. These methods have recently been reviewed by Mangouri and Milad[1], who classified them into two classes, one class consisting of methods involving direct titration with alkali, and the other titration with standard silver nitrate solution. These authors found most of the published methods to be wanting in accuracy unless modified, and they developed a method depending upon the precipitation of the barbiturate as its silver salt with silver nitrate solution and determination of the excess of silver. Apart from gravimetric determination of tablets and sodium salts, there are no pharmacopœial methods of assay of the barbiturates.

Barbituric acid derivatives give precipitates with other heavy metals besides silver and precipitation with a strong nitric acid solution of mercuric nitrate (Millon's Reagent) is commonly used as a preliminary qualitative test for their presence. This precipitate appears to be quite insoluble in excess of the cold reagent, but is appreciably soluble on boiling. It was considered feasible that a method of assay depending upon this precipitate could be developed. The solution of mercuric nitrate, however, is necessarily strongly acid and dilute neutral volumetric solutions are therefore difficult to obtain.

It was found however, that a solution of mercuric perchlorate also gives a precipitate with neutral solutions of the barbiturates and as this may be readily obtained in a dilute, almost neutral, solution, an attempt was made to develop a method of assay using this reagent. A solution of mercuric perchlorate is readily prepared in a 0·1M concentration by dissolving mercuric oxide in dilute perchloric acid solution[2], and the method developed consists essentially of adding a known excess of this solution to an aqueous solution of the barbiturate, filtering off the precipitate of mercuric barbiturate and determining the excess of mercury volumetrically.

Precipitation with this solution is not specific for the barbiturates and a precipitate is obtained with the sulphonamides, cinchophen, phenazone and carbromal. As, however, these drugs (except perhaps the last) are not usually found associated with the barbiturates, this fact would not appear to detract from the value of the method, at least in its application to pharmaceutical preparations.

Experimental

Mercuric Perchlorate Solution.—An approximately 0·1M solution was prepared by boiling an excess (25 g.) of mercuric oxide with 28 g. of

60 per cent. perchloric acid in 200 ml. of water, adjusting to 1 l. and filtering. This solution contains approximately 36·6 g. of mercuric perchlorate and remains stable indefinitely.

Determination of the Mercury.—Earlier determinations of the excess of mercury were carried out, using the process described in the British Pharmacopœia for the assay of mercuric chloride, i.e., reduction with alkaline formaldehyde and absorption of the precipitated mercury with excess of standard iodine. This method, however, did not prove completely successful, as widely varying results were often obtained. As it was found that the perchlorate ion differs from the chloride ion in not forming complexes in the presence of the thiocyanate ion, the ammonium thiocyanate method of assay was used and found to be completely satisfactory. It was found that the final oxidation of the solution by boiling with nitric acid before titration with ammonium thiocyanate solution was not essential, and the same titration figures were obtained with boiled solutions as with the unboiled ones. This was found to be so even in the presence of starch.

Precipitation of the Barbiturate.—Analysis of the precipitate obtained with a barbiturate containing an unsubstituted imido nitrogen indicated a monomolecular compound with the mercury, e.g. barbitone gave a precipitate containing 7·26 per cent. of nitrogen and 51·43 per cent. of mercury, $(C_8H_{10}O_3N_2)Hg$ requires N, 7·33 per cent., Hg, 52·3 per cent. Hence 2 l. of 0·1M mercuric perchlorate solution is equivalent to one molecule of the barbiturate.

As the barbituric acid derivatives themselves are insoluble in water, preliminary experiments were performed by adding the mercuric perchlorate solution to a boiling solution of the barbiturate in water. Owing to the slight solubility of the precipitate in boiling water, however, low results were obtained. This tendency was emphasised when the volume of water used to dissolve the barbiturate was increased. At high concentration also, low figures were obtained, probably due to complex formation[3], but this was only manifest in concentrations over 8·0 millimoles/l. and if the concentration was maintained between 2·5 millimoles and 6·25 millimoles/l., concordant results were obtained (Table I).

In the method finally adopted, mixing of the two solutions was carried out at room temperature at a dilution of about 0·2 g. in 150 ml. The effect of buffering the barbituric acid solution was also investigated, as

TABLE I

EFFECT OF CONCENTRATION OF BARBITURATE

	Amount added	Dissolved in ml.	Found	Percentage
	g.		g.	
Barbitone	0·1005	200	0·0995	98·97
	0·2010	200	0·1989	98·95
	0·3015	200	0·2947	97·74
Phenobarbitone	0·1000	200	0·0999	99·91
	0·2000	200	0·1998	99·68
	0·3000	200	0·2966	98·87

the sodium salts of the barbiturates are alkaline in reaction, and here again it was found that a wide variation of *p*H was possible without any appreciable effect on the results obtained (Table II). The precipitate was found to be appreciably soluble in excess of perchloric acid however, and no precipitate is obtained in the presence of excess of chloride ion or mineral acids.

TABLE II

EFFECT OF *p*H OF SOLUTION OF SODIUM PHENOBARBITONE

Amount added g.	Dissolved in ml.	*p*H	Found	Percentage
0·2438	50	8·6	0·2296	94·18
0·2438	50	8·0	0·2306	94·58
0·2438	50	7·0	0·2296	94·18
0·2438	50	6·0	0·2296	94·18
0·2438	50	5·0	0·2306	94·58

No difficulty was experienced in filtering the solution after precipitation, and the presence of starch had no effect on filtration if the solution was boiled to partially hydrolyse this. In order to avoid a long washing of the precipitate, complete filtration was avoided and an " aliquot part " method used throughout.

METHOD ADOPTED

Weigh out approximately 0·2 g. of the barbiturate (or its equivalent in powdered tablets) and dissolve in 50 ml. of boiling distilled water. Boil for a few minutes after solution has been achieved and add about 80 ml. of water and allow to cool to room temperature. Transfer the solution to a 200 ml. measuring flask and wash the container several times adding the washings to the contents of the flask, until the volume is about 150 ml. Now add slowly, with rotation of the contents of the flask, 25 ml. of the solution of mercuric perchlorate and allow the mixture to stand with frequent shaking for 15 minutes. Adjust the volume of the solution to 200 ml. and then filter through a double fluted filter paper into a dry 100 ml. measuring flask. Reject the first 50 ml. of the clear solution and then collect 100 ml. Transfer this filtrate to a conical flask, washing the measuring flask with 20 ml. quantities of 10 per cent. nitric acid solution, and adjust the volume of liquid to about 250 ml. Add 1 ml. of a saturated solution of ferric alum and titrate with N/10 ammonium thiocyanate. Repeat the operation without the barbiturate. The difference in the two titrations represents the number of ml. of mercuric perchlorate solution required for the barbiturate.

Each ml. of N/10 ammonium thiocyanate is equivalent to the molecular weight of the barbiturate/20,000, e.g.:—barbitone 0·00921 g.; barbitone sodium 0·0103 g.; phenobarbitone 0·0116 g.; phenobarbitone sodium 0·0127 g.

RESULTS

This procedure was applied to the Pharmacopœial barbiturates and their preparations. As a check on the method, the B.P. assay was used in

the case of the sodium salts and a Kjeldahl assay was carried out on the barbiturates themselves.

TABLE III

BARBITURATES

Compound	Perchlorate Method			B.P. Method or Kjeldahl
	Added g.	Found g.	Percentage	Percentage
Barbitone	0·2021 0·2029	0·2008 0·2019	99·35 99·50	99·75
Barbitone Sodium	0·2531 0·2522	0·2523 0·2513	99·68 99·64	99·59
Phenobarbitone	0·1984 0·2030 0·1796	0·1972 0·2018 0·1786	99·40 99·40 99·44	99·94
Phenobarbitone Sodium	0·2273 0·2223	0·2230 0·2198	98·10 98·87	99·12

TABLETS

Various makers' tablets were used and as checks on the method, the B.P. gravimetric assay was used throughout. In this application of the suggested procedure, certain advantages were seen over the B.P. assay. In the latter, some makers' tablets tended to give difficulty by the formation of emulsions in the preliminary extraction. Other makers' tablets on the other hand, were quite free from this difficulty. The starch vehicle gave no difficulty in the mercuric perchlorate method but in order to ensure rapid filtration from the mercury precipitate, 5 minutes' gentle boiling was used in order to partially hydrolyse the starch. After this, filtration was as rapid as in the absence of starch.

It will be noticed that in the majority of cases the perchlorate method gave slightly higher results than the Pharmacopœial method. No explanation of this was discovered but it may be that the starch present in the tablets is a disturbing factor. This is borne out to some extent by the fact that though no such discrepancy was observed with the sodium salts of the barbiturates it was observed with a factitious "tablet," i.e., a simple mixture of starch and barbiturate. These figures are given in Table IV, "Maker B," and Table V, "Maker C."

N-Substituted Barbiturates.—The assay outlined above was applied to methylphenobarbitone and hexobarbitone sodium. Satisfactory results were obtained only with the former. In the case of hexobarbitone sodium the precipitated mercury compound was appreciably soluble in water and results obtained were very low.

Methylphenobarbitone on the other hand, though closely related to hexobarbitone gives a practically insoluble mercury derivative, but owing to the almost complete insolubility of the methylphenobarbitone itself the method had to be modified in the following way:—Heat 0·4 to 0·5 g. of the methylphenobarbitone with 20 ml. of N/10 sodium hydroxide and

120 ml. of water to 60°C. until dissolved. While maintaining at this temperature add 10 ml. of 10 per cent. acetic acid followed immediately by 25 ml. of mercuric perchlorate solution. Allow the mixture to cool to room temperature, transfer to a measuring flask with washing, adjust to 200 ml., stand for 15 minutes with shaking and proceed as in the original method.

TABLE IV

TABLETS OF BARBITONE AND BARBITONE SODIUM

Compound	Maker	Perchlorate Method			B.P. Method
		Added	Found	Percentage	Percentage
Barbitone 5 grains	A	0·1665	0·1423	85·46	82·52
		0·2000	0·1704	85·20	
		0·1747	0·1491	85·34	
	B	0·2102	0·1308	62·22	61·28
		0·2107	0·1308	62·07	
	C	0·2005	0·1510	75·50	73·27
		0·2015	0·1528	75·83	
Barbitone Sodium 5 grains ...	A	0·2090	0·1463	70·00	67·36
		0·2193	0·1535	70·00	
	B	0·2000	0·1669	83·45	83·35
		0·2062	0·1730	83·89	
	C	0·1995	0·1607	80·55	77·69
		0·2034	0·1627	80·00	

TABLE V

TABLETS OF PHENOBARBITONE AND PHENOBARBITONE SODIUM

Tablet	Maker	Perchlorate Method			B.P.
		Added	Found	Percentage	Percentage
Phenobarbitone 1 grain... ...	A	0·2103	0·1183	56·25	53·60
		0·2006	0·1137	56·69	
		0·1892	0·1067	56·39	
	B	0·1949	0·1298	66·64	66·00
		0·2329	0·1554	66·71	
		0·2092	0·1392	66·53	
	C	0·2226	0·1392	62·53	61·23
		0·1990	0·1253	62·96	
Phenobarbitone Sodium 1 grain	A	0·1945	0·0965	49·61	48·16
		0·2253	0·1118	49·62	
½ grain	B	0·3967	0·2083	52·51	52·53
		0·3996	0·2108	52·78	
	C	0·2538	0·1067	42·03	40·30
		0·2461	0·1041	42·30	

In this case one molecule of mercury combines with two molecules of the barbiturate so that 1 ml. of N/10 ammonium thiocyanate is equivalent to 0·02464 g. of methylphenobarbitone. Results obtained are given in Table VI.

TABLE VI

METHYLPHENOBARBITONE

Added	Found	Percentage	B.P.
0·4200	0·4139	98·57	99·51
0·3785	0·3746	98·97	
0·4820	0·4762	98·91	

SUMMARY

1. A method of assay of Pharmacopœial barbiturates and their tablets is suggested depending upon their precipitation as a mercury compound with excess of a standard solution of mercuric perchlorate followed by determination of the excess of mercury.

2. The precipitates obtained with the various barbiturates vary in solubility in water, but it is only in the case of hexobarbitone that the solubility is sufficient to interfere seriously with the assay.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Capsaicin in Oleoresin of Capsicum, Colorimetric Determination of. H. North. (*Anal. Chem.*, 1949, **21,** 934. Vanillin is used instead of pure capsaicin as a standard in the determination which utilises the Folin and Denis phosphotungstic-phosphomolybdic acid reagent. The sample of oleoresin (approximately 1 g.) is transferred to a separating funnel using purified kerosene, sodium chloride dissolved in acetone-water is added and the mixture shaken gently. The lower layer is removed and the extraction of the oleoresin solution is continued to completion using the same solvent. The separated bulked extracts are clarified and filtered and an aliquot portion is evaporated to an oily sediment, cooled, and the residue is dissolved in 0·5 N sodium hydroxide solution. Sodium bicarbonate is added and the mixture is extracted with light petroleum which is then shaken with 0·5 N sodium hydroxide. After washing the light petroleum with water the extracts are bulked, made up to volume, and an aliquot taken for the colorimetric determination. This is performed in the normal manner with slight modifications. With samples poor in capsaicin, a slight difference in colour between the standard solution and the test solution may be seen, because traces of colour carried through from the oleoresin have an influence on the total colour; this does not, however, interfere with the usefulness of the method. For the determination of capsaicin in spice, 5 to 10 gm. dry material are extracted with acetone or ether in a Soxhlet apparatus; the extract is then tested as before. R. E. S.

Grote's Reagent for Sulphur Compounds. J. J. M. van Sonsbeek. (*Pharm. Weekbl.*, 1949, **84,** 433.) The preparation of Grote's reagent may be simplified by omitting the bromine, as follows: 2 g. of hydroxylamine hydrochloride is mixed with 7.2 ml. of 4N sodium hydroxide and 25 ml. of water, then with 2 g. of sodium nitroprusside. After gas evolution ceases, the solution is made up to 100 ml. and filtered. This formula, though somewhat less stable owing to the higher alkalinity, does not become turbid to the same extent as the original formula. The reaction for the -C=S grouping (a blue colour) proceeds best at *p*H 7, and is apparently due to $Na_3[Fe(CN)_5H_2O]$ formed by the replacement of the NO in the nitroprusside by H_2O. In the reaction for the -C-S-H and -C-S-S-C- groupings, a purple-red colour is produced in alkaline solution (*p*H about 10). For reliable results in this test it is necessary to add cyanide, which also reduces the -C-S-S-C- group to -C-S-H, so that it is not possible to distinguish between the latter two groupings. The reagent is therefore inferior to nitroprusside. With thiopental, neither Grote's reagent nor nitroprusside gave the expected reaction for -C-S-H. On the other hand, at a *p*H of 4 to 8 Grote's reagent gave a fine red colour, which would appear to be a useful identification reaction. G. M.

Hexachloro*cyclo*hexane (Benzene Hexachloride Infra-red Spectroscopic Analysis of Mixture of Stereoisomerides. L. W. Morrison. (*J. Soc. chem. Ind., Lond.*, 1949, **68,** 192.) More than 100 samples of mixtures of the stereoisomers can be analysed per week, the results

being accurate to within 0·5 to 2·5 per cent. of the correct result, by means of infra-red spectroscopy. The α, β and δ isomers are determined from a spectrogram of a solution of the sample in methyl acetate, and the γ isomer from that of a solution in nitromethane between 800 cm.$^{-1}$ and 900 cm.$^{-1}$. Absorption measurements are made at 745 cm.$^{-1}$ (β), 762 cm.$^{-1}$ (α) and 772 cm.$^{-1}$, (δ) on the methyl acetate trace and at 845 cm.$^{-1}$ (γ) on the nitromethane trace. A number of minor constituents occur in varying proportions in different samples, and consist of heptachloro*cyclo*hexanes, octachloro*cyclo*hexanes, *p*-dichlorobenzene, and ε-hexachloro*cyclo*hexane; the absorption bands of certain of these are listed. G. R. K.

Mercuric Chloride Tablets, Determination of. N. Silvestri. (*Boll. chim.-farm.*, 1949, **88**, 205.) The following is a quick and easy way of testing mercuric chloride tablets. Put one 2 g. or two 1 g. tablets, previously powdered, in a graduated 100 ml. flask and add 40 to 45 ml. of cold water. Without mixing add 50 ml. of 0·2 N sodium hydroxide, shake and make up to 100 ml. After a few minutes shake again and filter through a dense double filter. Reject the first 30 to 35 ml. which are turbid, measure 50 ml. of the clear filtrate and titrate the excess of sodium hydroxide with 0·2 N hydrochloric acid in the presence of methyl orange. The method is accurate to 0·1 per cent. It can also be used for pure mercuric chloride using 1 g. with 1 g. of sodium chloride. Heavy metals which would react with sodium hydroxide must be absent. H. D.

Potassium Iodide in Tincture of Iodine, Determination of. P. Mesnard. (*Bull. Soc. Pharm. Bordeaux*, 1949, **87**, 40.) By the following method, both iodine and potassium iodide may be determined in the same sample of tincture of iodine, using a single standard solution: 2.5 g. of the tincture is titrated with 0·1 N arsenious acid in presence of sodium bicarbonate and starch. The mixture is then treated with 5 ml. of a 5 per cent. solution of potassium iodate and 2 ml. of 20 per cent. sulphuric acid. The acidity is neutralised by the addition of 20 ml. of 20 per cent. solution of sodium bicarbonate, and it is again titrated. Five sixths of the last titration corresponds to the total iodine, and deduction of the first titration gives the amount of iodine corresponding to the iodide in the tincture. G. M.

Starch and Cellulose, Determination of, with Anthrone. F. J. Viles Jr., and L. Silverman. (*Anal. Chem.*, 1949, **21**, 950.) A solution of anthrone (0·05 to 0·20 per cent.) in concentrated sulphuric acid was added to an aqueous solution or suspension of the carbohydrate to be determined and mixed immediately; under controlled conditions the amount of green colour produced was found to be proportional to the carbohydrate content. Heat was produced on mixing the acid and water and was necessary to the reaction. The colour produced in this reaction was measured spectrophotometrically and variations in the age of the anthrone reagent, in the water content, in the anthrone concentration, in the age of the colour, and in the nature of the carbohydrate were studied. Although the spectrum transmittance curve shape was altered by these factors, the maximum of the absorption band remained at 625 mμ. Investigations into the effect of heat upon the reaction showed that consistent readings were obtained after 10 minutes of air cooling. Maximum colour development occurred with rapid cooling, and in hot water immersion tests the colour rapidly deteriorated. The colour produced in the reaction was found to be stable for from 5 minutes to 3 hours, after which slight fading occurred. Maximum sensitivity occurred with 0·16 per

cent. anthrone solution, although 0·1 per cent. solution was found to be the most satisfactory for the reaction; this solution, however, deteriorated on storage. Since the reagent was unstable the use of a single standard curve was not practicable and in order to obtain accurate results one or more known standards are required for each group of analyses. Details of the final method chosen are given. R. E. S.

Thiocyanate, Titration of. E. W. Hammock, D. Beavon and E. H. Swift. (*Anal. Chem.*, 1949, **21**, 970.) Values obtained using the iodine monochloride method for the determination of thiocyanate in acid solutions were found to be erratic and an investigation was undertaken into the causes of these variations. It was found that direct titration of soluble thiocyanates with iodate in hydrochloric acid solution to the iodine monochloride end-point, resulted in less than the stoichiometric volume of iodate being used owing to partial decomposition of the thiocyanate before oxidation. Experiments showed that by previous addition to the acid of approximately four-tenths the equivalent amount of iodine monochloride required for the oxidation of the thiocyanate the error is considerably decreased, provided that the initial acid is not too concentrated and that the mixture is not allowed to stand. Slowly reacting products of the acid decomposition of the thiocyanate, rather than atmospheric oxygen, were largely responsible for the error, as indicated by the dependence of the error on the time of standing especially in the more concentrated acid, and by the fact that titrated solutions showed a return of end-point on standing; in several cases solutions that had stood for several days and were then retitrated gave substantially correct titrations. A procedure was adopted therefore in which iodine monochloride was added to the hydrochloric acid before addition of the thiocyanate; a series of titrations were then made with iodate, permanganate and ceric sulphate solutions. It was found that quantitative determinations could be made by using this procedure and titrating with iodate; the titration was not quantitative however under similar conditions with permanganate or ceric sulphate, as negative errors resulted. R. E. S.

Thyroxine, Polarographic Determination of. E. T. Borrows, B. A. Hems and J. E. Page. (*J. chem. Soc.*, 1949, Supp. 1. S 204.) Methods hitherto proposed for the determination of thyroxine are discussed. The polarographic behaviour of 23 aromatic iodo-compounds has been examined and the conditions for the hydrolysis of iodinated casein has been studied, in consequence of which it has been found possible to detect 0·1 per cent. of thyroxine in 1·0 g. of iodinated protein polarographically. The polarographic method has the advantage that it can differentiate between thyroxine and 3 : 5-di-iodotyrosine and does not depend on the non-extraction of di-iodotyrosine by *n*-butyl alcohol. It is shown that chemical methods for the determination of thyroxine based on Leland and Foster's butyl alcohol extraction procedure (*J. biol. Chem.*, 1932, **95**, 165) give high results. The procedure recommended for determination of thyroxine in iodinated casein containing large amounts of di-iodotyrosine is as follows. Hydrolyse 1·0 g. of iodinated casein (containing about 5 mg. of thyroxine) by heating for 6 hours with 100 ml. of barium hydroxide solution (40 per cent.) added in 10 ml. portions. Cool, adjust the *p*H to 3·5 with dilute hydrochloric acid and extract with 50-ml. followed by 2 25-ml. quantities of *n*-butyl alcohol. Wash the butyl alcohol extract with 2 50-ml. quantities of a solution containing 1·6 per cent. of sodium hydroxide and 5 per cent. of sodium bicarbonate and evaporate to dryness under reduced

pressure. Dissolve the residue in 10 ml. of N sodium carbonate, transfer to a 20-ml. graduated flask, add 2·0 ml. of tetramethylammonium bromide solution (10 per cent.) and 4·0 ml of *iso*propyl alcohol and make up to 20 ml. with distilled water. Polarograph a 3·0 ml. oxygen-free portion of the final solution over the potential range of −0·6 to −2·0 volts. Measure the height in microamps of the first thyroxine step and of the combined thyroxine and di-iodotyrosine steps. Calibration curves of the diffusion currents obtained with thyroxine and di-iodotyrosine are shown in the paper. F. H.

FIXED OILS, FATS AND WAXES

Bacury (*Platonia insignis*) Seed Fat, Component Glycerides of. T. P. Hilditch and S. P. Pathak. (*J. chem. Soc.*, 1949, Supp. 1, S.87.) The seed fat of *Platonia insignis*, Mart. (family Gutteriferæ), is a yellowish solid becoming completely liquid at 51° to 52·5°C. After removal of free fatty acid (about 5 per cent.) the neutral fat was resolved into three groups by systematic crystallisation from ether and the component acids in each group were determined by ester fractionation. It was found that the chief component acids were palmitic (55 per cent.) and oleic (32 per cent.) with smaller proportions of stearic (6 per cent.) and hexadecenoic (3 per cent.) with probably traces of myristic, arachidic and linoleic acids. In spite of a total molar content of 35 per cent. of unsaturated acids, the fat contained over 20 per cent. of trisaturated glycerides (largely tripalmitin) thus causing it to comprise with the seed fats of *Laurus nobilis* and *Myristical malabarica* the only known exceptions to the "rule of even (or widest) distribution" of acyl groups amongst the glycerol molecules of a fat. A possible explanation is suggested for the departure in bacury fat from the generalisation followed in most seed fats that trisaturated glycerides are not encountered in appreciable amounts unless their content of oleic (or other unsaturated) acid is insufficient to provide one acyl group in each triglyceride molecule.

F. H.

Ergot, Oil of, Unsaponifiable matter of. E. Ruppol. (*J. Pharm. Belg.*, 1949, **4**, 55.) Although a large number of constituents have been reported in the unsaponifiable matter of ergot, many of these have not been characterised with sufficient certainty. The author treated 6g. of the unsaponifiable matter, from which most of the sterols had been removed. by chromatography on alumina. The products identified were cerevisterol, cerebrin, the "sterol C" of Vandermeulen (identified as dehydroergosterol), and another substance, which was apparently stigmasterol but was not identified with certainty. Questions which require further investigation are: complete analysis of cerebrin and its decomposition products; examination of the volatile oil; identity of the higher alcohols; constancy of composition of the sterol fraction. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalinum Verum, Isolation of, from *D. purpurea* and *D. lanata*. K. Mohr and T. Reichstein. (*Pharm. Acta Helvet.*, 1949, **24**, 246.) Digitalinum verum has till now been isolated only from the seeds of *D. purpurea*, of which it forms the main glycoside, and it has not been obtained crystalline. As no good method of preparation appears to be on record, the authors used the following process. The finely powdered seeds were defatted with light petroleum, and then extracted with hot alcohol (50 per cent.) until all bitter tasting substance

was removed. The extract was purified with lead hydroxide in the usual way, freed from alcohol *in vacuo*, and purified by shaking with ether and also with chloroform (chloroform treatment is desirable, but sometimes impracticable on account of emulsion formation). Digitonin was then removed by treatment with cholesterol, and after removal of the excess of cholesterol, the glycoside mixture was acetylated to form the hexa-acetate of digitalinum verum. This may be obtained crystalline from a mixture of benzene and ether. From 500 g. of seeds, 1·5 g. of the acetate was obtained. The acetate, like that of gitoxigenin, easily loses acetic acid when chromatographed on alumina. Careful saponification with potassium bicarbonate in aqueous methyl alcohol gives the free digitalinum verum in a crystalline state. With *D. lanata*, which contains more digitonin than *D. purpurea*, the digitonin should first be removed before the shaking out, by extraction with choloroform-alcohol. The yield in this case was 0·564 g. of pure hexa-acetate from 900 g. of seeds. G. M.

Maize Starch, Waxy, Amylose Component of. E. J. Bourne and S. Peat. (*J. chem. Soc.*, 1949, 5.) A sample of waxy maize starch was defatted by treatment with aqueous methyl alcohol and dioxan and then submitted to the action of hot aqueous acid, when it was converted completely into glucose. Hydrolysis using β-amylose (from soya bean) however, ceased at a conversion limit (to maltose) of 49 per cent. The blue-value of the starch was 0·10 compared with average values of 1·10 and 0·22 found respectively for amylose and amylopectin isolated from potato starch by the thymol method. The lower staining power of waxy maize starch relative to potato amylopectin is emphasised by the light absorption curves of their iodine complexes. Attempts to fractionate waxy maize starch using the amylose-precipitants thymol or cyclohexanol produced a small fraction (3 to 5 per cent.) which had blue-values of 0·17 and 0·14, and a limiting conversion into maltose of 51 per cent. and 49 per cent. respectively, thus yielding some indication that these fractions were enriched in amylose although the light absorption curves for these fractions were of amylopectin character. Fractionation using the aluminium hydroxide precipitation method wielded among others a fraction with a blue-value of 0·40 and a limit of β-amylose conversion of 59 per cent. and obviously similar in composition to potato starch. The properties of fractions isolated by this and other procedures are consistent with the view that this starch does contain the largely unbranched component amylose to the extent, however, of less than 2 per cent. The recent work of Pascu and Hillier (*Text. Research J.*, 1946, **16**, 243) is discussed although the authors do not depart from the view that amylose is a pre-formed component of the starches, including that of maize search.

R. E. S.

Potato Starch, Fractionation of, by means of Aluminium Hydroxide. E. J. Bourne, G. H. Donnison, S. Peal and W. J. Whelan. (*J. chem. Soc.*, 1949, 1.) Aqueous dispersions of potato starch (3 per cent.) were treated with varying amounts of hydrated aluminium sulphate (ranging from 0·3 g./g. of starch to 4·0 g./g. of starch) followed by a slight excess of ammonia. In each case a portion of the starch was not adsorbed and could be recovered from the supernatant liquid by precipitation with alcohol. This fraction, representing 1·2 to 10·3 per cent. of the starch, invariably had a higher blue value (varying from 1·11 to 1·36 in 6 experiments), than that of thymol-amylose. Exhaustive extraction of the aluminium hydroxide with boiling water gave fractions some of which consisted mainly of amylose, while others contained a large proportion of amylopectin. When the precipitate

remaining after exhaustive aqueous extraction had been redissolved in dilute alkali, neutralised, and dialysed to remove inorganic material, an amylopectin fraction was recovered by precipitation with alcohol which stained red-purple with iodine, and had blue value 0·18 to 0·22, comparable with that of an average amylopectin fraction separated from potato starch by the thymol method. The aluminium hydroxide method was tried for the separation of amylose fractions of high blue value as it was no convenient as the thymol method for the routine isolation of the amylopectin of starch. A number of factors, namely the temperature of precipitation and of "ageing" of the hydroxide, the time of "ageing" of the hydroxide, and the mode of preparation of the starch paste were examined with regard to their influence on the purity of the amylose fraction. A group of separations conducted on portions of the same starch paste, showed that when the aluminium hydroxide was both precipitated and "aged" at 30°C. the amylose fraction had a higher blue-value (1·03) than that obtained when the temperatures of precipitation and "ageing" were 30°C. and 14°C. respectively, or when both operations were carried out at 14°C. The time of "ageing" played an important part in the fractionation, as shown by separations in which the starch-aluminium hydroxide suspension was kept at 15°C. and aliquot portions were removed at intervals for the isolation of the amylose fraction; the blue-value of the product was 1·01 when no "ageing" occurred, although it rose to a maximum of 1·27 after "ageing" of the hydroxide suspension for 3 days, and diminished slightly to 1·22 after 7 days. "Ageing" at a higher temperature (30°C.) for 3 days considerably raised the blue value of the product. When a 3 per cent. paste was stirred at 100°C. and portions removed at intervals for aluminium treatment, the amylose fraction with maximum blue-value was isolated from the paste which had been boiled for 1 hour. Details are given of a method which was worked out for the isolation of amylose using aluminium nitrate; samples of amylose isolated by this technique were consistently of high blue value (1·35 to 1·40). The yield was somewhat variable (6 to 13 per cent.) and was always lower than the usual yield (*ca.* 20 per cent.) obtained with organic precipitants, presumably due to the difficulty of separating completely the amylose solution from the aluminium hydroxide gel. The light absorption curves of the polysaccharide-iodine complexes of a number of "amylose" fractions obtained are given: they confirm that adsorption on aluminium hydroxide effects a true fractionation of the starch. R. E. S.

ORGANIC CHEMISTRY

Dramamine, Chemistry of. J. W. Cusic. (*Science*, 1949, **109**, 574.) Attempts have been made, unsuccessfully, to obtain chemical compounds of antihistamines with methylxanthines. The methylxanthines do not form stable salts because of their low ionisation constants. 8-chlorotheophyllin does form stable salts and is used to prepare dramamine (β-dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate). A slight excess of the base is dissolved with the 8-chlorotheophyllinate in hot methyl ethyl ketone or ethyl alcohol. On cooling, an almost quantitative yield of salt is obtained with m.pt. 101° to 103°C.; empirical formula $C_{24}H_{30}O_3N_5Cl$. Found: Cl., 7·45, 7·46 and 7·51 per cent.; Basic N, 2·98, 2·98 per cent.; 8-chlorotheophylline 45·65, 45·62 per cent.; $C_{24}H_{30}O_3N_5Cl$ requires Cl, 7·55 per cent.; Basic N, 2·98 per cent.; 8-chlorotheophyllin, 45·67 per cent. A. D. O.

Fluorescent Compound from Adrenaline, Structure of. I. Ehrlèn. (*Farm. Revy.*, 1949, **48**, 485.) The formation of a fluorescent compound from adrenaline

via adrenochrome, has been used by the author for the fluorimetric determination of adrenaline. A polarographic study of the transformation of adrenochrome into the fluorescent compound indicated that the spontaneous transformation of adrenochrome in neutral or alkaline solutions was a intramolecular rearrangement with formation of an *o*-dihydroxy compound. The secondary cathodic wave, ascribed by Wiesner to reaction with an added reducing substance, also occurs in the absence of the latter, and is probably due to the conversion of an enol into a ketone. The fluorescent compound is probably 1-methyl-3-oxo-5 : 6-dihydroxy-2 : 3-dihydroindol. Actually indoxyl itself shows a resemblance to the fluorescent compound. Although the latter is insoluble in ether, it can be extracted from an aqueous solution at *p*H 4 by shaking with ether containing some alcohol. The spectrum of such a solution showed 4 maxima at respectively 232·0, 258·0, 294·0 and 415·0 mμ. G. M.

Thyroxine and Related Substances, Synthesis of. E. T. Borrows, J. C. Clayton and B. A. Hems. (*J. chem. Soc.*, 1949, Supp. 1, S 185.) DL-Tyrosine has been synthesised in 55 per cent. overall yield by condensation at 160°C. of *p*-hydroxybenzaldehyde with hydantoin using morpholine as catalyst, reduction of the *p*-hydroxybenzylidene-hydantoin quantitatively in alcohol with Raney nickel at 130°C. under 60 atmospheres pressure of hydrogen, and hydrolysis of the resultant *p*-hydroxybenzyl hydantoin on refluxing with 2 N sodium hydroxide solution. DL-3 : 5-Di-iodotyrosine was obtained in 86 per cent. yield on iodinating tyrosine in hot dilute hydrochloric acid with a solution of iodine monochloride in concentrated hydrochloric acid. The attempted iodination of thyronine and related diphenyl ethers are described and discussed, and contrary to a previous report it has been found possible to tetrazotise 2 : 6-diaminodiphenyl ethers and to replace the diazo-groups by iodine atoms. This reaction has been used to develop an alternative synthesis of thyroxine. Condensation of methyl 4-chloro-3 : 5-dinitrobenzoate with quinol monomethyl ether in the presence of potassium hydroxide at 150°C., gave in good yield the dinitromethoxyphenoxy benzoate which was reduced to the diamine catalytically using palladised charcoal. Tetrazotisation was achieved when a solution of the diamine in glacial acetic acid was run into a cold stirred solution of nitrosyl-sulphuric acid in concentrated sulphuric acid and on decomposition by aqueous potassium iodide solution yielded 3 : 5-di-iodo-4-(4′-methoxyphenoxy)benzoic acid This was converted to the acid chloride with thionyl chloride and thence to the hydrazide by heating with hydrazine hydrate in methyl alcohol. The toluene-*p*-sulphonyl derivative was formed with toluene-*p*-sulphonyl chloride in dry pyridine and on heating for 30 seconds in ethylene glycol with sodium carbonate gave the crude aldehyde which on condensation with hippuric acid gave 2-phenyl-4-[3′ : 5′-di-iodo-4′-(4″-methoxyphenoxy)benzylidene] oxazol-5-one identical with the oxazolone obtained by Harington and Barger in their synthesis of thyroxine. F. H.

Thyroxine, Synthesis from 2:6-Dinitrophenyl Ethers. E. T. Borrows, J. C. Clayton and B. A. Hems. (*J. chem. Soc.*, 1949, Supp. 1. S 199.) Thyroxine has been synthesised in 14 per cent overall yield from *p*-hydroxybenzaldehyde in the following manner. 5-(4′-Hydroxybenzyl)-hydantoin, obtained on condensation of hydantoin with *p*-hydroxybenzaldehyde in the presence of morpholine and catalytic reduction of the product using Raney nickel, gave 5-(3′ : 5′-dinitro-4′-hydroxybenzyl)hydantoin in 83 per cent. yield when added in portions to concentrated nitric acid at 25° to 30°C., this temperature being maintained for 2 hours. This was converted

to its toluene *p*-sulphonyl ester in dilute alkaline solution and on then heating with quinol monomethyl ether in pyridine gave 5-(3′: 5′-dinitro-4′-*p*-methoxyphenoxybenzyl)hydantoin which was reduced catalytically at elevated temperature in an autoclave to the diamine. In acetic acid of phosphoric acid the freshly prepared diamine added to a cooled stirred solution of nitrosylsulphuric acid in concentrated sulphuric acid gave a solution of the tetrazonium compound which on drop by drop addition to a solution of potassium tri-iodide gave 5-(3′: 5′-di-iodo-4′-*p*-methoxyphenoxybenzyl)-hydantoin. This was best demethylated by refluxing gently with hydriodic acid (57 per cent.) and glacial acetic acid and on hydrolysis by heating in stainless steel with 2N-sodium hydroxide solution for 15 hours 3:5-di-iodothyronine was obtained. Iodination to thyroxine was accomplished in concentrated ammonia using drop by drop addition of a solution of iodine in potassium iodide. F. H.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline, Separation of nor-Adrenaline from Natural. B. F. Tullar. (*Science*, 1949, **109**, 536.) Paper chromatography studies have shown that crystalline epinephrine U.S.P. derived from adrenal glands contains appreciable quantities of *l*-arterenol (*nor*-adrenaline) and direct chemical evidence has therefore been sought in support of the physiological observations that the adrenal medulla may elaborate more than one hormone having adrenaline-like properties. Examination of commercial samples of natural adrenaline and also of a sample from the bulk stock of U.S.P. Reference Standard epinephrine has led to the isolation from them of *l-nor*-adrenaline. *l-nor*-adrenaline, *l*-bitartrate (m.pt. 163° to 164°C, $[\alpha]_{D}^{25°C}$ -40·2°) and *l-nor*-adrenaline *d*-bitartrate (m.pt. 101° to 102°C. $[\alpha]_{D}^{25°C}$ -12°) and *l-nor*-adrenaline hydrochloride (m.pt. 147·5° to 148·5°C., $[\alpha]_{D}^{25°C}$ -39·8°) were prepared from the samples of natural adrenaline and identified with the corresponding salts prepared from synthetic *l-nor*-adrenaline. It is confirmed that natural adrenaline contains appreciable quantities of *l-nor*-adrenaline and the isolation of the latter in chemically pure form from biological material furnishes the final step of proof in establishing its hormonal nature. F. H.

nor-Adrenaline in Adrenal Medullary Tumours. P. Holton. (*Nature*, 1949, **163**, 217.) An acid extract of a portion of human suprarenal tumour, found histologically to be a typical phæochromocytoma consisting of chromaffin tissue, has been shown to contain both adrenaline and *nor*-adrenaline. Using the rat's uterus and the frog's perfused heart, which have been shown to be insensitive to *nor*-adrenaline, each ml. of the extract was found to be equivalent to 0·37 mg. of adrenaline. When tested on the rabbit's duodenum and the spinal cat's blood pressure, which are approximately equally sensitive to adrenaline and *nor*-adrenaline, the extract was found to be equivalent to 1 mg. of adrenaline. It was therefore concluded that each g. of tumour contained 3·7 mg. of *l*-adrenaline and 6·3 mg. of *nor*-adrenaline. The presence of both amines was confirmed by tests with polyphenolase, a mixture of the extract and enzyme on incubation giving a brown colour typical of *nor*-adrenaline during 10 minutes changing after 20 minutes to a rose-pink colour typical of adrenaline. By paper chromatography, using phenol as solvent and identifying the spots by spraying with potassium ferricyanide, adrenaline and *nor*-adrenaline were separated from the extract. Two

other adrenal medullary tumours examined, contained 5·3 mg. of *l-nor*-adrenaline and 0·32 mg. of l-adrenaline, and 5·5 mg. of *l-nor*-adrenaline and 0·37 mg. of *l*-adrenaline respectively per g. of tumour. It is suggested that since *nor*-adrenaline has slightly greater pressor activity than adrenaline, the attacks of high blood pressure caused by the tumours were probably mainly due to *nor*-adrenaline. F. H.

***l*-nor-Adrenaline in the Suprarenal Medulla.** U. S. von Euler and U. Hamberg. (*Nature*, 1949, **163**, 642.) The demonstration that *nor*-adrenaline constitutes the specific neurohormone (sympathin N) of adrenergic nerve fibres has directed attention to the occurrence of this substance in biological material. Suprarenal glands from cattle were extracted with trichloracetic acid (5 per cent.) and the acid removed with ether. The extracts were examined for the presence of *nor*-adrenaline, chemically, biologically and by paper chromatography. It has been shown that the rates of formation of adrenochrome and of *nor*-adrenochrome on oxidation of the parent amines with iodine depends on the *p*H. At *p*H 4 and an oxidation time of 1½ minutes the formation of adrenochrome is complete whereas only about 7 per cent. of the *nor*-adrenaline is then oxidised. At *p*H 6 an oxidation time of 3 minutes gives the sum of adrenochrome and *nor*-adrenochrome. Although the colorimetric method does not differentiate between *dl*- and *l-nor*-adrenaline, the results obtained agreed well with those obtained from comparison of the effect of extracts on the blood pressure of the cat with those of mixtures of adrenaline and *nor*-adrenaline, samples of extracts of whole glands containing the equivalent of 0·48 to 0·70 μg. of *nor*-adrenaline hydrochloride and 1·80 to 2·16 μg. of adrenaline hydrochloride per mg. of fresh tissue and a sample of adrenal medulla containing the equivalent of 2·68 μg. of *nor*-adrenaline hydrochloride and 11·3 μg. of adrenaline hydrochloride per mg. of fresh tissue. Paper chromatography using *n*-butyl alcohol saturated with N/1 hydrochloric acid as solvent gave a close correspondence between the migration of a suprarenal gland extract purified by adsorption to alumina and a mixture of *nor*-adrenaline and adrenaline. The results showed that the normal suprarenal gland from cattle contains *l-nor*-adrenaline in appreciable amounts as previously shown for adrenergic nerves and support Blaschko's concept (*J. Physiol.* 1942, **101**, 337) of adrenaline formation in the chromaffin cells. F. H.

Aureomycin and Blood Coagulation. D. I. Macht and R. Farkas. (*Science*, 1949, **110**, 305.) Experiments were made on rabbits and cats, and clinical tests were made on patients who had not received any previous medication. In all the experiments the drug was administered by stomach and the clotting time measured before administration and at various intervals afterwards. In both rabbits and cats the clotting time after administration of aureomycin showed a progressive diminution. This finding was confirmed in 14 human subjects following administration of one or two capsules of aureomycin of 250 mg. each, the usual clinical dosage. Tests made on both human subjects and lower animals revealed no difference in prothrombin time, thus indicating that the diminution in clotting time is due to other factors involved in blood coagulation. The authors suggest that in order to avoid the possibility of thrombo-embolic accidents during antibiotic therapy prophylactic measures by the use of anticoagulant drugs may be instituted. S. L. W.

Chloramphenicol (Chloromycetin), Enzymatic Hydrolysis of. G. N. Smith, C. S. Worrel, and B. L. Lilligren. (*Science*, 1949, **110**, 297.) In an

initial series of experiments with *E. coli*, *P. vulgaris* and *B. subtilis* it was found that chloramphenicol could be hydrolysed when introduced into actively growing broth cultures of these organisms. Subsequent experiments showed *P. vulgaris* and *B. subtilis* to be the best sources of the enzyme. Filtrates from cultures 2 to 4 weeks old actively destroyed chloramphenicol and a fairly active preparation of the enzyme could be obtained by concentration of the filtrate. The action of this enzyme, which has been tentatively designated enzyme A, is to hydrolyse the amide linkage of chloramphenicol and thus liberate the corresponding basic amine and dichloroacetic acid. The optimum conditions for the enzymatic hydrolysis have been found to be *p*H 7·5 and a temperature of 37·5 to 40·0°C. The rate of enzymatic hydrolysis increases with substrate concentration up to 2 mg./ml. One unit of enzyme activity has been arbitrarily chosen as that amount of the enzyme which will hydrolyse 1μg. of chloramphenicol in 1 hour at *p*H 7·5 and 37·5°C. The enzyme is probably very similar to other proteolytic enzymes that have been isolated from bacterial cells. It is neither a true papain nor a trypsin though it is probably more closely allied to the former group of enzymes than to the latter. S. L. W.

BIOCHEMICAL ANALYSIS

Bacitracin, Assay of. G. D. Darker, H. B. Brown, H. Free, B. Biro and J. T. Goorley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 156.) Two methods for assaying bacitracin, an antibiotic produced by an organism of the *Bacillus subtilis* group, are described. The turbidimetric assay is rapid, sensitive for samples containing from 0·2 to 0·8 units per ml., and shows an accuracy of ± 20 per cent. Strictly aseptic technique is not necessary. *Staphylococcus aureus* grown on Bacto-yeast broth is incubated with various dilutions of the sample and with a standard solution for 4½ hours at 38°C., when growth is stopped by steaming. The turbidity is determined in a Soleman Universal Spectrophotometer at 600 mμ wavelength. The amount of antibiotic activity, as measured by the turbidity, is given by the difference between the growth obtained in the bacitracin sample and that obtained with the organism alone. This is compared with the inhibition produced by the standard bacitracin solution. The second method, the cylinder-plate method, is suitable for solutions containing from 1·5 to 8 units of bacitracin per ml., and has an accuracy of ± 15 per cent. The procedure is the same as for penicillin, except that the inoculated plates with the cylinders holding the test-solutions should be kept at 4°C. for 6 to 10 hours before incubation, to allow for the slower diffusion rate of bacitracin. Methods for establishing standard curves and charts for the cylinder-plate assay are described.

L. H. P.

Penicillin. Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin. Part I. The Determination of Benzyl Penicillin by Precipitation with N-Ethylpiperidine. (*Analyst*, 1949, **74**, 79.) The method was that of Sheehan, Mader and Cram (*J. Amer. Chem. Soc.*, 1946, **68**, 2407) and the Food and Drug Administration of the United States, depending on the extraction of an acidified aqueous solution of the penicillin salt with amyl acetate and subsequent precipitation of the N-ethylpiperidinium salt from a mixture of amyl acetate and acetone. Details are given of the reagents and the tests for limits of impurities, together with the exact procedure to be adopted. Two standards were used, one the "M.U. Standard" or Manufacturers'

Ultimate Standard, and the "A.S.C. Standard" or Analysts' Sub-Committee Standard. After collaborative determinations it was found that, assuming the purity of the M.U. Standard to be 100 per cent. 98·6 per cent. recovery was obtained in the process; accordingly as the (weighted) mean assay result for the A.S.C. Standard was 96·1 per cent., after correction on a 98·6 per cent. recovery basis it was assigned a sodium benzyl penicillin content of 97·5 per cent. Results produced in microchromatographic examinations showed that the precipitation procedure gave a precipitate, which except with the already pure M.U. Standard, was not composed of the N-ethyl-piperidinium salt of pure benzyl penicillin; in the range 90 to 100 per cent. of benzyl penicillin, there was some agreement between the calculated benzyl penicillin content of the samples examined and the amount determined by the proposed method, indicating a possible compensation of errors. The Sub-Committee recommended that the procedure described be used as a tentative method in the examination of samples of penicillin consisting substantially of benzyl penicillin. In view of the appreciable error to which the method is subject and the number of variable factors, it was considered desirable that a standard penicillin should be assayed alongside the test sample and a correction applied equal to the difference between the benzyl penicillin content found for this standard and its actual value. The Sub-Committee, with the approval of the Ministry of Health, has standardised a quantity of sodium benzyl penicillin designated the "Benzyl Penicillin Gravimetric Standard" to which a value of 95·8 per cent. sodium benzyl penicillin has been assigned. R. E. S.

Streptomycin, Colorimetric Determination of, in Urine. M. J. Masquelier. (*Bull. Soc. Pharm. Bordeaux,* 1949, **87,** 53.) By treatment with acetylacetone, the methylglucosamine of streptomycin is converted into pyrrole derivatives which give a red colour with Ehrlich reagent. 1 ml. of solution, containing 100 to 500 μg. of streptomycin, is heated for 10 minutes on the water-bath with 1 ml. of a 2 per cent. aqueous solution of acetylacetone and 2 drops of sodium hydroxide. After cooling, there are added 2 ml. of alcohol (95 per cent.) and 2 ml. of a solution of 0.8 g. of *p*-dimethylaminobenzaldehyde in 30 ml. of alcohol (95 per cent.) and 30 ml. of hydrochloric acid. In order to allow for indole derivatives, etc., in the urine, another portion of the solution is treated similarly, but omitting the acetylacetone. The colour is compared with that obained with a standard solution of streptomycin. It is sometimes of advantage to treat the urine first with one tenth of its volume of basic lead acetate solution, removing excess of lead with sodium sulphate. The streptomycin passes into the filtrate and gives a purer colour. The concentration of streptomycin in the urine, after the administration of 1.5 g. in 24 hours, is generally about 100 to 500 μg. per ml. G. M.

Trichloroethylene in Blood, Estimation of. F. H. Brain and P. J. Helliwell. (*Biochem. J.,* 1949, **45,** 75.) New conditions are described for the quantitative production of the coloured compound from trichloroethylene and pyridine in the presence of alkali. The sample of blood is steam-distilled and collected in anisole, the anisole solution of trichloroethylene being added to dry, colourless, redistilled pyridine. The mixture is stirred mechanically for 10 minutes while being heated in a boiling water-bath; tetra-ethylammonium hydroxide is then added with stirring, and after cooling the two layers separated. The upper pyridine layer is removed by a pipette and delivered into an absorptiometer cell containing sodium hydroxide solu-

tion. The colour is measured immediately using a Spekker photoelectric absorptiometer with an Ilford spectrum blue-green filter No. 603. The colour began to fade in subdued light after 20 minutes; the absorptiometer was calibrated with standard solutions of trichloroethylene in anisole. Details are given of an efficient apparatus for the absorption of the trichloroethylene steam-distilled from blood. The addition of tetraethylammonium hydroxide was necessary owing to the sensitivity of the coloured material to carbon dioxide. The amount of alkali in the pyridine-anisole layer was so much reduced by the use of 10M sodium hydroxide that, in the process of transferring the coloured layer to the absorptiometer cell, sufficient carbon dioxide was absorbed to neutralise it, and the blue-red colour which is formed only in alkaline solution, changed to a pale yellow. Care was taken to ensure that there could be no variation in the amount of water taken up from the alkali solution by the pyridine-anisole layer by using always exactly 10M sodium hydroxide which had been checked by titration. A simple procedure for colour development was also investigated in which the pyridine-anisole solution and alkali were not stirred during heating. This gave reproducible absorptiometer readings proportional to the trichloroethylene concentration up to about 0·05 mg./5 ml. of anisole, but above this the absorption varied considerably from one estimation to another and was always too large to fit the proportionality of the lower concentrations. Using the method devised the concentration of trichloroethylene on approximately 1 ml. blood samples could be estimated to the nearest μg. at concentration levels between 1 and 12 mg. per 100 ml. R. E. S.

PHARMACY

GALENICAL PHARMACY

Emulsifying Agents. α-Monostearin and Sodium Stearate. H. H. G. Jellinek and H. A. Anson. (*J. Soc. Chem. Ind.*, 1949, **68**, 108.) A tensionmeter was devised to measure interfacial tensions by a capillary height method and with the apparatus described a number of systems were studied at 70° C. The areas (in Ångstrom units) occupied by each molecule at the interface were evaluated from the interfacial data obtained for the systems: (a) water—white oil containing α-monostearin against water; (b) water—white oil against water containing sodium stearate; and (c) water—white oil containing α-monostearin against water containing sodium stearate. The separation of phases at 70°C. of emulsions of water—white oil and water containing either α-monostearin in the oil or sodium stearate in water or both emulsifying agents, was studied under controlled conditions, the phase volume ratio being kept at 1 : 1. α-Monostearin was found to give water/oil emulsions but the addition of sodium stearate led to the formation of oil/water emulsions, the amount of sodium stearate necessary to cause the inversion being only slightly dependent on the concentrations of α-monostearin. A special apparatus is described for preparing the emulsions, and details are given of the methods used for the determination of emulsion type, together with micro-photographs and solid phase diagrams. Sodium stearate and α-monostearin together gave more stable emulsions than equivalent amounts of either emulsifying agent. R. E. S.

Injections, Bactericidal Action of. K. Alin and N. Diding. (*Farm. Revy.*, 1949, **48**, 545.) The autobactericidal action of a number of solutions

for injection was tested by inoculating such solutions with pure cultures. The results are summarised in the table below, which shows the results of sterility tests made 30 days after the inoculation.

Injection	Organism		
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*
Ascorbic acid	–	–	+
Adrenaline	–	–	–
Atropine	+	–	+
Carbachol	+	–	+
Dihydromorphinone	+	–	+
Ephedrine	–	–	+
Methadone	–	–	–
Morphine with scopolamine	+	–	+
Nicethamide	–	–	–
Oxicone (Eucodal)	–	–	+
Papaverine	–	–	+
Pentazol	–	–	–
Pethidine	–	–	–
Phenemal (phenobarbitone)	–	–	–
Phenopromine (Amphetamine)	–	–	–
Picrotoxin	+	–	+
Prostigmine	+	–	+
g-Strophanthin	–	–	–
Sulphur	–	–	–
Tetrapon	–	–	+

These results show the necessity of the addition of bacteriostatic substances to solutions dispensed in multiple dose containers. G. M.

Ringer's Solution, Isotonicity of. J. Michaels and K. Münzel. (*Pharm. Acta Helvet.* 1949, **24,** 199.) A comparison of experimentally observed depressions of freezing points, for Ringer's solution prep—according to various Pharmacopœias, showed that only the Swedish formula gave a solution having the correct value of 0·55 to 0·57° C., all of the others being hypotonic. For Ringer-Locke solution nearly all the formulæ gave the correct depression. There appears however to be no justification for a concentration of calcium and potassium different from that of Ringer's solution, and the following formula is recommended: sodium chloride, 8·5 g.; anhydrous potassium chloride, 0·3 g.; calcium chloride 0·3 g.; glucose, 1·0 g.; sodium bicarbonate, 0·5 g.; water, to 100 ml. The Ringer-lactate solution of the British Pharmacopœia, 1948, is hypotonic; it may be made isotonic by reducing the concentration of sodium chloride to 0·73 per cent. G. M.

Theophylline Ethylenediamine, Solution of, for Injection. J. Büchi and F. Hippenmeyer. (*Pharm. Acta. Helvet.*, 1949, **24,** 326.) Commercial theophylline ethylenediamine shows a considerable variation in the proportion of the two components. In order to determine the optimum proportions for a stable solution of relatively low *p*H value, a number of solutions were made up with different proportions of the two components. As a result the following two formulæ were recommended. Strong solution: theophylline (1 H_2O), 20·60 g.; ethylenediamine hydrate, 5·630 g.; water, to 100ml. Dilute solution: theophylline (1 H_2O), 2·06 g.; ethylenediamine hydrate, 0·510 g.; water, to 100 ml. These solutions contain respectively 126 and 139 per cent. of the amount of ethylenediamine corresponding to the formula $1\,2\,C_7H_8O_2N_4 \cdot 1C_2H_8N_2.2H_2O$; the *p*H values, after sterilisation, being 9·45 and 9·10. They show no tendency to crystallisation, and no appreciable change after 6 months' storage. The reaction is less alkaline than that of most commercial preparations. G. M.

PHARMACOLOGY AND THERAPEUTICS

Aureomycin in the Treatment of Late Cutaneous Syphilis. P. A. O'Leary, R. R. Kierland and W. E. Herrell. (*Proc. Mayo. Clin.*, 1949, **24,** 302.) Aureomycin administered orally produced healing in 2 patients with late nodulo-ulcerative lesions of syphilis. The dose was 250 mg. every 6 hours for 2 or 3 days, increased to 500 mg. to 1 g. 6-hourly. One patient received a total of 56·5 g. and the other a total of 60 g. Nausea, vomiting and diarrhœa were the only complications encountered. Encouraged by these results the authors have commenced the treatment of patients with various types of neurosyphilis with aureomycin administered orally and have found that the serologic changes in the cerebrospinal fluid are similar to those noted after penicillin. S. L. W.

***para*-Aminosalicylic Acid Therapy, Complications of.** J. M. Swanson. (*Lancet*, 1949, **257,** 175.) 5 out of 6 cases of rheumatoid arthritis treated with *para*-aminosalicylic acid developed hypoprothrombinæmia. 15 g. of the drug had been given daily in 5 doses at 3-hourly intervals. A. D. O.

Digitalis, Effect of the Rate of Injection on the Lethal Dose in Guinea-pigs. T. S. Miya and H. G. O. Holck. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 64.) Eighty four male guinea-pigs arranged in seven groups, were anæsthetised with urethane and injected with tincture of digitalis U.S.P. diluted with normal saline to contain 1·60, 2·26, 3·20, 4·53, 6·40, 9·05 and 12·80 per cent. of the tincture respectively. The average lethal dose was determined by the U.S.P. method for the assay of digitalis except that the injections were made into the jugular instead of the femoral vein. In general the average lethal dose increased as the concentration of the tincture decreased except that the highest concentration gave a slightly higher lethal dose than the next lowest. The end-point was difficult to determine because the respiration became irregular some time before the heart beats ceased, and the beats became sporadic before finally stopping. Comparison of the results with those of other workers suggest that the guinea-pig is less suitable for the assay of digitalis than the cat. G. R. K.

Dramamine for the Prevention of Airsickness. B. A. Strickland, Jr. and G. L. Hahn. (*Science*, 1949, **109,** 359.) 108 young men were treated with dramamine (β-dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate) and then taken on specially controlled flights in an aeroplane. 100 mg. of the drug was given 25 to 45 minutes before taking off. 31 men were airsick and, of a similar number of controls, 60 were sick. A. D. O.

Dramamine in the Prevention and Treatment of Motion Sickness. L. N. Gay and P. E. Carliner. (*Science*, 1949, **109,** 359.) Clinical trials of dramamine (β-dimethylamine ether benzohydryl ether 8-chlorotheophyllinate) were carried out on a troopship. In each case the dose was 100 mg. every 5 hours and before retiring. Seasickness was prevented in all but 2 of 134 men who received the drug at the time of departure, whereas 34 men comprising a control group were sick. In the latter group complete relief of symptoms were obtained 1 hour after commencing treatment. Out of 195 other cases of severe seasickness 187 were completely relieved of symptoms 30 minutes after treatment was started. Relapses occurred when a placebo was substituted for the drug and relief was obtained again when treatment recommenced. In another series of 359 cases, 372 recovered within 1 hour of commencing treatment. Rectal administration was equally as effective as oral treatment. There were no reactions to the drug. A. D. O.

Iron, Intravenous, and Anæmia of Pregnancy. A. D. T. Govan and J. M. Scott. (*Lancet*, 1949, **256**, 14.) A stable saccharated oxide of iron was given by intravenous injection to 25 pregnant women suffering from iron-deficiency anæmia. All showed a rapid response to treatment, the hæmoglobin increasing in almost all cases by 8 per cent. in the first week of treatment. During the first week injections were given daily, starting with a dose equivalent to 30 mg. of iron on the first day, 60 mg. on the second day, and 100 mg. thereafter, the injections being reduced at the end of a week to 100 mg. on alternate days. It apparently requires about 40 mg. of iron to increase the hæmoglobin by 1 per cent. in an anæmic pregnant woman. The solution appears to irritate the vessel wall, and rapid injection causes an immediate vasospasm, though only one case showed a severe reaction, probably due to vagal stimulation. A comparison of the hæmoglobin readings of the patients treated by intravenous injection and of 62 cases treated with iron by mouth showed a very striking difference, not only as regards time but also because in the patients treated intravenously succeeding increments of hæmoglobin increased instead of diminishing as in the patients treated with iron by mouth. S. L. W.

Morphine Derivatives: Chemical Constitution and Analgesic Action. F. W. Schueler, E. G. Gross and H. Holland. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 74.) In a review of the pharmacodynamic action of morphine and eighteen of its derivatives, the authors have concluded that the analgesic action may be due to the presence of both sympathomimetic and parasympathomimetic portions in the molecule connected by the same amino-nitrogen atom. Previous workers found that for a maximum muscarine-like action the average optimum limiting distance between the methyl group of the amino-nitrogen and the ether oxygen or carboxyl oxygen are respectively 5·3 Å and 7·0 Å; using Fisher-Hirschfelder-Taylor models the corresponding distances in the nineteen molecules under discussion varied between 5·0 and 9·0 Å. For sympathomimetic action the distance between the methyl groups of the amino-nitrogen and the aromatic carbon atom joining the ring to the alkyl carbon chain appears to be of paramount importance it varied between 4·5 and 6·5 Å in these molecules. It is suggested that the analgesic action of morphine and its analogues is due to the presence of both these autonomic nervous system stimulating portions and acts through a peripheral mechanism. The parasympathomimetic group may be active in stimulating the output of inhibitory action on cholinesterase and the sympathomimetic group may adrenaline by the suprarenal glands either by direct action or through the enhance the peripheral perineural vasoconstrictor response of the adrenaline either by its direct action or by preserving adrenaline. G. R. K.

Nisin, Some Recent Applications of. A. Hirsch and A. T. R. Mattick. (*Lancet*, 1949, **257**, 190.) Nisin is an antibiotic produced by *Streptococcus lactis*. It is mainly effective against Gram-positive organisms including the tubercle bacillus and has the properties of a polypeptide or protein of low molecular weight. Nisin is soluble in dilute acids and the solution is stable to heat, but it tends to precipitate at neutrality and is slowly labile under alkaline conditions. With *Strep. agalactiæ* and *Strep. pyrogenes* nisin is rapidly bactericidal. The intravenous LD50 in rabbits is between 20 and 23 mg./kg. The intramuscular LD50 is about 200 mg./kg. and the subcutaneous dose was greater than 1,000 mg./kg. Rabbits which had received a fatal intravenous dose died quickly and quietly, but guinea-pigs suffered very

violent spasms resembling "peptone shock." Necropsies showed that nisin was present in all the internal organs with the highest concentration in the lungs. Lesions at the site of subcutaneous injection contained almost all the nisin which had been injected and this probably explains the low toxicity by this route. The "peptone shock" symptoms of fatal doses of nisin are usually avoided when the injection was given slowly with a perfuser. No apparent effect on leucocytes and red blood cells could be observed. After initial saturation injections, satisfactory blood-nisin levels (against mycobacteria, 100 units/ml.) were obtained by 12 hourly injections. Blood levels of 1 to 10 units/ml. of serum in rabbits were obtained by oral doses. The *in vitro* activity of nisin against mycobacteria in the Dubos and Davis medium, was equivalent to that of streptomycin. Combinations of nisin and streptomycin or nisin and licheniformin were neither synergistic nor markedly additive, but a combination of all three was. A similar behaviour was shown by nisin, licheniformin, and sulphathiazole, and a more than a mere additive action occurred with nisin and sulphathiazole together. Sulphathiazole resistant cultures were sensitive to nisin, as were streptomycin cultures. Nisin resistant cultures remained susceptible to streptomycin. *In vivo* experiments with subcutaneous doses of crude nisin resulted in a reduction in the spread of infection. After intravenous treatment, histological examination revealed early tuberculosis but this could not be detected macroscopically as in the control animals. A.D.O.

Salicylates. Toxicity during Pregnancy. A. V. Jackson. (*J. Path. Bact.*, 1948, **60**, 587.) Pharmacological text-books commonly contain references to the danger of using large doses of salicylates during pregnancy but as there is little clinical support for this view the matter was investigated in rabbits and rats the placental permeability of which resembles that of man. It was found that salicylate readily passed the placental barrier in rabbits, the concentration in the fœtal serum being about two-thirds that in the mother. Even when doses sufficient to kill one out of four of the mothers were given most of the fœtuses survived. In rats, whenever the mother survived so also did the fœtuses. It is concluded that there is no special liability to abortion or fœtal death in salicylate poisoning until doses approaching maternal lethal levels are reached. No specific histological changes occur in salicylate poisoning and the harmful effects are thought to be referable to interference with intracellular enzyme activity. H. T. B.

Senna, Biological Assay of. L. C. Miller and E. B. Alexander. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 417.) The assay procedure described involves administering the preparation under test at three dosage levels selected with a view to producing laxative effects in approximately 25, 50 and 75 of three similar groups of mice. Concurrently, a suspension of a standard senna powder was given at three appropriate dosage levels. The number of mice receiving each dose of unknown and standard and showing laxative effects during the $5\frac{1}{2}$ hours after dosing were recorded and from these data the potency and confidence limits were calculated by conventional methods. The method is applicable to senna in its various pharmaceutical forms; the material used in this investigation consisted of proprietary preparations containing about 50 per cent. of sugar and senna extractives equivalent to 50 to 100 mg. of fresh drug per ml. Satisfactory dosage-response relationships were found in experiments on human beings and the potency ratios found on human subjects agreed well with those of mice. S. L. W.

Sodium Carboxymethylcellulose, Evaluation of Hydrophilic Properties of Bulk Laxatives containing. R. H. Blythe, J. J. Gulesich and H. L. Tuthill. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 59.) In a study to find a laxative agent superior to those now in use, the hydrophilic properties of standard bulk laxatives were compared with two new synthetic agents by new and modified methods *in vitro*. The significant properties evaluated were (a) the volume of water absorbed in various media (water and artificial gastric and intestinal juices), (b) the viscosity and texture of the gel formed, and (c) the ability of the gel to retain water. To avoid causing gastric discomfort the agent should swell in the alkaline intestinal tract but not in the acid gastric juice. To simulate *in vivo* conditions polyethylene glycol was used an an osmotic agent. Karaya, psyllium and the synthetic agents methylcellulose and sodium carboxymethylcellulose were compared. It was found that methylcellulose went slowly into solution when agitated in artificial gastric juice while sodium carboxymethylcellulose remained essentially insoluble. The synthetic substances proved superior to the natural gums in hydrophilic capacity and the formation of viscous, homogeneous solutions which permit uniform distribution and eliminate any tendency to blockage. It was concluded that sodium carboxymethylcellulose was the most promising substance of those tested because of these properties and its insolubility in artificial gastric juice. G. R. K.

BACTERIOLOGY AND CLINICAL TESTS

***p*-Aminobenzoic Acid and Related Compounds, Fungistatic Properties of.** G. W. K. Cavill and J. M. Vincent. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 189.) This paper deals with the relative fungistatic actions of the methyl to *n*-amyl *p*-aminobenzoates and of *o*-, *m*-, and *p*-aminobenzoic acids and their methyl esters The fungistatic activities were determined from the inhibition of the growth rates of *Aspergillus niger, Byssochlamys fulva* and *Penicillium roqueforti*. It was noted that the inhibition of *P. roqueforti* was unusually increased if the nitrogen was supplied as nitrate and not as ammonium. The results were treated in two ways. On the basis of the reciprocal of the concentration required for 50 per cent. inhibition (I_{50}), there was a general increase in activity in ascending the series methyl to *n*-amyl *p*-aminobenzoate, and also the *m*-isomer was markedly less effective than the corresponding *o*- or *p*-isomer. On the basis of an adsorption approach, using an adsorption equation relating inhibition to the Langmuir isotherm, the method proved of little use with *P. roqueforti*, but with *B. fulva* it seemed to permit a useful analysis of the activity of the esters, particularly in terms of increasing biological adsorbability. The esters tested against *A. niger* did not give the same clear cut picture. With both *B. fulva* and *A. niger* the low I_{50} values of the *m*-isomer could be explained on the basis of the adsorption approach. G. R. K.

***p*-Aminosalicylic Acid and Resistance to Streptomycin.** O. E. Graessle and J. J. Pietrowski. (*J. Bact.*, 1949, **57**, 459.) The effect of *p*-aminosalicylic acid on the development of resistance to streptomycin by *Mycobacterium tuberculosis* was investigated *in vitro*. The strain of *M. tuberculosis* used had an initial sensitivity of 0·8 unit/ml. By repeated exposure to the antibiotic the organism was rendered resistant to 20,000

[*Continued on p.* 63

BOOK REVIEWS

PHARMACOGNOSTISCHE COMMENTAAR OP DE BELGISCHE PHARMACOPEE IV EN HAAR EERSTE BIJVOEGSEL by Prof. Apoth. R. Dequeker. Part I, pp. XIII + 746, Part II, Atlas pp. 109. 3rd Edition. Laboratorium Pharmacognosee Artsenijkunde. Ed. van Evenstraat, Leuven, 1949.

This commentary on the Belgian Pharmacopœia IV and its First Supplement includes, in the text, 160 items. Sixteen crude drugs in the B.P. 1948 are absent from this book: these are agar, anethum, belladonna root, capsicum, cardamom, carum, catechu, coccus, colchici cormus, colocynth, creta, ipomœia, lemon peel, hypoglossi oleum, Indian podophyllum, prunus serotina. There are included, however, several commodities which are not "official" in Britain, but are commonly sold in the pharmacy.

Each drug in the text is considered under the following headings:—definition, synonyms, biological and geographical sources, concise description of the plant, cultivation, collection and preparation, macroscopical characters, microscopical characters, constituents, adulterants and assay, poisonous dose, pharmacodynamic properties, pharmaceutical preparations, storage. For each section the relevant quotation from the Pharmacopœia is printed in italics and is followed by notes and explanation. A large number of references to current literature are given under authors' names with detailed references as footnotes; in this way a large amount of useful documentation is provided, including references up to 1948. The chemical nature, and where possible, molecular constitution of the constituents are discussed and many structural formulæ are given.

In describing the drugs measurements are given for both macroscopical and microscopical details. Under microscopy, however, though reference is made to the use of palisade ratio, no numerical values are given and there is no reference to vein-islet number or to the very useful stomatal index. Under lycopodium, the number of spores per mg. is not given and there is no reference to its use for quantitative assays. The value of the length of trichome rib per mg. of nux vomica is not referred to and area measurements per mg. for certain leaves and sclerenchymatous layers are also omitted. The use of crude fibre values as criteria of purity seems to have been overlooked, whereas they are valuable for the examination of drugs such as ginger, clove and linseed.

The Atlas of figures, illustrating the text, consists of a well-drawn and carefully arranged selection of details from a number of well-known illustrated treatises and publications, including amongst others the Anatomischer Atlas of Tschirch and Osterle, the Codex Français 6, the Lehrbuch de Botanik of Strasburger, and the Pharmakognisticher Atlas of Flück, Schlumpf and Siegfried. In general the habit sketches of plants and the drawings of macroscopical details are good and accurate. The figure of *Coccus cacti* on pg. 24 is entirely erroneous and misleading and should be replaced by a new drawing made from the insect itself. The microscopical drawings, although faithfully reproduced from the originals, are often unsatisfactory in deails although they provide a general guide to the particulars which must be considered. The illustrations of many drugs, unofficial in Britain, will prove particularly useful to both pharmacists and analysts, especially such commodities as althæa root, cola seeds, lime flowers, santonica, black mustard, and bearberry leaves.

The book is in semi-stiff paper covers, it is printed on good paper and is well produced. There is a satisfactory index and reference to the monographs is greatly facilitated by printing, in italics at the outside top corner of each page, the names of the drug described on that page. T. E. WALLIS.

STRUCTURE AND PHARMACOLOGICAL ACTIVITY OF SUBSTANCES ACTING ON THE AUTONOMIC NERVOUS SYSTEM, by D. Bovet and F. Bovet-Nitti. Pp. 849. S. Karger, Basle and New York, 1949. 85 Swiss francs.

Dr. D. Bovet and F. Bovet-Nitti have produced a most interesting and informative book on the structure and pharmacological activities of drugs acting on the autonomic nervous system. The substances considered include adrenaline, acetylcholine and histamine, together with their antagonists. It is particularly useful to have a book of this sort written by those who have been responsible for so much of the original work described. The book includes a historical review of the development of each of these lines of work and discusses the various views as to the modes of action of the drugs concerned. The method of producing antisubstances to the various drugs by introducing oxygen or nitrogen atoms into the molecules between the side-chain and the ring structure is particularly well stressed, and each section dealing with these antagonists is treated in great detail. Under adrenaline, for example, the aromatic and heterocyclic synthetic antagonists and the alkaloidal sympatholytics occupy more than 100 pages. It is a book which will be both useful to the student and stimulating to the investigator. Since the chemical relationships of the substances are discussed in detail, it will be of great interest to all pharmacologists and chemists working in this field. The book is a comprehensive treatise on this section of pharmacology and contains a very complete list of references. G. A. H. BUTTLE.

ABSTRACTS (*continued from page* 61)

units/ml. of streptomycin. The organism was initially inhibited by 1 mg./ml. of *p*-aminosalicylic acid. Exposure to the compound for 120 days produced no increase in tolerence. When the organism was exposed to increasing amounts of streptomycin in the presence of 0·5 mg. of *p*-aminosalicylic acid during 120 days it was inhibited by as little as 1 unit/ml. of the antibiotic. It is suggested that the simultaneous use of *p*-aminosalicylic and streptomycin may provide an effective means of preventing the development of streptomycin resistance *in vivo*. H. T. B.

Penicillin Uptake by Bacterial Cells. E. A. Maass and M. J. Johnson. (*J. Bact.*, 1949, **57**, 415.) Two types of absorption occur when cells of *Staphylococcus aureus* are equilibrated with penicillin solution. There is a simple diffusion of penicillin into the intracellular fluid, continuing until the penicillin concentration inside the cell is equal to that outside. There is also a specific uptake of the antibiotic by the cell, and this absorbed penicillin is not removed by extensive washing. By using a bacterial paste, obtained by centrifuging a culture, so as to obtain a high concentration of bacterial cells, and radioactive penicillin G, it was shown that each cell specifically absorbs 750 molecules of penicillin. No evidence was obtained to show whether this uptake of penicillin is responsible for its bactericidal activity. H. T. B.

LETTER TO THE EDITOR

Liver Extracts

SIR,—At the British Pharmaceutical Conference this year I reported the Vitamin B_{12} content of various commercial liver extracts as shown by microbiological analysis, using *Lactobacillus lactis* Dorner[1]. The reference standard used was a dry liver extract supplied by Dr. Rickes of Messrs. Merck, U.S.A., and this was alleged to be equivalent to 0·4 μg of B_{12} per ml. In the discussion at the Conference[2] I stated that a commercial preparation of crystalline vitamin B_{12} assayed at three times the labelled strength when compared with Rickes' standard. Subsequent investigation in these Laboratories, using crystalline B_{12} as reference standard and *Lactobacillus lactis* Dorner and *Lactobacillus Leishmanni* as test organisms, has shown that the real value of the Rickes' liver extract was one-third of that declared. As a consequence of this apparent discrepancy, Dr. Rickes retested his stock of the same liver standard held by him in America, and he now informs me that the present value is one-third of that originally found to be present. It is fruitless at the moment to discuss possible causes of the apparent loss in potency of this liver standard. The net result, however, is that all the figures previously given by me which have been based on the Rickes' standard must be divided by three.

It will be sen that, with this correction, a large number of the liver extracts tested from manufacturers in both hemispheres show very low figures and if the sole active material in them were the indicated vitamin B_{12}, a considerable number of the liver extracts should be almost without activity for the treatment of pernicious anæmia. In some of the low value liver extracts examined, blending experiments were performed which provided no evidence for the presence of any inhibitors. Moreover, although inhibitors have been described by Shorb, such as folic acid, the concentration at which these known inhibitors are likely to be present in liver extracts is considerably under the effective inhibiting concentrations given by Dr. Shorb.

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