JOURNAL OF PHARMACY AND PHARMACOLOGY

VOLUME XIII, 1961

WITH THE

TRANSACTIONS

OF THE

BRITISH PHARMACEUTICAL CONFERENCE

HELD AT

PORTSMOUTH

SEPTEMBER 18 to 22, 1961

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

JOURNAL OF PHARMACY AND PHARMACOLOGY

TRANSACTIONS OF THE BRITISH PHARMACEUTICAL CONFERENCE

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 1 **January 1961**

CONTENTS

Research Papers PAGES

THE ANTI-ANAPHYLACTIC ACTIVITY OF ETHANOLAMINE AND CHOLINE. By W. G. Smith 1–11

STUDIES IN ORGANO-SELENIUM COMPOUNDS. PART I. Determination of Se. By D. Dingwall and W. D. Williams 12–19

[*Continued on page ii*

CONTENTS

PAGE

Research Papers—*continued*

THE EFFECTS OF CORTISONE AND HYDROCORTISONE ON THE PLASMA LEVELS OF CORTICOTROPHIN IN THE RAT, AFTER AN ACUTE STRESS. By A. M. Barrett 20–25

ANTITUBERCULOSIS AGENTS. PART VII. 4,4′-DIPYRIDYL SULPHONE AND RELATED COMPOUNDS. By A. M. Comrie and J. B. Stenlake .. 26–33

THE ACTIVITY OF POLYMETHYLENE-BIS-4-AMINOQUINALDINIUM SALTS AGAINST *Pityrosporum ovale* AND *Candida albicans*. By W. A. Cox and P. F. D'Arcy 34–38

THE PREPARATION AND PHARMACOLOGY OF SOME PHENOLIC CARBAMATES AND ALLOPHANATES. By J. H. Barnes, Marguerite V. A. Chapman, P. A. McCrea, P. G. Marshall and Pamela A. Walsh 39–48

THE ACTION OF PROCAINE AT THE NEUROMUSCULAR JUNCTION. By D. W. Straughan 49–52

MICELLAR SIZE OF MIXED LYSOLECITHIN-LECITHIN SOLS. By N. Robinson 53–57

Pharmacopoeias and Formularies

ADDENDUM 1960 TO THE BRITISH PHARMACOPOEIA 1958. Reviewed by Miles Weatherall 58

Book Review 59

Letters to the Editor

THE ESTIMATION OF ERGOMETRINE ON THE RAT UTERUS. By Jocelyn N. Pennefather 60–61

MODIFICATION OF HISTAMINE SENSITIVITY AFTER 48/80 TREATMENT. By Judith Ann Harvey 61–62

A REACTION BETWEEN PREDNISOLONE PHOSPHATE AND SODIUM METABISULPHITE. By P. F. G. Boon and M. J. Busse 62–63

THE EMULSIFYING PROPERTIES OF GUM ACACIA. By K. Wibberley .. 63–64

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 2 **February 1961**

CONTENTS

Research Papers PAGES

THE RELATION BETWEEN CHEMICAL STRUCTURE AND UNCOUPLING ACTIVITY IN CONGENERS OF SALICYLATE. By D. V. Brostoff, V. Moses and M. J. H. Smith 65–71

A NOTE ON THE APPLICATION OF THE FLASK COMBUSTION TECHNIQUE TO SULPHUR-CONTAINING SUBSTANCES. By C. Vickers and J. V. Wilkinson 72–74

[*Continued on page ii*

CONTENTS

Research Papers—(*continued*)

PAGE

THE FORMATION OF HISTAMINE IN THE RAT. By J. M. Telford and G. B. West 75–82

ANATOMICAL STUDIES IN THE GENUS *Rubus*. PART IV. ANATOMICAL VARIATIONS IN THE LEAVES OF CULTIVATED VARIETIES OF *R. idaeus* L. AND *R. loganobaccus* L. H. BAILEY, AND OF CERTAIN SPECIES OF BRAMBLE. By K. R. Fell and J. M. Rowson 83–92

CURARE-LIKE DRUGS AND CARDIOVAGAL SYNAPSES: COMPARATIVE STUDY *in vitro* ON ISOLATED GUINEA-PIG VAGUS-HEART PREPARATION. By D. Della Bella, F. Rognoni and K. Gopal 93–97

A PHYTOCHEMICAL SURVEY OF MALAYA. PART II. ALKALOIDS. By A. K. Kiang, Bryce Douglas and Francis Morsingh 98–104

ANALGESIC AND ANTIPYRETIC PROPERTIES OF SOME ASPIRIN DERIVATIVES. By L. C. Weaver, Alice B. Richards and H. E. Martin .. 105–110

A NOTE ON BACTERIOLOGICAL TOXICITY TESTS OF SILICONE RUBBERS FOR MEDICAL AND PHARMACEUTICAL USES. By I. H. Riley and H. I. Winner 111–114

POLYHYDROXY (CATECHOLIC) PHENOLIC ACIDS—STUDIES OF THEIR METABOLISM IN MAN. By S. L. Tompsett 115–120

NOTES ON ORIGIN AND SIGNIFICANCE OF THE PEROXIDE VALUE OF ANHYDROUS LANOLIN. By E. W. Clark and G. F. Kitchen .. 121–122

Letters to the Editor

THE EFFECTS OF TUBOCURARINE, DECAMETHONIUM, SUXAMETHONIUM EDROPHONIUM AND NEOSTIGMINE UPON FLUX OF CALCIUM-47 IN FROG SKELETAL MUSCLE. By K. Ahmad and J. J. Lewis 123–127

TOLERANCE TO TREMORINE. By L. Decsi, M. Várszegi and J. Méhes 127

ANTIFERTILITY AGENTS. By Martin L. Black 127

HYPOGLYCEMIC AGENT FROM ONIONS. By H. D. Brahmachari and K. T. Augusti 128

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 3 **March 1961**

CONTENTS

Research Papers PAGES

THE EFFECT OF *ORTHO* SUBSTITUTION ON THE HYDROLYSIS OF BENZOYLCHOLINE. By J. Thomas and J. R. Stoker 129–138

THE ANTIPEPTIC ACTIVITY OF SULPHATED POLYSACCHARIDES. By W. Anderson 139–147

THE DETERMINATION OF OESTRADIOL DIPROPIONATE IN OILS. By D. W. Snair and L. A. Schwinghamer 148–153

THE ANATOMY OF THE LEAF OF *Symphytum officinale* L. By (Miss) J. M. Peck and K. R. Fell. 154–165

ABSORPTION AND ELIMINATION OF GRISEOFULVIN FROM THE ALIMENTARY TRACT OF THE RAT. By B. Davis, K. J. Child and E. G. Tomich 166–171

AUTOXIDATION, AND ITS INHIBITION, IN ANHYDROUS LANOLIN. By E. W. Clark and G. F. Kitchen 172–183

New Apparatus

A NON-CORRODIBLE VALVE FOR USE IN AUTOMATIC CONTROL OF FLOW OF PHYSIOLOGICAL FLUIDS. By S. A. Hemes and J. B. Roberts .. 184–186

Pharmacopoeias and Formularies

THE NATIONAL FORMULARY OF THE UNITED STATES OF AMERICA, ELEVENTH EDITION, 1960. Reviewed by K. R. Capper 187–189

Book Review 190

Letter to the Editor

STUDIES WITH A RAT HEPATOMA. By Lalitha Kameswaran and G. B. West 191–192

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 4 **April 1961**

CONTENTS

Review Articles PAGES

THE PHARMACOLOGY OF MEMBRANES

THE PASSAGE OF SUBSTANCES ACROSS BIOLOGICAL MEMBRANES. By Poul Kruhøffer 193–203

THE EFFECT OF DRUGS ON CELL MEMBRANES WITH SPECIAL REFERENCE TO LOCAL ANAESTHETICS. By Jens Chr. Skou 204–217

Research Papers

THE MECHANISM OF THE ANTI-INFLAMMATORY ACTIVITY OF SALICYLATE. By V. Marks, M. J. H. Smith and A. C. Cunliffe.. 218–223

THE PHARMACOGNOSY OF THE ROOT OF *RAUWOLFIA LIGUSTRINA* ROEM. AND SCHULT. By J. E. Adderson, W. C. Evans and G. E. Trease 224–239

A NOTE ON IRRADIATED HEPARIN: SOME BIOLOGICAL AND CHEMICAL PROPERTIES. By S. S. Adams, B. V. Heathcote and P. E. Macey 240–243

A NEW ANALEPTIC: 5,5-DIETHYL-1,3-OXAZIN-2,4-DIONE (DIOXONE) I. TOXICITY AND CONVULSANT ACTIVITY. By G. Maffii, V. M. Dezulian and B. Silvestrini 244–253

Letters to the Editor

HISTAMINE RELEASERS AND HISTAMINE SENSITIVITY. By Paul S. Guth, Joseph W. Byron and Julian L. Ambrus 254–255

CARCINOID TUMOURS AND PINEAPPLES. By D. W. Bruce 256

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 5 **May 1961**

CONTENTS

PAGES

Research Papers

AMINO-ACID DECARBOXYLASE ACTIVITIES IN RAT HEPATOMA. By D. Mackay, J. F. Riley and D. M. Shepherd 257–261

HYDROGENATION OF DIGITALIS GENINS AND ANHYDROGENINS. By B. T. Brown and S. E. Wright 262–267

[*Continued on page ii*

CONTENTS

Research Papers—(*continued*)

PAGE

SOME OBSERVATIONS ON THE CURARISING ACTIVITY OF *Gonioma kamassi*, E. MAY. Preliminary Observations. By J. A. Lock .. 268–278

THE ISOLATION OF CYMARIN AND PERIPLOCYMARIN FROM THE SEEDS OF *Castilloa elastica* CERV. By Miss Gillian R. Adams and S. Wilkinson 279–282

THE CHEMICAL ASSAY OF DIGITALIS. By J. G. Bhatt, H. Brindle and A. D. Macdonald 283–289

A PHARMACOLOGIC STUDY OF SOME NUCLEOSIDES AND NUCLEOTIDES. By Paul V. Buday, C. Jelleff Carr and Tom S. Miya 290–299

PHARMACOGNOSY OF INDIAN SUBSTITUTES OF MALE FERN. I. *Dryopteris ramosa* (HOPE) C. CHR. AND *D. chrysocoma* (*Christ*) C. CHR. By P. N. Mehra and T. C. Mittal 300–308

A NEW ANALEPTIC: 5,5-DIETHYL-1,3-OXAZIN-2,4-DIONE (DIOXONE). II. ANTAGONISM TO SOME C.N.S. DEPRESSANTS. By G. Maffii and M. G. Serralunga 309–317

Letters to the Editor

BANANA AND GASTRIC SECRETION. By R. K. Sanyal, P. K. Das, S. Sinha and Y. K. Sinha 318–319

MODIFICATION OF QUANTITATIVE COLORIMETRIC ESTIMATION OF GLUTETHIMIDE IN TOXICOLOGY. By S. E. Phang, M. C. Dutt and Thng Soon Tee 319–320

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 6 **June 1961**

CONTENTS

Review Article PAGES

LYSOLECITHIN. By N. Robinson, M.Sc., Ph.D., A.R.I.C. .. 321–354

Research Papers

16α,17α-ISOPROPYLIDENEDIOXY-6α-METHYLPREGNA-1,4-DIENE-3,20-DIONE (B.D.H. 3144). A NEW ANTI-INFLAMMATORY AGENT. By C. Bianchi, A. David, B. Ellis, V. Petrow (Mrs.), S. Waddington-Feather and (Miss) E. J. Woodward 355–360

A FURFURAL REAGENT OF HIGH SPECIFICITY FOR THE DETECTION OF CARBAMATES ON PAPER CHROMATOGRAMS. By M. S. Moss and J. V. Jackson 361–364

PHYTOCHEMICAL STUDIES ON THE GENUS *Crotalaria*. PART I. A PHYTOCHEMICAL INVESTIGATION OF THE SEEDS OF *Crotalaria sericea* RETZ. By C. K. Atal and M. L. Sethi 365–369

CHROMATOGRAPHIC SEPARATION OF THE DEGRADATION PRODUCTS OF PRALIDOXIME IODIDE (PYRIDINE-2-ALDOXIME METHIODIDE). By Robert I. Ellin and David E. Easterday 370–373

THE EFFECT OF TRICHLOROACETIC ACID ON THE PAPER CHROMATOGRAPHY OF TISSUE AMINES. By B. Robinson and D. M. Shepherd 374–377

New Apparatus

AN AUTOMATIC SYRINGE FOR USE WITH ISOLATED ORGAN BATHS. By J. A. Lock 378–379

Book Review 380

Letters to the Editor

HYPOGLYCAEMIC AGENTS FROM INDIAN INDIGENOUS PLANTS. By H. D. Brahmachari and K. T. Augusti 381–382

5-HYDROXYTRYPTAMINE IN PINEAPPLES. By J. M. Foy and J. R. Parratt 382–383

THE EFFECT OF GALLAMINE, CARBACHOL, NICOTINE, RYANODINE AND PROTOVERATRINE A AND B UPON FLUX OF CALCIUM-47 IN FROG SKELETAL MUSCLE. By K. Ahmad and J. J. Lewis.. 383–384

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 7 **July, 1961**

CONTENTS

Research Papers PAGES

PHARMACOLOGICAL AND BIOCHEMICAL EFFECTS OF 5-HYDROXYTRYPTAMINE IN ADRENALECTOMISED RATS. By S. Garattini, L. Lamesta, A. Mortari, V. Palma and L. Valzelli 385–388

SPECTROFLUOROMETRIC ANALYSIS OF CARDIOTONIC STEROIDS. By D. Wells, B. Katzung and F. H. Meyers 389–395

THE ANTITUSSIVE ACTIVITY OF GLYCYRRHETINIC ACID AND ITS DERIVATIVES. By Doreen M. Anderson and W. G. Smith .. 396–404

THE EFFECT OF PIPETTING ON THE CONCENTRATION OF HOMOGENEOUS SPORE SUSPENSIONS. Part II. By H. N. Gerrard, M. S. Parker and G. S. Porter 405–411

A NOTE ON THE ANTIDIURETIC EFFECT OF SMALL AMOUNTS OF ISOPRENALINE IN RATS. By J. B. Farmer and Mary F. Lockett 412–415

THE BIOLOGICAL ASSAY OF THYROACTIVE MATERIALS BY THE GOITRE PREVENTION TECHNIQUE. By G. S. Wiberg and N. R. Stephenson 416–421

AFRICAN *RAUWOLFIA* SPECIES. PART I. THE STRUCTURE OF THE ROOT AND STEM OF *RAUWOLFIA VOLKENSII* STAPF. By W. E. Court 422–434

TREATMENT OF POISONING BY ANTICHOLINESTERASE INSECTICIDES IN THE RAT. By D. M. Sanderson 435–442

Book Reviews 443–444

Letters to the Editor

A SENSITIVE METHOD FOR THE ASSAY OF ACETYLCHOLINE. By L. C. Blaber and A. W. Cuthbert 445–446

EFFECT OF HERSAPONIN AND ACORUS OIL ON NORADRENALINE AND 5-HYDROXYTRYPTAMINE CONTENT OF RAT BRAIN. By C. L. Malhotra, K. Prasad, N. S. Dhalla and P. K. Das. 447–448

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.
Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.
Annual Subscription **63s.** Single Copies **7s. 6d.**
17 BLOOMSBURY SQUARE, LONDON, W.C.1
Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 8 **August, 1961**

CONTENTS

Review Article PAGES

RELEASE AND METABOLISM OF THE NEUROHYPOPHYSIAL HORMONES. By S. E. Dicker, M.D., Ph.D., D.Sc. 449–469

Research Papers

STUDIES ON THE KINETICS OF FUNGICIDAL ACTION. PART I. THE EFFECT OF CONCENTRATION AND TIME ON THE VIABILITY OF *Penicillium notatum* SPORES IN SOLUTIONS OF PHENOL. By N. M. Chauhan and V. Walters 470–478

THE EFFECT OF FASTING, COLD STRESS AND ACTH ADMINISTRATION ON THE BLOOD SUGAR AND LIVER GLYCOGEN LEVELS OF NORMAL AND ADRENALECTOMISED RATS AND MICE. By B. P. Block .. 479–484

IPOMOEA—AN ANATOMICAL STUDY. By E. J. Shellard 485–491

THE EXCRETION OF SOME AZO DYES IN RAT BILE. By A. J. Ryan and S. E. Wright 492–495

ABSORPTION STUDIES WITH A SUSTAINED RELEASE TABLET. By John Sjögren and Ivan Östholm 496–503

METHOD FOR THE QUANTITATIVE ESTIMATION OF STRYCHNINE AND BRUCINE IN NUX VOMICA BY PAPER ELECTROPHORESIS. By D. K. Bardhan and Diptish Chakravarty 504–506

Pharmacopoeias and Formularies

THE DISPENSATORY OF THE UNITED STATES OF AMERICA. 1960 EDITION. VOLUME 2. NEW DRUG DEVELOPMENTS 507–508

Letters to the Editor

AN INTERFERING GLYCOSIDE IN THE CHEMICAL ASSAY OF SENNA. By C. Vickers 509

ABSENCE OF SPASM IN SENSITIVE ASSAY FOR ACETYLCHOLINE. By A. T. Birmingham 510

THE INCORPORATION OF ^{14}C FROM $NaH^{14}CO_3$ INTO THE SOLUBLE METABOLIC INTERMEDIATES OF ANIMAL TISSUES. By C. Bryant, A. K. Huggins and M. J. H. Smith 511–512

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 9 **September, 1961**

CONTENTS

Research Papers PAGES

PIRINITRAMIDE (R 3365), A POTENT ANALGESIC WITH UNUSUAL CHEMICAL STRUCTURE. By Paul A. J. Janssen 513–530

PAPER CHROMATOGRAPHIC SEPARATION OF THE PHLOROGLUCINOL DERIVATIVES FROM *DRYOPTERIS* SPECIES. By Aneri Penttilä and Jacobus Sundman 531–535

[*Continued on page ii*

CONTENTS

Research Papers—(*continued*)

PAGES

THE ANALGESIC ACTION OF CHLORMEZANONE IN MICE. By Mary F. Lockett and U. G. PATEL 536–542

NEUROMUSCULAR BLOCKING AGENTS. PART VIII. LINEAR BIS- AND TRIS-ONIUM ETHERS. By J. J. Lewis, D. E. McPhail, T. C. Muir and J. B. Stenlake 543–547

PHARMACOLOGICAL AND BIOCHEMICAL EFFECTS OF SOME RESERPINE DERIVATIVES. By S. Garattini, L. Lamesta, A. Mortari and L. Valzelli 548–553

THE ANTIBACTERIAL ACTIVITY OF NEW DERIVATIVES OF 4-AMINOQUINOLINE AND 4-AMINOQUINALDINE. By D. Caldwell, W. A. Cox, P. F. D'Arcy and L. R. Rowe 554–564

THE OXYTOCIC ACTIVITY OF DL-5-HYDROXYTRYPTOPHAN. By Ž. Lovašen, Z. Supek and M. Randič. 565–566

HYPOGLYCAEMIC AGENTS. PART I. By B. G. Boggiano, V. Petrow, O. Stephenson and A. M. Wild 567–574

Book Review 575–376

NOTICE TO CONTRIBUTORS

GENERAL. Original research papers or review articles are accepted on the understanding that the material has not been previously published. The title page should bear the name of the contributor and the name and address of the laboratory where the work was done.

PREPARATION OF TEXT. Authors should consult a current issue of the Journal and conform to the typographical conventions, use of headings, lay-out of tables, and citation of references. Texts must be typewritten in double spacing on quarto or foolscap sheets with a one-and-a-half inch margin. The top copy and one carbon copy should be sent. The following style should be used: Summary, introduction, experimental methods, results, discussion, acknowledgements, references. The summary should be brief but self-explanatory, giving results and conclusions described in the paper, and should be in the form of an abstract suitable for use as such by abstracting journals.

REFERENCES—CHANGE IN STYLE

References should be arranged according to the HARVARD system. In the text the surname of the author(s) and the date of publication are given thus:

Beleslin and Varagić (1958) described . . . or

. . . has been described (Beleslin and Varagić, 1958).

At the end of the paper the references are arranged in alphabetical order of the first author of each paper, and should include each author's name and initials, year of publication in parenthesis, title of publication (underlined) abbreviated as in the *World List of Scientific Periodicals*, the volume number, and the first and last page number of the paper, e.g.

Beleslin, D. and Varagić, V. (1958). *Brit. J. Pharmacol.*, **13**, 321-325.

References to books should follow the pattern: name of author(s), year of publication, title (underlined), editor's name or number of edition, place of publication, and publisher, e.g.

Goodman, L. S. and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 213, New York: Macmillan.

TABLES (for each copy of the text) should be typed on separate sheets, their headings should describe their content and they should be understandable without reference to the text.

ILLUSTRATIONS. Illustrations, which need not be duplicated, are usually limited to 6 in number. They will normally appear in print between 2 in. and 4 in. in width (base line). Line illustrations such as graphs or apparatus should be clearly and boldly drawn at least twice the size of the reproduction required, in black waterproof ink on white paper, Bristol Board, faintly blue-lined paper or blue tracing cloth, at a thickness which will produce a suitable line when the scale is reduced. Kymograph records and photographs should be selected to allow for similar reductions. Lettering and numbering should be reduced to the minimum and inserted lightly and clearly in pencil. Curves based on experimental data should carry clear and bold indications of the experimentally determined points, which should be marked by using, preferably, circles, crosses, triangle or squares. Legends, descriptions or captions for all figures should be typed on separate sheets of paper and fastened to the typescript of the paper or attached to the individual figures. The author's name, the title of the paper and the number of the figure should be written on the back of each illustration. The approximate position of each illustration should be marked in the text. All illustrations should be understandable without reference to the text.

SYMBOLS AND ABBREVIATIONS. Authors should refer to the current number of the Journal for information. The attention of authors is drawn particularly to the following symbols: m = (milli) = 10^{-3} and μ = (micro) = 10^{-6}; ml. (millilitres) should always be used and not c.c.; μg. (micrograms) and not γ; per cent and not %.

NOMENCLATURE OF PLANTS, ANIMALS AND MICRO-ORGANISMS. Binominal Latin names, the generic names only with an initial capital letter, should be used in accordance with international usage. They should be underlined in the text. The first mention should be in full and the subsequent references abbreviated, but ambiguities should be avoided.

REPRINTS. 50 reprints are supplied free of charge to the author. A further 10 reprints are supplied free to each co-author. Additional reprints may be purchased.

COPYRIGHT. Contributions are accepted for publication on the condition that an exclusive licence to publish in any periodical form is granted by the contributor, in so far as he can grant tne same, to the *Journal of Pharmacy and Pharmacology*.

The proper abbreviation for this Journal is *J. Pharm. Pharmacol.*

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 10 **October, 1961**

CONTENTS

Review Article PAGES

INFLAMMATION AND THE INFLAMMATORY MECHANISMS. By Professor N. Jancsó, M.D., M.Hungarian Acad. Sci. 577–594

Research Papers

EFFECTS OF SALICYLATE ADMINISTRATION ON REPUTED INDICES OF ADRENAL CORTICAL ACTIVITY IN THE RAT. By G. F. Blane and K. A. Munday 595–600

ANTITRICHOMONAL AGENTS. 5-NITROTHIAZOLES, 5-NITROPYRIDINES AND 5-NITROPYRIMIDINES. By R. M. Michaels and R. E. Strube 601–609

ANTITRICHOMONAL AGENTS. TRIAZEN DERIVATIVES. By R. E. Strube and R. M. Michaels 610–612

THE BREAKDOWN OF ATROPINE BY BACTERIA. By W. Kedzia, J. Lewon and T. Wiśniewski 613–616

THE MECHANISM OF THE LOCAL INFLAMMATORY REACTION INDUCED BY COMPOUND 48/80 AND DEXTRAN IN RATS. By A. Jori, A. P. Bentivoglio and S. Garattini 617–619

THE STABILITY OF ANTIBACTERIALS IN POLYETHYLENE GLYCOL MIXTURES. By L. V. Coates, Melanie M. Pashley and K. Tattersall 620–624

A COMPARISON OF ACTIVITY OF QUININE AND QUINIDINE ON THE ISOLATED ELECTRICALLY STIMULATED RAT VENTRICLE STRIP. By L. Molinengo and G. Segre 625–630

A NOTE ON THE INFLUENCE OF A METABOLITE OF ADRENALINE ON WATER DIURESIS IN RATS. By D. Roberts and Mary F. Lockett 631–633

Pharmacopoeias and Formularies

THE EXTRA PHARMACOPOEIA SUPPLEMENT, 1961. Reviewed by Edward G. Feldmann 634–635

Letters to the Editor

ON THE CHEMICAL STRUCTURE OF LYSOLECITHINS. By A. Petter, W. Schlemmer and K. Zipf 636

PEROXIDE-SULPHURIC ACID TEST AS AN INDICATION OF THE RIPENESS AND PHYSIOLOGICAL ACTIVITY OF CANNABIS RESIN. By Lj Grlić 637–638

NEW GLYCOSIDES FROM SENNA. By J. K. Crellin, J. W. Fairbairn, C. A. Friedman and H. A. Ryan 639–640

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 11 **November, 1961**

CONTENTS

Research Papers PAGES

SOME PHARMACOLOGICAL PROPERTIES OF A NEW INTRAMUSCULAR IRON PREPARATION. By P. O. Svärd 641–649

MECHANISM OF ABSORPTION OF TWO INTRAMUSCULAR IRON PREPARATIONS. By P. O. Svärd and S. Lindvall 650–653

MULTIPLE EFFECTS OF γ-RESORCYLIC ACID ON INTERMEDIARY METABOLISM. By A. K. Huggins, C. Bryant and M. J. H. Smith 654–662

MICELLE FORMATION BY LECITHIN IN SOME ALIPHATIC ALCOHOLS. By P. H. Elworthy and D. S. McIntosh 663–669

THE PATTERN, SENSITIVITY AND PRECISION OF THE RESPONSE TO INSULIN IN RANDOM BRED, INBRED AND HYBRID STRAINS OF MICE. By Annie M. Brown 670–678

SLEEPING TIME RESPONSES OF MICE—RANDOM BRED, INBRED AND F_1 HYBRIDS—TO PENTOBARBITONE SODIUM. By Annie M. Brown .. 679–687

THE EFFECT OF TEMPERATURE ON THE ACTION OF PENICILLIN ON *ESCHERICHIA COLI*. By W. B. Hugo and A. D. Russell 688–693

SOME 1-ALKYL-3-AROYL UREAS. By (Miss) Y. M. Beasley, V. Petrow and O. Stephenson 694–697

A NOTE ON THE ANTIBACTERIAL ACTION OF SOME HALOGEN SUBSTITUTED CHALKONES. By S. Ambekar, S. S. Vernekar, S. Acharya and S. Rajagopal 698–699

Book Review 700

Letters to the Editor

AMPHETAMINE TOXICITY IN AGGREGATED MICE. By Beryl M. Askew 701–703

NEUROMUSCULAR BLOCKING ACTION OF SUCCINYLDICHOLINE STEREOISOMERS. By E. Lesser 703–704

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor: J. R. Fowler, B.Pharm., F.P.S.

Annual Subscription **63s.** Single Copies **7s. 6d.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Telegrams: Pharmakon, Westcent, London. *Telephone:* HOLborn 8967

Vol. XIII No. 12 **December, 1961**

CONTENTS

Review Article PAGES

THE MODE OF ACTION OF PENICILLIN. By W. B. Hugo, B.Pharm., Ph.D., F.P.S. and A. D. Russell, B.Pharm., Ph.D., M.P.S. .. 705–722

Research Papers

POLYMORPHISM OF CORTISONE ACETATE. By R. K. Callow and Olga Kennard 723–733

[*Continued on page ii*

Research Papers—*continued*

ANALGESIC ACTION OF CHLORPROMAZINE AND RESERPINE IN RELATION TO THAT OF MORPHINE. By J. Garcia Leme and M. Rocha e Silva 734–742

A NOTE ON AN EFFECT OF PYROGALLOL ON DUODENAL MOTILITY. By Ivan Izquierdo and Juan A. Izquierdo 743–746

THE IDENTIFICATION AND DETERMINATION OF SOME PHENOLIC ACIDS IN URINE USING TWO-DIMENSIONAL PAPER CHROMATOGRAPHY. By S. L. Tompsett 747–751

THE NEUROMUSCULAR BLOCKING ACTION OF TETRAETHYLAMMONIUM. By J. Thomas and G. A. Starmer 752–758

THE ISOLATION AND IDENTIFICATION OF 1-ETHINYLCYCLOHEXYL CARBAMATE AND ITS METABOLITE FROM TOXICOLOGICAL SPECIMENS. By Lee Kum-Tatt 759–765

Book Review 766

Letter to the Editor

PAPER AND GAS CHROMATOGRAPHIC ANALYSIS OF CANNABIS. By C. G. Farmilo and T. W. McConnell Davis 767–768

JOURNAL OF PHARMACY AND PHARMACOLOGY

TRANSACTIONS OF THE

BRITISH PHARMACEUTICAL CONFERENCE

EDITORS: D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C.
D. TRAIN, M.C., Ph.D. F.P.S., F.R.I.C., M.I.Chem.E.
PRESS EDITOR: J. R. FOWLER, B.Pharm., F.P.S.
17 BLOOMSBURY SQUARE, LONDON, W.C.1.

Vol. XIII Supplement **December 1961**

CONTENTS

PAGES

Report of Proceedings .. .. 1 *T*–8 *T*

Chairman's Address

ANALYSIS ANALYSED. By D. C. Garratt .. .. 9 *T*–22 *T*

Symposium on Skin Medication

TOPICAL MEDICATION IN RELATION TO SKIN PHYSIOLOGY. By F. J. Ebling, M.Sc., Ph.D. .. .. 23 *T*–34 *T*

SKIN MEDICATION. By A. Jarrett, M.B., F.R.C.P. .. .. 35 *T*–42 *T*

PHARMACEUTICAL FORMULATION IN SKIN MEDICATION. By J. W. Hadgraft, F.P.S., F.R.I.C. .. .. 43 *T*–56 *T*

Conference Lecture

INTERFERON: A ROUND UNVARNISH'D TALE. By Alick Isaacs, M.D. .. .. 57 *T*–61 *T*

Science Papers

THE STABILITY OF SOLUTIONS OF THE INTERNATIONAL PYROGEN REFERENCE PREPARATION. By C. H. R. Palmer and T. D. Whittet .. .. 62 *T*–66 *T*

THE REMOVAL OF AIR DURING AUTOCLAVE STERILISATION OF FABRICS USING LOW PRESSURE STEAM. By G. R. Wilkinson and F. G. Peacock .. .. 67 *T*–71 *T*

IMPROVEMENT OF HEATING OF BOTTLED FLUIDS DURING AUTOCLAVE STERILISATION USING LOW PRESSURE STEAM. By G. R. Wilkinson and F. G. Peacock .. .. 72 *T*–74 *T*

A SLOPE-RATIO DESIGN FOR ACCELERATED STORAGE TESTS. By J. P. R. Toothill .. .. 75 *T*–86 *T*

ETHYLENE OXIDE STERILISATION—SOME EXPERIENCES AND SOME PRACTICAL LIMITATIONS. By A. Royce and C. Bowler .. 87 *T*–96 *T*

THE PREPARATION AND *in Vitro* EVALUATION OF SOME MODIFIED ALUMINIUM HYDROXIDES AS GASTRIC ANTACIDS. By B. K. Davison and R. E. Schaffer .. .. 97 *T*–102 *T*

CONTENTS

Science Papers—(*continued*) PAGES

NEUROMUSCULAR BLOCKING AGENTS. PART IX. SOME SHORT-ACTING LINEAR *NNN*-TRIS-ONIUM ESTERS. By Fiona MacLeod Carey, J. J. Lewis, J. B. Stenlake and W. D. Williams .. .. 103 *T*–106 *T*

THE ANTI-INFLAMMATORY AND ANTIDIURETIC ACTIONS OF FRACTIONS OBTAINED FROM XANTHOGLABROL. By R. Best and R. S. H. Finney .. .. 107 *T*–110 *T*

THE DETERMINATION OF HEXACHLOROPHANE AND OTHER PHENOLS IN PHARMACEUTICAL PREPARATIONS BY A Δ E SPECTROPHOTOMETRIC METHOD. By D. A. Elvidge and B. Peutrell .. 111 *T*–116 *T*

THE SPECTROPHOTOMETRIC DETERMINATION OF THALIDOMIDE IN BODY FLUIDS. By J. N. Green and B. C. Benson .. .. 117 *T*–121 *T*

THE EFFECT OF A SULPHATED POLYSACCHARIDE UPON THE DIFFUSION OF PEPSIN THROUGH MUCIN. By W. Anderson .. 122 *T*–125 *T*

ION-EXCHANGE CHROMATOGRAPHY ON ALIGINIC ACID OF CERTAIN B-GROUP VITAMINS. By J. S. Foster and J. W. Murfin .. 126 *T*–132 *T*

ON THE STANDARDISATION OF THYROID B.P. By C. A. Johnson and K. L. Smith .. .. 133 *T*–135 *T*

COMPARISON OF BIOLOGICAL AND CHEMICAL ASSAY OF THYROID. By F. W. Webb .. .. 136 *T*–143 *T*

THE STRENGTH OF COMPRESSED TABLETS. III. THE RELATION OF PARTICLE SIZE, BONDING AND CAPPING IN TABLETS OF SODIUM CHLORIDE, ASPIRIN AND HEXAMINE. By E. Shotton and D. Ganderton .. .. 144 *T*–152 *T*

A NEW METHOD FOR THE DETERMINATION OF BARBITURATES. By I. M. Roushdi, H. Abdine and A. Ayad .. .. .. 153 *T*–155 *T*

THE FLASK COMBUSTION TECHNIQUE IN PHARMACEUTICAL ANALYSIS: MERCURY-CONTAINING SUBSTANCES. By C. Vickers and J. V. Wilkinson .. .. 156 *T*–160 *T*

4-ALKOXYPIPERIDINES RELATED TO REVERSED ESTERS OF PETHIDINE. By A. F. Casy and A. H. Beckett .. .. 161 *T*–163 *T*

A SPECIFIC SOLVENT-EXTRACTION METHOD FOR THE DETERMINATION OF TRACE QUANTITIES OF FLUORIDE. By C. A. Johnson and M. A. Leonard .. .. 164 *T*–168 *T*

ENTRAINMENT AND FLOODING IN VERTICAL STILL-HEADS. By A. J. Evans, E. Shotton and D. Train .. .. .. 169 *T*–182 *T*

STUDIES ON BACTERIAL POPULATIONS IN SOLUTIONS OF PHENOLS. PART II. THE INFLUENCE OF CELL-EXUDATE UPON THE SHAPE OF THE SURVIVOR-TIME CURVE. By H. S. Bean and V. Walters 183 *T*–194 *T*

THE ASSAY OF ENZYME ACTIVITY BY THE PLATE-DIFFUSION TECHNIQUE. By D. V. Carter and G. Sykes .. .. .. 195 *T*–199 *T*

THE INFLUENCE OF THE VARIATIONS IN SOLUBILISING PROPERTIES OF POLYSORBATE 80 ON THE VITAMIN A PALMITATE: POLYSORBATE 80: GLYCEROL: WATER SYSTEM. By P. F. G. Boon, C. L. J. Coles and M. Tait .. .. 200 *T*–204 *T*

PHASE EQUILIBRIA IN SYSTEMS CONTAINING COMMERCIAL NON-IONIC SURFACE-ACTIVE AGENTS AND BENZENE. By B. A. Mulley 205 *T*–206 *T*

SOME OBSERVATIONS ON THE FLOW PROPERTIES OF BARIUM SULPHATE SUSPENSIONS. By J. E. Carless and N. C. Chenoy 207 *T*–212 *T*

RESEARCH PAPERS

THE ANTI-ANAPHYLACTIC ACTIVITY OF ETHANOLAMINE AND CHOLINE

By W. G. Smith

From the Research Laboratory in Biochemical Pharmacology, Department of Pharmacy, Sunderland Technical College

Received October 11, 1960

Ethanolamine, *N*-methylethanolamine, *N*-dimethylethanolamine and choline have anti-anaphylactic activity in actively sensitised guinea-pigs. Administered alone their activity is slight, but they considerably potentiate the limited protection afforded by mepyramine. Ethanolamine has been shown to inhibit the release of the slow reacting substance of anaphylaxis in guinea-pig lung subjected to anaphylaxis *in vitro*.

Various authors have described an anti-allergic factor in peanut oil, lecithin fractions obtained from soya beans, and egg yolk (Coburn and Moore, 1943; Coburn, Graham and Haninger, 1954; Long and Martin, 1956). This factor has been identified as *N*-2-hydroxyethyl-palmitamide (Kuehl, Jacob, Ganley, Ormond and Meisinger, 1957) and shown to suppress passive joint anaphylaxis in the guinea-pig (Ganley, Grassle and Robinson, 1958). These last authors also stated that ethanolamine, *N*-methylethanolamine, *N*-dimethylethanolamine and choline were active in the same test. It has since been reported that *N*-2-hydroxyethyl-palmitamide and ethanolamine hydrochloride suppress passive anaphylaxis in the mouse (Ganley and Robinson, 1959) and that ethanolamine, *N*-dimethylethanolamine and choline diminish anaphylactoid oedema induced in rats (Cronheim and Toekes, 1959).

Ethanolamine and its *N*-methyl derivatives became the subject of anti-anaphylactic studies in this laboratory when it was noted that ethanolamine and choline salts of glycyrrhetinic acid had greater activity against Arthus reactions in the guinea-pig than that reported for the disodium salt of glycyrrhetinic acid hydrogen succinate by Brown, Christie, Colin Jones, Finney, MacGregor, Morrison Smith, Smith, Sullivan, Tarnoky, Turner, Watkinson and Wotton (1959). In the present study, ethanolamine and its *N*-methyl derivatives were examined as antagonists of anaphylaxis induced in actively sensitised guinea-pigs. In this species, both histamine and the slow reacting substance of anaphylaxis (SRS-A) are liberated from sensitised lung by antigen (Brocklehurst, 1953; 1955; 1956; 1960). The experiments were designed to show inhibition of the effects of histamine and SRS-A and also changes in the amounts of these substances released during anaphylaxis.

METHODS

Anaphylactic Shock in vivo

Guinea-pigs of either sex weighing 200 to 250 g. were obtained from Mr. Donald Harrodine of March, Cambs, and sensitised to commercial

egg albumin (G. T. Gurr, London) by the subcutaneous injection of 100 mg. in 1 ml. normal saline. They were fed on Diet 18 pellets (Oxo Ltd.) and received 50 mg. of ascorbic acid each morning in drinking water contained in amber glass bottles. Overnight the animals were given tap water.

Three weeks after the sensitising dose of antigen, the animals were subjected to anaphylactic shock by the technique developed by Herxheimer (1952). Each animal was placed in a 10 in. diameter glass vessel and exposed to an aerosol of antigen. The aerosol was produced by applying air at a pressure of 5 lb./sq. in. to a Riddostat inhaler (Riddell Products Ltd., London) containing a 5 per cent w/v solution of egg albumin in distilled water. The animal was removed from the aerosol at a point immediately before the onset of convulsions. This point was usually characterised by a powerful convulsive expiration and contraction of the abdominal muscles. The time in seconds required to reach this point was termed the "collapse time" and assumed to be similar to the "pre-convulsion time" described by Herxheimer (1952). Confirmation that shock had occurred was obtained by listening for coughs and examining the retinae for signs of central cyanosis. If the shocked animal was not removed from the aerosol at its collapse time, it was likely to die almost immediately. After the collapse time had been measured, a mixture of 95 per cent oxygen and 5 per cent carbon dioxide was applied to severely distressed animals. In this way, animals were saved which otherwise might have died, and overestimation of the collapse time avoided.

After the first exposure to antigen, more antibody is formed in the animal and this newly-formed antibody can be removed by a second exposure to antigen. Hence, the amount of antibody formed, the severity of the shock, and magnitude of the collapse time all depend on the interval between successive exposures. In the present experiments, the time interval for each animal which gave approximately the same collapse time for every re-exposure to antigen was 7 days. The collapse time obtained under these conditions was designated the "normal collapse time".

Increase in the collapse time following treatment with a drug indicates a protective effect. In measuring the protective effect of a number of antihistamine drugs, Armitage, Herxheimer and Rosa (1952) compared a preconvulsion time obtained on the day of treatment with the mean of two preconvulsion times obtained for exposures to antigen made several days before and on several days after drug treatment. The interval between these successive antigen exposures was usually 3 or 5 days. A percentage protection was calculated according to the formula: per cent protection $= 100\ (1 - C/T)$, C = mean of two normal preconvulsion times, and T = preconvulsion time observed on day of treatment. When results were calculated in this way, 100 indicated complete protection (T = 00), and 50 indicated tolerance of twice the normal amount of antigen (T = 2C). In these experiments protection has been expressed as T/C, which is referred to as the "protection ratio", and corresponds approximately to the number of lethal doses of antigen which a protected animal can tolerate.

Anaphylactic Shock in vitro

Anaphylactic shock was induced in intact guinea-pig lungs undergoing perfusion through the pulmonary artery with Tyrode solution at 37° as described by Brocklehurst (1960). The perfusate was collected for 30 minutes after antigen administration, centrifuged to remove blood cells, and then examined for histamine and SRS-A.

Estimation of Histamine and SRS-A in Perfusates From Anaphylactic Guinea-pig Lung

These were performed on guinea-pig ileum suspended in 2 ml. of aerated Tyrode solution at 37° as described by Brocklehurst (1960).

Results of *in vivo* Experiments

Normal Sensitivity of the Animals

A group of nine animals was exposed to antigen at weekly intervals for 3 weeks. The mean of the last two of these three exposures for each animal in the group is given as the normal collapse time in Table I. This

TABLE I

THE SENSITIVITY TO ANTIGEN (EXPRESSED AS A PROTECTION RATIO) OF A GROUP OF NINE GUINEA-PIGS EXPOSED TO AEROSOLISED ANTIGEN AT WEEKLY INTERVALS

Animal No.	Normal collapse time in sec.	Protection ratio									
1	100	1·22	1·76	0·99	1·05	4·00	1·06	2·35	1·23	1·12	0·85
2	130	1·27	0·83	0·89	0·96	0·65	1·29	0·73	0·57	0·59	0·49
3	89	1·27	1·07	1·04	0·90	0·76	0·83	0·90	0·94	0·97	0·65
4	90	0·88	0·73	1·21	0·99	0·35	1·14	1·11	1·77	2·56	1·83
5	103	1·20	0·89	1·02	1·04	0·91	1·40	0·91	0·97	1·43	1·39
6	107	0·88	0·67	0·75	0·75	0·44	1·34	0·62	0·51	0·94	0·60
7	83	0·95	0·97	1·16	0·91	1·23	1·37	1·58	1·19	1·53	1·51
8	110	1·21	0·55	0·70	0·55	0·58	0·51	0·83	0·54	0·68	0·81
9	143	1·07	0·91	1·01	0·92	0·68	0·98	0·88	0·94	1·50	1·17
Mean		1·11	0·93	0·97	0·89	1·07	1·10	1·10	0·98	1·25	1·03
S.D.		0·03	0·12	0·03	0·02	1·28	0·09	0·28	0·16	0·36	0·22

S.D. = Standard deviation

Table shows the results of exposing these animals to antigen at weekly intervals for ten consecutive weeks. Their tolerance to antigen is expressed as a protection ratio calculated from their normal collapse times. It can be seen that in any one week the mean protection ratio for the whole group does not vary outside the limits 0·89 $\pm$0·02 to 1·25 $\pm$ 0·36. The week to week variation of any individual animal is, however, larger.

The Protection Afforded by Antihistamines

The results obtained by Armitage, Herxheimer and Rosa (1952) show that antihistamines afford a definite but limited protection against aerosol induced anaphylactic shock. In fact, none of the antihistamines which they investigated gave a protection substantially greater than that represented by a protection ratio of 4. This was verified in a group of nine animals exposed to antigen at weekly intervals in which the protection

afforded by various doses of mepyramine, administered intramuscularly as the maleate 1 hr. before antigen exposure, was examined on alternate weeks. The results are shown in Fig. 1. It can be seen that doses in the range 1 to 3 mg./kg. afford a maximum protection. The value of the

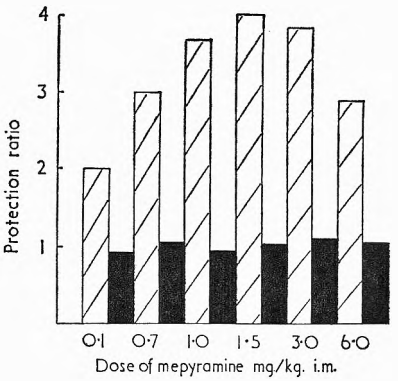


FIG. 1. The protective effects of various doses of mepyramine determined in a group of 9 animals exposed to aerosolised antigen at weekly intervals. Shaded columns show the effects of mepyramine. The figures indicate the dose in mg./kg. mepyramine base administered as mepyramine maleate intramuscularly 1 hr. before antigen exposure. Solid columns indicate the effect of exposing the animals without pretreatment on alternate weeks.

protection ratio is close to 4. Lower or higher doses afford less protection. These findings agree with those of Armitage and others (1952) for mepyramine maleate.

The Constancy of Antihistamine Protection

A further group of nine animals was exposed weekly to antigen for eleven consecutive weeks. On alternate weeks the protection afforded

TABLE II

THE PROTECTIVE EFFECT OF MEPYRAMINE (EXPRESSED AS A PROTECTION RATIO) IN A GROUP OF NINE GUINEA-PIGS EXPOSED TO AEROSOLISED ANTIGEN AT WEEKLY INTERVALS

Animal No.	Normal collapse time in sec.	Protection ratio										
		N	T	N	T	N	T	N	T	N	T	N
41	130	1·12	8·78	1·22	8·02	1·22	3·96	1·14	5·22	1·16	3·83	1·11
42	90	1·05	7·23	1·05	2·73	1·05	3·20	0·97	4·03	0·95	4·04	0·99
43	180	1·04	8·19	0·96	3·93	0·99	4·43	1·00	3·69	1·17	5·10	1·21
44	124	1·10	5·09	1·14	3·88	1·19	4·00	1·19	4·03	1·21	4·05	1·24
45	130	1·12	6·12	0·91	6·62	1·00	4·70	1·03	4·25	1·08	4·00	1·10
46	90	1·02	3·73	1·09	4·07	1·09	3·83	1·22	6·51	1·38	4·11	1·38
47	94	0·93	3·63	1·11	4·68	1·17	7·75	1·18	3·95	1·25	4·00	1·29
48	88	1·05	4·04	1·03	3·96	1·09	4·02	1·06	4·20	1·05	4·17	1·15
49	90	0·98	3·81	1·05	4·02	1·06	4·18	1·06	4·29	1·11	3·92	1·14
Mean		1·04	5·62	1·06	4·66	1·09	4·45	1·09	4·46	1·15	4·14	1·19
S.D.		0·004	4·13	0·008	2·59	0·007	1·70	0·008	0·77	0·015	0·14	0·013

S.D. = Standard deviation

N = exposure without drug pre-treatment. T = exposure after 1 mg./kg mepyramine base administered as mepyramine maleate intramuscularly one hr. before exposure to antigen.

by 1 mg./kg. of mepyramine was examined. The results are given in Table II. For the whole group of nine animals the mean protection ratio calculated for that dose of mepyramine ranged from 4·14 $\pm$ 0·14 to 5·62 $\pm$ 4·13.

The Activity of the Ethanolamine-choline Series

The limited protection afforded by mepyramine to guinea-pigs undergoing anaphylactic shock under the conditions of these experiments might be due to the inability of mepyramine to protect animals from bronchoconstriction induced by SRS-A. It was, therefore, decided to investigate

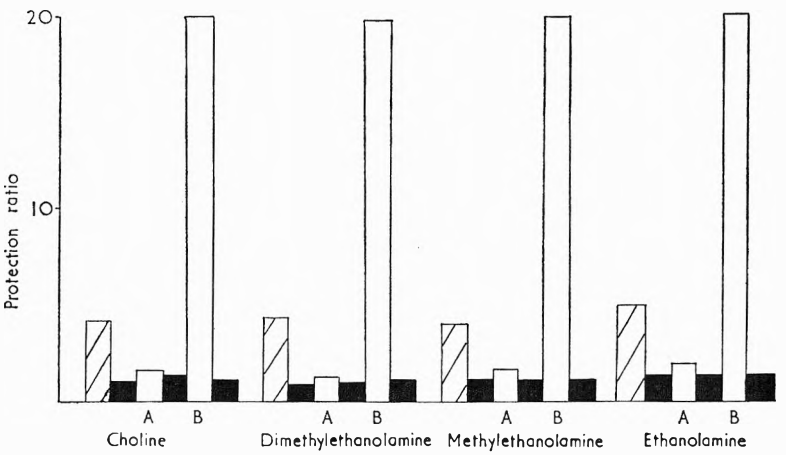


FIG. 2. The protective effects of choline, dimethylethanolamine, methyl ethanolamine and ethanolamine in groups of 9 guinea-pigs exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. mepyramine base. The open columns show the effect of test material. A shows the effect of test material administered alone at 20 mg./kg. B shows the effect of test material at 20 mg./kg. administered simultaneously with 1 mg./kg. mepyramine base. The solid columns show the effect of exposing the animals without pretreatment on alternate weeks. All substances were administered intramuscularly one hr. before exposure to antigen. Mepyramine was administered as the maleate and other materials as hydrochlorides.

the ethanolamine-choline series both alone and simultaneously with a maximum protection dose of mepyramine, in the expectation that an SRS-A antagonist would potentiate the limited protection afforded by mepyramine alone. The results of the first experiments are shown in Fig. 2.

Protection ratios up to a value of 20 were determined. No attempt was made to estimate protection ratios of a higher order because of the time involved in making the observations in groups of nine animals. For an animal having a normal collapse time of 3 min., a protection ratio of 20 indicates tolerance to antigen of 1 hr. None of the four substances had much activity when given alone, but all of them potentiated the action of mepyramine. In a further experiment using promethazine as the antihistamine a similar result was obtained. Peak antihistamine

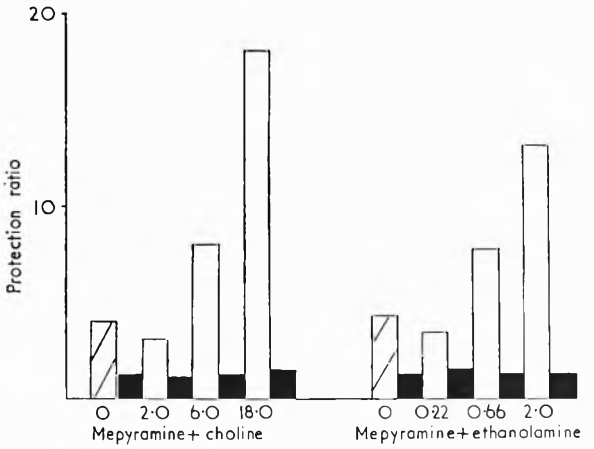


FIG. 3. The effect of various doses of ethanolamine and choline when administered simultaneously with mepyramine to groups of 9 guinea-pigs exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. of mepyramine. The open columns show the effect of administering test material simultaneously with 1 mg./kg. of mepyramine. Figures indicate dosage of test material in mg./kg. Solid columns show the effect of exposing the animals to pretreatment on alternate weeks. All substances were administered intramuscularly 1 hr. before exposure to antigen. Mepyramine was administered as the maleate, ethanolamine as the hydrochloride and choline as the chloride.

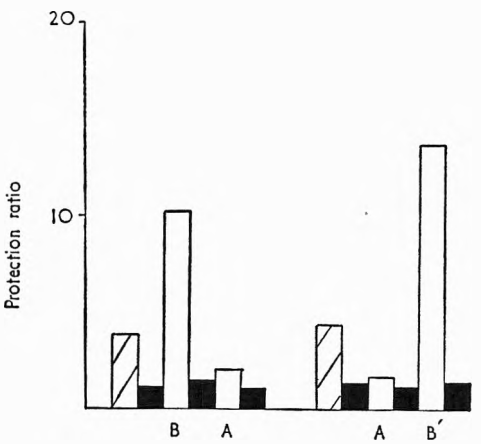


FIG. 4. The effect of orally administered ethanolamine on two groups of 9 animals exposed to aerosolised antigen at weekly intervals. The shaded columns show the effect of 1 mg./kg. mepyramine given intramuscularly. The open columns show the effects of ethanolamine, A shows the effect of administering 500 mg./kg. of ethanolamine orally. B is the effect of 1 mg./kg. mepyramine administered intramuscularly at the same time as 500 mg./kg. of ethanolamine orally. All drugs were administered 1 hr. before exposure to antigen. The solid columns record the effects of exposing the animals to antigen without pretreatment on alternate weeks.

activity observed with a group of nine animals corresponded to a protection ratio of 4·00 ± 0·21 and was achieved with 0·25 mg./kg. promethazine, administered as the hydrochloride 1 hr. before exposure to antigen. Protection ratios of 20 were observed after the simultaneous administration of promethazine 0·25 mg./kg. and ethanolamine 20 mg./kg.

Using two groups of nine animals a quantitative comparison was made of the ability of ethanolamine and choline to potentiate mepyramine. The results are shown in Fig. 3. Ethanolamine had between six and nine times the activity of choline.

The activity of ethanolamine after oral administration was then examined. The results are shown in Fig. 4 from which it can be concluded that orally administered ethanolamine potentiates the effect of intramuscularly administered mepyramine.

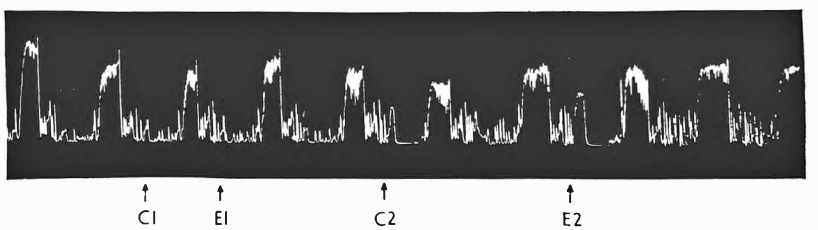


FIG. 5. The effect of ethanolamine and choline on the isolated guinea-pig ileum at 37° in a bath of 2 ml. capacity containing Tyrode with mepyramine and atropine each at a concentration of 10^{-6} g./ml. Unlabelled contractions were produced by SRS-A in Tyrode perfusate from sensitised guinea-pig lung shocked *in vitro*. C1 = Choline 100 μg./ml. E1 = Ethanolamine 100 μg./ml. C2 = Choline 1 mg./ml. E2 = Ethanolamine 1 mg./ml. Choline and ethanolamine were administered as chloride and hydrochloride respectively and left in contact with the tissue for 30 sec. They were removed from the bath before eliciting the next contraction. Spasmogen contact time = 2 min. Dose interval = 5 min.

RESULTS OF *in vitro* EXPERIMENTS

Pharmacological Antagonism of Histamine and SRS-A

The results of the above experiments suggested that ethanolamine had pharmacological actions antagonistic to SRS-A rather than histamine. Tests using the isolated guinea-pig ileum showed that neither ethanolamine nor choline in both concentrations up to 1 mg./ml. antagonised histamine. Fig. 5 shows that neither substance antagonised SRS-A.

Inhibition of SRS-A Release During Anaphylaxis

It seemed possible that ethanolamine inhibited histamine or SRS-A release or both during anaphylaxis. Experiments were made using lungs from groups of four sensitised animals which were perfused and shocked *in vitro*. Comparisons were made between the histamine and SRS-A content of perfusates derived from shocked but otherwise untreated animals (controls) and shocked animals previously treated with ethanolamine. In all, four such experiments were made using a total of 32 animals.

Each experiment with eight animals (four controls) was made in a single day. The histamine and SRS-A assays were made with freshly prepared perfusate, since SRS-A is not stable. The time factor thus precluded the use of more elaborate experimental designs for the assay procedures. The results of one typical experiment are given in Figs. 6 and 7.

Fig. 6 compares the histamine equivalent of perfusate P4 from a control animal with perfusate Q4 from an animal which had received three daily doses of 200 mg./kg. ethanolamine (given intramuscularly as the hydrochloride) on the days immediately preceding anaphylactic shock. Perfusate P4 in a 1 in 200 dilution and Perfusate Q4 in a 1 in 200 dilution gave responses approximately equivalent to 0·002 μg. histamine per ml. (H) indicating that pretreatment with ethanolamine did not inhibit histamine release during subsequent anaphylaxis.

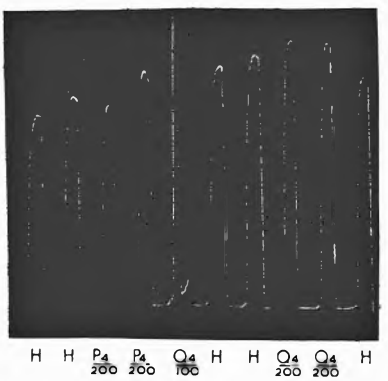


FIG. 6. An approximate estimate of the histamine content of perfusates P4 and Q4. Guinea-pig ileum. 37°. Tyrode. 2 ml. bath. H = Histamine 0·001 μg./ml. P4/200 = Perfusate P4 at a 1 in 200 dilution. Q4/200 = Q4 at a 1 in 200 dilution. Q4/100 = Perfusate Q4 in a 1 in 100 dilution followed immediately by a wash. For the origins of perfusates P4 and Q4 see text. Spasmogen contact time = 30 sec. (except Q4). Dose interval = 3 min.

Fig. 7 shows an experiment in which the same two series of perfusates (P and Q) were compared for SRS-A content on the same piece of isolated guinea-pig ileum. The tissue was first tested for histamine sensitivity (H, 2H and H/2) and then mepyramine and atropine were added to the Tyrode solution. Ten min. later histamine blockade was demonstrated by adding a large dose of histamine (100H) to the bath. The response induced by changing the Tyrode in the bath (T) is shown for comparison. Then, whereas the activity of the Q perfusates from animals pretreated with ethanolamine was observed to be negligible, the activity of the P perfusates from control animals was found to be appreciable. It was concluded that ethanolamine is capable of inhibiting the release or formation of SRS-A, or both, in guinea-pig lung undergoing anaphylaxis.

DISCUSSION

Since an antigen-antibody reaction in sensitised guinea-pig lung leads to the liberation of histamine and SRS-A, both of these substances can be

expected to contribute to the syndrome of anaphylaxis observed in a sensitised animal inhaling an aerosol of the specific antigen. Under these conditions antagonism of the released histamine by pretreatment with mepyramine or promethazine affords only a limited degree of protection. The increased measure of protection observed after the simultaneous administration of an antihistamine and ethanolamine might therefore

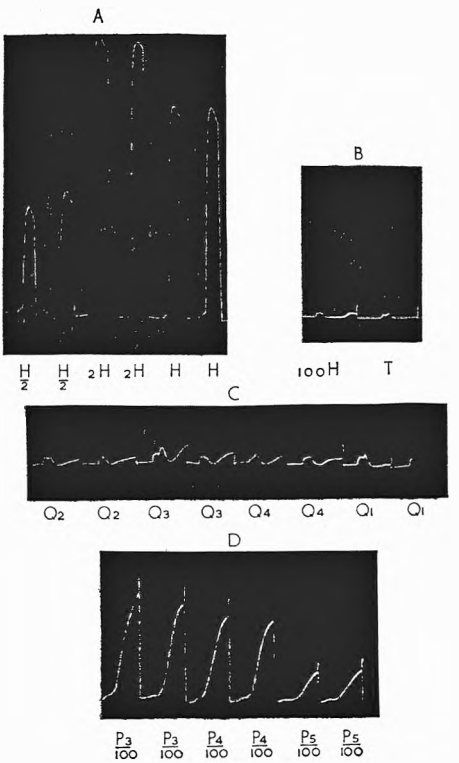


FIG. 7. A comparison of the SRS.A contents of two series of anaphylactic guinea-pig lung perfusates (P and Q). Guinea-pig ileum 37° 2 ml bath. Tyrode solution. Between A and B mepyramine and atropine at concentrations of 10^{-6} g./ml. were added to the wash fluid. H = Histamine 0·001 μg./ml. T = response to changing Tyrode in bath. P1/100, Q1/100, etc. = responses to 1 in 100 dilutions of perfusate. For origins of the perfusates see text. Dose interval = 3 min. before adding mepyramine and atropine to the Tyrode but 5 min. thereafter. Spasmogen contact time = 30 sec. before mepyramine and atropine and 60 sec. thereafter.

indicate protection from the pharmacological actions of both histamine and SRS-A. Exploration of such a hypothesis led to the observation that pretreatment with ethanolamine could inhibit the release of SRS-A in sensitised guinea-pig lungs subjected to anaphylaxis *in vitro*. However, the dose of ethanolamine required in these experiments was appreciably greater than that required to potentiate mepyramine in animals subjected to anaphylaxis *in vivo*. This apparent anomaly is worthy of comment.

In the *in vivo* experiments it has been demonstrated that a single intramuscular dose of as little as 2 mg./kg. can significantly potentiate 1 mg./kg. of mepyramine. In the *in vitro* experiments reported here three daily doses of 200 mg./kg. of ethanolamine were necessary before inhibition of SRS-A release could be demonstrated unequivocally. There are a number of possible explanations. Under the *in vivo* shock conditions, it can be calculated from the amount of antigen released as aerosol per minute and the volume of air containing antigen which is breathed by the experimental animal per minute, that the amount of antigen actually inhaled during the normal collapse time is about 1 or 2 μg. Because of antigen losses on the walls of the apparatus and in the upper parts of the respiratory system of the guinea-pig, exact quantitative determination of the dose of antigen reaching the lungs is difficult. The estimation has been attempted unsuccessfully by other authors (see references in Winter and Flataker, 1955). By comparison, the minimum amount of antigen (1 mg.) necessary for the liberation of histamine and SRS-A in the *in vitro* experiments is large. In addition, the route of administration was different. In one experiment antigen was administered via the air passages and in the other by the capillaries. Whereas in the first, administration was virtually continuous and ceased on reaching a predetermined end-point, in the other experiment antigen probably reached its reaction site in a sudden high concentration. There are thus a number of reasons why the dose of ethanolamine used in the *in vivo* experiments is not the same as that used to establish its mechanism of action.

The mechanism whereby ethanolamine inhibits the release of SRS-A is not yet known. However, the metabolic fate of ethanolamine has previously been studied in rats by Stetten (1941), Pilgeram, Gal, Sassenrath and Greenberg (1953), and Pilgeram, Hamilton and Greenberg (1957). Studies with ^{15}N- and ^{14}C-labelled ethanolamine have shown that it is rapidly incorporated into tissue phospholipids either as ethanolamine itself or after conversion to choline or serine. The remainder is metabolised to carbon dioxide and urea. The observed inhibition of SRS-A release during anaphylaxis in sensitised guinea-pig lung is thus most likely related to an effect of ethanolamine on phospholipid synthesis or phospholipid turnover in that tissue. This possibility is now the subject of further investigations.

Acknowledgements. The author wishes to thank Miss Doreen M. Anderson for her assistance with some of the experimental work, Professor J. M. Robson for advice and a number of helpful discussions, Professor R. T. Williams for a discussion on ethanolamine metabolism and its relevance to this work, Professor E. E. Turner and Dr. S. Gottfried of Biorex Laboratories Ltd., for a supply of pure crystalline ethanolamine hydrochloride and their interest and assistance.

REFERENCES

Armitage, P., Herxheimer, H., and Rosa, L. (1952). *Brit. J. Pharmacol.*, **7**, 625–636.
Brocklehurst, W. E. (1953). *J. Physiol.*, **120**, 16–17P.
Brocklehurst, W. E. (1955). *Ibid.*, **128**, 1P.

Brocklehurst, W. E. (1956). *Ciba Foundation Symposium on Histamine*, p. 175–179, London: Churchill.
Brocklehurst, W. E. (1960). *J. Physiol.*, **151**, 416–435.
Brown, H. M., Christie, B. G. B., Colin-Jones, E., Finney, R. S. H., MacGregor, W. G., Morrison-Smith, J., Smith, W. G., Sullivan, F. M., Tarnoky, A. L., Turner, E. E., Wotton, D. E. M., and Watkinson, G. (1959). *Lancet*, **2**, 492–493.
Coburn, A. F., and Moore, L. V. (1943). *Amer. J. Dis. Child.*, **65**, 744–756.
Coburn, A. F., Graham, C. D., and Haninger, J. (1954). *J. exp. Med.*, **100**, 425–435.
Cronheim, G. E., and Toekes, I. M. (1959). *J. Pharmacol.*, **127**, 167–170.
Ganley, O. H., Graessle, O. E., and Robinson, H. J. (1958). *J. Lab. clin. Med.*, **51**, 709–714.
Ganley, O. H., and Robinson, H. J. (1959). *J. Allergy*, **30**, 415–419.
Herxheimer, H. (1952). *J. Physiol.*, **117**, 251–255.
Kuehl, F. A., Jun., Jacob, T. A., Ganley, O. H., Ormond, R. E., and Meisinger, M. A. P. (1957). *J. Amer. chem Soc.*, **79**, 5577–5578.
Long, D. A., and Martin, A. J. P. (1956). *Lancet*, **1**, 464–467.
Pilgeram, L. O., Hamilton, R. E., and Greenberg, D. M. (1957). *J. biol. Chem.*, **227**, 107–113.
Pilgeram, L. O., Gal, E. M., Sassenrath, E. N., and Greenberg, D. M. (1953). *Ibid.*, **204**, 367–377.
Stetten, D. (1941). *Ibid.*, **140**, 143–152.
Winter, C. A., and Flataker, L. (1955). *J. exp. Med.*, **101**, 17–24.

STUDIES IN ORGANO-SELENIUM COMPOUNDS

PART I. DETERMINATION OF Se

BY D. DINGWALL AND W. D. WILLIAMS

From the School of Pharmacy, Royal College of Science and Technology, Glasgow

Received August 31, 1960

Selenium in some organo-selenium compounds has been determined colorimetrically by conversion to selenious acid and hence to a selenium sol stabilised with chlorpromazine hydrochloride. Non-ionic, cationic and anionic stabilisers have been compared with chlorpromazine hydrochloride but only cetomacrogol appeared to be as efficient.

WITH the development of studies in organo-selenium compounds in this School a simple method for the determination of selenium became essential. The available methods are generally based upon oxidation of selenium to selenious acid which can be determined by volumetric (Yoshimura, 1957; Bradt and Lyons, 1926), gravimetric (Banks and Hamilton, 1939; Drew and Porter, 1929), or colorimetric procedures. The most sensitive of the colorimetric methods appears to be that described by Hoste and Gillis (1955) involving the formation of a coloured piazselenol with diaminobenzidine. Relatively less sensitive reactions are those using such reducing agents as sulphurous acid, hydrazine and stannous chloride (De Meio, 1948) and ascorbic acid (Yoshimura, 1957), and one using codeine sulphate (Gortner and Lewis, 1939). Selenious acid in the presence of reducing agents yields golden brown sols which rapidly deposit red selenium, and for quantitative work a stabiliser must be present. Many substances have been used for this purpose, among them acacia (Robinson, Dudley, Williams and Byers, 1934), gelatin (Yoshimura, 1957), glycerol (Dolique, Giroux and Pérahia, 1946), mucilage from *Plantago psyllium* seeds (Gutbier, Huber and Eckert, 1923), saponin (Gutbier and Rhein, 1923) and starch (van der Meulen, 1934) in colorimetric and volumetric procedures.

A chance observation led to the discovery that chlorpromazine hydrochloride, and similar phenothiazine derivatives, stabilised selenium sols to give a stable colour suitable for spectrophotometric measurement at 420 mμ. The use of a pure chemical entity as a reagent offered a material advantage over complex colloidal material in the preparation of standard solutions and as glycerol proved unsatisfactory, chlorpromazine hydrochloride was used in preference to such substances as starch or gelatin. When it became evident that a complex of selenium and chlorpromazine was not involved (cf. palladium chloride and chlorpromazine, as described by Ryan, 1959), the efficiency of the reagent was compared with that of other substances.

EXPERIMENTAL

Reagents. Sulphuric acid. Nitric acid. Hydrochloric acid. Ascorbic acid solution, 1 per cent w/v. Chlorpromazine hydrochloride solution, 2 per cent w/v. Sodium hydroxide solution, 20 per cent w/v.

Standard selenium dioxide. Selenium dioxide (reagent grade, 50 g.) was treated with nitric acid (25 ml.) in an evaporating dish and heated in a heating mantle until the removal of nitric acid was complete. The surface-crust of colourless needle crystals (24·5 g.) was easily separated

TABLE I

PERCENTAGE OF SeO_2 WHEN DETERMINED BY VARIOUS CHEMICAL METHODS

Material	Method			
	Potassium permanganate (volumetric)*	Iodine/thiosulphate (volumetric)** (Method B)	Gravimetric (procedure A)†	Gravimetric (hydrazine)††
Original	99·6 99·6	94·6 94·4	93·0 93·1 96·3	91·7
Standard	99·6 99·6	99·7 99·7	99·6 100·0	99·2 99·3

* Vogel (1951a). ** Vogel (1951b). † Vogel (1951c). †† Gutbier, Metzner and Lohmann (1904).

from an amorphous grey residue by means of a spatula, stored in a desiccator and used for the preparation of standard selenious acid solutions. Analyses of original and standard selenium dioxide are recorded in Table I.

Reaction time. The extinction of the sols obtained when selenious acid was treated with ascorbic acid solution (1 ml.) reached a maximum within

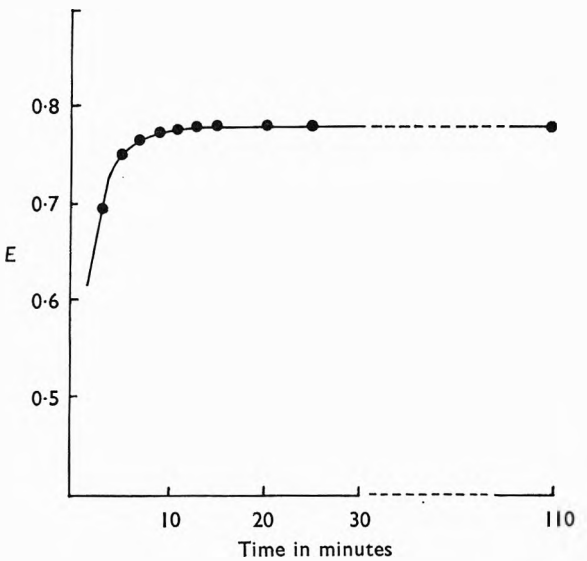


FIG. 1. Development and stability of the extinction of a selenium sol.

20 min., indicating the reaction was complete. A standard reaction time of 30 min. was therefore adopted. Fig. 1 illustrates the results obtained in the presence of chlorpromazine hydrochloride solution (1 ml.) as stabiliser.

Effect of Acidity on the Stability and Extinction of Se Sols

Nitric acid (traces) present. (*a*) Weighed quantities of selenium dioxide were examined as described in the Method for the Determination of Selenium (below) omitting the neutralisation with sodium hydroxide and addition of hydrochloric acid. The development of the colour when chlorpromazine hydrochloride and cetomacrogol were used as stabilisers was slow and the results were variable.

(*b*) Aliquot portions of the selenious acid solutions obtained in (*a*) above were carefully neutralised and treated as described in the Method for the Determination of Selenium (p. 15) from the words "add hydrochloric acid (1 drop) . . .". No abnormality was observed in the development

TABLE II

EFFECT OF A MODERATE EXCESS OF ACID ON EXTINCTIONS

Excess of hydrochloric acid ..	As in method	1 drop	2 drops
Extinction	0·883	0·878	0·886

TABLE III

EFFECT OF SODIUM SULPHATE ON EXTINCTIONS

Extinction (Na_2SO_4 absent)	0·344	0·528	0·684	0·844
Extinction (Na_2SO_4 present)	0·356	0·538	0·714	0·895

TABLE IV

EFFECT OF TEMPERATURE ON EXTINCTIONS

Temperature (° C.) ..	13	22	25
Extinction	0·292	0·289	0·284

of the colour or the constancy of the readings. A similar result was obtained when cetomacrogol solution (1 per cent, 1 ml.) was used in place of chlorpromazine hydrochloride solution.

(*c*) A solution of selenious acid obtained during one of the assays was examined in the presence of one and two drops of hydrochloric acid in excess of that stated in the Method for the Determination of Selenium. The results are recorded in Table II.

Nitric acid absent. Aliquot portions of a selenious acid solution (0·208 mg./ml.) were treated with sulphuric acid (0·2 ml.), cetomacrogol (0·01 per cent, 1 ml.), ascorbic acid solution (1 ml.) and made up to 10 ml. with water. The extinctions reached a maximum within 30 min. and remained stable.

Effect of Sodium Sulphate on the Extinction of Se Sols

Colours were developed from selenious acid solutions in the presence of sodium sulphate (anhydrous, 0·5 g.) and in its absence. The results are recorded in Table III.

Effect of Temperature on the Extinction of Se Sols

Colours were developed from a selenious acid solution at 13°, 22° and 25°. The results are recorded in Table IV.

Comparison of Stabilisers

Varying quantities of stabilisers were added to slightly acid solutions of selenious acid and a sol developed by the addition of ascorbic acid solution (1 ml.). The sols were adjusted to 10 ml. with water and the extinctions were measured after 30 min. at 420 mμ in 1 cm. cells. The results are recorded in Table V.

TABLE V

EXTINCTIONS OF SELENIUM SOLS IN THE PRESENCE OF STABILISERS

Stabiliser	SeO_2 (mg.)	ml. of 1 per cent solution of stabiliser						
		0·005	0·01	0·2	0·5	0·6	1·0	2·0
Chlorpromazine hydrochloride	1·0	—	—	Turbid	Unstable	0·860	0·832	0·820
Cetomacrogol 1000 B.P.C.	0·604	0·490	0·490	0·480	0·476	—	0·468	0·463
Cetrimide	0·604	—	0·479	—	—	—	0·474	—
Gelatin	0·604	—	—	0·530	0·520	—	0·504	—
Sodium dodecylsulphate	0·604	—	Turbid	Turbid	—	—	0·468	—
Starch	0·604	—	—	0·695	0·620	—	0·567	—
Glycerol	0·5	—	Turbid at all concentrations even up to 1 ml. in 10 ml. of final mixture.					

TABLE VI

EFFECT OF ORGANIC MATTER ON THE RECOVERY OF SELENIUM

Organic compound	SeO_2 weighed (mg.)	SeO_2 found (mg.)	Recovery (per cent)
Glucose	3·260	3·21	98·5
Glucose	2·350	2·35	100·0
Methylene blue	3·360	3·32	98·8
Phenothiazine	3·348	3·28	98·0
Phenothiazine	3·310	3·32	100·3
Phenobarbitone	3·040	3·06	100·7
Thiouracil	2·990	2·97	99·3
	1·038	1·04	100·2

Method for the Determination of Selenium

Dissolve an accurately weighed quantity of the organic compound, equivalent to about 2–3 mg. of Se, in sulphuric acid (1 ml.) and nitric acid (2 ml.) in a Kjeldahl flask of about 40 ml. capacity. Boil off the nitric acid carefully to leave a colourless residue (7–15 min.)* If the residue acquires a red or brown tint towards the end of the reaction add 1 drop of nitric acid and remove the excess by heating. Cool the residue, dilute with water (10 ml.) and neutralise with sodium hydroxide solution using 1 drop of phenolphthalein solution as indicator. Cool the mixture to 20°, transfer quantitatively to a 25 ml. graduated flask, make up to volume and mix well. Transfer 5 ml. of the solution to a 10 ml. graduated flask, add hydrochloric acid (1 drop) from a teat pipette to make slightly acid, chlorpromazine hydrochloride solution (1 ml.) water (2 ml. if necessary

* The digestion can also be carried out by electrical heating as used in the normal micro-Kjeldahl process but a period of 2 hours is necessary to remove the nitric acid.

to give a clear solution) and ascorbic acid solution (1 ml.). Make up to volume with water, mix well and measure the extinction of the sol at 420 mμ in a 1 cm. cell after 30 min. using a blank of reagents treated in the same way. Calculate the percentage of selenium in the compound by reference to a calibration curve prepared by using known amounts of selenium dioxide in the presence of sodium sulphate (anhydrous, 0·5 g.) and hydrochloric acid (1 drop) in a final volume of 10 ml.

Calibration Curve

The curve obtained showed no deviation from Beer's law up to the maximum amount of selenium dioxide used (1·0 mg.).

Effect of Organic Matter on the Recovery of Selenium

Accurately weighed quantities of selenium dioxide were treated as described in the Method for the Determination of Selenium in the presence of 7–10 mg. of organic compounds. The results are recorded in Table VI.

Discussion

Standard Selenium Dioxide

The required product for the formation of a colour is selenious acid, H_2SeO_3, which is formed when selenium dioxide dissolves in water. Sublimation of the dioxide always gave traces of red selenium in the product and proved unsatisfactory in preparing a standard. The method using nitric acid, however, gave colourless crystals and was similar to that of Lenher (1898) except that condensing funnels were omitted.

The standard gave satisfactory analyses when examined by four methods whereas the apparent content of SeO_2 in the original material depended upon the method used. The results are deemed to be of sufficient interest to be given in Table I.

Oxidation of Organic Matter

Oxidation of the organic compounds with a mixture of sulphuric and nitric acids proceeded smoothly and without the significant loss of selenium which is liable to occur when such an oxidation mixture is used (Fogg and Wilkinson, 1956; Gorsuch, 1959). This is no doubt explained by the very much smaller quantity of organic matter present (less than 10 mg.) as compared with 0·2–10 g. when traces of selenium are determined in vegetable material (Gorsuch, 1959; Williams and Lakin, 1935). Perchloric acid, which yields quantitative recovery of selenium (Fogg and Wilkinson, 1956), could not be used in this instance because selenious acid is oxidised further to selenic acid which is not reduced under the conditions of the assay. Control experiments with selenium dioxide in the presence of glucose and compounds which are difficult to oxidise gave reasonable recoveries of Se (Table V).

Reduction of Selenious Acid

Reduction of selenious acid to selenium is more conveniently carried out by ascorbic acid rather than by hydrazine, stannous chloride or sulphurous acid.

Although reduction can be carried out in strongly acid media (approximately N) the results of the experiments described under Effect of Acidity on the Stability and Extinction of Se Sols showed that the presence of appreciable traces of nitric acid in the final solution slowed the rate of colour formation and gave erratic results. Neutralisation of the oxidation mixture was therefore essential in order that some degree of control could be exercised over the final acidity. One and two drops of hydrochloric acid in excess of that used in the method caused no marked change in extinction and allowed a reasonable latitude in the size of drops.

Colour

The colour is attributed to colloidal selenium rather than to a complex of selenium and chlorpromazine because it is similar to that obtained when other reagents are used. This is confirmed by the precipitation of selenium on addition of ethanol to the reaction mixture.

The sols showed no absorption maximum and the nominal wavelength of 420 mμ was selected because this region is conveniently obtained on a filter absorptiometer if a spectrophotometer is not available. Further, the extinction of the blank in chlorpromazine hydrochloride is small in this region.

Stabilisers

Three of the stabilisers were quickly eliminated from consideration by the appearance of the final sols. Starch and gelatin gave slightly opalescent sols which showed a marked fall in extinction when the concentration of colloid was increased. These disadvantages could well give rise to difficulties when different batches of stabiliser are used. Cetrimide was more satisfactory but the Tyndall effect was still evident under the assay conditions. The remarkably small effect of increasing cetrimide concentration is of interest (Table IV). Although highly efficient in slightly acid, salt-free solutions it could not be used without preliminary neutralisation of the oxidation mixture.

Cetomacrogol 1000 B.P.C. was very efficient (Table V) and a determined effort was made to confirm it as the stabiliser of choice. Encouraging results were obtained in the presence of sulphuric acid, but, as shown in the experimental section, neutralisation of the oxidation mixture could not be avoided. As a consequence, the selenium sols showed an obvious Tyndall effect at low concentrations of cetomacrogol and an increased concentration was necessary to give the degree of clarity obtained when chlorpromazine hydrochloride was used. Thus cetomacrogol under the assay conditions lost two possible advantages, viz., elimination of one step in the assay and a low concentration of reagent. In addition, the composition of cetomacrogol may vary slightly from batch to batch so that a check on the effect of using different batches of material would be necessary before suggesting its use in the colorimetric assay. Consequently there appeared to be no advantage in reverting to cetomacrogol as stabiliser although it was realised that other analysts, bearing in mind such factors as cost and nature of materials, might well prefer it.

Chlorpromazine hydrochloride was therefore retained as stabiliser and no difference in results was noted when using different batches of reagent. A concentration effect was observed as with other stabilisers but it was not regarded so seriously since standard solutions can be reproduced more easily than with colloids.

Sodium dodecylsulphate was not examined in detail but was included to show the effect of an anionic surface-active agent.

Salt and Temperature Effects

As might be expected, the presence of sodium sulphate affected the extinction of the sols (Table II). It must therefore be included when

TABLE VII

SELENIUM IN ORGANIC COMPOUNDS

Indent. No.	Type of compound	Se (per cent) Found	Theory
1	Amino-selenazolone, hydrochloride	39·4	39·64
2	Amino-selenazolone, picrate	20·2	20·13
3	Amino-selenazolone, hydrobromide	30·8	30·66
4	Amino-selenazolone, picrate	19·2	19·45
5	Amino-selenazolone, hydrobromide	29·0	29·09
6	Amino-selenazolone, picrate	18·6	18·78
7	Se-Benzyl iso-selenourea hydrochloride	31·4	31·70
8	Selenothiazine	31·6	32·0
9	Selenothiazine	27·7	28·15
10	Selenothiazine	24·5	24·1
11	Benzyldiselenide	45·9	46·19
12	Selenazolidine	41·6	41·11
13	Selenazolidine	43·9	44·34
14	Selenazolidine	37·8	38·30
15	Selenazolidine	40·9	41·11

preparing the calibration curve. The effect of temperature (Table III) indicated that a wide range for development of colour should be avoided and solutions were generally used at about 20°.

RESULTS

The results obtained on synthetic compounds are given in Table VII. With the exception of compounds 7 and 11 (Table VII) only the class of compound is given but satisfactory elemental analyses were obtained for all.

In view of the results in Table VI those in Table VII would appear to be reliable figures for the content of Se in the organic compounds.

The method has the advantage over the volumetric method of Gould (1951) in that it is applicable to compounds which contain iodine. Any iodate produced in the oxidation step is reduced by the chlorpromazine.

Acknowledgements. We wish to thank Dr. J. B. Stenlake for helpful discussion, Mrs. D. Harvey and Miss M. Buchanan for technical assistance and Smith, Kline and French Ltd., for the supply of compounds 8, 9 and 10 and the award of a maintenance grant to one of us (D.D.).

REFERENCES

Banks, C. K., and Hamilton, C. S. (1939). *J. Amer. chem. Soc.*, **61**, 2306–2308.
Bradt, W. E., and Lyons, R. E. (1926). *Ibid.*, **48**, 2642–2646.
De Meio, R. H. (1948). *Analyt. Chem.*, **20**, 488–489.

Dolique, R., Giroux, J., and Pérahia, S. (1946). *Bull. Soc. Chim. Fr.*, **42**, 48–51.
Drew, H. D. K., and Porter, C. R. (1929). *J. chem. Soc.*, 2091–2095.
Fogg, D. N., and Wilkinson, N. T. (1956). *Analyst*, **81**, 525–531.
Gorsuch, T. T. (1959). *Ibid.*, **84**, 150–151.
Gortner, R. A., Jr., and Lewis, H. B. (1939). *Ind. Engng Chem.* (*Anal.*), **11**, 198–200.
Gould, E. S. (1951). *Analyt. Chem.*, **23**, 1502–1503.
Gutbier, A., Huber, I., and Eckert, P. (1923). *Kolloid-Z.*, **32**, 255–262.
Gutbier, A., Metzner, G., and Lohmann, J. (1904). *Z. anorg. Chem.*, **41**, 291–304.
Gutbier, A., and Rhein, V. (1923). *Kolloid-Z.*, **33**, 35–36. Through *Chem. Abstr.*, 1923, **17**, 3437.
Hoste, J., and Gillis, J. (1955). *Anal. Chim. Acta*, **12**, 158–161.
Lenher, V. (1898). *J. Amer. chem. Soc.*, **20**, 559–560.
Robinson, W. O., Dudley, H. C., Williams, K. T., and Byers, H. G. (1934). *Ind. Engng Chem.* (*Anal.*), **6**, 274–276.
Ryan, J. A. (1959). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 240–243.
Van der Meulen, J. H. (1934). *Chem. Weekbl.*, **31**, 333–335. Through *Chem. Abstr.*, 1934, **28**, 6079.
Vogel, A. I. (1951a). *Quantitative Inorganic Analysis*, 2nd ed., p. 290. London: Longmans, Green and Co.
Vogel, A. I. (1951b). *Ibid.*, p. 291.
Vogel, A. I. (1951c). *Ibid.*, p. 442.
Williams, K. T., and Lakin, H. W. (1935). *Ind. Engng Chem.* (*Anal.*), **7**, 409–410.
Yoshimura, C. (1957). *J. chem. Soc., Japan*, **78**, 5–6.

THE EFFECT OF CORTISONE AND HYDROCORTISONE ON THE PLASMA LEVELS OF CORTICOTROPHIN IN THE RAT, AFTER AN ACUTE STRESS

By A. M. Barrett

*From the Department of Pharmacology, School of Pharmacy, University of London Brunswick Square, London, W.C.*1

Received August 30, 1960

Pretreatment of intact female rats with cortisone and hydrocortisone acetates produces a graded inhibition of ACTH release in response to acute stress as shown by changes in plasma ACTH levels. The inhibition was greater with hydrocortisone and was dependent upon the time and dose relations.

Until comparatively recently, most of the evidence that has been marshalled into the various theories of ACTH regulation has been of an indirect nature. The predominant type of experimental approach has been one in which the test animal is subjected to some stressful stimulus and then at an arbitrary interval secondary changes within the animal are examined. Such indices as eosinopenia (McDermott, Fry, Brobeck and Long, 1950), lymphocytopenia (Colfer, de Groot and Harris, 1950), thymic involution (Bruce, Parkes and Perry, 1952), altered urinary steroid excretion patterns (Liddle, Richard and Peterson, 1955), and adrenocortical changes (Sayers and Sayers, 1947) are all secondary or indirect indices of ACTH activity. Normally responses have been measured from 1 to 6 hours after the application of the stress: however, this time interval is even longer when morphological or histological changes have been used as indices of adrenocorticotrophic acitivity.

It cannot be denied that these indices reflect chages due to disturbed adrenocortical activity. But, they possess a distinct disadvantage in that they do not convey precise information on the dynamics of ACTH release from the adenohypophysis. With the development of techniques permitting the quantitative estimation of ACTH in plasma or blood, it is now possible to measure the rate and magnitude of ACTH release under a wide variety of experimental conditions.

The ability of adrenocortical hormones to inhibit the stress-induced release of ACTH has been observed in many laboratories (Barrett, 1959). However, there are various discrepancies in the literature over this phenomenon which may well be due to the fact that secondary indices of pituitary adrenocorticotrophic activity have been used. Barrett and Hodges (1956) reported the stress of laparotomy under ether anaesthesia to cause an elevation of plasma ACTH in the rat which was maximal ten minutes after the application of the stimulus. This finding has been confirmed in the present work and used to demonstrate the inhibitory action of cortisone and hydrocortisone on the release of ACTH in response to stress.

Experimental

Materials and Methods

Wistar rats were fed on a cube diet and water and maintained at a constant temperature of 70° F. Hypophysectomised rats were kept on a

normal diet with the addition of 5 per cent glucose in their drinking water. Male rats (120–140 g.) were used for assay purposes, and females (160–200 g.) for the plasma for assay.

Cortisone acetate (saline suspension) and hydrocortisone acetate were administered to female rats subcutaneously. Dilutions with normal saline were prepared immediately before use and injections were given in volumes of 1 ml. and in doses equivalent to 2 and 20 mg./100 g. weight. Control injections of normal saline were given in similar volumes. Deoxycortone acetate was dissolved in arachis oil and injected subcutaneously into male rats in volumes of 20 mg./100 g. weight. Deoxycortone (DCA) treated animals were used 16–24 hr. later for the assay of ACTH in plasma by the method of Hodges (1955).

Lyophilised adrenocorticotrophic hormone (Armour, 1 unit/mg.) was dissolved in normal saline immediately before use. ACTH was injected intravenously into both DCA-treated and hypophysectomised male rats in

TABLE I

THE CONCENTRATION OF ACTH (MU/100 ML.) IN THE PLASMA OF STRESSED NORMAL FEMALE RATS: COMPARISON BETWEEN ASSAYS PERFORMED ON DCA-TREATED AND HYPOPHYSECTOMISED ANIMALS

Type of assay animal	Treatment	No. of donor rats	Dose per 100 g. assay rat	No. of assay rats	Mean ascorbic acid depletion (mg./100 g. adrenal ± S.E.)	Conc. ACTH mU/100 ml. plasma (95 per cent fiducial limits)
DCA-treated	Standard ACTH	—	0·15 mU	12	44 ± 9	14·6 (12·7–19·2)
		—	0·60 mU	12	121 ± 6	
	Plasma from intact female rats, 10 min. after stress	32	3·0 ml.	12	103 ± 14	
Hypophysectomised	Standard ACTH	—	0·15 mU	12	53 ± 9	14·5 (12·5–17·8)
		—	0·60 mU	12	133 ± 6	
	Plasma from intact female rats, 10 min. after stress.	32	3·0 ml.	12	115 ± 7	

Depletion calculated from saline injected controls. DCA-treated 452 ± 7 mg. per cent; hypophysectomised 501 ± 6 mg. per cent.

doses of 0·15 and 0·60 milliunits (mU.) in volumes of 3 ml./100 g. weight. Control injections of normal saline were given intravenously in similar volumes.

Experimental procedures. The stressful stimulus was laparotomy under ether anaesthesia. Ten min. after commencement of exposure to ether the animals were decapitated and blood collected from the trunk portion into heparinised tubes by a funnel which had been rinsed in heparin solution. The blood was centrifuged at 3,000 r.p.m. for 15 min. and the plasma collected.

Plasma obtained from each group of stressed rats was pooled and injected intravenously into groups of either DCA-treated or hypophysectomised rats. Hypophysectomy was by the parapharyngeal approach. The hypophysectomised rats were used for ACTH assay 24 hr. after

removal of their pituitary glands according to the method of Sayers, Sayers and Woodbury (1948), as modified by Munson, Barry and Koch (1948).

The concentration of adrenal ascorbic acid in all DCA-treated and hypophysectomised rats was determined (Roe and Kuether, 1943) 1 hr. after the intravenous injections had been administered. The ACTH activity was estimated from the adrenal ascorbic acid depletion produced in test and standard ACTH injected groups obtained by subtraction of these values from that for the control, saline injected group. The results and

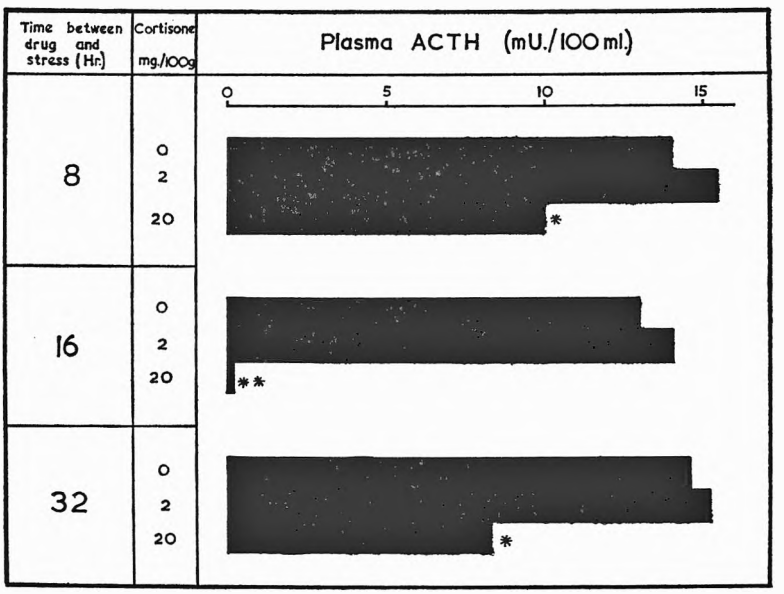


FIG. 1. ACTH concentrations in the plasma of normal rats and rats pre-treated with cortisone acetate, 10 min. after stress. The value expressed by each horizontal column represents an estimate of the ACTH concentration in the pooled plasma from 16 animals assayed in a group of six DCA-treated rats. An asterisk indicates that the result is significantly ($P = 0.05$) different from non-steroid injected control value: a double asterisk indicates that the difference is highly significant ($P = 0.01$).

their fiducial limits ($P = 0.95$) were calculated by the method of Gaddum (1953) for a $2 + 1$ assay and were expressed as mU. ACTH/100 ml. plasma.

The effect of cortisone and hydrocortisone. Six groups of 16 female rats were injected with cortisone. Three of these groups received a dose of 2 mg./100 g. and three groups a dose of 20 mg./100 g.; similar groups of animals were injected with the same dose levels of hydrocortisone. These rats were exposed to the stressful stimulus 8, 16 or 32 hr. after the steroid injection. Further groups of 16 female rats, which had received control injections of saline alone at the appropriate time intervals, were stressed in a similar manner. Pooled plasma from each group of rats was assayed for ACTH activity, and the plasma ACTH concentrations were calculated.

RESULTS

The summarised results for two methods for the bioassay of ACTH are compared in Table I. When mobilisation of endogenous ACTH in the assay animal was prevented by pre-treatment with DCA the concentration of ACTH in the plasma of stressed female rats was found to be 14·6 mU. ACTH/100 ml. With hypophysectomised assay animals a value of 14·5 mU. ACTH/100 ml. of plasma was obtained. The results showed that DCA-treated animals compared favourably with hypophysectomised rats in the bioassay of plasma ACTH.

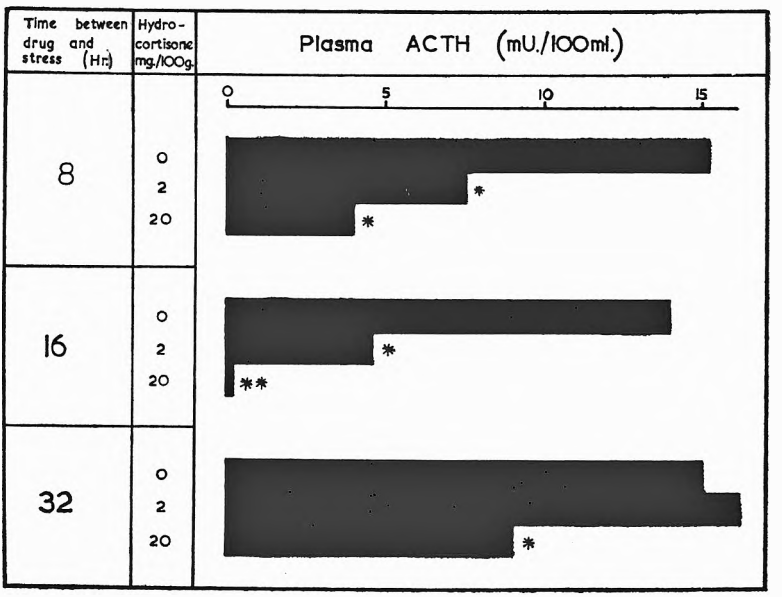


FIG. 2. ACTH concentrations in the plasma of normal rats and rats pre-treated with hydrocortisone acetate, 10 min. after stress. The value expressed by each horizontal column represents an estimate of the ACTH concentration in the pooled plasma of 16 animals assayed in a group of six DCA-treated rats. An asterisk indicates that the result is significantly ($P = 0{\cdot}05$) different from non-steroid injected control value: a double asterisk indicates that the difference is highly significant ($P = 0{\cdot}01$).

DCA-treated animals were then used for ACTH estimations as this technique prevented the release of ACTH in the assay rat due to any stressful stimuli associated with the assay technique.

The effects of pre-treatment with cortisone and hydrocortisone on plasma ACTH concentrations 10 min. after stress are summarised in Figs. 1 and 2. In all experiments where rats were subjected to stress, without pre-treatment with either cortisone or hydrocortisone, a steady value of about 14 mU./100 ml. of plasma ACTH was found. As shown in Fig. 1, cortisone at a dose of 20 mg./100 g. produced a significant reduction in the plasma ACTH level after stress at 8, 16 or 32 hr. after administration whereas at a dose of 2 mg. there was no significant change. But, as shown in Fig. 2, hydrocortisone was active at the lower dose at 8 and 16 hr.

but not at 32 hr. The high dose of hydrocortisone was active at all times. Both cortical hormones afforded a complete inhibition of ACTH release 16 hr. after injection, at the higher doses. Eight hr. after administration hydrocortisone appeared to be twice as effective as cortisone. In general hydrocortisone was more active and quicker acting than cortisone.

Discussion

A comparison has been described between two methods for the assay of plasma ACTH. Results obtained in DCA-treated animals were in close agreement with those found using hypophysectomised rats. It has been argued that the DCA-blocking technique is neither specific nor fully effective since the ability of corticosteroids to inhibit pituitary adrenocorticotrophic activity is relative rather than absolute (Miahle-Voloss and Stutinsky, 1956). But, with a dose of DCA of 20 mg./100 g. the release of endogenous ACTH was totally inhibited in the assay animal.

In the present work it has been shown that under certain conditions complete inhibition of ACTH discharge in response to stress can be obtained with both cortisone and hydrocortisone. However, the effective doses were large, confirming earlier observations from indirect indices of ACTH release (Hodges, 1954; Hodges and Vernikos, 1958). Hydrocortisone was active earlier than cortisone and was about twice as potent as a pituitary inhibitor. This is in contrast to the report of Sayers and Sayers (1947) which ascribed equal potency to the two compounds in this respect.

There has been much controversy about the amounts and relative potencies of the various steroids necessary to inhibit pituitary ACTH discharge. This may well be due to the widely different techniques used. Routes of administration, time intervals between injection and testing, stressful stimuli, and acetate or free alcohol steroid preparations have all varied and contributed to the confusion. The relative potencies must also be influenced by the relative solubilities of the steroids in the vehicle used compared with the tissue fluids surrounding the site of administration.

Sayers and Sayers (1947) have shown that subcutaneous administration of both cortisone and hydrocortisone was effective in reducing the adrenal ascorbic acid depletion observed in untreated rats after various stimuli. They used doses less than 1 mg./100 g. weight and gave the drugs immediately before stressing. Another report described similar experiments but was unable to confirm that cortisone had any effect on the release of ACTH in response to stress, at similar dose levels (Fortier, Yrarrazaval and Selye, 1951). Other workers have found that the stress-induced secretion of ACTH can be prevented only by very large doses of cortical hormones and by allowing a longer time interval between injection and submission to stress (Hodges, 1954; Abelson and Baron, 1952). Hodges (1954) showed that cortisone was most effective as a pituitary inhibitor at the same time and dose relations as have been found in the present experiments. But using adrenal ascorbic acid depletion as a secondary index, he also found that cortisone did not completely prevent the depletion observed in untreated controls. The technique described here does not necessarily provide evidence that ACTH discharge is completely inhibited by cortisone

and hydrocortisone at the high dose, 16 hr. after administration. At present the method is not sufficiently sensitive to detect the normal circulating level of ACTH in the non-stressed animal. There may therefore be a large increase in plasma ACTH levels of acutely stressed rats which had pre-treatment with cortisone 16 hr. previously which is not detectable by this method. Very low concentrations of ACTH are effective in producing a response in the adrenal cortex (Renold, Jenkins, Forsham and Thorn, 1952) and this may account for the discrepancy between the two investigations.

In the present experiments DCA was used successfully to produce complete inhibition of pituitary adrenocorticotrophic activity. The dose used was 20 mg./100 g. administered at least 16 hr. before testing. By the same route of administration it was necessary to give the same doses of both cortisone and hydrocortisone in order to effect complete inhibition of ACTH 16 hr. later. ACTH is primarily concerned with the control of the secretion of glucocorticoids and it is surprising that DCA is equally effective at the same dose as those of cortisone and hydrocortisone. The experiments suggest that the ability of cortical hormones to suppress ACTH release is not necessarily related to their physiological activity. Dr. Farrell tells me that the glucocorticoid activity of hydrocortisone is approximately one hundred times that of DCA whereas for electrolyte balance DCA is twenty-five times more potent than hydrocortisone. The relation between physiological activity and pituitary adrenocorticotrophic inhibitory activity of the various steroids is not clear and deserves further study.

REFERENCES

Abelson, D. and Baron, D. N. (1952). *Lancet*, **2**, 663–664.
Barrett, A. M. (1959). Ph.D. Thesis, University of London.
Barrett, A. M. and Hodges, J. R. (1956). *J. Endocrinol.*, **14**, 292–296.
Bruce, H.M., Parkes, A. S. and Perry, W. L. M. (1952). *Ibid.*, **8**, xvi.
Colfer, H. F., de Groot, J. and Harris, G. W. (1950). *J. Physiol.*, **111**, 328–334.
Fortier, C., Yrarrazaval, S. and Selye, H. (1951), *Amer. J. Physiol.*, **163**, 466–468.
Gaddum, J. H. (1953). *J. Pharm. Pharmacol.*, **5**, 345.
Hodges, J. R. (1954). *J. Endocrinol.*, **10**, 173–178.
Hodges, J. R. (1955). *Ibid.*, **12**, 152–158.
Hodges, J. R. and Vernikos, J. (1958). *Brit. J. Pharmacol.*, **13**, 98–102.
Liddle, G. W., Richard, J. E. and Peterson, R. E. (1955). *Endocrinology*, **57**, 594–598.
McDermott, W. V., Fry, E. G., Brobeck, J. R. and Long, C. N. H. (1950). *Yale J. Biol. Med.*, **23**, 652–666.
Miahle-Voloss, C. and Stutinsky, F. (1956). *C.R. Acad. Sci., U.R.S.S.*, **243**, 189.
Munson, P. L., Barry, A. G. and Koch, F. C. (1948). *J. clin. Endocrinol.*, **8**, 586.
Renold, A. E., Jenkins, D., Forsham, P. H. and Thorn, G. W. (1952). *Ibid.*, **12**, 763.
Roe, J. H. and Kuether, C. A. (1943). *J. biol. Chem.*, **147**, 399–407.
Sayers, G. and Sayers, M. A. (1947). *Endocrinology*, **40**, 265–273.
Sayers, M. A., Sayers, G. and Woodbury, L. A. (1948). *Ibid.*, **42**, 379–393.

ANTITUBERCULOSIS AGENTS

PART VII.* 4,4′-DIPYRIDYL SULPHONE AND RELATED COMPOUNDS

BY A. M. COMRIE AND J. B. STENLAKE

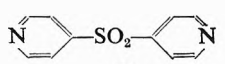
From The School of Pharmacy, The Royal College of Science and Technology, Glasgow

Received September 30, 1960

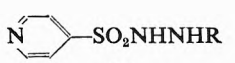
Pyridine-4-sulphinic acid, 4,4′-dipyridyl sulphone and other related 4-pyridyl sulphides and 4-pyridyl sulphones have been prepared.

BOTH 2,2′-dipyridyl sulphone (Dewing, Gray, Platt and Stevenson, 1952) and 3,4′-dipyridyl sulphone (Goldberg and Teitel, 1954) are known, but 4,4′-dipyridyl sulphone (I), the analogue of dapsone (4,4′-diaminodiphenyl sulphone), has not been investigated. Although, Ochai, Itai, and Yoshino (1944) prepared 4,4′-dipyridyl sulphide 1,1′-dioxide and 4,4′-dipyridyl sulphone 1,1′-dioxide, no attempt was made to convert the latter to 4,4′-dipyridyl sulphone. The synthesis of this and other related sulphones is now reported.

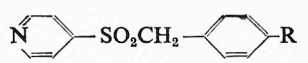
In a recent investigation of sulphonyl analogues of isoniazid and cognate compounds attempts to prepare *N*-alkyl derivatives (II, R = alkyl) were unsuccessful (Comrie and Stenlake, 1958b). Condensation of the sodio derivative of pyridine 4-sulphonhydrazide (II, R = H) with benzyl chloride gave benzyl 4-pyridyl sulphone (III, R = H) in some 10 per cent yield instead of the required 1-benzyl-2-pyridine-4′-sulphonylhydrazine (II, R = $C_6H_5CH_2$). Sulphone formation could be readily explained



(I)



(II)



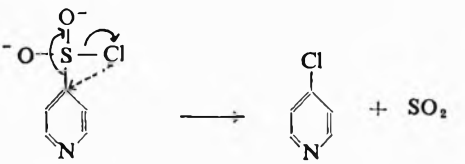
(III)

assuming decomposition of the pyridine-4-sulphonhydrazide to sodium pyridine-4-sulphinate (McFadyen and Stevens, 1936), and condensation of the latter with the alkyl halide. Since a similar condensation of sodium pyridine-3-sulphinate with 4-bromopyridine had already been reported in the preparation of 3,4′-dipyridyl sulphone (Goldberg and Teitel, 1954), the isolation of pyridine-4-sulphinic acid was attempted.

Pyridine-4-sulphonhydrazide is unstable even in cold aqueous solution (Comrie and Stenlake, 1958b), but at 40° decomposition was accompanied by a brisk evolution of gas, and on pouring the resulting acidic solution into acetone pyridine-4-sulphinic acid was obtained as yellow needles. It was characterised by analysis, by formation of a sodium salt and by oxidation to pyridine-4-sulphonic acid (Comrie and Stenlake, 1958a). Condensation of the sodium sulphinate and benzyl chloride gave the same benzyl sulphone still in poor yield. Attempted condensations with 4-chloropyridine to obtain 4,4′-dipyridyl sulphone (I), however, gave only a dark blue non-crystalline product either with the reactants alone or in the presence of a trace of iodine and copper as catalyst (Burton and Davy, 1947).

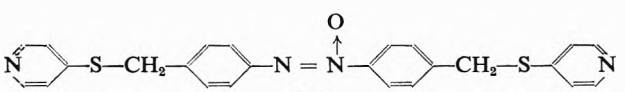
* Part VI, Comrie, Mital and Stenlake, *J. med. pharm. Chem.*, 1960, **2**, 171–177.

Oxidation of 4,4′-dipyridyl sulphide was next considered. This substance had been obtained by King and Ware (1939) as a by-product in the chlorination of pyrid-4-thione during an attempt to prepare pyridine-4-sulphonamide *via* pyridine-4-sulphonyl chloride. The latter is thermolabile (King and Ware, 1939; Comrie and Stenlake, unpublished work) decomposing to give sulphur dioxide and 4-chloropyridine, with cleavage of the C–S bond as depicted by Kwart and Miller (1958).



4,4′-Dipyridyl sulphide is therefore almost certainly formed from unreacted pyrid-4-thione and 4-chloropyridine formed by the above desulphonation, a general method for sulphide formation. Direct condensation of pyrid-4-thione and 4-chloropyridine gave 4,4′-dipyridyl sulphide in good yield (71 per cent). Oxidation of the sulphide with hydrogen peroxide in glacial acetic acid gave only starting material, and a viscous liquid from which no crystalline product could be isolated. Potassium dichromate, as used in the oxidation of 2,2′-dipyridyl sulphide (Dewing, Gray, Platt and Stephenson, 1942), was also unsuccessful, but with cold potassium permanganate in dilute acetic acid oxidation proceeded smoothly to give 4,4′-dipyridyl sulphone in 72 per cent yield, the manganese dioxide formed during the reaction being removed by the addition of 30 per cent hydrogen peroxide (Takahashi, Shibasaki and Uchibayashi, 1954). Removal of manganese dioxide with sulphur dioxide (King and Ware, 1939) proved unsatisfactory probably due to overheating during evaporation of the solution to dryness (Burton and Davy, 1947).

Condensation of benzyl chloride and pyrid-4-thione, followed by oxidation of the resulting benzyl 4-pyridyl sulphide with potassium permanganate gave benzyl 4-pyridyl sulphone in greatly improved yield (60 per cent). Pyrid-4-thione and *p*-nitrobenzyl bromide gave the expected sulphide which was readily oxidised to 4-nitrobenzyl-4′-pyridyl sulphone (III, R = NO_2). Reductions of both 4-nitrobenzyl-4′-pyridyl sulphide and sulphone at a platinum catalyst were anomalous, and gave products possessing none of the properties of primary aromatic amines. The nitro-sulphide gave 4,4″-azoxybenzyl-4′-pyridyl sulphide (IV), characterised by analysis, formation of a dipicrate, and ultra-violet absorption spectrum, whilst the nitro-sulphone gave 4-hydroxylaminobenzyl-4′-pyridyl sulphone (III, R = NHOH). The latter structure was



(IV)

assigned on the basis of the hydrogen uptake (2 moles), analysis, and its ability to reduce ammoniacal silver nitrate in the cold. Similar incomplete

catalytic hydrogenations of aromatic nitro compounds are known, as for example that of 2-nitrofluorene to 2,2′-azoxyfluorene (Campbell and Temple, 1957), and 4-nitroquinoline-1-oxide to 4-hydroxylaminoquinoline-1-oxide (Ochiai, Ohta and Nomura, 1957). As with this latter compound, the insolubility of 4-hydroxylaminobenzyl 4′-pyridyl sulphone made a molecular weight determination impracticable. The partial hydrogenations described above probably follows from the low solubility of the isolated products, which are deposited from solution as the reaction proceeds. 4-Aminobenzyl-4′-pyridyl sulphide and 4-aminobenzyl-4′-pyridyl sulphone (III, R = NH_2) were obtained by reduction of the corresponding nitro compounds using iron powder and hydrochloric acid in boiling ethanol (Campbell and Temple, 1957). Both compounds were pale yellow when freshly prepared but on standing the colour intensified. Toxicity associated with the NH_2 group can often be decreased by acylation and Schiff base formation, without seriously reducing antibacterial activity (Buttle, Dewing, Foster, Gray and Stephenson, 1938), hence the acetyl derivatives (which are more stable) and several Schiff bases of the amino sulphide and amino sulphone were prepared.

Biological Results

We are indebted to Dr. S. R. M. Bushby of the Wellcome Research Laboratories for the *in vitro* examination of 4,4′-dipyridyl sulphide and sulphone, 4-acetylaminobenzyl-4′-pyridyl sulphide and sulphone, 4-aminobenzyl-4′-pyridyl sulphide, 4,4″-azoxybenzyl 4′-pyridyl sulphide against *Myco. tuberculosis* var. *hominis* H37Rv in Peizer and Schecter medium, and in presence of 33 per cent lysed blood. None of the compounds showed significant tuberculostatic activity compared with isoniazid.

The chemical similarity of pyridine-4-sulphinic acid and *p*-aminobenzoic acid prompted an examination of the former as an antimetabolite. We wish to express our thanks to Dr. E. O. Morris of the Microbiology Department of this College for screening this compound against a selection of Gram-positive and Gram-negative organisms. Antibacterial activity was negligible at concentrations below 1 in 1,000.

Experimental

Melting points, which are uncorrected, were determined on a hot-stage microscope. We wish to thank Mr. W. McCorkindale, Dr. A. C. Syme and Miss M. Buchanan for the micro-analyses.

Pyridine-4-sulphinic acid. A solution of pyridine-4-sulphonhydrazide (0·865 g.) in water (5 ml.) was heated on a water bath at 40° to 50° until effervescence ceased (*ca.* 10 min.). The yellow acidic solution was slowly added to acetone (100 ml.) and left at room temperature for several hr. *Pyridine-4-sulphinic acid* (0·45 g., 63 per cent) separated as yellow needles, m.p. 140–141° after washing with acetone and drying *in vacuo*. Found: C, 42·6; H, 3·7; N, 9·6; S, 21·9 per cent. Equiv. (titration) 145. $C_5H_5NO_2S$ requires C, 42·0; H, 3·5; N, 9·8; S, 22·4 per cent. Equiv. 143.

Sodium pyridine-4-sulphinate. Pyridine-4-sulphinic acid (0·72 g.) was suspended in ethanol (10 ml.) and a solution containing sodium (0·12 g.) dissolved in ethanol (10 ml.) added. The mixture was gently warmed till homogeneous and the alcohol removed under reduced pressure. The residue was washed with ethanol (2 ml.) and crystallised from ethanol to give sodium pyridine-4-sulphinate (0·58 g., 68 per cent) as small white needles. Found: Na, 13·7 per cent. $C_5H_4NO_2S$ Na requires Na, 13·9 per cent.

Pyridine-4-sulphonic acid. An aqueous solution of pyridine-4-sulphinic acid was treated dropwise with 30 per cent hydrogen peroxide until the yellow colour was discharged. The solution was concentrated and diluted with ethanol to give pyridine-4-sulphonic acid m.p. 330° (decomp.) on standing. (Comrie and Stenlake (1958a) give pyridine-4-sulphonic acid m.p. 333°, decomp.).

Benzyl 4-pyridyl sulphone (method a). Pyridine-4-sulphinic acid (0·72 g.) in ethanol (10 ml.) was converted into a solution of the sodium salt as before and benzyl chloride (0·63 g.) in ethanol (10 ml.) added. The mixture was refluxed on a water bath for 3 hr., concentrated progressively, and sodium chloride removed by filtration until the product (0·12 g., 10 per cent) m.p. 169–170° crystallised (Comrie and Stenlake (1958b) give m.p. 169–170°). *Picrate,* needles m.p. 190–191° (from methanol). Found: C, 46·6; H, 3·3; N, 11·6 per cent. $C_{18}H_{14}N_4O_9S\frac{1}{2}CH_3OH$ requires C, 46·4; H, 3·4; N, 11·7 per cent.

4,4′-Dipyridyl sulphide. Pyrid-4-thione (5·55 g.) in hot ethanol (40 ml.) was allowed to react with 4-chloropyridine (prepared by the method of Wibaut and Brockman, 1939) (5·7 g.) in ethanol (15 ml.). The deep-orange coloured solution was heated on a water bath till pale-yellow (*ca.* 15 min.), cooled and left overnight at 0°. The yellow crystals were separated and a further yield of crude product obtained by concentrating the filtrate. The crude solid was dissolved in water (10 ml.) basified with 20 per cent sodium hydroxide and extracted with ether (3 × 50 ml.) each extract being washed with water (5 ml.). The combined ether solution was dried ($CaCl_2$) and filtered, and the ether removed leaving a red oil which quickly set to a crystalline mass in a vacuum desiccator. Recrystallisation from light petroleum (b.p. 40–60°) gave 4,4′-dipyridyl sulphide (6·7 g., 71 per cent) as colourless needles m.p. 72°. Found: C, 63·6; H, 4·3; N, 14·9 per cent. Calc. for $C_{10}H_8N_2S$, C, 63·8; H, 4·3; N, 15·0 per cent. *Dipicrate* needles m.p. 228° (from methanol). Found: C, 40·9; H, 2·5; N, 18·0 per cent. Calc. for $C_{22}H_{14}N_8O_{14}S$, C, 40·9; H, 2·2; N, 17·3 per cent. (King and Ware (1939) give 4,4′-dipyridyl sulphide m.p. 72° and dipicrate m.p. 229°).

4,4′-Dipyridyl sulphone. 4,4′-Dipyridyl sulphide (1·88 g.) was dissolved in cold 20 per cent acetic acid (20 ml.) and 5 per cent potassium permanganate slowly added shaking after each addition, until an excess of reagent was considered to be present (*ca.* 35 ml. of reagent was added over a period of 45 min.). The mixture was left for a few hr. at room temperature and the precipitated manganese dioxide removed by the careful addition of 30 per cent hydrogen peroxide. The white solid

remaining suspended in the solution was removed by filtration and washed with water. The filtrate was neutralised with dilute ammonia, extracted with ether (4 × 50 ml.) filtered, and the ether solution dried (KOH). The volume was reduced to 10 to 15 ml. and the ether decanted from more solid which had separated. The solids were combined and crystallised from water to give 4,4′-*dipyridyl sulphone* (1·42 g.) as beautiful long lustrous needles m.p. 145°. Found: C, 54·5; H, 3·7; N, 12·4; S, 15·1 per cent. $C_{10}H_8N_2O_2S$ requires C, 54·6; H, 3·7; N, 12·7; S, 14·5 per cent. The ether was taken down to dryness leaving a pale-yellow oil (0·2 g.) which solidified on standing. Recrystallisation from light petroleum (b.p. 40–60°) gave 4,4′-dipyridyl sulphide m.p. 72° (mixed m.p. with starting material undepressed). The yield of 4,4′-dipyridyl sulphone allowing for recovery of unchanged starting material, 72 per cent.

4,4′-*Dipyridyl sulphone dihydrochloride.* Mixing cold ethanolic solutions of 4,4′-dipyridyl sulphone and hydrogen chloride, and washing the white crystalline solid with ethanol gave the *dihydrochloride* m.p. 138° (decomp.). Found: C, 41·4; H, 3·9; N, 9·5 per cent. $C_{10}H_{10}Cl_2N_2O_2S$ requires C, 41·0; H, 3·4; N, 9·6 per cent. The *monopicrate* separated in needles m.p. 175–176° on mixing methanolic soutions of 4,4′-dipyridyl sulphone and picric acid. Found: C, 42·7; H, 2·6; N, 15·6 per cent. $C_{16}H_{11}N_5O_9S$ requires C, 42·8; H, 2·5; N, 15·6 per cent.

Benzyl 4-*pyridyl sulphide hydrochloride.* A mixture of pyrid-4-thione (0·555 g.) and benzyl chloride (0·63 g.) in ethanol (15 ml.) was refluxed for 10 min. and the solution concentrated till crystallisation commenced. On standing *benzyl* 4-*pyridyl sulphide hydrochloride* (0·76 g., 64 per cent) separated. It was washed with ether and crystallised from ethanol in needles m.p. 196–198°. Found: C, 59·8; H, 4·6 per cent. $C_{12}H_{12}ClNS$ requires C, 60·6; H, 5·1 per cent. *Benzyl* 4-*pyridyl sulphide* was liberated from the hydrochloride by addition of dilute ammonia and extracted into ether (50 ml.). The ether was removed and the residue crystallised from aqueous ethanol to give the *product* (0·55 g., 54 per cent) as needles or prisms m.p. 70–71°. Found: C, 71·8; H, 5·8; N, 6·9 per cent. Calc. for $C_{12}H_{11}NS$, C, 71·6; H, 5·5, N, 7·0 per cent. (Stevenson, Cranham Cummings and Brookes (1956) give m.p. 69–71°). *Picrate* needles m.p. 170° (from methanol). Found: C, 50·4; H, 3·3; N, 12·8 per cent. $C_{18}H_{14}N_4O_7S$ requires C, 50·2; H, 3·3; N, 13·0 per cent.

Benzyl 4-*pyridyl sulphone* (*method b*). Benzyl 4-pyridyl sulphide (0·201 g.) in 20 per cent acetic acid (10 ml.) was oxidised with 5 per cent potassium permanganate as described under 4,4′-dipyridyl sulphone. The brown solid remaining suspended in solution after removal of manganese dioxide, was filtered washed with water and crystallised from ethanol (charcoal) giving glistening plates of *benzyl* 4-*pyridyl sulphone* (0·14 g., 60 per cent) m.p. 169–170°. Melting point when mixed with the product obtained in *method a*, undepressed. Picrate m.p. 190–191°.

4-*Nitrobenzyl* 4′-*pyridyl sulphide hydrobromide.* Pyrid-4-thione (1·11 g.) and *p*-nitrobenzyl bromide (2·16 g.) in ethanol (50 ml.) was refluxed on a water bath for 10 min. and the solution concentrated till crystallisation commenced. Recrystallisation of the solid which had separated

on standing for several hr., gave *4-nitrobenzyl 4'-pyridyl sulphide hydrobromide* (2·8 g., 85 per cent) as pale-yellow needles m.p. 220–221°. Found: C, 44·7; H, 3·8; N, 8·0 per cent. $C_{12}H_{11}BrN_2O_2S\frac{1}{2}C_2H_5OH$ requires C, 44·6; H, 4·0; N, 8·0 per cent.

4-Nitrobenzyl 4'-pyridyl sulphide. The hydrobromide obtained in the previous experiment was dissolved in warm water (50 ml.) and the solution made alkaline with dilute ammonia. The solid separating was filtered, washed with water and the filtrate extracted with ether (4 × 50 ml.). The ether was removed and the residue combined with the solid obtained on basifying with ammonia. Recrystallisation from methanol gave 4-nitrobenzyl 4'-pyridyl sulphide (2·0 g.) as pale yellow needles or prisms m.p. 98°. Found: C, 58·6; H, 4·3; N, 11·3 per cent. Calc. for $C_{12}H_{10}N_2O_2S$, C, 58·6; H, N, 4·1; 11·4 per cent. (Stevenson and others (1956) give m.p. 97–98·5°). Yield (calculated on pyrid-4-thione) 81 per cent. *Picrate*, needles m.p. 198–199° (from methanol). Found: C, 45·7; H, 2·8; N, 14·3 per cent. $C_{18}H_{13}N_5O_9S$ requires C, 45·5; H, 2·8; N, 14·7 per cent.

4-Nitrobenzyl 4'-pyridyl sulphone. 4-Nitrobenzyl 4'-pyridyl sulphide (1·23 g.) was dissolved in 33 per cent acetic acid (30 ml.) and oxidised with potassium permanganate as before. Addition of 30 per cent hydrogen peroxide gave a light brown precipitate which recrystallised from ethanol (charcoal) giving long pale-yellow needles of *4-nitrobenzyl 4'-pyridyl sulphone* (0·82 g., 59 per cent) m.p. 185–186°. Found: C, 52·0; H, 3·7; N, 10·0 per cent. $C_{12}H_{10}N_2O_4S$ requires C, 51·8; H, 3·6; N, 10·1 per cent. *Picrate*, needles m.p. 191° (from methanol). Found: C, 42·8; H, 2·6; N, 13·4 per cent. $C_{18}H_{13}N_5O_{11}S$ requires C, 42·7; H, 2·6; N, 13·8 per cent.

4,4''-Azoxybenzyl 4'-pyridyl sulphide. 4-Nitrobenzyl 4'-pyridyl sulphide (0·82 g.) in ethanol (100 ml.) was hydrogenated at room temperature and atmospheric pressure using Adams' platinum oxide catalyst (50 mg.) till no further uptake of gas occurred. The solid which had crystallised from solution was redissolved by boiling and the catalyst removed by filtration. The solvent was removed under reduced pressure and the residue recrystallised from a large volume of ethanol to give *4,4''-azoxybenzyl 4'-pyridyl sulphide* (0·5 g., 68 per cent) as orange needles m.p. 206–208°. Found: C, 65·1; H, 4·9; N, 12·5 per cent. $C_{24}H_{20}N_4OS_2$ requires C, 64·9; H, 4·5; N, 12·6 per cent. *Dipicrate* m.p. 210–212° (from ethanol). Found: C, 47·8; H, 3·2; N, 15·2 per cent. $C_{36}H_{26}N_{10}O_{15}S_2$ requires C, 47·9; H, 2·9; N, 15·5 per cent.

4-Aminobenzyl 4'-pyridyl sulphide. Finely divided iron powder (1·0 g.) was added to a boiling solution of 4-nitrobenzyl 4'-pyridyl sulphide(1·23g.) in ethanol (80 ml.). Concentrated hydrochloric acid (10 ml.) was added dropwise over a period of *ca.* 30 min. and the mixture refluxed for a further 1½ hr. before taking down to dryness under reduced pressure. The residue was dissolved in water (10 ml.), made alkaline with dilute ammonia and extracted with ether (4 × 50 ml.). The ethereal solution was filtered and concentrated till crystallisation commenced. On standing *4-aminobenzyl 4'-pyridyl sulphide* (0·51 g., 48 per cent) separated as pale-yellow glistening plates, m.p. 167–168° when dried *in vacuo*. Found:

C, 66·6; H, 5·4; N, 12·9 per cent. $C_{12}H_{12}N_2S$ requires C, 66·7; H, 5·6; N, 13·0 per cent. *Monopicrate* rosettes m.p. 170° (dried at 100°) (from ethanol). Found: C, 48·8; H, 3·8; N, 15·5 per cent. $C_{18}H_{15}N_5O_7S$ requires C, 48·5; H, 3·4; N, 15·7 per cent.

4-*Acetylaminobenzyl 4′-pyridyl sulphide.* 4-Aminobenzyl 4′-pyridyl sulphide (0·216 g.) was dissolved in acetic anhydride (2 ml.) and heated on a water bath for 5 min. The solvent was removed under reduced pressure and the solid residue recrystallised from methanol (charcoal) to give 4-*acetylaminobenzyl 4′-pyridyl sulphide* (0·165 g., 64 per cent) as long needles m.p. 157°. Found: C, 64·8; H, 5·6; N, 10·7 per cent. $C_{14}H_{14}N_2OS$ requires C, 65·1; H, 5·5; N, 10·85 per cent. *Picrate* needles m.p. 179° resolidifying in spikes and remelting at 199° (from ethanol). Found: C, 49·7; H, 4·2; N, 14·2 per cent. $C_{20}H_{17}N_5O_8S\frac{1}{2}C_2H_5OH$ requires 49·4; H, 4·0; N, 13·7 per cent.

4-*Hydroxylaminobenzyl 4′-pyridyl sulphone.* 4-Nitrobenzyl 4′-pyridyl sulphone (0·93 g.) was dissolved in ethanol (150 ml.) by the aid of heat. The solution was cooled and hydrogenation carried out as described under 4,4″-azoxybenzyl 4′-pyridyl sulphide. The catalyst was removed as before and on concentrating the filtrate the *product* separated as a yellow micro-crystalline solid (0·51 g., 58 per cent) which did not melt below 330°. Found: C, 54·9; H, 4·5; N, 10·5 per cent. $C_{12}H_{12}N_2O_3S$ requires C, 54·5; H, 4·6; N, 10·6 per cent.

4-*Aminobenzyl 4′-pyridyl sulphone.* 4-Nitrobenzyl 4′-pyridyl sulphone (0·93 g.) was reduced with iron powder and concentrated hydrochloric acid in boiling ethanol as described under 4-aminobenzyl 4′-pyridyl sulphide and the reaction mixture concentrated till solid started to separate. After standing for several hr. the solid was removed and washed liberally with ethanol. It was suspended in water (10 ml.) made alkaline with dilute ammonia and extracted with ether (4 × 50 ml.). The ether solution was filtered and the volume reduced to *ca.* 100 ml. On standing the *product* separated as the hemihydrate in pale-yellow shining needles m.p. 159–160°. Yield, 0·39 g., 42 per cent. Found: C, 56·25; H, 5·4; N, 11·2 per cent. $C_{12}H_{12}N_2O_2S\frac{1}{2}H_2O$ requires C, 56·0; H, 5·1; N, 10·9 per cent. The *dipicrate* separated in sheaves m.p. 174–175° (decomp.) (from ethanol). Found: C, 41·3; H, 3·1; N, 14·2 per cent. $C_{24}H_{18}N_8O_{16}SC_2H_5OH$ requires C, 41·5; H, 3·2; N, 14·9 per cent.

4-*Acetylaminobenzyl 4′-pyridyl sulphone.* 4-Acetylaminobenzyl 4′-pyridyl sulphide (0·258 g.) was dissolved in 33 per cent acetic acid (35 ml.) and oxidised with potassium permanganate as described under 4-nitrobenzyl 4′-pyridyl sulphone, giving 4-*acetylaminobenzyl 4′-pyridyl sulphone* (0·14 g., 48 per cent) as shining needles of the hemihydrate m.p. 210–211°. Found: 56·6; H, 5·1; N, 9·4 per cent. $C_{14}H_{14}N_2O_3S\frac{1}{2}H_2O$ requires C, 56·2; H, 5·05; N, 9·4 per cent. The same product was obtained in 42 per cent yield by acetylating 4-aminobenzyl 4′-pyridyl sulphone by the method described under 4-acetylaminobenzyl 4′-pyridyl sulphide. *Picrate* prisms m.p. 200–201° (from methanol). Found: C, 45·8; H, 3·3; N, 13·4 per cent. $C_{20}H_{17}N_5O_{10}S$ requires C, 46·2; H, 3·3; N, 13·5 per cent.

The following Schiff bases were prepared by mixing methanolic solutions containing equimolecular proportions of 4-aminobenzyl 4′-pyridyl sulphide and the appropriate carbonyl compound, and recrystallising the product from methanol.

Yields are given in parenthesis.

4-*Benzylideneaminobenzyl 4′-pyridyl sulphide*, aggregates of pale-yellow prisms m.p. 158–159° (89 per cent). Found: C, 75·7; H, 5·4; N, 9·25 per cent. $C_{19}H_{16}N_2S$ requires C, 75·0; H, 5·3; N, 9·2 per cent.

4-(o-*Hydroxybenzylideneamino*)*benzyl 4′-pyridyl sulphide*, orange-yellow needles m.p. 197–198° (washed with methanol) (69 per cent). Found: C, 71·7; H, 5·4; N, 8·6 per cent. $C_{19}H_{16}N_2OS$ requires C, 71·25; H, 5·0; N, 8·75 per cent.

4-(p-*Hydroxybenzylideneamino*)*benzyl 4′-pyridyl sulphide* (commences to char *ca.* 240° but does not melt below 340°) small pale-yellow prisms (75 per cent). Found: C, 71·45; H, 5·2; N, 9·1 per cent. $C_{19}H_{16}N_2OS$ requires C, 71·25; H, 5·0; N, 8·75 per cent.

Schiff bases of 4-aminobenzyl 4′-pyridyl sulphone were similarly prepared.

4-*Benzylideneaminobenzyl 4′-pyridyl sulphone*, glistening pale-yellow plates m.p. 208–209° (85 per cent). Found: C, 67·75; H, 4·9; N, 8·6 per cent. $C_{19}H_{16}N_2O_2S$ requires C, 67·8; H, 4·8; N, 8·3 per cent.

4-(o-*Hydroxybenzylideneamino*)*benzyl 4′-pyridyl sulphone*, pale-yellow shining plates m.p. 206–207° (77 per cent). Found: C, 65·3; H, 4·6; N, 8·4 per cent. $C_{19}H_{16}N_2O_3S$ requires C, 64·8; H, 4·6; N, 8·0 per cent.

4-(p-*Hydroxybenzylideneamino*)*benzyl 4′-pyridyl sulphone hemihydrate*, bright-yellow prisms m.p. 182–183° (80 per cent). Found: C, 63·1; H, 5·15; N, 8·6 per cent. $C_{19}H_{16}N_2O_3S\frac{1}{2}H_2O$ requires C, 63·2; H, 4·7; N, 7·8 per cent.

4-*Cinnamylideneaminobenzyl 4′-pyridyl sulphone hemihydrate* yellow shining plates m.p. 193° (75 per cent). Found: C, 67·9; H, 5·2; N, 7·7 per cent. $C_{21}H_{18}N_2O_2S\frac{1}{2}H_2O$ requires C, 67·9; H, 5·15; N, 7·5 per cent.

References

Burton, H., and Davy, W. A. (1947). *J. chem. Soc.*, 52–55.

Buttle, G. A. H., Dewing, T., Foster, G. E., Gray, W. H., Smith, S., and Stephenson, D. (1938). *Biochem. J.*, **32**, 1101–1110.

Campbell, N., and Temple, A. F. (1957). *J. chem. Soc.*, 207–212.

Comrie, A. M., Mital, H. C., and Stenlake, J. B. (1960). *J. med. pharm. Chem.*, **2**, 171–177.

Comrie, A. M., and Stenlake, J. B. (1958a). *J. chem. Soc.*, 1853–1854.

Comrie, A. M., and Stenlake, J. B. (1958b). *Ibid.*, 3514–3518.

Dewing, T., Gray, W. H., Platt, B. C., and Stephenson, D. (1942). *Ibid.*, 239–244.

Goldberg, M. W., and Teitel, S. (1954). U.S. 2,761,866.

King, H., and Ware, L. C. (1939). *J. chem. Soc.*, 873–877.

Kwart, H., and Miller, L. J. (1958). *J. Amer. chem. Soc.*, **80**, 884–887.

McFadyen, J. S., and Stevens, T. S. (1936). *J. chem. Soc.*, 584–587.

Ochiai, E., Ohta, A., and Nomura, H. (1957). *Pharm. Bull. Japan*, **5**, 310–313.

Ochiai, E., Itai, T., and Yoshino, K. (1944). *Proc. Imp. Acad. (Tokyo)*, **20**, 141–145.

Stevenson, H. A., Cranham, J. E., Cummings, A. W., and Brookes, R. F. (1956). Brit. Pat. 758,658.

Takahashi, T., Shibasaki, J., and Uchibayashi, M. (1954). *Pharm. Bull. Japan*, **2**, 30–34.

Wibaut, J. P., and Brockman, F. W. (1939). *Rec. trav. chim.*, **58**, 885–894.

THE ACTIVITY OF POLYMETHYLENE-BIS-4-AMINOQUINALDINIUM SALTS AGAINST *PITYROSPORUM OVALE* AND *CANDIDA ALBICANS*

By W. A. Cox and P. F. D'Arcy

From the Research Division, Allen & Hanburys Limited, Ware, Hertfordshire

Received September 14, 1960

A homologous series of polymethylene-bis-4-aminoquinaldinium salts has been shown to possess marked fungistatic activity against the potential pathogens *Candida albicans* and *Pityrosporum ovale*. The peak of antifungal activity against *C. albicans* occurs at a chain length $(CH_2)_{12}$, whilst against *P. ovale* the most active member of the series has a chain length $(CH_2)_6$. The decamethylene member (dequalinium), shows a potent and rapid fungicidal activity against both yeasts; quantitative data, from experiments involving short periods of drug-yeast contact, indicates that this activity of dequalinium is a function of the concentration of the quaternary compound and of the time of exposure.

The polymethylene-bis-4-aminoquinaldinium salts (B.A.Q.D.) have been reported by Babbs, Collier, Austin, Potter and Taylor (1956) to have potent antibacterial activity, of this series the decamethylene member, dequalinium, has been found to be highly effective as a topical antibacterial agent (Babbs and others, 1956; Collier, Cox, Huskinson and Robinson, 1959).

In this present work dequalinium and some other members of this series have been examined for their activity against two yeasts, *Pityrosporum ovale* and *Candida albicans.*

Pityrosporum ovale has been isolated from human scurf and although regarded by some workers to be the causative agent of dandruff (Sabouraud, 1902; Macleod and Dowling, 1928; Barber, 1948; Reddish, 1952), it is held by others to be a mere harmless saprophyte (Whitlock, 1953; Rocha, Silva, Lima and Goto, 1952). However, Hughes and Hamilton (1958) have recently shown that this fungus is the cause of an allergy to human scurf, and in fact responsible, to a considerable degree, for the conditions of eczema, rhinorrhoea and asthma frequently found in association with dandruff.

Candida albicans has long been known as one of the more common causes of superficial fungus infections, and is responsible for the varied conditions commonly classified under the general terminology of moniliasis.

Experimental

Materials and Methods

Fungistatic Activity

P. ovale. Serial twofold dilutions of test compounds were prepared in malt extract broth with an added fat source (0·05 per cent sterile cream). Inoculations were made with a standard suspension of the fungus grown on malt extract agar with added cream. The tube dilutions were incubated for 7 days at 37° and the minimum inhibitory concentration was determined for each compound both by visual observation and by subculture.

C. albicans. Double strength aqueous solutions of the compounds were prepared and mixed with double strength Sabouraud's broth (Glucose 4 per cent; A. & H. Eupeptone No. 2 1·0 per cent). Twofold serial dilutions were made each of a final volume of 2 ml., these were sterilised by autoclaving at 10 lb./sq. in. for 10 min. Tubes were inoculated with 0·02 ml. of an 18-hr. broth culture of *C. albicans*, grown at 37° and standardised to an arbitrary opacity (approx. 500,000 cells/ml.); the tubes were then incubated at 37° and the minimum inhibitory concentration for each compound was determined visually after 7 days.

Fungicidal Activity

P. ovale. The fungus was cultured for 3 days at 37° on slopes of tauroglycocholate agar (sodium tauroglycocholate 10 per cent; Oxoid mycological peptone 5 per cent; agar 1·5 per cent, adjusted to pH 5·0). A washed cell suspension was prepared in sterile distilled water to a

TABLE I

FUNGISTATIC ACTIVITY OF B.A.Q.D. SERIES AGAINST *P. ovale* AND *C. albicans*

Polymethylene chain length	M.I.C. μg./ml. base	
	P. ovale	*C. albicans*
B.A.Q.D. 4	4·4	>100
6	2·2	50
8	6·3	2·5
10	8·8	1·25
12	—	0·32
14	25	0·63
16	—	2·5
18	35	10
20	100	10

standard turbidity. The number of viable units/ml. of suspension was determined by plating out after suitable dilution following the technique described in detail below.

Culture suspensions (approximately 5×10^5 viable cells/ml.) were mixed with solutions of test compounds for contact times of 1, 5 or 30 min.; samples were then withdrawn and inactivated by Lubrol W (polyethylene oxide condensate), 1 ml. of the sample was diluted × 10 by a 1 per cent solution of this inactivator, and plated out on to tauroglycocholate agar plates following the technique of Miles and Misra (1938). The plates were incubated at 37° for 48–60 hr. and the colonies of fungi counted. The fungicidal activity of a specific concentration of test compound at a stated time of contact was expressed as a mean percentage kill.

C. albicans. The yeast was grown on Sabouraud's agar at 37° for 18 hr.; the growth was then suspended in water, centrifuged (R.C.F. 600 *g.*/10 min.) and resuspended in distilled water to produce a standard opacity of approximately 1×10^7 viable cells/ml. (Wellcome opacity tube 5).

An inoculum of 1·0 ml. of this washed cell culture was added to 9·0 ml. of an aqueous solution of the test compound; samples of 1·0 ml. were withdrawn after contact times of 1, 5 or 30 min. and were inactivated by pipetting into 9 ml. of a 2·0 per cent oxgall solution (Difco). The number

of surviving yeast cells were determined by a plating out technique in Sabouraud's agar; the number of resulting colonies was counted after incubation at 37° for 3 days.

Fungicidal activity was expressed by the percentage loss of viability when compared with the control count of the standardised yeast suspension.

RESULTS

The fungistatic activity of compounds B.A.Q.D. 4, 6, 8, 10, 14, 18 and 20 against *P. ovale* and compounds B.A.Q.D. 4, 6, 8, 10, 12, 14, 16, 18 and 20 against *C. albicans* is summarised in Table I. The compounds are listed according to the length of their polymethylene chain, and the minimum inhibitory concentration for each member of the series is expressed in μg./ml. calculated as the base.

TABLE II

FUNGICIDAL ACTIVITY OF B.A.Q.D. 10 AGAINST *P. ovale*

Concentration per cent	Contact min.	Mean kill per cent*
0·01	1	100
0·01	5	100
0·01	30	100
0·005	1	98·083
0·005	5	99·833
0·005	30	100
0·004	1	92·115
0·004	5	98·404
0·004	30	100
0·0025	1	87·886
0·0025	5	98·688
0·0025	30	100
0·001	1	33·579
0·001	5	61·599
0·001	30	100

* *P. ovale* suspension 5×10^5 viable cells/ml.

There is a well defined gradation of activity within the B.A.Q.D. series against both fungal species. Potent fungistatic activity against *P. ovale* is shown by compounds B.A.Q.D. 4, 6, 8 and 10 and is maximal with the hexamethylene member (B.A.Q.D. 6). Compounds B.A.Q.D. 8, 10, 12, 14 and 16 exhibit high activity against *C. albicans*, and this effect is maximal with compound B.A.Q.D. 12.

Fungicidal tests were confined to a study of the activity of the decamethylene member of the series. Dequalinium was selected from those compounds showing good fungistatic activity because of its potency against other microbial species (Babbs and others, 1956, Collier and others, 1959).

The fungicidal activity of dequalinium against *P. ovale* and against *C. albicans* is shown in Table II and in Table III respectively; fungicidal activity is expressed by the figure for the mean percentage kill. In the former tests the compound was examined at concentrations from 0·001 to 0·01 per cent and in the latter 0·001 to 0·02 per cent at drug-organism contact times of 1, 5 and 30 min.

The results of the fungicidal tests show that dequalinium is 100 per cent effective against *P. ovale* at a concentration of 0·01 per cent for a contact time of 1 min., and has the same activity against *C. albicans* at a concentration of 0·02 per cent for 1 min. This fungicidal action is also evident at lower concentrations of the compound when the contact time is

TABLE III

FUNGICIDAL ACTIVITY OF B.A.Q.D. 10 AGAINST *C. albicans*

Concentration per cent	Contact min.	Mean kill per cent*
0·02	1	100
0·02	5	100
0·02	30	100
0·01	1	99·702
0·01	5	99·999
0·01	30	100
0·005	1	99·162
0·005	5	99·947
0·005	30	99·977
0·0025	1	71·833
0·0025	5	96·660
0·0025	30	99·979
0·001	1	25·882
0·001	5	61·176
0·001	30	84·705

* *C. albicans* suspension 1 × 10^8 viable cells/ml.

increased. Although both fungal species show a very similar degree of sensitivity to dequalinium, it would appear that the compound, at low concentrations, is more effective against *P. ovale* than against *C. albicans.*

DISCUSSION

Candida species and *P. ovale* have been regarded by most taxonomists as being sufficiently closely related to be included together in the sub-family Cryptococcoideae of the yeasts, although below this level of classification the morphology and physiology of *C. albicans* and *P. ovale* are different in a number of respects; they differ, for example, in their ability to ferment sugars and to utilise ammonium salts as a source of nitrogen. Certain species of *Candida* notably *C. lipolytica* and *C. rugosa* appear to lie intermediate between *C. albicans* and *P. ovale* in that they utilise fats for growth and are unable to ferment sugars. In view of these points of dissimilarity between the two species studied it is interesting to note that the peak of fungistatic activity of the B.A.Q.D. series is n = 6 against *P. ovale* and n = 12–14 against *C. albicans.* This peak of activity against *P. ovale* is surprising in view of the previous studies on the bacteriostatic and fungistatic activity of this series which reveal a general pattern of maximal activity between the decyl and the hexadecyl members (Babbs and others, 1956; Cox and D'Arcy, 1959). It would seem likely that this difference in the most effective length of the polymethylene chain could be attributed to either an essential difference in the distribution of the receptor groups, susceptible to these quaternaries, or differences in lipid solubility between *P. ovale* and other fungi including *C. albicans.*

Dequalinium, which showed good activity against both species in the fungistatic tests, was examined for fungicidal activity because it has been shown to be effective in the treatment of local infections with *C. albicans* (Coles, Grubb, Mathuranayagam and Wilkinson, 1958; Stockdale and Banks, 1959; Roddie, 1958; Levinson, 1959), and in initial clinical investigations has proved highly effective in cases of seborrhoea and infective dandruff (Colin-Jones private communication). Some initial laboratory observations on the effects of dequalinium salts on the growth of *P. ovale* have been published elsewhere (Cox, D'Arcy, Hedge and Wilkinson, 1960).

Dequalinium has potent fungicidal activity against both species, the slightly more potent effect against *P. ovale* is in direct contrast to the fungistatic results, in which dequalinium is about eight times more active against *C. albicans* than against *P. ovale*.

This anomaly may be partly explained by the inactivation of this type of quaternary ammonium compound by the fat source in the media, which is essential for the growth of *P. ovale*. Because of this, it was in fact, necessary to perform the fungistatic tests on this series of compounds in media, in which the constituents did not neutralise antifungal activity. Media containing bile salts (Martin Scott, 1952), and fatty acids (oleic acid) were found to be unsatisfactory whilst a malt extract media with added cream was found to be the most suitable.

Acknowledgement. We thank Miss C. Bedford for her technical assistance.

The strain of *P. ovale* used in these experiments was kindly supplied by the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine; and the strain of *C. albicans* by Dr. R. W. Riddell.

References

Babbs, M., Collier, H. O. J., Austin, W. C., Potter, M. D. and Taylor, E. P. (1956). *J. Pharm. Pharmacol.*, **8**, 110–119.

Barber, H. W., in MacKenna, R. M. B. (1948). *Modern Trends in Dermatology.* London: Butterworths Scientific Publications.

Coles, R. B., Grubb, C., Mathuranayagam, D. and Wilkinson, D. S. (1958). *Brit. med. J.*, **2**, 1014–1016.

Collier, H. O. J., Cox, W. A., Huskinson, P. L. and Robinson, F. A. (1959). *J. Pharm. Pharmacol.*, **11**, 671–680.

Cox, W. A. and D'Arcy, P. F. (1959). *J. gen. Microbiol.*, **20**, i–ii.

Cox, W. A., D'Arcy, P. F., Hedge, M. J. and Wilkinson, G. R. (1960). *J. Soc. cosmet. Chem.*, **11**, 37–42.

Hughes, W. H. and Hamilton, E. D. (1958). *Brit. med. J.*, **2**, 542–544.

Levinson, D. R. (1959). *Practitioner*, **183**, 195–197.

Macleod, J. M. H. and Dowling, G. B. (1928). *Brit. J. Dermatol.*, **40**, 139–148.

Martin Scott, I. (1952). *Ibid.*, **64**, 257–273.

Miles, A. A. and Misra, S. S. (1938). *J. Hyg. Camb.*, **38**, 732–749.

Reddish, G. F. (1952). *J. Soc. cosmet. Chem.*, **3**, 90–98.

Rocha, G. L., Silva, C., Lima, A. O. and Goto, M. (1952). *J. invest. Dermatol.*, **19**, 289–292.

Roddie, T. W. (1958). *Med. J. Malaya.*, **13**, 171–172.

Sabouraud, R. (1902). *Maladies du Cuir Chevelu*, Paris.

Stockdale, C. R. and Banks, L. L. (1959). *Practitioner*, **182**, 215–217.

Whitlock, F. A. (1953). *Brit. med. J.*, **1**, 484–487.

THE PREPARATION AND PHARMACOLOGY OF SOME PHENOLIC CARBAMATES AND ALLOPHANATES

By J. H. Barnes, Marguerite V. A. Chapman, P. A. McCrea, P. G. Marshall and Pamela A. Walsh

From British Schering Research Laboratories Ltd., Alderley Edge, Cheshire

Received September 1, 1960

A series of alkyl-substituted phenyl carbamates has been screened for analgesic activity by two methods and the ED50 of a representative number has been determined by one of them. An attempt has been made to correlate the analgesic activity with chemical constitution and the following points emerge: (i) at least one *m*-alkyl group is essential for significant activity, (ii) where only one substituent is present, maximal activity occurs when this is ethyl, (iii) two *o*-substituents abolish the activity, (iv) 2,4,5-trimethylphenyl carbamate (compound 686) possesses the highest activity, (v) the most active compounds are in general also the most toxic, compound 688 being a notable exception, (vi) *o*-substitution by a group larger than methyl tends to reduce activity, (vii) substitution on the carbamate N, except by hydroxyl, strongly reduces the activity. The toxicity of some of the compounds could not be attributed to their anticholinesterase activity.

Some years ago the reassessment of salicylamide and its related compounds as analgesics was undertaken, in the hope that this might lead to the development of an improved drug of the aspirin type. This led to the appraisal of other amides, for example, phenoxyacetamide, and to the synthesis of a large series of phenolic carbamates, the first member of which, *o*-chlorophenyl carbamate, had shown significant activity by the screening test then employed by us. Most of the members of this series are carbamates of mono- and polyalkyl phenols.

Chemical

The following are new intermediates which were obtained by standard methods.

3,5-*Dimethylphenol* n-*butyrate*. A solution of 3,5-dimethylphenol (122 g.) in pyridine (200 ml.) was cooled in ice and stirred whilst butyric anhydride (173·8 g., 1·1 mole) was added during 30 minutes. After standing overnight the mixture was heated for 1 hr. at 100°, cooled and poured on ice and hydrochloric acid. The ester was extracted with ether, and the extract washed with water, dried and evaporated. The residue was distilled, b.p. 127° at 14 mm. Found: C, 74·5; H, 8·2. $C_{12}H_{16}O_2$ requires C, 75·0; H, 8·3 per cent.

2-n-*Butyryl*-3,5-*dimethylphenol* was prepared by Fries re-arrangement (1 mole $AlCl_3$ in CS_2) of 3,5-dimethylphenol *n*-butyrate. It crystallised from light petroleum (b.p. 40–60°), m.p. 55–58·5°. Found: C, 74·3; H, 8·4. $C_{12}H_{13}O_2$ requires C, 75·0; H, 8·3 per cent.

2-n-*Butyl*-3,5-*dimethylphenol* was prepared by Clemmensen reduction of 2-*n*-butyryl-3,5-dimethylphenol. It crystallised from light petroleum

TABLE I

A SUMMARY OF THE NEW CHLOROFORMATES

Phenyl chloroformate	Boiling point	Formula	Found			Required		
			C	H	Cl	C	H	Cl
3,5-Dimethyl-	96–97° at 12 mm.	$C_9H_9ClO_2$	58·6	5·1	19·3	58·6	4·9	19·2
3-Ethyl-5-methyl	107–108° at 11 mm.	$C_{10}H_{11}ClO_2$	61·1	5·7	17·5	60·5	5·5	17·9
3,4-Dimethyl-	101° at 13 mm.	$C_9H_9ClO_2$	58·6	4·9	19·4	58·6	4·9	19·2
2,5-Dimethyl-	89–90° at 11 mm.	$C_9H_9ClO_2$	59·2	4·8	19·2	58·6	4·9	19·2
2,6-Dimethyl-	83–83·5° at 11 mm.	$C_9H_9ClO_2$	—	—	18·5	—	—	19·2
2,3-Dimethyl-	94° at 12 mm.	$C_9H_9ClO_2$	58·9	5·0	19·0	58·6	4·9	19·2
2,3,5-Trimethyl-	108° at 12 mm.	$C_{10}H_{11}ClO_2$	61·0	6·0	17·4	60·5	5·5	17·9
2-Phenyl-	86–90° at 0·05 mm., m.p. 59–62·5°, except light petroleum	$C_{13}H_9ClO_2$	—	—	15·8	—	—	15·3
p-Phenyl-	132–137° at 0·4 mm., m.p. 35–40°, except light petroleum	$C_{13}H_9ClO_2$	67·6	3·9	14·8	67·1	3·9	15·3

TABLE II

A SUMMARY OF THE NEW ALLOPHANATES

Phenyl allophanate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
3-Ethyl-	1	155–158° decomp.	Methanol	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·7	57·7	5·8	13·5
2,5-Dimethyl- ..	1	183–184° decomp.	Ethyl acetate	$C_{10}H_{12}N_2O_3$	57·6	6·1	13·7	57·7	5·8	13·5
3,5-Dimethyl- ..	2	199° decomp.	Ethanol	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·1	57·7	5·8	13·5
3,4-Dimethyl- ..	2	167° decomp.	Ethanol	$C_{10}H_{12}N_2O_3$	58·0	5·9	13·1	57·7	5·8	13·5
2,3-Dimethyl- ..	2	200° decomp.	Ethyl acetate	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·1	57·7	5·8	13·5
3-Ethyl-5-methyl-	2	168° decomp.	Ethanol	$C_{12}H_{14}N_2O_3$	59·7	6·2	12·2	59·5	6·3	12·6

(b.p. 30–40°), m.p. 64·5–66°. Found: C, 80·6; H, 9·7. $C_{12}H_{18}O$ requires C, 80·9; H, 10·1 per cent.

2-n-*Propyl*-3,5-*dimethylphenol* was prepared by Clemmensen reduction of the corresponding propiophenone. It crystallised from *n*-hexane, m.p. 53·5–54°. Found: C, 80·6; H, 9·9. $C_{11}H_{16}O$ requires C, 80·5; H, 9·8 per cent.

4-n-*Propyl*-2,5-*dimethylphenol* was prepared by Clemmensen reduction of the corresponding propiophenone. It has b.p. 132° at 18 mm. Found: C, 80·5; H, 10·0. $C_{11}H_{16}O$ requires C, 80·5; H, 9·8 per cent.

Preparation of chloroformates. A 20 per cent w/v solution of sodium hydroxide (220 ml.) was added to a solution of the phenol (1 g. mole) and phosgene (100 g.) in carbon tetrachloride (500 ml.) at −5° with vigorous stirring for 45 min. The organic layer was separated, washed with N sodium hydroxide and water, and dried over anhydrous sodium sulphate. The solvent was removed on the steam bath and the residual chloroformate was distilled *in vacuo*.

Table I lists the new chloroformates.

Preparation of Allophanates

Method 1. Cyanic acid vapour, from the thermal depolymerisation of cyanuric acid, was passed into an ethereal solution of a phenol until the theoretical weight increase had occurred. The crystalline precipitate was collected the next day (Blohm and Becker, 1952).

Method 2. Blohm and Becker (1952) found that ethyl and 3-chloropropyl chloroformates yield allophanates on heating with urea. Aryl chloroformates gave good yields of allophanates by this method. A phenyl chloroformate (1 mole) and urea (2 moles) protected from moist air were heated on the water bath until the urea dissolved and the mass re-solidified. After a further 3 hr. heating the mass was extracted with water and ether. The residue was crude allophanate. 2,6-Dimethylphenyl chloroformate did not react with urea.

Table II lists the new allophanates prepared.

Preparation of Carbamates

Method 1. A solution of the crude chloroformate, prepared as previously described, in carbon tetrachloride was reacted with 2 moles of ammonia or the appropriate amine to give the carbamate.

Method 2. 0·2 mole of a phenol was dissolved in dry carbon tetrachloride (100 ml.), dry powdered sodium cyanate (13 g.) was added, and the mixture was stirred whilst a solution of trichloracetic acid (33 g.) in carbon tetrachloride (80 ml.) was added. Stirring was continued at 55° for 6 hr. After cooling, water was added with stirring and the organic layer was separated, washed with N sodium hydroxide and water, and dried over anhydrous sodium sulphate. The solvent was evaporated and the residue was crystallised from the appropriate solvent.

Table III lists the phenyl carbamates prepared.

A number of miscellaneous carbamates are given in Table IV.

TABLE III

A LIST OF THE PREPARED PHENYLCARBAMATES

Phenyl carbamate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
3-Methyl-*	1	139°	Ethyl acetate	$C_8H_9NO_2$	64·2	6·0	9·1	63·6	6·0	9·3
3-n-Propyl-	1	108–9°	Cyclohexane	$C_{10}H_{13}NO_2$	67·0	7·1	7·9	67·1	7·3	7·8
3-Ethyl-	2	103–4°	50 per cent. aq. methanol	$C_9H_{11}NO_2$	65·5	6·7	8·8	65·5	6·7	8·5
3-n-Butyl-	2	110–112°	Cyclohexane	$C_{11}H_{15}NO_2$	69·0	8·0	7·1	68·4	7·8	7·3
3-n-Pentadecyl-	2	97°	Benzene	$C_{23}H_{37}NO_2$	76·1	10·6	4·3	76·2	10·7	4·0
4-n-Propyl-	2	104–6°	Cyclohexane	$C_{10}H_{13}NO_2$	67·1	7·5	7·9	67·1	7·3	7·8
2-Allyl-	2	117–8°	Cyclohexane	$C_{10}H_{11}NO_2$	68·1	6·0	8·0	67·8	6·2	7·9
2,3-Dimethyl-	1	138–141°	Benzene	$C_9H_{11}NO_2$	66·0	6·6	8·2	65·5	6·7	8·5
2,5-Dimethyl-	2	109–110°	Cyclohexane	$C_9H_{11}NO_2$	65·9	6·6	8·5	65·5	6·7	8·5
2,6-Dimethyl-	1	177° decomp.	Ethanol	$C_9H_{11}NO_2$	65·7	6·7	8·8	65·5	6·7	8·5
2-Ethyl-5-methyl-	2	106–7°	Cyclohexane	$C_{10}H_{13}NO_2$	67·5	7·6	8·0	67·1	7·3	7·8
2-n-Propyl-5-methyl	2	105–6°	Cyclohexane	$C_{11}H_{15}NO_2$	68·5	7·7	7·0	68·4	7·8	7·3
2-n-Butyl-5-methyl-	2	87–87·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·5	8·0	7·0	69·6	8·2	6·8
3-Methyl-4-ethyl-	2	99–100°	Cyclohexane	$C_{10}H_{13}NO_2$	67·0	7·5	7·9	67·1	7·3	7·8
3-Methyl-5-ethyl	2	100–101°	Benzene	$C_{10}H_{13}NO_2$	67·1	6·9	7·5	67·1	7·3	7·8
3-Methyl-4-n-propyl-	2	117–118°	Cyclohexane	$C_{11}H_{15}NO_2$	68·4	7·8	—	68·4	7·8	—
2,3,4-Trimethyl-	2	162–164°	Benzene	$C_{10}H_{13}NO_2$	67·7	7·2	7·8	67·1	7·3	7·8
2,3,5-Trimethyl-	1	137–139°	Benzene, light petroleum	$C_{10}H_{13}NO_2$	66·9	7·0	8·2	67·1	7·3	7·8
3,4,5-Trimethyl-	1	163–166°	Ethanol	$C_{10}H_{13}NO_2$	67·0	7·3	8·2	67·1	7·3	7·8
2,5-Dimethyl-4-ethyl	2	138–140°	Benzene	$C_{11}H_{15}NO_2$	68·4	7·5	6·7	68·4	7·8	7·3
2,5-Dimethyl-4-n-propyl	2	132–133·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·4	7·8	7·0	69·6	8·2	6·8
2,3-Dimethyl-6-ethyl-	2	132·5–134·5°	Carbon tetrachloride	$C_{11}H_{15}NO_2$	68·7	8·2	7·0	68·4	7·8	7·3
2-Ethyl-3,5-dimethyl-	1	110·5–112°	Cyclohexane	$C_{11}H_{15}NO_2$	68·3	7·5	7·3	68·4	7·8	7·3
2-Ethyl-4,5-dimethyl-	1	142·5–143·5°	Ethanol	$C_{11}H_{15}NO_2$	—	—	7·15	68·4	7·8	7·3
2-n-Propyl-3,5-dimethyl-	1	128–128·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·9	8·0	6·7	69·6	8·2	6·8
2,3,4,5-Tetrameth	2	177–178·5°	Toluene	$C_{11}H_{15}NO_2$	68·9	7·4	7·0	68·4	7·8	7·3
2,3,5,6-Tetrameth	2	191–192°	Toluene	$C_{11}H_{15}NO_2$	68·2	7·8	7·0	68·4	7·8	7·3
2,3,5-Trimethyl-6-ethyl-	2	150·5–152°	Benzene	$C_{12}H_{17}NO_2$	69·6	8·2	6·8	69·6	8·2	6·8
3-Methoxy-	2	136–138°	Ethyl acetate	$C_8H_9NO_3$	57·6	5·5	8·1	57·5	5·4	8·4
4-Methoxy-	2	127–129°	Ethyl acetate	$C_8H_9NO_3$	57·9	5·5	8·3	57·5	5·4	8·4
4-t-Butyl-	2	118–119°	Cyclohexane	$C_{11}H_{15}NO_2$	68·7	7·9	7·2	68·4	7·8	7·3
2-Phenyl-	1	143°	Ethanol	$C_{13}H_{11}NO_2$	73·7	5·1	6·4	73·3	5·2	6·6
3-Phenyl-	2	157–158°	Benzene	$C_{13}H_{11}NO_2$	72·8	5·1	6·9	73·3	5·2	6·6
4-Phenyl-	1	170°	Ethanol	$C_{13}H_{11}NO_2$	73·5	5·3	7·1	73·3	5·2	6·6
3-Chloro-	2	135–137°	Benzene	$C_7H_4ClNO_2$	49·0	3·6	7·9	49·0	3·5	8·2
4-Chloro-	2	142°	Ethyl acetate	$C_7H_4ClNO_2$	49·0	3·3	8·0	49·0	3·5	8·2
2-Methyl-4-chloro-	2	153°	Ethyl acetate	$C_8H_6ClNO_2$	51·5	4·0	7·8	51·8	4·3	7·6
3-Methyl-4-chloro-	2	150–152°	Ethyl acetate	$C_8H_6ClNO_2$	51·8	4·4	7·8	51·8	4·3	7·6
4-Methyl-2-chloro-	2	155°	Benzene	$C_8H_6ClNO_2$	52·4	4·5	7·4	51·8	4·3	7·6
2,4-Dichloro-	2	129°	Benzene	$C_7H_3Cl_2NO_2$	41·0	2·5	6·8	40·8	2·4	6·8
2,4,5-Trichloro-	2	131–132°	Benzene	$C_7H_4Cl_3NO_2$	35·5	1·5	5·9	35·0	1·7	5·8
3-Methyl-(*N*-methyl)	1	74–75°	Light petroleum (60–80°)	$C_9H_{11}NO_2$	65·9	6·8	8·1	65·5	6·7	8·5
3-Methyl-(*N*-ethyl)	1	b.p. 1140/0·2 mm.	—	$C_{10}H_{13}NO_2$	67·3	7·2	7·9	67·1	7·3	7·8
3-Methyl-(*N*-n-butyl)	1	52–53°	Light petroleum (40–60°)	$C_{13}H_{17}NO_2$	69·9	8·5	6·6	69·6	8·2	6·8
3-Methyl-(*N*-*m*-tolyl)	1	71–72°	Light petroleum (60–80°)	$C_{15}H_{15}NO_2$	74·7	6·3	6·1	74·7	6·2	5·8
3-Methyl-(*NN*-dimethyl)	1	b.p. 147°/16 mm.	—	$C_{10}H_{13}NO_2$	67·5	7·4	8·0	67·1	7·3	7·8
2,3-Dimethyl-(*N*-methyl)	1	110–112°	Cyclohexane	$C_{10}H_{13}NO_2$	67·3	7·6	7·9	67·1	7·3	7·8
2,3,5 Trimethyl-(*N*-hydroxy)	1	173·5–174·5°	Benzene	$C_{10}H_{13}NO_3$	62·0	6·6	6·8	61·6	6·7	7·2

* cf. Avenarius (1923)

TABLE IV

A SUMMARY OF THE MISCELLANEOUS CARBAMATES

Carbamate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
4-*m*-Tolyloxycarbonyl-morpholine	1	82–83°	Cyclohexane	$C_{12}H_{15}NO_3$	65·5	7·1	6·2	65·2	6·8	6·3
1-*m*-Tolyloxycarbonyl-piperidine	1	63–63·5°	Light petroleum (60–80°)	$C_{13}H_{17}NO_2$	71·6	7·9	6·9	71·3	7·8	6·4
β-Naphthyl	2	156–157°	Benzene	$C_{11}H_9NO_2$	70·7	5·2	7·2	70·6	4·8	7·5
5-Hydroxytetralin ..	2	138–140°	Cyclohexane	$C_{11}H_{13}NO_2$	69·5	6·9	7·4	69·2	6·8	7·3
4-Hydroxyindane ..	2	139·5–140·5°	Benzene-cyclohexane	$C_{10}H_{11}NO_2$	68·0	6·1	8·3	67·9	6·2	7·9
5-Hydroxyindane ..	1	147–148°	Cyclohexane	$C_{10}H_{11}NO_2$	68·0	6·2	7·9	67·9	6·2	7·9

Pharmacology

Methods

All mice used were female albinos of Schofield strain.

Analgesic activity. Compounds were first screened by the hot-plate method of Woolfe and Macdonald (1944) with minor modifications. The mice were tested on the hot surface (temperature 54°) and any mice failing to respond before 15 sec. were discarded. Drugs were given orally as a suspension in 5 per cent acacia to groups of 20 mice, which were placed individually on the hot surface at intervals of 5, 10, 20 and 40 min. after dosing. The mean percentage increase in reaction time over the control time at each interval was calculated for each group and the final potency was expressed as the sum of these percentages. The numerical value obtained naturally varied according to the average control time, thus, the lower the control time the higher the possible numerical value. This test can therefore not be regarded as by any means quantitative, and the potency is described merely as negligible, low, moderate, or high.

For quantitative assessment of potency the tail-clip method decribed by Bianchi and Franceschini (1954) was used. By this means it was possible to determine the ED50 of a representative series of the most interesting compounds at 15 min. after oral administration.

Acute toxicity. Groups of not less than ten mice weighing between 20–25 g. were given three logarithmically graded doses in 5 per cent acacia. The percentage mortality was noted over a 5-day period. LD50 values were estimated by the method of Bliss (1938).

Chronic toxicity. Groups of 20 mice were given oral doses of the drug (compound 688), at doses of 1/20 and 1/60 of the lower limit of error of the LD50 daily excluding week-ends and public holidays for approximately 4 months. Weight gains were recorded, treated statistically and compared with the controls. Differential blood counts were made on the animals at the end of the experiment, and histological sections of a representative selection of organs from test and control animals were made after post-mortem examination.

Blood pressure and respiration. Guinea pigs weighing approximately 1 kg. were used, and anaesthetised with urethane, and the drug was injected in distilled water or saline into the cannulated jugular vein in a dose of 50 μg. for blood pressure effect and 100 μg. for effect on respiration.

Anticholinesterase activity. This was determined by the method of Buckles and Bullock (1956).

Results

Analgesic tests. Table V gives ratings to the activities of selected compounds in the Woolfe-Macdonald test. These agree qualitatively with those obtained by the Bianchi tail-clip method. The latter are given in Table VI, which records the ED50 of a representative number of compounds 15 min. after oral administration. Observations on these two tests are made under the discussion of results.

Comparison of the activity of morphine, pethidine, and Compound 688. Woolfe and Macdonald found pethidine to show little activity in their test; in our test pethidine shows little activity and is less active than morphine, and much less so than compound 688.

The results are summarised in Table VII.

Toxicity Tests

Acute toxicity. The oral, and in some cases i.v., LD50 values for selected compounds are given in Table V.

TABLE V

ANALGESIC ACTIVITY OF A SERIES OF PHENOLIC CARBAMATES IN THE WOOLFE-MACDONALD SCREENING TEST

Compound No.	Phenol carbamate	Potency	Toxicity	
			Oral (LD50 mg./kg.)	Intravenous (LD50 mg./kg.)
613	3-Methyl-	Moderate	1116·0 (932·4–1337·0)	105·1 (97·2–113·5)
631	3-Ethyl-	High	143·6 (108·2–190·5)	20·88 (15·59–70·42)
641	4-Isopropyl-	Negligible	—	—
642	3,5-Dimethyl-	High	421·9 (337·3–527·8)	60·29 (51·59–70·42)
654	3,4-Dimethyl-	Very high	112·9 (85·0–149·8)	24·1 (20·4–28·5)
658	2,5-Dimethyl-	Very high	155·8 (142·8–169·9)	33·9 (23·12–49·71)
661	2,3,5-Trimethyl-	Very high	145·5 (100·3–211·2)	29·93 (24·77–36·17)
663	3-Ethyl-5-methyl-	High	374·4 (333·9–420·0)	—
666	3,5-Dimethyl-(allophanate)	Inactive	—	—
669	3-Methyl-4-ethyl-	High	39·82 (29·85–53·13)	—
678	2-n-Propyl-5-methyl-	Low	—	—
680	2,6-Dimethyl-	Low	—	—
682	2,3-Dimethyl-(allophanate)	Negligible	—	—
688	2,3-Dimethyl-	High	864·4 (645·7–1157·0)	—
686	2,4,5-Trimethyl-	Very High	35·8 (27·9–46·0)	—
690	2,5-Dimethyl-4-ethyl-	Very high	23·13 (20·49–26·10)	—
696	2,3,5-Trimethyl-6-ethyl-	Negligible	—	—
699	2,3,6-Trimethyl-	Negligible	—	—
700	2,3,5,6-Tetramethyl-	Negligible	—	—
703	2,4-Dimethyl-	Negligible	—	—
709	2,3,4,5-Tetramethyl-	Very high	—	—
714	2,3,4-Trimethyl-	High	—	—
727	2,3,5-Trimethyl-(*N*-methyl)	Negligible	—	—
729	2,3,5-Trimethyl-(*N*-hydroxy)	High	220·9 (161·0–302·9)	—
730	3-Methyl-4-ethyl-(*N*-acetyl)	Negligible	—	—
732	2,3-Dimethyl-(*N*-hydroxy)	High	—	—
734	2,4,5-Trimethyl-(*N*-hydroxy)	High	86·3 (71·64–104·0)	—

Chronic toxicity. Compound 688 (2,3-dimethylphenyl carbamate) was chosen for this test. The average weight gains showed no significant differences from those of the controls. There were three deaths at each dose level during the 17-week experimental period, apparently unconnected with the experimental conditions. The blood counts (average) and haemoglobin contents are given in Table VIII.

The cause of the apparently highly significant increase in leucocytes on the *low* dose of the drug is doubtful. However, since no abnormalities

attributable to the drug were reported in the histological examination of liver, kidney, and spleen, and owing to the frequency of the occurrence of subcutaneous abscesses, it is probable that these phenomena are unconnected with the drug. Moreover, persistently high leucocyte counts

TABLE VI

ORAL ANALGESIC ACTIVITY OF PHENOLIC CARBAMATES BY THE BIANCHI TAIL-CLIP METHOD

Compound*	Analgesic activity (ED50 mg./kg.)
613	58·0
631	12·2
642	17·4
654	3·5
658	10·6
661	2·58
663	16·0
686	1·34
688	8·8
690	1·76
696	No effect at 100
699	No effect at 100
700	No effect at 100
703	No effect at 100
709	1·60
714	16·6

* For chemical formulae see Table V.

TABLE VII

COMPARISON OF THE ANALGESIC ACTIVITY OF MORPHINE, PETHIDINE AND COMPOUND 688 ADMINISTERED BY MOUTH

Compound	Dose mg./kg.	No of tests	Index of potency
688*	10	8	343
688	5	4	100
Morphine HCl	10	4	206
Morphine HCl	5	2	92
Pethidine HCl	10	4	130
Pethidine HCl	5	2	51

* For chemical formula see Table V.

TABLE VIII

AVERAGE BLOOD COUNTS AND HAEMOGLOBIN CONTENTS OF MICE ON CHRONIC TOXICITY TEST OF COMPOUND 688*

	Erythrocytes millions/cu. mm.	Leucocytes thousands/cu. mm.	Haemoglobin (Sahli) per cent
Controls	12·72	14·28	113
Mice receiving 32 mg./kg. ..	11·52	14·70	120
Mice receiving 10 mg./kg. ..	11·82	23·50	123

* For chemical formula see Table V.

have been obtained in both control and test animals in other chronic toxicity tests, and may be associated with the particular mouse stock used.

Anticholinesterase activity. Table IX shows the results of a comparison of compounds 688 and 709 with neostigmine in the inhibition of the pseudocholinesterase of horse serum.

Compound 688, one of the less toxic carbamates selected for clinical trial, and compound 709 which was highly toxic and apparently one

of the most potent analgesics of the series, both possessed anticholinesterase activity. This activity is, however, negligible compared with that of neostigmine, and is much less than that of some *N*-substituted carbamates. The toxicity is therefore unlikely to be due to anticholinesterase activity.

Blood pressure and respiration. Intravenous injection of compound 688 produced a definite but inconsistent effect on the blood-pressure, the fall produced by 50 μg. being sometimes equivalent to that produced by 0·5 μg. of acetylcholine. The above doses of acetylcholine and compound 688 caused a slight rise in blood-pressure after injection of 0·25 mg. of atropine, further injection of which blocked the effect. Intravenous injection of 100 μg. produced no effect on respiration.

TABLE IX

ANTICHOLINESTERASE (PSEUDOCHOLINESTERASE) ACTIVITY OF TWO PHENOLIC CARBAMATES COMPARED WITH THAT OF NEOSTIGMINE

Compound	No. of tests	Inhibition per cent
Neostigmine (0·0002 mg./ml. in water) ..	6	87·8
709 (0·0002 mg./ml. suspended in water) ..	2	16·0
709 (0·5 mg./ml. in water)	1	86·3
709 (0·0002 mg./ml. in acetone)	1	7·2
709 (0·0004 mg./ml. in acetone)	1	10·3
709 (0·005 mg./ml. in acetone)	3	70·3
688 (0·005 mg./ml. in acetone)	2	23·6

For chemical formulae of compounds 709 and 688 see Table V. The activity of compound 709 is evidently $> 0{\cdot}0004$ of that of neostigmine.

DISCUSSION

The results of the analgesic tests by both the Woolfe–Macdonald and Bianchi tail-clip methods reveal that only the carbamates of 3-alkylphenols exhibit significant analgesic activity as determined by these tests. An attempt to correlate the effect of the orientation of substituent alkyl groups with analgesic activity was based on the activity of *m*-tolyl carbamate (compound 613). In 3-monoalkyl derivatives, maximum activity appeared at ethyl and thereafter activity fell off with increase in the size of the alkyl substituent until, with *m*-n-pentadecyl phenyl carbamate, only negligible activity remained. The activity of *m*-tolyl carbamate may be increased by the introduction of further alkyl substituents. Two *o*-substituents reduced the activity to negligible proportions, as in compounds 680, 696, 699 and 700. Introduction of two t-butyl groups (compound 701) also greatly depressed activity. The most active compound was 2,4,5-trimethylphenyl carbamate (compound 686); others with comparable activity are 2,5- and 3,4-dimethyl-, 2,3,5-trimethyl-, and 2,3,4,5-tetramethylphenyl carbamates, together with 2,5-dimethyl-4-ethylphenyl carbamate. Substitution of the carbamate group by any other radical than hydroxyl strongly reduced the activity. None of the allophanates exhibited significant activity. It is clear that under our conditions the Woolfe–Macdonald test is not adaptable to quantitative assays, but that the ED50 can be readily determined using the Bianchi test. In general the most active compounds are also very toxic, for example, compounds 686 and 690. The least toxic compound proved to be 613 with an

oral LD50 in mice of 1116 mg./kg. Compound 688, with an oral LD50 of 864 mg./kg., appeared to possess the best therapeutic ratio and was therefore selected for further pharmacological and clinical trial, to be reported elsewhere. Unfortunately it had little value in the treatment of pathological pain in man and this fact, combined with the results of a comparison with morphine and pethidine using the Woolfe–Macdonald test, suggests that this test alone is inadequate to assess a morphine-like analgesic. Our results were confirmed independently in another laboratory.

References

Avenarius, R. (1923). *Angew. Chem.*, **36**, 165–168.
Bianchi, C., and Franceschini, J. (1954). *Brit. J. Pharmacol.*, **9**, 280–284.
Bliss, C. I. (1938). *Quart. J. Pharm.*, **11**, 192–216.
Blohm, H. W., and Becker, E. I. (1952). *Chem. Rev.*, **51**, 471–504.
Buckles, J., and Bullock, K. (1956). *J. Pharm. Pharmacol.*, **8**, 946–955.
Woolfe, G., and Macdonald, A. D. (1944). *J. Pharmacol.*, **80**, 300–307.

THE ACTION OF PROCAINE AT THE NEUROMUSCULAR JUNCTION

By D. W. Straughan

From the Department of Pharmacology, King's College, London, W.C.1

Received September 1, 1960

The mode of action of procaine at the neuromuscular junction has been studied in the rat isolated phrenic nerve diaphragm preparation. Procaine had no effect on the response to indirect stimulation in concentrations below 8×10^{-5}. But 2×10^{-5} procaine reduced the neostigmine potentiated twitch to its normal size, and caused a significant reduction in the amount of acetylcholine released by a period of tetanus. This is thought to be due to a selective local anaesthetic action on the fine motor nerve terminals.

Numerous reports have shown that procaine can cause neuromuscular blockade. This is usually attributed to its curare-like action (Fulton, 1921; Peterson, 1955), but qualitative differences between the action of curare and procaine at the neuromuscular junction, suggested that procaine might in addition interfere with the release of acetylcholine from motor nerve endings (Harvey, 1939; Jaco and Wood, 1944; Bülbring, 1946). The present experiments were undertaken therefore to see if this suggestion could be demonstrated by the direct measurement of acetylcholine release.

Methods

The method for studying acetylcholine release has been described previously (Straughan 1959; 1960). A single side of a rat diaphragm with its attached phrenic nerve was suspended flat in a glass diaphragm bath containing 3 ml. of Krebs solution with 5×10^{-6} neostigmine methylsulphate. The nerve was stimulated with supramaximal rectangular pulses, 0·03 millisec. in duration for 20 min. periods at a rate of 25/sec. Immediately after the end of stimulation the bath fluid was removed for assay. Up to six successive periods of tetanus with 10 min. rest between them, can be applied to each diaphragm, and the acetylcholine release has been shown to be steady under these conditions. The temperature was kept constant at 37° ± 0·25° in each experiment.

Samples of bath fluid containing acetylcholine were assayed on the rat blood pressure preparation, this has been fully described previously (Straughan, 1953; 1959; 1960).

Results

The effect of procaine on the response to indirect stimulation. A rat diaphragm was set up in the manner described by Bülbring (1946), in Krebs solution and stimulated indirectly through the phrenic nerve at 8/min. with supramaximal rectangular pulses. The addition of procaine to the bath fluid in concentrations 1×10^{-5}, 2×10^{-5} and 4×10^{-5} had no effect on the size of the muscle twitch. When the procaine

concentration was increased to 8×10^{-5} a small diminution in twitch height developed gradually over 10 min. Complete abolition of the twitch response to nerve stimulation developed over 10 min. at a procaine concentration of $1{\cdot}6 \times 10^{-4}$.

Concentrations of procaine which were without effect on twitch height caused an immediate reduction to its previous size of the twitch potentiated by neostigmine methylsulphate (1×10^{-6}). The same effect occurs with procaine and neostigmine in the cat gastrocnemius muscle (Jaco and Wood 1944). These observations are illustrated in Fig. 1.

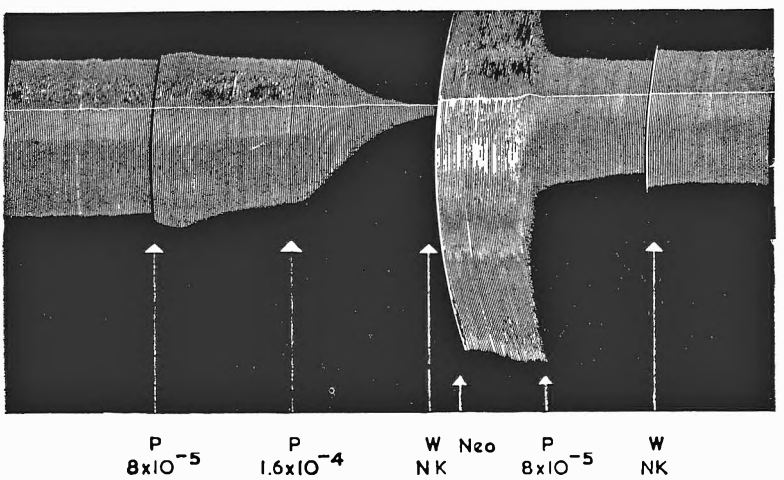


FIG. 1. The action of procaine on the indirect twitch before and after neostigmine. Rat isolated diaphragm preparation stimulated through phrenic nerve at 8/min. At P, procaine added to bath fluid for 10 min. At WNK preparation washed with plain Krebs. At Neo, neostigmine methylsulphate ($1 + 10^{-6}$) added to bath for 10 min.

The effect of procaine on the release of acetylcholine. A standard procedure was adopted; after immersing the preparation for 30 min. in 5×10^{-6} neostigmine methylsulphate, two successive periods of tetanus were applied and the bath fluid collected each time for assay. The diaphragm was now allowed to equilibrate for 15 min. to a known

TABLE I

THE EFFECT OF PROCAINE ON ACETYLCHOLINE RELEASE FROM THE RAT DIAPHRAGM
ACETYLCHOLINE IN NG. BASE/20 MIN. AT 25/SEC.

EACH FIGURE IS THE MEAN OF TWO SUCCESSIVE PERIODS OF STIMULATION

Expt. 1	No procaine	2×10^{-5} procaine	4×10^{-5} procaine
1	62	53	—
2	59	52	—
3	44	28	—
4	38	21	—
5	37	33	—
6	48	41	33
7	42	30	19
8	61	—	34
9	44	—	32
10	49	—	29

concentration of procaine, two further periods of tetanus were applied and the samples collected. This was repeated in a concentration of increased procaine. All the samples were assayed individually and a mean acetylcholine release figure calculated from the two values obtained at each particular concentration of procaine.

The results showed that even a concentration of procaine as small as 2×10^{-5} caused a significant reduction in the amount of acetylcholine released from the rat diaphragm preparation by a period of tetanus. Further increases in the procaine concentration brought about an even greater reduction in the amount of acetylcholine released, so that in one diaphragm the mean release in normal Krebs fluid was 64 ng. base/20 min. at 25/sec., the addition of procaine 4×10^{-5} now caused the mean release to fall to 37 ng. base/20 min. at 25/sec. and when the procaine concentration was increased to 8×10^{-5} the mean acetylcholine release fell even further, to 15 ng. base. The results from ten other typical experiments are shown in Table I.

Discussion

These results suggest that procaine in doses which have no curarising effect reduces acetylcholine release. This observation is supported by results reported by Harvey (1939). He observed that procaine was less curarising than tubocurarine, dose for dose, in that it reduced the twitch response of the cat tibialis muscle to injected acetylcholine less than did tubocurarine. A tetanus applied to the motor nerve produced a partial relief of the neuromuscular blockade produced by procaine, but exaggerated a tubocurarine block.

The observation that 2×10^{-5} procaine reduced the indirect twitch of the rat diaphragm only after it had been potentiated by neostigmine, can be explained as follows. Because of the high safety factor for neuromuscular transmission by acetylcholine, the reduction in release which follows small doses of procaine is insufficient to impair transmission. But, when neostigmine is present acetylcholine accumulates at the end plate, repetitive firing occurs, and the twitch height is potentiated. The reduced amount of acetylcholine now released after a small dose of procaine, is then just sufficient to maintain the potentiated twitch height at its former size.

It is clear from the results that procaine interferes with release during high frequency nerve stimulation. There are good reasons for believing that this action of procaine is a general one and not just due to the conditions of stimulation. Firstly, Furukawa (1957) showed by electrophysiological methods that procaine reduced the acetylcholine release in frog muscle even at low rates of stimulation. Secondly, the results suggest that the release from motor nerve endings in the rat diaphragm can be reduced by half without impairment of transmission as judged by the twitch response at low rates of stimulation. This is consistent with current knowledge of a high safety factor for neuromuscular transmission by acetylcholine. Kuffler (1942) showed that the end plate potential in

frog muscle could be reduced to 30 or 40 per cent of its original height before transmission failed. Thirdly the qualitative differences seen between curare and procaine at low rates of stimulation by Jaco and Wood (1944) were seen also at high rates of stimulation by Bülbring (1946).

It remains a possibility that high frequencies of stimulation exaggerate the action of procaine on acetylcholine release, perhaps by increasing the extent of presynaptic failure of nervous transmission already present under these conditions (Krnjević and Miledi, 1958).

The action of procaine on acetylcholine release may be attributed to its local anaesthetic properties, since cocaine also appears to reduce acetylcholine release in the rat diaphragm, Bülbring (1946). Harvey (1939) suggested that procaine might interfere with release by acting on the terminal branches of the motor nerve. This is the most likely site of action since the terminals are of fine diameter and are unmyelinated which should make them preferentially sensitive to the action of local anaesthetics (Gasser and Erlanger, 1929; Matthews and Rushworth, 1957).

References

Bülbring, C. (1946). *Brit. J. Pharmacol.*, **1**, 38–61.
Fulton, J. F. (1921). *Amer. J. Physiol.*, **57**, 153–170.
Furukawa, J. (1957). *Jap. J. Physiol.*, **7**, 199–212.
Gasser, A. S., and Erlanger, J. (1929). *Amer. J. Physiol.*, **88**, 581–591.
Harvey, A. M. (1939). *Bull. John Hopkins Hosp.*, **54**, 223–238.
Jaco, N. T., and Wood, D. R. (1944). *J. Pharmacol.*, **82**, 63–73.
Krnjević, K., and Miledi, R. (1958). *J. Physiol.*, **140**, 440–461.
Kuffler, S. W. (1942). *J. Neurophysiol.*, **5**, 18–26.
Matthews, P. B. C., and Rushworth, C. (1957). *Ibid.*, **135**, 263–269.
Peterson, C. G. (1955). *Anesthesiology*, **16**, 678–698.
Straughan, D. W. (1958). *J. Pharm. Pharmacol.*, **10**, 783–784.
Straughan, D. W. (1959). Ph.D. Thesis, University of London.
Straughan, D. W. (1960). *Brit. J. Pharmacol.*, **15**, 417–424.

MICELLAR SIZE OF MIXED LYSOLECITHIN-LECITHIN SOLS

By N. Robinson

*From the Department of Physical Chemistry, School of Pharmacy, University of London, Brunswick Square, London, W.C.*1

Received September 7, 1960

Lysolecithin and lecithin can be expected to occur together in biological systems—aggregates formed by mixed lysolecithin-lecithin sols were found to have a molecular weight of nearly 1·5 million and to be unsymmetrical, probably closest to a rod shape of length 1,500 Å. The particles were thought to be mixed micelles and not solubilised lecithin within the micelles of the highly surface-active lysolecithin according to the classical Hartley concept.

Lysolecithin is completely soluble in water and possesses considerable surface activity. Robinson and Saunders (1959) have shown it to form large symmetrical aggregates in water having a micellar weight of 100,000.

Lecithin possesses an additional unsaturated fatty acid chain occupying the α-position of the glycerol nucleus. This confers on lecithin a greater lipophilic character which causes the substance to form a dispersion in water. Lecithin forms large asymmetric particles (Robinson, 1960) in water having a micellar weight of $2{\cdot}7 \times 10^6$.

Since lysolecithin is an enzymatic breakdown product of lecithin these substances would be expected to occur together in biological systems. It is thought that the co-existence of these phosphatides will be in the formation of mixed micelles. The effect of the presence of the lecithin component on the micellar size and shape of lysolecithin aggregates, which will have some solubilising effect on the lecithin component, has been studied by the light-scattering method.

Experimental

Materials

The preparation of lecithin from the yolks of eggs has been reported (Robinson, 1960).

Lysolecithin was prepared by Saunder's (1957) modification of the method of Hanahan, Rodbell and Turner (1954). Analytical figures for the phosphatides are given in Table I below.

TABLE I

Analytical figures for the phosphatides

	N (per cent)	P (per cent)	$[\alpha]_D^{20}$	Double bonds per molecule from iodine uptake
Lecithin	1·73	3·82	+7·6	2·3
Lysolecithin	2·83	5·98	+2·26	0·12

Preparation of Mixed Sols

Measured quantities of ethanolic solutions of known concentrations of lysolecithin and lecithin were placed in the same conical flask and gently

warmed to obtain a clear solution. This was then evaporated to dryness under vacuum leaving a film of intimately mixed phosphatides. A measured volume of ion-exchanged water was added to the residue and the flask attached to an automatic shaking machine and placed in a water bath at 40°. The flask was shaken in the water bath until the sol gave a minimum turbidity.

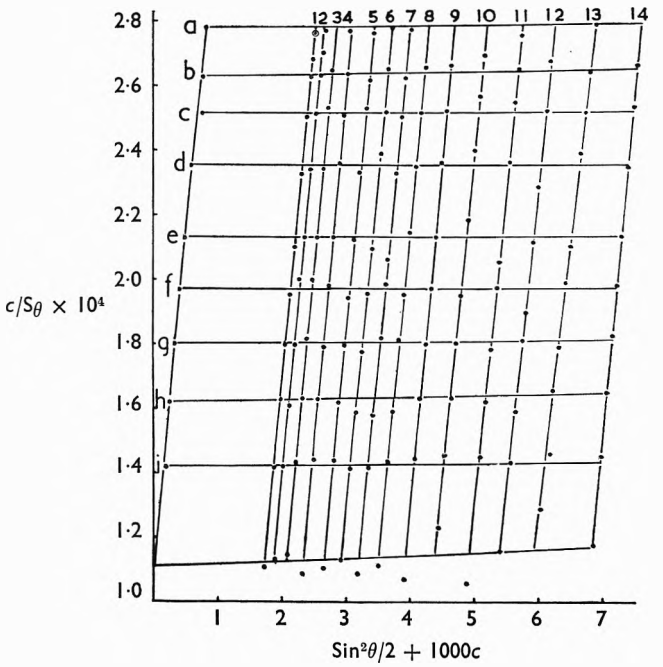


FIG. 1. Zimm plot for mixed lysolecithin-lecithin sols at 20°. Figures along the top of the plot denote total phosphatide concentrations (c) in g. ml.$^{-1}$ × 10^3 as follows.

1.	1·72	6.	2·90	11.	4·89
2.	1·88	7.	3·17	12.	5·40
3.	2·08	8.	3·51	13.	6·04
4.	2·32	9.	3·92	14.	6·84
5.	2·63	10.	4·44		

Letters denote angles (θ) of scatter to incident beam.

a.	$\theta = 130°$	f.	$\theta = 80°$
b.	$\theta = 120°$	g.	$\theta = 70°$
c.	$\theta = 110°$	h.	$\theta = 60°$
d.	$\theta = 100°$	i.	$\theta = 50°$
e.	$\theta = 90°$		

Preliminary experiments on the turbidity of mixed lysolecithin-lecithin sols showed that sols having a mol ratio of lecithin to total phosphatide of less than 0·11 were optically clear, indicating that the lecithin component was completely solubilised. Sols having a mol ratio of lecithin to total phosphatide of 0·10 were prepared and used in the light-scattering studies.

Apparatus. The light-scattering apparatus has been previously described (Robinson and Saunders, 1959; Robinson, 1960).

Results and Discussion

Measurements were made in pure water which is close to the isoelectric point of lecithin (found experimentally by Chain and Kemp (1934) to be pH 6·7). The isolectric point of lysolecithin is probably very close to that of lecithin and the system in water was considered to possess minimum charge effects.

Diffusion studies of mixed phosphatide sols having a mol ratio of lecithin to total phosphatide of 0·5 or less followed the theory for a single solute (Thomas, 1958) indicating that, in selected ratios of mixed phosphatide sols, the micelles were fairly uniform in size and composition. These findings, probably due to the closely related structures of the lecithins, enabled the concentration term (c) to be taken as the sum of the two components.

The specific refractive index increment for the mixed lysolecithin-lecithin sols using the Raleigh interference refractometer method previously described (Robinson and Saunders, 1959) was 0·1361. The depolarisation of 90° scatter was 0·0331.

Light scattered by sols having total phosphatide concentrations between 6·84 and 1·72 $\times$ 10^{-3} g. ml.$^{-1}$ was examined between the angles 50° and 130° to the incident beam. Values for $c/S\theta$, where c is the concentration in g. ml.$^{-1}$ and $S\theta$ the scatter at angle θ to the incident beam, were plotted against $\sin^2\frac{\theta}{2} + 1{,}000\,c$ by the method of Zimm (Fig. 1). From the intercept $\left[\frac{c}{S\theta}\right]$ $c = 0$, $\theta = 0$ the molecular weight was calculated to be 1,484,000.

Since the shape of the particles was unknown the reciprocal of $P(\theta)$ (the particle scattering factor) was plotted against $\sin^2\frac{\theta}{2}$ (Stacey, 1956); by plotting theoretical curves with the same initial slope for a sphere, rod, random and polydisperse coil a comparison for the closest fit indicated that the particles were probably closest to a rod (Fig. 2), having a maximum dimension of 1,530 Å.

Results show that the molecular weight of the mixed micelles was nearly fifteen times greater than the micellar weight of the lysolecithin component although this substance constituted a mol fraction of nine-tenths of total phosphatide content. The dominating influence of lecithin to form asymmetric aggregates resulted in the mixed micelles showing a greater dissymmetry ($z = 2{\cdot}27$) than the dissymmetries shown by lysolecithin ($z = 1{\cdot}08$) and lecithin ($z = 1{\cdot}44$) independently; the shape was also closest to that of the lecithin micelles. The symmetrical lysolecithin micelle has been shown to possess a maximum dimension of less than 400 Å—this was extended in the presence of lecithin to the high value of 1,500 Å.

The shape and dimension of the particles suggest that the lecithin was not actually solubilised within the lipophilic region of the lysolecithin micelle in the conventional manner—this is restricted somewhat by the large hydrophilic phosphorylcholine head group of the lecithin molecule. Furthermore, the symmetry of the lysolecithin micelle would probably

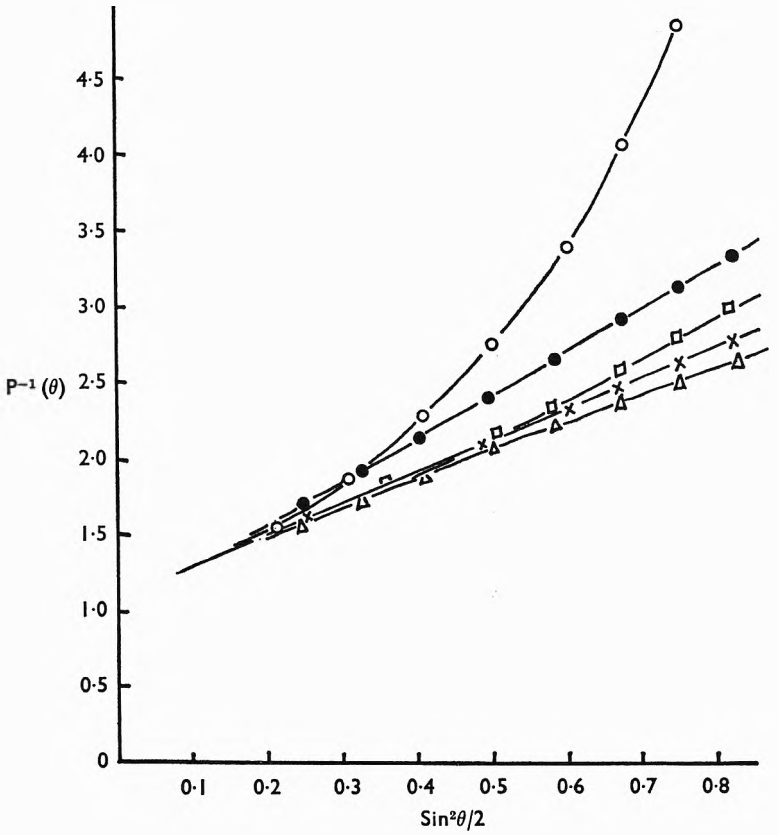


FIG. 2. $P^{-1}(\theta)$ functions for the sols and for models.
× Mixed lysolecithin-lecithin sols.
○ Sphere.
△ Rod.
□ Monodisperse coil.
● Polydisperse coil.

not have been disturbed significantly on solubilisation of a mol fraction of lecithin amounting to one-tenth of total phosphatide. It is more probable that the surface of a lysolecithin micelle was impregnated with single lecithin molecules orienting themselves in a manner adjacent to the lysolecithin molecules, though not without causing considerable distortion of the spherical shape. The particles of the sol were thus regarded as evenly distributed mixed micelles rather than macromolecules composed of a lecithin nucleus within a lysolecithin sheath surrounded by an aqueous medium.

If the assumption is made that the particles were composed of the mole fractions of phosphatide components used in the preparations, then the numbers of lysolecithin and lecithin monomers present in the mixed micelles were 2,630 and 280 respectively. This represents a 14-fold increase in the number of lysolecithin monomers in the mixed micelle over those present in a micelle of lysolecithin alone.

The increase in depolarisation of this system compared with pure lysolecithin sols resulted mainly from an increase in anisotropy, since the turbidity of the mixed sols was low and hence the secondary scatter minimised.

Acknowledgement. I thank Dr. L. Saunders for his continued interest throughout this work.

References

Chain, E. and Kemp, I. (1934). *Biochem. J.*, **28**, 2052–2055.
Hanahan, D. J., Rodbell, M. and Turner, L. D. (1954). *J. biol. Chem.*, **206**, 431–441.
Robinson, N. (1960). *Trans. Faraday Soc.*, **56**, Part 8, 1260–1264.
Robinson, N. and Saunders, L. (1959). *J. Pharm. Pharmacol.*, **11**, Suppl. 115*T*–119*T*.
Saunders, L. (1957). *Ibid.*, **9**, 834–839.
Stacey, K. A. (1956). *Light-Scattering in Physical Chemistry*, London: Butterworths Scientific Publications.

PHARMACOPOEIAS AND FORMULARIES

Addendum 1960 to the British Pharmacopoeia 1958*

Reviewed by Miles Weatherall

The 1960 Addendum to the 1958 edition of the British Pharmacopoeia does not contain any great surprises. There are forty-nine new monographs, but most of the substances introduced appear in more than one form, and some are new forms of existing drugs, so that only sixteen substances are introduced. These are bemegride, busulphan, chorhexidine, chlorothiazide, halothane, hydroxychloroquine, liothyronine, mercaptopurine, novobiocin, phytomenadione, poliomyelitis vaccine, potassium perchlorate, probenecid, pyridostigmine, tolbutamide and tripelennamine. Monographs dealing with new preparations of substances already represented in the Pharmacopoeia cover sodium amylobarbitone (injection, capsules and tablets), corticotrophin (gelatin injection and zinc hydroxide injection), diphtheria and tetanus vaccine, hydrocortisone (hydrogen succinate, sodium succinate and sodium succinate injection), naphazoline hydrochloride, phenoxymethyl penicillin capsules, piperazine citrate, soluble compound codeine tablets and typhoid-paratyphoid A and B and cholera vaccine. Revisions particularly affect the monographs on B.C.G. vaccine and smallpox vaccine, but in all forty-two monographs are the subject of some sort of change.

The revisions are inevitably less interesting than the innovations, but the Pharmacopoeia Commission are discreetly silent about the reasons for their selection. The B.P. contains "descriptions of and standards for . . . medicines, preparations, materials and articles used in the practice of medicine, surgery, or midwifery" but the selection is far from comprehensive and the inclusion of a medicine in the B.P. is generally regarded as giving it the seal of official approval. It would be nice to see the criteria for selection explicitly stated. This is not to doubt but to applaud the wisdom of the Commission, who have a formidable problem in separating wheat from chaff. Whether the reasons could be stated compactly is perhaps another matter.

The new edition becomes official on March 1, 1961.

* Pp. xxi + 83 (including index). Published for the General Medical Council by The Pharmaceutical Press, London. 30s. (Postage, U.K. 1s.; Overseas, 1s. 10d.).

BOOK REVIEW

HANDBUCH DER PAPIERCHROMATOGRAPHIE. By I. M. Hais and K. Macek. Vol. II. Bibliographie und Anwendungen. Pp. xxiv + 726 (including Index). VEB Gustav Fischer Verlag, Jena, 1960. DM.44.00.

When the first massive volume of this pair of books on chromatography was published last year (for review see *J.P.P.*, October, 1959, p. 637), the authors promised a second volume dealing with more specific methods. This has now appeared under the subtitle: Bibliography and Applications.

The contents consists of ten thousand numbered references with their titles arranged under subject headings, complemented by separate indices of authors and individual compounds.

While in the last fifteen years paper chromatography has emerged as a major tool in analytical technique, many of its users are unaware that it was applied many years before its official recognition in 1952 when Martin and Synge received their Nobel Prize. The authors have uncovered many references which they have brought together in a historical section. Here one can find such interesting information as the fact that Bailey in the *J. chem. Soc.*, **33**, 304, described a method to detect cadmium by the use of filterpaper as early as 1878. Tsewett, well known as the originator of chromatography is quoted and references to all his early papers can be found. But the references are by no means restricted to those of historical value. One doubts whether any relevant paper escaped the scrutiny of the authors: the thorough searching and collating has created an impressive document. It extends to the year 1956 after which date no papers are included. This is no great drawback since most techniques had been worked out by that time. And as all references give the full titles of papers as well as the names of the authors, the user is handed a quick guide to the possible usefulness of the given reference.

A check of half a dozen subjects has shown that no relevant works have been omitted. In fact, many from unusual sources have been included. The authors express their gratitude, for example, to a compiler of Japanese papers. The titles of many of these are those which one might wish to consult although the language barrier may present a great difficulty even if the journal is available.

Most of us remain confined to those English language papers to which the majority of libraries subscribe, and whilst in some scientific fields whole Russian journals are translated into English it will be many years before it will be taken for granted that one can consult, for example, Naturwissenschaften or Biochimija in a form which is intelligible to all scientists—a further argument for language standardization in scientific literature, whether it be in Interlingua, Esperanto or even English!

Nevertheless this reference book will be of the greatest value to research workers, whether multi- or monolingual, as the text is confined to a minimum, and two-thirds of the papers referred to are in English. When embarking on the chromatography of a new compound or substance this book will provide a rapid and accurate list of work already undertaken by other workers, and it will without a doubt save considerable time in literature research. It is a book that will prove very popular in the chemical library.

B. REIFF.

LETTERS TO THE EDITOR

The Estimation of Ergometrine on the Rat Uterus

SIR,—Studies of the metabolism of ergometrine in these laboratories (Slaytor, Pennefather and Wright, 1959) prompted a search for a means of estimating its oxytocic acitivity by a method more sensitive than that of Vos (1943). The rat oestrous uterus has been used successfully for the estimation of 5-hydroxytryptamine (Erspamer, 1953) and oxytocin, but it has not been applied to the estimation of ergometrine; I have now found it suitable for this purpose.

Virgin female rats (180 g.) were treated with an intramuscular injection of stilboestrol (100 μg. in peanut oil). Uteri were removed 30 hr. later, and one

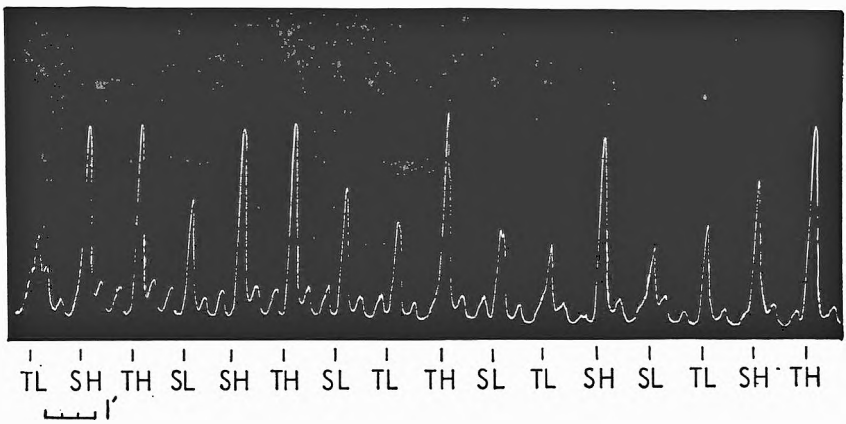


FIG. 1. Record showing contractions elicited by ergometrine on an isolated rat oestrous uterus. SH = 20 ng., SL = 10 ng. TH = 0·2 ml. of a solution of ergometrine of assumed strength 100 ng./ml. TL = 0·1 ml. of the same solution. Doses were given in a 4 × 4 Latin Square sequence.

horn suspended in a 10 ml. organ bath containing the solution described by Gaddum, Peart and Vogt (1949), maintained at 29° and oxygenated, and isotonic contractions recorded.

The assay design for ergometrine maleate solutions was similar to that described in the B.P. 1958 for the assay of Oxytocin Injection. If certain precautions were always followed, the height of contraction could be taken as the response, and tachyphylaxis rarely developed. Uteri were relatively refractory to the first few applications of ergometrine; at this stage, and throughout the assay, it was important not to use doses exceeding 200 ng. to avoid the possibility of prolonged insensitivity to subsequent doses. When responses were obtained, the drug was washed out of the bath as soon as each contraction reached its maximum. This precaution lessened the possibility of tachyphylaxis, and reproducible responses were produced by doses as low as 10 ng. Doses were given at 3 to 4 min. intervals.

Estimates within 5 per cent of actual strength were obtained with three of the four test solutions of ergometrine. The estimate of the strength of the fourth solution was within 8 per cent of the actual strength. The percentage limits of error (P=0·95) for all assays were within 85 and 120 per cent of actual strength. The precision of an estimate could be increased by combining results obtained

in several assays in the manner described in the B.P. 1958. Fig. 1 is a record of an assay.

This method of estimation has three advantages. Firstly, it is sensitive enough to permit the assay of concentrations of ergometrine as low as 100 ng./ml. Secondly, the use of height of contraction as the response rather than latency as in earlier methods (Vos, 1943; Foster and Stewart, 1948) increases the accuracy of measurement. Finally, the absence of tachyphylaxis permits the use of a standard assay design and consequently simplifies calculation of results.

Acknowledgements. I am indebted to Drug Houses of Australia Ltd. for a grant, and to Burroughs Wellcome & Co. (Australia) Ltd. for a gift of ergometrine maleate.

JOCELYN N. PENNEFATHER.

Department of Pharmacology,
University of Sydney,
Sydney, N.S.W., Australia.

November 4, 1960.

REFERENCES

British Pharmacopoeia (1958). 940, 908. London: The Pharmaceutical Press.
Erspamer, V. (1953). *Arch. int. Pharmacodyn.*, **93**, 293–316.
Foster, G. E., and Stewart, G. A. (1948). *Quart. J. Pharm. Pharmacol.*, **21**, 211–218.
Gaddum, J. H., Peart, W. S., and Vogt, M. (1949). *J. Physiol.*, **108**, 467–481.
Slaytor, M., Pennefather, J., and Wright, S. E. (1959). *Experientia*, **15**, 111–113.
Vos, B. J. (1943). *J. Amer. pharm. Ass., Sci. Ed.*, **32**, 138–141.

Modification of Histamine Sensitivity after 48/80 Treatment

SIR,—Feldberg and Talesnik (1953) and Parratt and West (1957) have shown that in the rat treatment with the histamine liberator, compound 48/80 causes a prolonged fall in the levels of histamine in tissues.

Female rats were treated with a single intraperitoneal dose of 48/80 (2 mg./kg. found to be the LD33). They were killed after the times shown in Table I. Histamine sensitivity was determined on the isolated oestrous uterus which was stimulated electrically by a method similar to that described by Csapo and Corner (1952) for the rabbit uterus. Stimulation and recording characteristics were constant throughout the course of these experiments.

TABLE I

DOSES OF HISTAMINE (μG./10 ML.) PRODUCING 20 PER CENT INHIBITION OF UTERINE CONTRACTIONS IN 48/80 TREATED RATS

Time after 48/80 (days)	Dose of histamine	Mean dose
0·25	100, 100, 120	107
1	20, 20, 40	27
2	2, 5	4
4	4	4
6	2, 5, 10	6
8	4, 5, 8, 8	6
12	4, 6, 8, 8	7
20	2, 2	2
28	5, 7	6
40	20, 20	20
50	20, 20	20

As on the spontaneously contracting rat uterus, the action of histamine against electrically-induced contractions was inhibitory. The doses of histamine which when added to the organ bath (10 ml. capacity) produced 20 per cent inhibition of the height of contraction was taken as the index of sensitivity.

Determinations of the histamine sensitivities of 11 untreated rats gave a value of 27 μg. $\pm$ 3 (mean $\pm$ S.D.). Table I shows the histamine sensitivies of rats after 48/80 treatment.

It can be seen that the sensitivity to histamine decreased 6 hr. after treatment. However, 2 days later there was observed an increase in sensitivity which persisted for 28 days. 40 to 50 days after treatment sensitivity decreased towards the control figure for untreated rats. The sensitivity of the uteri to the inhibitory action of adrenaline was unaltered throughout the course of these experiments.

These results show that 48/80 treatment selectively modifies the sensitivity of the uterus to applied histamine. There is a similarity in the period during which increased sensitivity persisted in these experiments and the period during which tissue levels of histamine remain low after 48/80 treatment. Feldberg and Talesnik (1953) have shown that after 48/80 treatment the tissue content of histamine is lowered, remaining low up to and sometimes beyond 50 days after treatment. It may be that there is an inverse relationship between histamine sensitivity and histamine tissue content. Paton (1957) has observed that those species like the rat with high tissue histamine levels are relatively insensitive to the effects of injected histamine, whereas those species like the guinea-pig with low histamine levels are more susceptible.

Acknowledgements. I am indebted to Burroughs Wellcome & Co. (Australia) Ltd. for a grant, and for a gift of 48/80; and to Dr. M. J. Rand for suggesting this idea to me.

JUDITH ANN HARVEY

Department of Pharmacology,
University of Sydney,
Sydney, N.S.W., Australia.

November 4, 1960.

REFERENCES

Csapo, A. I. and Corner, G. W. (1952). *Endocrinology*, **51**, 378–385.
Feldberg, W., and Talesnik, L. J. (1953). *J. Physiol.*, **120**, 550–568.
Parratt, J. R., and West, G. B. (1957). *Ibid.*, **137**, 179–192.
Paton, W. D. M. (1957). *Pharmacol. Rev.*, **9**, 269–328.

A Reaction between Prednisolone Phosphate and Sodium Metabisulphite

SIR,—The reactivity of bisulphites with certain substances of pharmaceutical interest has been previously noted (Higuchi and Schroeter, 1959).

We observed the pH values of certain aqueous experimental preparations containing prednisolone phosphate and sodium metabisulphite to rise for several days after preparation. A close examination of the ultra-violet absorption spectra of their aqueous dilutions showed slight bathochromic shifts (1–2 mμ); increased amounts of sodium metabisuphite produced greater shifts. The maximum shift attainable was 8 mμ, when λ_{max} occurred at 255 instead of at the normal wavelength of 247 mμ. A recently developed enzyme method for prednisolone phosphate (Boon, 1960), which is specific for phosphoric esters of solvent extractable alcohols, when applied to fresh mixtures of prednisolone phosphate and sodium metabisulphite gave the expected values. After the spectral change had occurred, the prednisolone phosphate contents, as determined by the enzyme method, were unexpectedly low. In a series of solutions of constant prednisolone phosphate and various metabisulphite contents there was after equilibration a rank correlation between the shift in λ_{max} and the deficit of

prednisolone phosphate. Paper chromatographic evidence suggested the presence of a new compound more water-soluble than prednisolone phosphate. Aqueous solutions of a reaction-product isolated by freeze-drying an aqueous prednisolone phosphate solution saturated with sulphur dioxide had λ_{max} at 255 mμ. The infra-red spectrum of the solid gave no evidence for presence of a $\triangle^{1,4}$ compound, indicating the probability that only one double bond was conjugated with the 3-keto group in Ring A.

Two solutions, one containing 3·3 per cent prednisolone disodium phosphate and 5 per cent sodium metabisulphite, the other 2·7 per cent and 2 per cent, respectively, had rat-liver glycogen responses, after equilibration, consistent with the prednisolone phosphate contents determined by the enzyme method, which showed deficits of 90 per cent and 61 per cent respectively. The extent of the reaction is markedly dependent on pH, proceeding most readily at acid pH values. It is apparently completely and rapidly reversed in the pH range 10 to 12, since brief contact with alkali reverses the spectral shift and restores the deficit revealed by the enzymatic determination.

Acknowledgement. We are indebted to Mr. E. A. Woollett for determining the rat-liver glycogen responses.

P. F. G. Boon.
M. J. Busse.

Glaxo Laboratories Ltd.,
Greenford,
Middlesex.

November 16, 1960.

References

Boon, P. F. G. (1960). *J. Pharm. Pharmacol.*, **12**, *Suppl.*, 159*T*–163*T*.
Higuchi, T. and Schroeter, L. S. (1959). *J. Amer. pharm. Ass., Sci. Ed.*, **48**, 535–540.

The Emulsifying Properties of Gum Acacia

Sir,—In the discussion of the paper "The Emulsifying Properties of Gum Acacia" presented to the British Pharmaceutical Conference at Newcastle upon Tyne (Shotton and Wibberley, 1960) I postulated that a seven times washed emulsion of heptane if subjected to further homogenisation would crack, as there would be insufficient acacia to stabilise the new interfacial area produced. I have now submitted the seven times washed emulsion of heptane to a second homogenisation, using the same hand-operated homogeniser. A more effective machine was not used as the volume of emulsion available was small.

The results are much as predicted. The emulsion issuing from the homogeniser had deteriorated, and globules of oil were clearly seen. On standing, the emulsion separated into three layers: an upper layer of heptane, a middle layer of emulsion, and the aqueous phase. It was not possible to separate quantitatively the heptane layer produced by the breaking of the emulsion, but a substantial fraction of the heptane separated after a few minutes, and the quantity increased on standing until only a small amount of emulsion remained.

Examination of the emulsion under the microscope immediately after homogenisation showed that the globule size range was increased from the 1 to 25 μ of the original washed emulsion to 1 to 150 μ or more, and the larger globules were more numerous. After 7 days the number of the large globules in the residual emulsion had decreased.

The events that took place on the second homogenisation seemed to be as follows. Many of the smaller globules passed unchanged through the hand homogeniser and remained as stable oil droplets. Most of the remaining globules were ruptured by shear, lost their stabilising film completely and coalesced to form the oil phase of the system. The remainder were not completely stripped of the protective film, and coalesced to form the large globules above 50 μ which on long standing cracked. This ability to produce large globules during processing gives some idea of the speed at which a ruptured acacia film is able to reform. The reduction of the surface area: volume ratio would allow some losses of film without reducing the amount of acacia available for film formation below the critical levels. Breaking of the globules at a later stage must be attributed to mechanical failure of the film when the globules are distorted by compression during creaming.

K. WIBBERLEY.

Department of Pharmaceutics,
School of Pharmacy,
Brunswick Square,
London, W.C.1.

December 5, 1960.

REFERENCE

Shotton, E. and Wibberley, K. (1960). *J. Pharm. Pharmacol.*, **12**, *Suppl.*, 105*T*-107*T*.