

2 7

JOURNAL OF PHARMACY
AND PHARMACOLOGY

VOLUME XIV, 1962

WITH THE
TRANSACTIONS
OF THE
BRITISH PHARMACEUTICAL CONFERENCE
HELD AT
LIVERPOOL
SEPTEMBER 10 to 14, 1962

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

ห้องสมุด กรมวิทยาศาสตร์

JOURNAL OF PHARMACY
AND PHARMACOLOGY
VOLUME XIV, 1962

SUBJECT INDEX*

A

- Acetylcholine and Acetylcholinesterase, New General Concept of the Neurohumoral Functions of (Koelle), 65.
- Acetylcholine, Synthesis of, by Acetone-dried Powders from the Brains of Normal and Thiamine-deficient Rats (Bhagat and Lockett), 37.
- Acetylcholinesterase and Acetylcholine, New General Concept of the Neurohumoral Functions of (Koelle), 65.
- Adrenaline and Noradrenaline in Urine, Method for the Estimation of (Atkinson and Wynne), 794.
- Adrenal Glands and Aortic Walls of Rats, Note on the Influence of Chlorpromazine and Diethazine on the Catecholamine Stores in (Davis and others), 735.
- Adrenergic Blocking Agents and Chlorpromazine, Effect of, on Blood Pressure Increase by Vasopressin and Angiotensin (Supek and others), 284.
- Adrenergic Neurone Blocking Action of Dimethylphenylpiperazinium (Wilson), 700.
- Adrenocortical Steroids, Effects of Prolonged Administration of Some, in the Rat (D'Arcy and Howard), 294.
- African *Rauwolfia* Species. Part II. The Structure of the Root and Stem of *Rauwolfia mombasiana* Stapf. (Court), 22.
- Age, Effect of, on the Viability of *Penicillium notatum* Spores in Water and Solutions of Phenol (Chauhan and Walters), 130 T.
- Aldehydes, Oxidation of, in Aqueous Solutions of Cetomacrogol (Carless and Mitchell), 46.
- Alimentary Tract, Absorption of Bretylium and Related Quaternary Ammonium Salts from (Boura and McCoubrey), 647.
- Alkaloidal Studies on *Datura leichhardtii* Muell. ex Benth. (Evans and Stevenson), 107T.
- Alkaloids, Diterpene, Isolation and Study of Two New (Singh and Chopra), 288.
- Alkaloids of the Genus *Datura*, Section *Brugmansia*. Part I. *D. cornigera* Hook. (Evans and Pe Than), 147.
- Alkaloids, Steroidal, Biological Activity in (Alauddin and Martin-Smith), 469.
- Alkanolamides of 3-Dimethylamino-propionic Acid, 1-Methylhexahydronicotinic Acid and Arecaidine, Dissociation Constants of (Chilton and Stenlake), 367.
- Alkanolamides Sterically Related to Ergometrine (Chilton and Stenlake), 350.
- Alloxan Diabetes, Effects of Orally Effective Hypoglycaemic Agents from Plants on (Brahmachari and Augusti), 617.
- Aluminium Oxide-Silicic Acid Double Column, Analysis of Oil of Peppermint by (Karawya and Wahba), 611.
- Amines, Sympathomimetic, Dexamphetamine and other Related, Antagonism of Guanethidine by (Day and Rand), 541.
- m*-Aminophenol, Direct Colorimetric Determination of Small Quantities of, in Sodium Aminosalicylate (Bičan-Fister), 280.
- α -Aminophenylacetic Acids, *N*-Substituted, Local Anaesthetic Properties of Esters of (Shapero and Edwards), 119.
- Amitriptyline and Imipramine and their Monomethyl Derivatives, Effect of, on Reserpine Activity (Garrattini and others), 509.
- Ampicillin, Activity of, against *Escherichia coli* (Turner and Russell), 395.
- Anaesthetic Activity, Local, in Diethylaminoacetyl Derivatives of Substituted Benzylamines (Collins and Large), 48T.
- Anaesthetic Properties, General, Absence of, in a Number of Terpenoid Hemisuccinates (Ahmad and others), 467.
- Anaesthetic Properties, Local, of Esters of *N*-Substituted α -Aminophenylacetic Acids (Shapero and Edwards), 119.
- Anaesthetic Steroid, Sex Difference in Sensitivity of GFF Mice to (Atkinson and others), 698.
- Anaesthetics, Local, Anti-veratrinic Activity of some (Sharma and Arora), 515.

* Page numbers followed by an italic *T* refer to the Supplement containing the Transactions of the British Pharmaceutical Conference.

SUBJECT INDEX

- Anaesthetics, Local, Synthesis of *o*-Substituted 2-Diethylaminoethyl Benzoates as Potential (Thomas and Canty), 587.
- Anaphylaxis and Heparin (Dhar and Sanyal), 393.
- Anaphylaxis *in vivo*, Effect of Hydrocortisone on Changes induced in Lipid Metabolism in Guinea-pig Lung Tissue by (Goadby and Smith), 739.
- Aneurine *see* Thiamine
- Angiotensin and Vasopressin, Effect of Adrenergic Blocking Agents and of Chlorpromazine on Blood Pressure Increase by (Supek and others), 284.
- Animal Strain Selection and Conditioning, Influence of, on Biological Experiments and Assays, Symposium on, 397-428.
- Animals, Experimental, Conditioning of (D'Arcy), 411.
- Anticoagulant, Calophyllolide, a Complex Coumarin from *Calophyllum inophyllum* Linn. (Arora and others), 534.
- Anticonvulsant Action of Procaine and Five Congeners against Experimentally Induced Convulsions (Kapila and Arora), 253.
- Anticonvulsant Activity and Synthesis of Some *N*-Phenethylacetamides (Sidhu and others), 125.
- Anticonvulsant Drugs, an Apparatus for Testing, by Electroshock Seizures in Mice (Cashin and Jackson), 44*T*.
- Anti-inflammatory Activity of Musk (Mishra and others), 830.
- Anti-inflammatory Agents, 6-Methylcortisone Acetate 3-Enol Ethers—A New Group of (David and others), 127.
- Anti-inflammatory Drugs, Effects of, on some Aspects of Intermediary Metabolism (Bryant and Smith), 182.
- Antiveratrinic Activity of some Local Anaesthetics (Sharma and Arora), 515.
- Aorta and Spleen, Effects in Rabbits of Thyroidectomy and Treatment with Triiodothyronine on the Sensitivity to Noradrenaline and the Content of Noradrenaline in (Macmillan and Rand), 257.
- Aortic Walls and Adrenal Glands of Rats, Note on the Influence of Chlorpromazine and Diethazine on the Catecholamine Stores in (Davis and others), 735.
- Aqueous Suspensions, Retention of, on Leaf Surfaces (Challen), 707.
- Arecaidine, Dissociation Constants of Alkanolamides of (Chilton and Stenlake), 367.
- Aryloxyaliphatic Acids, Action of, on the Permeability of Blood Vessels (Northover and Verghese), 615.
- Auricles of the Rabbit, Note on the Action of Gallamine on (Laity and Garg), 371.

B

- Bacterial Spores on Paper Carriers, Apparatus for Testing the Resistance to Wet Heat of (Cook and Brown), 61.
- Bacterial Spores, Studies on the Post-irradiation Oxygen Effect in (Tallentire and Dickinson), 127*T*.
- Ball Milling, Wet, Particle Size Distribution of Marble on (Barnett and James), 111*T*.
- Barium Sulphate, A New Deflocculant and Protective Colloid for (Anderson), 64.
- Bases, Organic, in Pharmaceutical Preparations, use of Tetraphenylboron for the Determination and Characterisation of (Johnson and King), 77*T*.
- Bath, Modified Dual Unit, for Isolated Tissues (Brittain and Rowe), 191.
- Behavioural Responses to a Buzzer, Conditioned and Unconditioned (Izquierdo), 316.
- Benzaldehyde, Emulsified and Solubilised, Oxidation of (Carless and Swarbrick), 97*T*.
- Benzocaine, Ethyl Benzoate and Diethyl Phthalate in Cetrimide Solutions, Hydrolysis of (Mitchell), 172.
- Benzoylcholine, Effect of *Ortho* Substitution on the Pharmacology of (Thomas and Buckley), 225.
- Benzylamines, Substituted, Local Anaesthetic Activity in Diethylaminoacetyl Derivatives of (Collins and Large), 48*T*.
- N*-Benzyl-*N'*-*N''*-dimethylguanidine (BW 467C60), Estimation of, in Urine and some Observations on its Reaction with Hypobromite (McCoubrey), 798.
- N*-Benzyl-*N'*-*N''*-dimethylguanidine, a new Hypotensive Drug, Distribution and Excretion of, in Cats (Boura and others), 722.
- Benzyl Violet, 4B, Chronic Toxicity of, in Rats (Mannell and others), 378.
- Bersama abyssinica* Fres. Sub-species *abyssinica*, Cardiotonic Substances from (Lock), 496.
- Biogenic Amines, Note on the use of Cellulose Phosphate Cation-exchange Paper for the Separation of (Roberts), 746.

SUBJECT INDEX

- 4,4'-Biphenylenebis[carbonylmethyl-(2-hydroxyethyl)dimethylammonium Bromide] *see* Hemicholinium Compound (HC-3).
- Blood Griseofulvin, Common Drugs that may Invalidate Spectrophotoflometric Assays of (Child, and others), 374.
- Blood Pressure Increase by Vasopressin and Angiotensin, Effect of Adrenergic Blocking Agents and of Chlorpromazine on (Supek and others), 284.
- Blood Vessels, Action of Aryloxyaliphatic Acids on the Permeability of (Northover and Verghese), 615.
- Book Reviews, 63, 188, 322, 539, 619, 833.
- Bretylum, Biochemical Properties of (McCoubrey), 727.
- Bretylum and Cocaine, Effect of, on Noradrenaline Depletion (Callingham and Cass), 385.
- Bretylum and Related Quaternary Ammonium Salts, Absorption of, from the Alimentary Tract (Boura and McCoubrey), 647.
- Brilliant Blue FCF, Chronic Toxicity of, in Rats (Mannell and others), 378.
- Brilliant Green and Crystal Violet, Controlled Potential Reduction of, at the Stirred Mercury Cathode (Butler and Martin), 1037.
- British Pharmaceutical Conference 1962. Transactions, 17-131 T, as a Supplement. Conference Lecture, 20T-30T; Report of Proceedings, 1T-9T; Science Papers, 31T-131T; Symposium on Drug Addiction, 9T-19T.
- Brugmansia* Section of the Genus *Datura*, Alkaloids of. Part I. *D. cornigera* Hook (Evans and Pe Than), 147.
- Butylated Hydroxyanisole and Hydroxytoluene, Tritiated, Urinary Excretion of, in the Rat (Golder and others), 268.
- C**
- Calciphyllactic Challenging Potency of Various Iron Compounds, Comparative Study of (Strebel and others), 658.
- Calcium, Determination of, in Heavy Magnesium Carbonate using Glyoxal Bis(2-hydroxyanil) (Leonard), 63T.
- Calophyllolide, a Complex Coumarin Anticoagulant from *Calophyllum inophyllum* Linn. (Arora and others), 534.
- Calophyllum inophyllum*, Linn., Calophyllolide, a Complex Coumarin Anticoagulant from (Arora and others), 534.
- Capillary Permeability Changes induced by *Echis carinatus* (Saw-scaled Viper) Venom in the Rat Effect of Hydrocortisone on (Somani and Arora), 535.
- Capillary Permeability, Mechanism of Increased, Induced by *Echis carinatus* (Saw-scaled Viper) Venom; A Possible new Approach to the Treatment of Viperine Snake Poisoning (Somani and Arora), 394.
- Cardiotonic Action of Hamycin (Arora), 320.
- Cardiotonic Substances from *Bersama abyssinica* Fres. Sub-species *abyssinica* (Lock), 496.
- Cardiotoxicity of Isoprenaline in Rats, Influence of Environmental Changes on (Balazs and others), 750.
- Cats, Distribution and Excretion by, of a new Hypotensive Drug, *N*-Benzyl-*N'*-*N''*-dimethylguanidine (Boura and others), 722.
- Cat, Spinal, Pressor Action of Guanethidine in (Bartlet), 91.
- Catechol and Pyrogallol, Effect of, on Isolated Smooth Organs (Izquierdo and Jurio), 190.
- Catechol and Pyrogallol, Spasmolytic Actions of, on the Isolated Guinea-pig Ileum (Johnson), 272.
- Catecholamine Stores in the Adrenal Glands and the Aortic Walls of Rats, Note on the Influence of Chlorpromazine and Diethazine on (Davis and others), 735.
- Catecholamines and some other Biogenic Amines, Note on the use of Cellulose Phosphate Cation-exchange Paper for the Separation of (Roberts), 746.
- Catecholamines, Concentration of, in the Turtle Heart and Vagal Escape (Friedman and Bhagat), 764.
- Catha edulis* Forsk, Anatomical Study of (Shadan and Shellard), 110.
- Cellulose Phosphate Cation-exchange Paper, Note on the use of, for the Separation of Catecholamines and some other Biogenic Amines (Roberts), 746.
- Cetomacrogol, Oxidation of Aldehydes in Aqueous Solutions of (Carless and Mitchell), 46.
- Cetrimide Solutions, Hydrolysis of Ethyl Benzoate, Diethyl Phthalate and Bezocaine in (Mitchell), 172.
- Chlorpromazine and Adrenergic Blocking Agents, Effect of, on Blood Pressure Increase by Vasopressin and Angiotensin (Supek and others), 284.

SUBJECT INDEX

- Chlorpromazine and Diethazine, Note on the Influence of, on Stores of Catecholamine in the Adrenal Glands and the Aortic Walls of Rats (Davis and others), 735.
- Choline, Phosphorylation of Anti-adrenergic Quaternary Ammonium Salts Related to (Copp and others), 641.
- Chronic Toxicity Studies on Food Colours. V. Observations on the Toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B in Rats (Mannell and others), 378.
- Cocaine and Bretylium, Effect of, on Noradrenaline Depletion (Callingham and Cass), 385.
- Codeine, Morphine and Nalorphine, Note on the Paper Chromatographic Separation of (Street), 56.
- Colloid, Protective and Deflocculant for Barium Sulphate (Anderson), 64.
- Complexometric Method for the Determination of some Sulphonamides (Abdine and Abdel Sayed), 761.
- Computer, Simple Mechanical, for Relating the Contractile Responses of Tissues (Paterson), 825.
- Conditioned and Unconditioned On- and Off-behavioural Responses to a Buzzer (Izquierdo), 316.
- Conditioning of Experimental Animals (D'Arcy), 411.
- Contact Sensitisation, Mechanism of (Schild), 1.
- Coronary Occlusion, Ectopic Ventricular Arrhythmia after, in the Indian Domestic Pig (Arora and Sivappa), 315.
- Coumarin Anticoagulant from *Calophyllum inophyllum* Linn. (Arora and others), 534.
- Crystal Violet and Brilliant Green, Controlled Potential Reduction of, at the Stirred Mercury Cathode (Butler and Martin), 103T.
- Curare-like Drugs and Vagal Synapses: Comparative Study *in vitro*, on the Isolated Vagus-stomach Preparation of the Rat (Della Bella and others), 701.
- Cyanocobalamin, Hydrolytic Destruction of Thiamine in the Presence of (Heathcote and Wills), 232.
- Cyclizine Hydrochloride and Ergotamine Tartrate, Determination of Ergotamine in Preparations Containing (Caws and Lawrence), 59T.
- D**
- Datura cornigera* Hook., Alkaloids of (Evans and Pe Than), 147.
- Datura cornigera*, Hook., Morphology and Histology of Seeds of (Wellendorf), 157.
- Datura* Genus, Section *Brugmansia*, Alkaloids of. Part I. *D. cornigera* Hook. (Evans and Pe Than), 147.
- Datura leichhardtii* Muell. ex Benth. Part I. Anatomy of Leaf and Stem (Evans and Stevenson), 664.
- Datura leichhardtii* Muell. ex Benth., Studies on Part II, Alkaloidal Constituents (Evans and Stevenson), 107T.
- Deflocculant and Protective Colloid for Barium Sulphate (Anderson), 64.
- Detergents, Synthetic Non-ionic, Surface Activity of a Series of (Elworthy and Macfarlane), 100T.
- Dexamphetamine and other Related Sympathomimetic Amines, Antagonism of Guanethidine by (Day and Rand), 541.
- Diabetes, Alloxan, Effects of Orally Effective Hypoglycaemic Agents from Plants on (Brahmachari and Augusti), 617.
- Diethazine and Chlorpromazine, Note on the Influence of, on Stores of Catecholamine in the Adrenal Glands and the Aortic Walls of Rats (Davis and others), 735.
- Diethylaminoacetyl Derivatives of Substituted Benzylamines, Local Anaesthetic Activity in (Collins and Large), 48T.
- 2-Diethylaminoethyl Benzoates, Synthesis of *o*-Substituted, as Potential Local Anaesthetics (Thomas and Canty), 587.
- Diethyl Phthalate, Ethyl Benzoate, and Benzocaine, in Cetrimide Solutions, Hydrolysis of (Mitchell), 172.
- Digitalis, Assay of, in Pigeons by Intra-peritoneal Administration (Kušević), 96.
- Digitoxin Metabolite C (Digoxin Metabolite B), Identification of, with Digoxigenin Di-digitoxoside (Wright), 613.
- Digoxigenin Di-digitoxoside, Identification of Digoxin Metabolite B, with (Wright), 613.
- Digoxin Metabolite B (Digitoxin Metabolite C), Identification of with Digoxigenin Di-digitoxoside (Wright), 613.
- N*-(3,4-Dimethoxyphenethyl)-3(or 4)-hydroxycyclohexane Carboxamide, Derivatives of, as Potential Reserpine Analogues (Chodneker and others), 756.
- 3-Dimethylaminobenzoic Acid, Derivatives of, as Potential Reserpine Analogues (Chodnekar and others), 756.

SUBJECT INDEX

- 3-Dimethylaminopropionic Acid, Dissociation Constants of Alkanolamides of (Chilton and Stenlake), 367.
- Dimethylphenylpiperazinium, Adrenergic Neurone Blocking Action of (Wilson), 700.
- Dispersed Phase Concentration and Electrical Resistance, Relationship Between, in Oil in Water Emulsions (Harrison and James), 503.
- Dissociation Constants of Some Compounds Related to Lysergic Acid. Part II. Ergometrine, Ergometrinine, and Alkanolamides of 3-Dimethylaminopropionic Acid, 1-Methylhexahydroindolizidine and Arecaidine (Chilton and Stenlake), 367.
- Diterpene Alkaloids, Isolation and Study of Two New (Singh and Chopra), 288.
- Diuretic Agents, Studies in the Field of. Part VI. Some Sulphamoylbenzoic Acids (Jackman and others), 679.
- Dog, Acute Myocardial Infarction in, Effectiveness of 5-Hydroxytryptamine in Ectopic Ventricular Tachycardia Resulting from (Kapila and Arora), 831.
- Drug Addiction, Symposium on 9T-19T.
- Drug Addiction (Symposium Papers), (Macdonald), 9T; (Johnston), 16T; (Thomas), 17T.
- Drugs, Anticonvulsant, Apparatus for Testing, by Electroshock Seizures in Mice (Cashin and Jackson), 44T.
- Drugs, Anti-inflammatory, Effect of, on some Aspects of Intermediary Metabolism (Bryant and Smith), 182.
- Drugs, Pharmacogenetics—A Study of Inherited Variability in the Response to (Clarke), 20T.
- Drug-Plasma Binding Measured by Sephadex (Barlow and others), 550.
- Drugs and Rat Pregnancy (West), 828.

E

- Echis carinatus* (Saw-scaled Viper) Venom, Effect of Hydrocortisone on Capillary Permeability Changes induced by, in the Rat (Somani and Arora), 535.
- Echis carinatus* Venom, Mechanism of Increased Capillary Permeability Induced by: A Possible new Approach to the Treatment of Viperine Snake Poisoning (Somani and Arora), 394.
- Ectopic Ventricular Arrhythmia after Coronary Occlusion in the Indian Domestic Pig (Arora and Sivappa), 315.

- Electrical Resistance and Dispersed Phase Concentration, Relation Between, in Oil in Water Emulsions (Harrison and James), 503.
- Electroshock Seizures in Mice, Apparatus for Testing Anticonvulsant Drugs by (Cashin and Jackson), 44T.
- Emetine, Spectrophotofluorometric Determination of, in Animal Tissues (Davis and others), 249.
- Emulsions, Oil in Water, Relationship between Electrical Resistance and Dispersed Phase Concentration in (Harrison and James), 503.
- Environmental Changes, Influence of, on the Cardiotoxicity of Isorenaline in Rats (Balazs and others), 750.
- Enzyme-inhibiting Action of Tetrahydroaminoacridine and its Structural Fragments (Kaul), 243.
- Ergometrine, Alkanolamides Sterically Related to (Chilton and Stenlake), 350.
- Ergometrine, Biological Assay of Oxytocin in the Presence of (Berde and Stürmer), 169.
- Ergometrine, Dissociation Constant of (Chilton and Stenlake), 367.
- Ergometrinine, Dissociation Constant of (Chilton and Stenlake), 367.
- Ergotamine, Determination of, in Preparations Containing Ergotamine Tartrate and Cyclizine Hydrochloride (Caws and Lawrence), 59T.
- Ergotamine Tartrate and Cyclizine Hydrochloride, Determination of Ergotamine in Preparations containing (Caws and Lawrence), 59T.
- Escherichia coli*, Activity of Ampicillin against (Turner and Russell), 395.
- Escherichia coli*, Note on the Activity of Six Penicillins Against (Russell), 390.
- Ethiopian Khat (Leaf of *Catha edulis* Forsk.), Anatomical Study of (Shadan and Shellard), 110.
- Ether, Effects of, on Potassium Flux in Skeletal Muscle Preparations (Lewis and others), 539.
- 4-Ethoxycarbonyloxy-3,5-dimethoxybenzoic Acid, Derivatives of, as Potential Reserpine Analogues (Chodnekar and others), 756.
- Ethyl Benzoate, Diethyl Phthalate and Benzocaine in Cetrimide Solutions, Hydrolysis of (Mitchell), 172.

F

- Films, Interfacial, of Potassium Arabate, some Physical Properties of (Wibberley), 87T.

SUBJECT INDEX

Food Colours, Studies on the Chronic Toxicity of. V. Observations on the Toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B in Rats (Mannell and others), 378.

Freeze-Drying, Recent Advances in (Greaves), 621.

Fruits of *Illicium verum* and *Illicium anisatum* L., Colour Test to Distinguish between (Phang and others), 108.

Fungicidal Action, Studies on the Kinetics of. Part II. Effect of Temperature on the Viability of *Penicillium notatum* Spores in Water and Solutions of Phenol (Chauhan and Walters), 605.

Fungistatic Activity of Methyl and Propyl Hydroxybenzoates and a Mixture of These against *Penicillium spinulosum* (Gerrard and others), 103.

G

Gallamine, Note on the Action of, on Isolated Rabbit Auricles (Laity and Garg), 371.

Gastric Secretion in the Guinea-pig, Histamine-stimulated, effect of a sulphated Polysaccharide on the Acidity and Volume of (Anderson and others), 119T.

Gastrointestinal Tract, Some Factors Influencing the Absorption of Griseofulvin from (Duncan and others), 217.

Glyoxal Bis(hydroxyanil), Determination of Calcium in Heavy Magnesium Carbonate using (Leonard), 63T.

Griseofulvin in Blood, Common Drugs that may Invalidate Spectrophotofluorometric Assays for (Child and others), 374.

Griseofulvin, Some Factors Influencing the Absorption of, from the Gastrointestinal Tract (Duncan and others), 217.

Guanethidine, Antagonism of, by Dexamphetamine and other Related Sympathomimetic Amines (Day and Rand), 541.

Guanethidine, Pressor Action of, in the Spinal Cat (Bartlett), 91.

Guinea Green B, Chronic Toxicity of, in Rats (Mannell and others), 378.

Guinea-pig, Histamine-stimulated Gastric Secretion in, Effect of a Sulphated Polysaccharide on the Acidity and Volume of (Anderson and others), 119T.

Guinea-pig Ileum, Isolated, Spasmodic Actions of Pyrogallol and Catechol on (Johnson), 272.

Guinea-pig Lung Tissue, Changes Induced in Lipid Metabolism in, by Anaphylaxis *in vivo*. Effects of Hydrocortisone on (Goadby and Smith), 739.

H

Hamycin, Cardiotonic Action of (Arora), 320.

Heart, Turtle, Concentration of Catecholamines in, and Vagal Escape (Friedman and Bhagat), 764.

Hemicholinium Compound (HC-3), 4,4'-Biphenylenebis [carbonyl-methyl-(2-hydroxyethyl)dimethylammonium Bromide], some Effects of on Neuromuscular Transmission in the Cat (Evans and Wilson), 34T.

Heparin and Anaphylaxis (Dhar and Sanyal), 393.

Heparin, Assay of Protamine Sulphate for its Capacity to Neutralise (Birkinshaw and Smith), 95T.

Hepatoma, Rat, Inhibition of L-Histidine Decarboxylase from (Robinson and Shepherd), 9.

Histamine-stimulated Gastric Secretion in the Guinea-pig, Effect of a Sulphated Polysaccharide on the Acidity and Volume of (Anderson and others), 119T.

L-Histidine Decarboxylases of Guinea-pig Kidney and Rat Hepatoma, Inhibition of (Robinson and Shepherd), 9.

Holarrhena antidyenterica see Kurchi.

Hydrocortisone, Effect of, on Capillary Permeability Changes induced by *Echis carinatus* (Saw-scaled Viper) Venom in the Rat (Somani and Arora), 535.

Hydrocortisone, Effects of, on the Changes in Lipid Metabolism Induced in Guinea-pig Lung Tissue by Anaphylaxis *in vivo* (Goadby and Smith), 739.

Hydroxyanisole and Hydroxytoluene, Butylated, Urinary Excretion of Tritiated, in the Rat (Golder and others), 268.

p-Hydroxybenzanilide, Derivatives of, as Potential Reserpine Analogues (Chodnekar and others), 756.

5-Hydroxyindoleacetic Acid, Effect of Administration of Water or Isotonic NaCl Solution on the Urinary Excretion of, in the Rat (Bertaccini and Erspamer), 687.

Hydroxytoluene and Hydroxyanisole, Butylated, Urinary Excretion of Tritiated, in the Rat (Golder and others), 268.

SUBJECT INDEX

- 5-Hydroxytryptamine, Effectiveness of, in Ectopic Ventricular Tachycardia Resulting from Acute Myocardial Infarction in the Dog (Kapila and Arora), 831.
- Hypertensive and Hypotensive Principles from West Indian Medicinal Plants (Durand and others) 562.
- Hypobromite, Some Observations on the Reaction of *N*-Benzyl-*N'*-*N''*-dimethylguanidine with (McCoubrey), 798.
- Hypoglycaemic Agents, New Oral (Luthra and Tayal), 396.
- Hypoglycaemic Agents. Part II. (Hayman and others), 451; Part III. (Hayman and others), 522.
- Hypoglycaemic Agents from Plants, Orally Effective (Brahmachari and Augusti), 254.
- Hypoglycaemic Agents from Plants, Effects of Orally Effective, on Alloxan Diabetes (Brahmachari and Augusti), 617.
- Hypotensive Drug, new, *N*-Benzyl-*N'*-*N''*-dimethylguanidine, Distribution and Excretion of, by Cats (Boura and others), 722.
- Hypotensive and Hypertensive Principles from some West Indian Medicinal Plants (Durand and others), 562.

I

- Illicium anisatum* and *Illicium verum*, Colour Test to Distinguish between the Fruits of (Phang and others), 108.
- Illicium verum* and *Illicium anisatum* L., Colour Test to Distinguish between the Fruits of (Phang and others), 108.
- Imipramine, Amitriptyline and their Monomethyl Derivatives, Effects of, on Reserpine Activity (Garrattini and others), 509.
- Imipramine, Effects of, on Reserpine Toxicity in Adrenalectomised Rats (Montanari and Stockham), 126.
- Infarction, Acute Myocardial, in the Dog, Effectiveness of 5-Hydroxytryptamine in Ectopic Ventricular Tachycardia Resulting from (Kapila and Arora), 831.
- Infra-red Spectroscopy, Analysis of Poldine Methyl Methosulphate by (Rapson and others), 667.
- Injection, Water for, by Ion-exchange (Cook and Saunders), 837.
- Interfacial Films of Potassium Arabate, some Physical Properties of (Wibberley), 877.
- Ion-exchange, Water for Injection by (Cook and Saunders), 837.

- Iron Compounds, Calciphylactic Challenging Potency of Various, Comparative Study of (Strebe and others), 658.
- Irradiation, Ultrasonic, of some Phospholipid Sols (Saunders and others), 567.
- Isoniazid in Mixtures with Sodium *p*-aminosalicylate, Modified Procedure for the Determination of (Lee Kum-Tatt and Ho Yan-Hon), 123.
- Isoprenaline, Influence of Environmental Changes on the Cardio-toxicity of, in Rats (Balazs and others), 750.
- Isoprenaline, Note on the Stability of Solutions of (West and Whittet), 937.

K

- Khat, Ethiopian (Leaf of *Catha edulis* Forsk.), Anatomical Study of (Shadan and Shellard), 110.
- Kidney, Guinea-pig, Inhibition of L-Histidine Decarboxylase from, (Robinson and Shephard), 9.
- Kinetics of Fungicidal Action Part II, Studies on. Effect of Temperature on the Viability of *Penicillium notatum* Spores in Water and Solutions of Phenol (Chauhan and Walters), 605.
- Kurchi, *Holarrhena antidysenterica*, *Wrightia tinctoria* Bark as an Adulterant of (Atal and Sethi), 41.

L

- Lanolin, Anhydrous, Peroxide Value of (Clark and Kitchen), 318.
- Lanolin, Note on the Peroxide Value of (Anderson and Wood), 186.
- Leaf Surfaces, Retention of Aqueous Suspensions on (Challen), 707.
- Letters to the Editor, 64, 125, 190, 253, 315, 393, 467, 534, 613, 698, 764, 828.
- Liothyronine *see also* Triiodothyronine.
- Liothyronine and Thyroxine in Enzymatic Hydrolysates of Pork Thyroid, Chemical Determination of (Devlin and Stephenson), 597.
- Lipid Metabolism, Changes Induced in, in Guinea-pig Lung Tissue by Anaphylaxis *in vivo*, Effects of Hydrocortisone on (Goaddy and Smith), 739.
- Liquorice, Solubilising Properties of (James and Standford), 445.
- Local Anaesthetic Activity in Diethylaminoacetyl Derivatives of Substituted Benzylamines (Collins and Large), 487.

SUBJECT INDEX

- Local Anaesthetics, Anti-veratrinic Action of some (Sharma and Arora), 515.
- Local Anaesthetic Properties of Esters of *N*-Substituted α -Aminophenylacetic Acids (Shapero and Edwards), 119.
- Local Anaesthetics, Synthesis of *o*-Substituted 2-Diethylaminoethyl Benzoates as Potential (Thomas and Canty), 587.
- Lysergic Acid, Dissociation Constants of some Compounds Related to, Part II. Ergometrine, Ergometrinine and Alkanolamides of 3-Dimethylaminopropionic Acid, 1-Methylhexahydronicotinic Acid and Arecaidine (Chilton and Stenlake), 367.
- Lysophosphatidylethanolamine, Surface Activity of (Robins and Thomas), 128.
- ### M
- Magnesium Carbonate, Heavy, Determination of Calcium in, Using Glyoxal Bis(hydroxyanil) (Leonard), 637.
- Marble, Wet, Particle Size Distribution of, on Wet Ball Milling (Barnett and James), 1117.
- Mast-Cells, Function of (West), 619.
- Medicinal Chemistry, Quaternary Ammonium Compounds in (D'Arcy and Taylor), Part I, 129: Part II (with all references), 193.
- Medicinal Plants, West Indian, Pharmacological Screening of some (Feng and others), 556.
- Mercury Cathode, Stirred, Controlled Potential Reduction of of Brilliant Green and Crystal Violet at the (Butler and Martin), 1037.
- Metabolism, Intermediary, Effects of Anti-inflammatory Drugs on some aspects of (Bryant and Smith), 182.
- 6-Methylcortisone Acetate 3-Enol Ethers — A New Group of Anti-inflammatory Agents (David and others), 127.
- 1-Methylhexahydronicotinic Acid, Dissociation Constants of Alkanolamides of (Chilton and Stenlake), 367.
- 5-Methyl-3-phenyl-4-isoxazolyl Penicillin, Nature and Resistance of, to Hydrolysis by Penicillinase (Citri and Garber), 784.
- Methyl and Propyl Hydroxybenzoates, and a Mixture of These, Fungistatic Activity of, against *Penicillium spinulosum* (Gerrard and others), 103.
- Mice, Apparatus for Testing Anticonvulsant Drugs in, by Electroshock Seizures (Cashin and Jackson), 447.
- Mice, Bioassay Method for Investigating Purgative Activity using, Observations on the use of (Brittain and others), 715.
- Mice, GFF, Sex Difference in Sensitivity of, to an Anaesthetic Steroid (Atkinson and others), 698.
- Mice, Strain Variation in (Brown), 406.
- Monomolecular Layers of Synthetic Phosphatides (van Deenen and others), 429.
- Morphine, Codeine and Nalorphine, Note on the Paper Chromatographic Separation of (Street), 56.
- Musk, Anti-inflammatory Activity of (Mishra and others), 830.
- Myasthenia Gravis, Investigation of the Metabolism of Neostigmine in Patients with (Scott and others), 317.
- Myasthenic-like Features of the Neuromuscular Transmission Failure produced by Triethylcholine (Bowman and others), 377.
- ### N
- Nalorphine, Codeine and Morphine, Note on the Paper Chromatographic Separation of (Street), 56.
- Neostigmine, Investigation of the Metabolism of, in Patients with Myasthenia Gravis (Scott and others), 317.
- Neurohumoral Functions of Acetylcholine and Acetylcholinesterase, New General Concept of the (Koelle), 65.
- Neuromuscular Blocking Action of Suxamethonium on the Rat Diaphragm (Whittaker), 177.
- Neuromuscular Blocking Action of Suxamethonium on the Rat Diaphragm, Effect of Lowered Temperature on (Whittaker), 803.
- Neuromuscular Transmission in the Cat, Some Effects of a Hemicholinium Compound (HC-3) on (Evans and Wilson), 347.
- Neuromuscular Transmission Failure produced by Triethylcholine, Myasthenic-like Features of (Bowman and others), 377.
- Neurone Blocking Action, Adrenergic, of Dimethylphenylpiperazinium (Wilson), 700.
- New Apparatus, 61, 464, 825.
- Nitrogen Atoms, Biological Activity in Steroids Possessing (Alauddin and Martin-Smith), Part I, 325; Part II, 469.
- Nitrogenous Steroids, Synthetic, Biological Activity in (Alauddin and Martin-Smith), 325.

SUBJECT INDEX

- Noradrenaline and Adrenaline in Urine, Method for the Estimation of (Atkinson and Wynne), 794.
- Noradrenaline Depletion, Effect of Bretlyium and Cocaine on (Callingham and Cass), 385.
- Noradrenaline, Sensitivity to and Content of, in Aorta and Spleen, Effects in Rabbits of Thyroidectomy and Treatment with Triiodothyronine on (Macmillan and Rand), 257.
- ### O
- Oestrus Cycle, Response of the Pig Uterus to Oxytocin at Different Stages in (Knifton), 427.
- Organ Bath, Modified Dual Unit for Isolated Tissues (Brittain and Rowe), 191.
- Organic Bases, in Pharmaceutical Preparations, use of Tetraphenylboron for the Determination and Characterisation of (Johnson and King), 777.
- Oxygen Effect, Postirradiation, in Bacterial Spores, Studies on (Tallentire and Dickinson), 1277.
- Oxytetracycline and Tetracycline, Bacteriostatic Actions of (Jones and Morrison), 808.
- Oxytocin in the Presence of Ergometrine, Biological Assay of (Berde and Stürmer), 169.
- Oxytocin, Response of Pig Uterus to, at different Stages in the Oestrus Cycle (Knifton), 427.
- ### P
- Paper Carriers, Apparatus for Testing the Resistance to Wet Heat of Bacterial Spores on (Cook and Brown), 61.
- Particle Size Distribution of Marble on Wet Ball Milling (Barnett and James), 1117.
- Penicillin-induced Spheroplasts, Effects of pH on the Stability of (Hugo and Russell), 256.
- Penicillin, 5-Methyl-3-phenyl-4-isoxazolyl, Nature and Resistance of, to Hydrolysis by Penicillinase (Citri and Garber), 784.
- Penicillins, Note on the Activity of Six, against *Escherichia coli* (Russell), 390.
- Penicillinase, Nature and Resistance of 5-Methyl-3-phenyl-4-isoxazolyl Penicillin to Hydrolysis by (Citri and Garber), 784.
- Penicillium notatum* Spores, Effect of Age on the Viability of, in Water and solutions of Phenol (Chauhan and Walters), 1307.
- Penicillium notatum* Spores, Effect of Temperature on the Viability of, in Water and Solutions of Phenol (Chauhan and Walters), 605.
- Penicillium spinulosum*, Fungistatic Activity of Methyl and Propyl Hydroxybenzoates and a Mixture of These against (Gerrard and others), 103.
- Peppermint Oil, Analysis of, by an Aluminium Oxide-Silicic Acid Double Column (Karaway and Wahba), 611.
- Permeability of Blood Vessels, Action of Aryloxyaliphatic Acids on (Northover and Verghese), 615.
- Peroxide Value of Anhydrous Lanolin (Clark and Kitchen), 318.
- Peroxide Value of Lanolin, Note on (Anderson and Wood), 186.
- pH, Effect of, on the Stability of Penicillin-induced Spheroplasts (Hugo and Russell), 256.
- Pharmacogenetics—A Study of Inherited Variability in the Response to Drugs (Clarke), 207.
- N-Phenethylacetamides, Synthesis and Anticonvulsant Activity of some (Sidhu and others), 125.
- Phenindione, Spectrophotometric Method for the Estimation of (Bose and Vijayvargiya), 58.
- Phenol Solutions and Water, Effect of Temperature on the Viability of *Penicillium notatum* Spores in (Chauhan and Walters), 605.
- Phenol Solutions and Water, Effect of Age on the Viability of *Penicillium notatum* Spores in (Chauhan and Walters), 1307.
- Phenolphalein, Colorimetric Determination of (Allen and others), 737.
- 4-Phenyl-1,2,3,6-tetrahydropyridines, Some N-Substituted Derivatives of (Petrov and others), 16.
- Phosphate Sols, Flocculation and Conductivity of (Thomas), 456.
- Phosphatides, Synthetic, Monomolecular Layers of (van Deenen and others), 429.
- Phospholipid Sols, Ultrasonic Irradiation of some (Saunders and others), 567.
- Phrenic Nerve Diaphragm Preparation of Rat, Effect of Deficiency and Small Excess of Thiamine on (Bhagat and Lockett), 161.
- Picrotoxin, Stability of Aqueous Solutions of, to Light (Ramwell and Shaw), 321.
- Pig, Indian Domestic, Ectopic Ventricular Arrhythmia after Coronary Occlusion in (Arora and Sivappa), 315.

SUBJECT INDEX

- Pig Uterus, Response of, to Oxytocin at Different Stages in the Oestrus Cycle (Knifton), 42T.
- Pigeons, Assay of Digitalis in, by Intra-peritoneal Administration (Kuševic), 96.
- Plants, Medicinal West Indian, Pharmacological Screening of some (Feng and others), 556; Simple Hypotensive and Hypertensive Principles from (Durand and others), 562.
- Plasma-Drug Binding Measured by Sephadex (Barlow and others), 550.
- Plethysmographic Apparatus, Modified, for Recording Changes in the Rat Paw (Harris and Spencer), 464.
- Podophyllum hexandrum* Royle, Morphology and Anatomy of the Leaf of (Fell and Ellis), 573.
- Polarographic Assay of Streptomycin (Goodey and others), 122T.
- Poldine Methy. Methosulphate, Analysis of, by Infra-red Spectroscopy (Rapson and others), 66T.
- Polysaccharide, Sulphated, Effect of, on the Acidity and Volume of Histamine-stimulated Gastric Secretion in the Guinea-pig (Anderson and others), 119T.
- Postirradiation Oxygen Effect in Bacterial Spores, Studies on (Tallentire and Dickinson), 127T.
- Potassium Arabate, some Physical Properties of Interfacial Films of (Wibberley), 87T.
- Potassium Flux, Effects of Ether on, in Skeletal Muscle Preparations (Lewis and others), 539.
- Pregnancy and Drugs in the Rat (West), 828.
- Procaine and Five Congeners, Anticonvulsant Activity of, in Experimentally Induced Convulsions (Kapila and Arora), 253.
- Propyl and Methyl Hydroxybenzoates and a Mixture of These, Fungistatic Activity of, against *Penicillium spinulosum* (Gerrard and others), 103.
- Protamine Sulphate, Assay of, for its Capacity to Neutralise Heparin (Birkinshaw and Smith), 95T.
- Purgative Activity, Observations on the use of a Mouse Bioassay Method for Investigating (Brittain and others), 715.
- Pyrogallol and Catechol, Effect of, on Isolated Smooth Organs (Izquierdo and Jurio), 190.
- Pyrogallol and Catechol, Spasmolytic Actions of, on the Isolated Guinea-pig Ileum (Johnson), 272.

Q

- Quaternary Ammonium Compounds in Medicinal Chemistry (D'Arcy and Taylor), Part I, 129; Part II, 193.
- Quaternary Ammonium Salts Related to Breylium, Absorption of, from the Alimentary Tract (Boura and McCoubrey), 647.
- Quaternary Ammonium Salts, Related to Choline, Anti-adrenergic, Phosphorylation of (Copp and others), 641.

R

- Rabbit Auricles, Isolated, Note on the Action of Gallamine on (Laity and Garg), 371.
- Rabbits, Effects in, of Thyroidectomy and Treatment with Triiodothyronine, on the Sensitivity to Noradrenaline and the Content of Noradrenaline in Aorta and Spleen (Macmillan and Rand), 257.
- Rats, Adrenalectomised, Effect of Imipramine on Reserpine Toxicity in (Montanari and Stockham), 126.
- Rats, Aortic Walls and Adrenal Glands of, Note on the Influence of Chlorpromazine and Diethazine on the Catecholamine Stores in (Davis and others), 735.
- Rats, Chronic Toxicity of Food Colours in (Mannell and others), 378.
- Rat Diaphragm, Effect of Lowered Temperature on the Neuromuscular Blocking Action of Suxamethonium on (Whittaker), 803.
- Rat Diaphragm, Neuromuscular Blocking Action of Suxamethonium on (Whittaker), 177.
- Rat, Effect of Hydrocortisone on Capillary Permeability Changes induced by *Echis carinatus* Venom in (Somani and Arora), 535.
- Rat, Effects of Prolonged Administration of some Adrenocortical Hormones in (D'Arcy and Howard), 294.
- Rat, Isolated Vagus-stomach Preparation of, Comparative Study *in vitro* of Curare-like Drugs and Vagal Synapses on (Della Bella and others), 701.
- Rat Paw, Modified Plethysmographic Apparatus for Recording Volume Changes in (Harris and Spencer), 464.
- Rat Phrenic Nerve Diaphragm Preparation, Effect of Deficiency and Small Excess of Thiamine on (Bhagat and Lockett), 161.
- Rat Pregnancy and Drugs (West), 828.

SUBJECT INDEX

- Rats, Thiamine-deficient and Normal, Synthesis of Acetylcholine by Acetone-dried Powders from the Brains of (Bhagat and Lockett), 37.
- Rauwolfia mombasiana* Stapf., Structure of the Root and Stem of (Court), 22.
- Rauwolfia* Species, African. Part II. The Structure of the Root and Stem of *Rauwolfia mombasiana* Stapf. (Court), 22.
- Reserpine Activity, Effect of Imipramine, Amitriptyline and their Monomethyl Derivatives on (Garrattini and others), 509.
- Reserpine Analogues, Potential, Part III. Derivatives of 4-Ethoxycarbonyloxy-3,5-dimethoxybenzoic Acid, 3-Dimethyl aminobenzoic Acid, *p*-Hydroxybenzanilide and *N*-(3,4-Dimethoxyphen-ethyl)-3 (or 4)-Hydroxycyclohexane Carboxamide (Chodnekhar and others), 766.
- Reserpine Toxicity, Effect of Imipramine on, in Adrenalectomised Rats (Montanari and Stockholm), 126.

S

- Selenium Analogues of Biologically Active Sulphur Compounds (Dingwall), 765.
- Sensitisation, Contact, Mechanism of (Schild), 1.
- Sephadex, Drug-Plasma Binding Measured by (Barlow and others), 550.
- Silicic Acid-Aluminium Oxide Double Column, Analysis of Oil of Peppermint by (Karawya and Wahba), 611.
- Skeletal Muscle Preparations, Effects of Ether on Potassium Flux in (Lewis and others), 539.
- Snake Poisoning, Viperine, A Possible new Approach to the Treatment of (Somani and Arora), 394.
- Sodium Aminosalicylate, Determination of Small Quantities of *m*-Aminophenol in (Bičan Fister), 280.
- Sodium *p*-Aminosalicylate, Mixtures of with Isoniazid, Modified Procedure for the Determination of Isoniazid in (Lee Kum-Tatt and Ho Yan-Hon), 123.
- Spasmodic Actions of Pyrogallol and Catechol on the Isolated Guinea-pig Ileum (Johnson), 272.
- Spectroscopy, Infra-red, Analysis of Poldine Methyl Methosulphate by (Rapson and others), 667.
- Spheroplasts, Penicillin-induced, Effect of pH on the Stability of (Hugo and Russell), 256.
- Spleen and Aorta, Effects in Rabbits of Thyroidectomy and Treatment with Triiodothyronine on the Sensitivity to Noradrenaline and the Content of Noradrenaline in (Macmillan and Rand), 257.
- Spores, Bacterial, on Paper Carriers, Apparatus for testing the Resistance to Wet Heat of (Cook and Brown), 61.
- Spores, Bacterial, Studies on the Post-irradiation Oxygen Effect in (Tallentire and Dickinson), 1277.
- Spores of *Penicillium notatum*, Effect of Age on the Viability of, in Water and Solutions of Phenol (Chauhan and Walters), 1307.
- Spores of *Penicillium notatum*. Effect of Temperature on the Viability of, in Water and Solutions of Phenol (Chauhan and Walters), 605.
- Stability of Solutions of Isoprenaline, Note on (West and Whittet), 937.
- Strain Variation in Mice (Brown), 406.
- Strain Variation in Relation to Pharmacological Testing, Practical Aspects of (Brown and Hughes), 399.
- Steroidal Alkaloids, Biological Activity in (Alauddin and Martin-Smith), 469.
- Steroids, Adrenocortical, Effects of Prolonged Administration of Some in the Rat (D'Arcy and Howard), 294.
- Steroid, Anaesthetic, Sex Difference in Sensitivity of GFF Mice to (Atkinson and others), 698.
- Steroids Possessing Nitrogen Atoms, Biological Activity in (Alauddin and Martin-Smith), Part I, Synthetic Nitrogenous Steroids, 325. Part II, Steroidal Alkaloids, 469.
- Steroids, Synthetic Nitrogenous, Biological Activity in (Alauddin and Martin-Smith), 325.
- Streptomycin, Polarographic Assay of (Goodey and others), 1227.
- o*-Substituted 2-Diethylaminoethyl Benzoates as Local Anaesthetics (Thomas and Canty), 587.
- o*-Substitution, Effect of, on the Pharmacology of Benzoylcholine (Thomas and Buckley), 225.
- Sulphamoylbenzoic Acids as Diuretic Agents, Synthesis of (Jackman and others), 679.
- Sulphonamides, New Complexometric Method for the Determination of (Abdine and Abdel Sayed), 761.
- Sulphur Compounds, Biologically Active, Selenium Analogues of (Dingwall), 765.
- Sulphuric Acid, Dilutions of (Betts), 698.

SUBJECT INDEX

- Surface Activity of a Series of Synthetic Non-ionic Detergents (Elworthy and Macfarlane), 100*T*.
- Suxamethonium, Effect of Lowered Temperature on the Neuromuscular Blocking Action of, on the Rat Diaphragm (Whittaker), 803.
- Suxamethonium, Neuromuscular Blocking Action of, on the Rat Diaphragm (Whittaker), 177.
- Sympathomimetic Amines, Dexamphetamine and other Related, Antagonism of Guanethidine by (Day and Rand), 541.
- Symposium on Drug Addiction, British Pharmaceutical Conference, 9*T*-19*T*.
- Symposium on the Influence of Animal Strain Selection and Conditioning on Biological Experiments and Assays, 397-428. Chairman's Address (Lane-Petter), 397-398; Introductory Papers, 399-415; Discussion, 416-428.
- ### T
- Tachycardia, Ectopic Ventricular, Resulting from Acute Myocardial Infarction in the Dog, Effectiveness of 5-Hydroxytryptamine in (Kapila and Arora), 831.
- Temperature, Effect of, on the Viability of *Penicillium notatum* Spores in Water and Solutions of Phenol (Chauhan and Walters), 605.
- Temperature, Lowered, Effect of, on the Neuromuscular Blocking Action of Suxamethonium on the Rat Diaphragm (Whittaker), 803.
- Terpenoid Hemisuccinates, Absence of General Anaesthetic Properties in a Number of (Ahmad and others), 467.
- Tetracycline and Oxytetracycline, Bacteriostatic Actions of (Jones and Morrison), 808.
- Tetrahydroaminoacridine, Estimation and Urinary Excretion of (Kaul), 237.
- Tetrahydroaminoacridine, and its Structural Fragments, Enzyme-inhibiting Action of (Kaul), 243.
- 1,2,3,6-Tetrahydropyridine, some Derivatives of (Petrov and Stephenson), 306.
- Tetraphenylboron, use of, for the Determination and Characterisation of Organic Bases in Pharmaceutical Preparations (Johnson and King), 77*T*.
- Thiamine-deficient and Normal Rats, Synthesis of Acetylcholine by Acetone-dried Powders from the Brains of (Bhagat and Lockett), 37.
- Thiamine, Effect of Deficiency and Small Excess of, on the Rat Phrenic Nerve Diaphragm Preparation (Bhagat and Lockett), 161.
- Thiamine, Hydrolytic Destruction of, Especially in the Presence of Cyanocobalamin (Heathcote and Wills), 232.
- Thiotepa, Estimation of, In Urine (Raine), 614.
- Thyroid, Pork, Chemical Determination of Liothyronine and Thyroxine in Enzymatic Hydrolysates of (Devlin and Stephenson), 597.
- Thyroid from Various Species, Comparison of the Thyroxine: Triiodothyronine Contents and Biological Activity of (Wiberg and others), 777.
- Thyroidectomy and Treatment with Triiodothyronine, Effects in Rabbits of, on the Sensitivity to Noradrenaline and the Content of Noradrenaline in Aorta and Spleen (Macmillan and Rand), 257.
- Thyroxine and Liothyronine in Enzymatic Hydrolysates of Pork Thyroid, Chemical Determination of (Devlin and Stephenson), 597.
- Thyroxine: Triiodothyronine Contents and Biological Activity of Thyroid from Various Species, Comparison of (Wiberg and others), 777.
- Tissues, Animal, Spectrophotofluorometric Determination of Emetine in (Davis and others), 249.
- Tissues, Contractile Responses of, Simple Mechanical Computer for Relating (Paterson), 825.
- Tissues, Isolated, Modified Dual Organ Bath for (Brittain and Rowe), 191.
- Toxicity Studies on Food Colours, V. Observations on the Chronic Toxicity of Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B in Rats (Mannell and others), 378.
- Trichloroethyl Phosphate, Determination of, in Pharmaceutical Preparations (Boon), 116*T*.
- Triethylcholine, Myasthenic-like Features of the Neuromuscular Transmission Failure Produced by (Bowman and others), 37*T*.
- Triiodothyronine: Thyroxine Contents and Biological Activity of Thyroid from Various Species, Comparison of (Wiberg and others), 777.
- 3,4,5-Triiodothyronine *see also* Liothyronine.
- Triiodothyronine Treatment and Thyroidectomy, Effects in Rabbits of, on the Sensitivity to Noradrenaline and the Content of Noradrenaline in Aorta and Spleen (Macmillan and Rand), 257.

SUBJECT INDEX

Turtle Heart, Concentration of Catecholamines in, and Vagal Escape (Friedman and Bhagat), 764.

U

Ultrasonic Irradiation of some Phospholipid Sols (Saunders and others), 567.

Unconditioned and Conditioned On-and-Off-behavioural Responses to a Buzzer (Izquierdo), 316.

Urinary Excretion of 5-Hydroxyindoleacetic Acid in the Rat, Effect of the Administration of Water or Isotonic NaCl Solution on (Bertaccini and Erspamer), 687.

Urinary Excretion of Tritiated Butylated Hydroxyanisole and Hydroxytoluene in the Rat (Golder and others), 268.

Urine, Estimation of *N*-Benzyl-*N''*-dimethylguanidine (BW 467C60), and some Observations on the Compound's Reaction with Hypobromite (McCoubrey), 798.

Urine, Estimation of Theotepa in (Raine), 614.

Urine, Method for the Estimation of Adrenaline and Noradrenaline in (Atkinson and Wynne), 794.

Uterus of Pig, Response of, to Oxytocin at Different Stages in the Oestrus Cycle (Knifton), 427.

V

Vagal Synapses and Curare-like Drugs: Comparative Study *in vitro* on the Isolated Vagus-stomach Preparation of the Rat (Della Bella and others), 701.

Vagus-stomach Preparation of the Rat, Isolated. Comparative Study *in vitro* of Curare-like Drugs and Vagal Synapses (Della Bella and others), 701.

Vasopressin and Angiotensin, Effect of Adrenergic Blocking Agents and Chlorpromazine on Blood Pressure Increase by (Supek and others), 284.

Venom of *Echis carinatus*, Effect of Hydrocortisone on Capillary Permeability Changes induced by, in the Rat (Somani and Arora), 535.

Venom of *Echis carinatus*, Mechanism of Increased Capillary Permeability Induced by: A Possible new Approach to the Treatment of Viperine Poisoning (Somani and Arora), 394.

Ventricular Arrhythmia, Ectopic, after Coronary Occlusion in the Indian Domestic Pig (Arora and Sivappa), 315.

Viperine Snake Poisoning, a Possible new Approach to the Treatment of (Somani and Arora), 394.

W

Water for Injection by Ion-exchange (Cook and Saunders), 837.

Water or Isotonic NaCl Solution, Effect of Administration of, or the Urinary Excretion of 5-Hydroxyindoleacetic Acid in the Rat (Bertaccini and Erspamer), 687.

Water and Solutions of Phenol, Effect of Age on the Viability of *Penicillium notatum* Spores in (Chauhan and Walters), 1307.

Water and Solutions of Phenol, Effect of Temperature on the Viability of *Penicillium notatum* Spores in (Chauhan and Walters), 605.

West Indian Medicinal Plants, Pharmacological Screening of some (Feng and others) 556.

Wrightia tinctoria Bark, an Adulterant of Kurchi (Atal and Sethi), 41.

แผนกห้องสมุด กรมวิทยาศาสตร์
กระทรวงอุตสาหกรรม

INDEX OF AUTHORS*

A

- Abdel Sayed, W. S. (*see* Abdine, H.), 761.
 Abdine, H. and Abdel Sayed, W. S., 761.
 Ahmad, K., Carey, F. M., Khatoom, T., Lewis, J. J. and Martin-Smith, M., 467.
 Alauddin, M. and Martin Smith, M., 325, 469.
 Allen, J., Gartside, B. and Johnson, C. A., 73*T*.
 Allmark, M. G. (*see* Mannell, W. A.), 378.
 Anderson, C. A. and Wood, G. F., 186.
 Anderson, W., 64.
 Anderson, W., Marcus, R. and Watt, J., 119*T*.
 Arora, H. R. K., 320.
 Arora, R. B., Mathur, C. N. and Seth, S. D. S., 534.
 Arora, R. B. (*see* Kapila, K.), 253, 831.
 Arora, R. B. (*see* Mishra, R. K.), 832.
 Arora, R. B. (*see* Sharma, V. N.), 515.
 Arora, R. B. and Sivappa, D. S., 315.
 Arora, R. B. (*see* Somani, P.), 394, 535.
 Atal, C. K. and Sethi, P. D., 41.
 Atkinson, R. and Wynne, N. A., 794.
 Augusti, K. T. (*see* Brahmachari, H. D.), 254, 617.
 Austin, K. W. (*see* Rapson, H. D. C.), 66*T*.

B

- Balazs, T., Murphy, J. B. and Grice, H. C., 750.
 Barlow, C. F., Firemark, H. and Roth, L. J., 550.
 Barnett, M. I. and James, K. C., 111*T*.
 Bartlet, A. L., 91.
 Bayne, A. J. (*see* Wiberg, G. S.), 777.
 Bedford, C. J. (*see* Child, K. J.), 374.
 Berde, B. and Stürmer, E., 169.
 Bertaccini, G. and Erspamer, V., 687.
 Betts, T. J., 698.
 Bhagat, B. and Lockett, M. F., 37, 161.
 Bhagat, B. (*see* Friedman, A. H.), 764.
 Bičan-Fister, T., 280.
 Birkinshaw, V. K. and Smith, K. L., 95*T*.
 Boon, P. F. G., 116*T*.
 Bose, B. C. and Vijayvargiya, R., 58.
 Boura, A. L. A., Duncombe, W. G., Robson, R. D. and McCoubrey, A., 722.
 Boura, A. L. A. and McCoubrey, A., 647.
 Bowman, W. C., Hemsworth, B. A. and Rand, M. J., 377.
 Brahmachari, H. D. and Augusti, K. T., 254, 617.
 Brittain, R. T., D'Arcy, P. F. and Grimshaw, J. J., 715.
 Brittain, R. T. and Rowe, D. J., 191.

- Brown, A. M., 406.
 Brown, D. M. and Hughes, B. O., 399.
 Brown, M. R. W. (*see* Cook, A. M.), 61.
 Bryant, C. and Smith, M. J. H., 182.
 Buckley, D. (*see* Thomas J.), 225.
 Bullock, K. (*see* Gerrard, H. N.), 103.
 Butler, C. G. and Martin, F. P., 103*T*.

C

- Callingham, B. A. and Cass, R., 385.
 Canty, J. (*see* Thomas, J.), 587.
 Carey, F. M. (*see* Ahmad, K.), 467.
 Carless, J. E. and Mitchell, A. G., 46.
 Carless, J. E. and Swarbrick, J., 97*T*.
 Carter, J. R. (*see* Wiberg, G. S.), 777.
 Cashin, C. H. and Jackson, H., 44*T*.
 Cass, R. (*see* Callingham, B. A.), 385.
 Caws, A. C. and Lawrence, B. E., 59*T*.
 Challen, S. B., 707.
 Chauhan, N. M. and Walters, V., 605, 130*T*.
 Child, K. J., Bedford, C. J., and Tomich, E. G., 374.
 Chilton, J. and Stenlake, J. B., 350, 367.
 Chodnekhar, M. S., Sharp, L. K. and Linnell, W. H., 756.
 Chopra, K. L. (*see* Singh, N.), 288.
 Citri, N. and Garber, N., 784.
 Clark, E. W. and Kitchen, G. F., 316.
 Clarke, C. A., 20*T*.
 Collins, R. F. and Large, B. J., 487.
 Cook, A. M. and Brown, M. R. W., 61.
 Cook, A. M. and Saunders, L., 837.
 Copp, F. C., Jones, T. S. G., and McCoubrey, A., 641.
 Couling, T. E. (*see* Goodey, R.), 122*T*.
 Court, W. E., 22.
 Cutmore, E. A. (*see* Rapson, H. D. C.), 667.

D

- D'Arcy, P. F., 411.
 D'Arcy, P. F. and Howard, E. M., 294.
 D'Arcy, P. F. and Taylor, E. P., 129, 193.
 D'Arcy, P. F. (*see* Brittain, R.), 715.
 David, A., Kirk, D. N., Petrow, V., Williamson, D. M. and Woodward, E. J., 127.
 Davis, B., Dodds, M. G. and Tomich, E. G., 249.
 Davis, R. A., Kaul, C. L. and Lockett, M. F., 735.
 Day, M. D. and Rand, M. J., 541.
 Della Bella, D., Rognoni, F. and Teotino, U. M., 701.
 Devlin, W. F., and Stephenson, N. R., 597.
 Devlin, W. F. (*see* Wiberg, G. S.), 777.
 de Haas, G. H. (*see* van Deenen, L. L. M.), 429.

*Page numbers followed by an italic *T* refer to the Supplement containing the Transactions of the British Pharmaceutical Conference.

INDEX OF AUTHORS

- Dhar, H. L., and Sanyal, R. K., 393.
 Dickinson, N. A. (*see Tallentire, A.*), 127T.
 Dingwall, D., 765.
 Dodds, M. G. (*see Davis, B.*), 249.
 Duncan, W. A. M., Macdonald, G. and Thornton, M. J., 217.
 Duncombe, W. G. (*see Boura, A. L. A.*), 722.
 Durand, E., Ellington, E. V., Feng, P. C., Haynes, L. J., Magnus, K. E. and Philip, N., 562.
 Dutt, M. C. (*see Phang, S. E.*), 108.

E

- Edwards, K. B. (*see Shapero, M.*), 119.
 Ellington, E. V. (*see Durand, E.*), 562.
 Ellis, S. and Fell, K. R., 573.
 Elworthy, P. H. and Macfarlane, C. B., 100T.
 Erspamer, V. (*see Bertaccini, G.*), 687.
 Evans, E. R. and Wilson, H., 34T.
 Evans, W. C. and Pe Than, M., 147.
 Evans, W. C. and Stevenson, N. A., 664, 107T.

F

- Fell, K. R. (*see Ellis, S.*), 573.
 Feng, P. C., Haynes, L. J., Magnus, K. E., Plimmer, J. R. and Sherratt, H. S. A., 556.
 Feng, P. C. (*see Durand, E.*), 562.
 Firemark, H. (*see Barlow, C. F.*), 550.
 Friedman, A. H. and Bhagat, B., 764.

G

- Gammack, D. (*see Saunders, L.*), 567.
 Garber, N. (*see Citri, N.*), 784.
 Garg, B. K. (*see Laity, J. L. H.*), 371.
 Garrattini, S., Giachetti, A., Jori, A., Pieri, L. and Valzelli, L., 509.
 Gartside, B. (*see Allen, J.*), 73T.
 Gerrard, H. N., Parker, M. S., and Bullock, K., 103.
 Giachetti, A. (*see Garrattini, S.*), 509.
 Gjuriš, V. (*see Supek, Z.*), 284.
 Goadby, P. and Smith, W. G., 739.
 Golder, W. S., Ryan, A. J. and Wright, S. E., 268.
 Goodey, R., Couling, T. E. and Hart, J. E., 122T.
 Greaves, R. I. N., 621.
 Grice, H. C. (*see Balazs, T.*), 750.
 Grice, H. C. (*see Mannell, W. A.*), 378.
 Grimshaw, J. J. (*see Brittain, R.*), 715.

H

- Harris, J. M. and Spencer, P. S. J., 464.
 Harrison, I. H. and James, K. C., 503.
 Hart, J. E. (*see Goodey, R.*), 122T.
 Hayman, D. F., Petrow, V., and Stephenson, O., 522.

- Hayman, D. F., Petrow, V., Stephenson, O. and Thomas, A. J., 451.
 Haynes, L. J. (*see Durand, E.*), 562.
 Haynes, L. J. (*see Feng, P. C.*), 556.
 Heathcote, J. and Wills, B. A., 232.
 Hemsworth, B. A. (*see Bowman, W. A.*), 37T.
 Houtsmuller, U. M. T. (*see van Deenen, L. L. M.*), 429.
 Howard, E. M. (*see D'Arcy, P. F.*), 294.
 Ho Yan-Hon (*see Lee Kum-Tatt*), 123.
 Hughes, B. O. (*see Brown, D. M.*), 399.
 Hugo, W. B. and Russell, A. D., 256.

I

- Izquierdo, I., 316.
 Izquierdo, J. A., and Jurio, A. V., 190.

J

- Jackman, G. B., Petrow, V., Stephenson, O. and Wild, A. M., 679.
 Jackson, H. (*see Cashin, C. H.*), 44T.
 Jaleel, S. (*see Sidhu, G. S.*), 125.
 James, K. C. and Standford, J. B., 445.
 James, K. C. (*see Barnett, M. I.*), 111T.
 James, K. C. (*see Harrison, I. H.*), 503.
 Johnson, C. A. and King, R. E., 77T.
 Johnson, C. A. (*see Allen, J.*), 73T.
 Johnson, E. S., 272.
 Johnston, J. M., 16T.
 Jones, J. G. and Morrison, G. A., 808.
 Jones, T. S. G. (*see Copp, F. C.*), 641.
 Jori, A. (*see Garrattini, S.*), 509.
 Jurio, A. V. (*see Izquierdo, J. A.*), 190.

K

- Kapila, K. and Arora, R. B., 253, 831.
 Karawya, M. S. and Wahba, S. K., 611.
 Kaul, C. L. (*see Davis, R. A.*), 735.
 Kaul, P. N., 237, 243.
 Khatoom, T. (*see Ahmad, K.*), 467.
 King, R. E. (*see Johnson, C. A.*), 77T.
 Kirk, D. N. (*see David, A.*), 127.
 Kitchen, G. F. (*see Clark, E. W.*), 316.
 Knifton, A., 42T.
 Koelle, G. B., 65.
 Kušević, V., 96.

L

- Laity, J. L. H. and Garg, B. K., 371.
 Lane-Petter, W., 397.
 Large, B. J. (*see Collins, R. F.*), 48T.
 Lawrence, B. E. (*see Caws, A. C.*), 59T.
 Lee Kum-Tatt and Ho Yan-Hon, 123.
 Leonard, M. A., 63T.
 Lewis, J. J., Mir, N. J. and Muir, T. C., 539.
 Lewis, J. J. (*see Ahmad, K.*), 467.
 Linnell, W. H. (*see Chodnekhar, M. S.*), 756.
 Lock, J. A., 496.

INDEX OF AUTHORS

Lockett, M. F. (*see* Bhagat, B.), 37, 161.
 Lockett, M. F. (*see* Davis, R. A.), 735.
 Luthra, P. N. and Tayal, J. N., 396.

M

McCoubrey, A., 727, 798.
 McCoubrey, A. (*see* Boura, A. L. A.), 647, 722.
 McCoubrey, A. (*see* Copp, F. C.), 641.
 Macdonald, A. D., 9T.
 Macdonald, G. (*see* Duncan, W. A. M.), 217.
 Macfarlane, C. B. (*see* Elworthy, P. H.), 100T.
 Macmillan, W. H. and Rand, M. J., 257.
 Magnus, K. E. (*see* Durand, E.), 562.
 Magnus, K. E. (*see* Feng, P. C.), 556.
 Mannell, W. A., Grice, H. C. and Allmark, M. G., 378.
 Marcus, R. (*see* Anderson, W.), 119T.
 Marijan, N. (*see* Supek, Z.), 284.
 Martin, F. P. (*see* Butler, C. G.), 103T.
 Martin-Smith, M. (*see* Ahmad, K.), 467.
 Martin Smith, M. (*see* Alauddin, M.), 325, 469.
 Mathur, C. N. (*see* Arora, R. B.), 534.
 Mir, N. J. (*see* Lewis, J. J.), 539.
 Mishra, R. K., Arora, R. B. and Seth, S. D. S., 830.
 Mitchell, A. G., 172.
 Mitchell, A. G. (*see* Carless, J. E.), 46.
 Montanari, R. and Stockham, M. A., 126.
 Morrison, G. A. (*see* Jones, J. G.), 808.
 Muir, T. C. (*see* Lewis, J. J.), 539.
 Mulder, E. (*see* van Deenen, L. L. M.), 429.
 Murphy, J. B. (*see* Balazs, T.), 750.

N

Northover, B. J. and Verghese, J., 615.
 Nowell, P. T. (*see* Scott, C. A.), 31T.

P

Parker, M. S. (*see* Gerrard, H. N.), 103.
 Paterson, G., 825.
 Perrin, J. (*see* Saunders, L.), 567.
 Pe Than, M. (*see* Evans, W. C.), 147.
 Phang, S. E., Dutt, M. C. and Thng Soon Tee, 108.
 Philip, N. (*see* Durand, E.), 562.
 Pieri, L. (*see* Garrattini, S.), 509.
 Plimmer, J. R. (*see* Feng, P. C.), 556.
 Petrow, V. and Stephenson, O., 306.
 Petrow, V., Stephenson, O., and Thomas, A. J., 16.
 Petrow, V. (*see* David, A.), 127.
 Petrow, V. (*see* Hayman, D. F.), 451, 522.
 Petrow, V. (*see* Jackman, G. B.), 679.

R

Raine, D. N., 614.
 Ramwell, P. W. and Shaw, J. E., 321.

Rand, M. J. (*see* Bowman, W. C.), 37T.
 Rand, M. J. (*see* Day, M. D.), 541.
 Rand, M. J. (*see* Macmillan, W. H.), 257.
 Rapson, H. D. C., Austin, K. W. and Cutmore, E. A., 66T.
 Roberts, M., 746.
 Robins, D. C. and Thomas, I. L., 128.
 Robinson, B. and Shepherd, D. M., 9.
 Robson, R. D. (*see* Boura, A. L. A.), 722.
 Rognoni, F. (*see* Della Bella, D.), 701.
 Roth, L. J. (*see* Barlow, C. F.), 550.
 Rowe, D. J. (*see* Brittain, R. T.), 191.
 Russell, A. D., 390.
 Russell, A. D. (*see* Hugo, W. B.), 256.
 Russell, A. D. (*see* Turner, T. D.), 395.
 Ryan, A. J. (*see* Golder, W. S.), 268.

S

Sanyal, R. K. (*see* Dhar, H. L.), 393.
 Sattur, P. B. (*see* Sidhu, G. S.), 125.
 Saunders, L., Perrin, J. and Gammack, D., 567.
 Saunders, L. (*see* Cook, A. M.), 83T.
 Schild, H. O., 1.
 Scott, C. A., Nowell, P. T. and Wilson, A., 31T.
 Selye, H. (*see* Strebel, R.), 658.
 Seth, S. D. S. (*see* Arora, R. B.), 534.
 Seth, S. D. S. (*see* Mishra, R. K.), 830.
 Sethi, P. D. (*see* Atal, C. K.), 41.
 Shadan, P. and Shellard, E. J., 110.
 Shaper, M. and Edwards, K. B., 119.
 Sharma, V. N. and Arora, R. B., 515.
 Sharp, L. K. (*see* Chodnekar, M. S.), 756.
 Shaw, J. E. (*see* Ramwell, P. W.), 321.
 Shellard, E. J. (*see* Shadan, P.), 110.
 Shepherd, D. M. (*see* Robinson, B.), 9.
 Sherratt, H. S. A. (*see* Feng, P. C.), 556.
 Sidhu, G. S., Sattur, P. B. and Jaleel, S., 125.
 Singh, N. and Chopra, K. L., 288.
 Sivappa, D. S. (*see* Arora, R. B.), 315.
 Smith, K. L. (*see* Birkinshaw, V. K.), 95T.
 Smith, M. J. H. (*see* Bryant, C.), 182.
 Smith, W. G. (*see* Goadby, P.), 739.
 Somani, P. and Arora, R. B., 394, 535.
 Spencer, P. S. J. (*see* Harris), 464.
 Standford, J. B. (*see* James, K. C.), 445.
 Stenlake, J. B. (*see* Chilton, J.), 350, 367.
 Stephenson, N. R. (*see* Devlin, W. F.), 597.
 Stephenson, N. R. (*see* Wiberg, G. S.), 777.
 Stephenson, O. (*see* Hayman, D. F.), 451, 522.
 Stephenson, O. (*see* Jackman, G. B.), 679.
 Stephenson, O. (*see* Petrow, V.), 16, 306.
 Stevenson, N. A. (*see* Evans, W. C.), 107T.
 Stockham, M. A. (*see* Montanari, R.), 126.
 Strebel, R., Vašků, J. and Selye H., 658.
 Street, H. V., 56.
 Stürmer, E. (*see* Berde, B.), 169.

INDEX OF AUTHORS

Supek, Z., Uroič, B., Gjuriš, V., and Marijan, N., 284.
 Swarbrick, J. (*see* Carless, J. E.), 97T.

T

Tallentire, A. and Dickinson, N. A., 127T.
 Tayal, J. N. (*see* Luthra, P. N.), 396.
 Taylor, E. P. (*see* D'Arcy, P. F.), 129, 193.
 Teotino, U. M. (*see* Della Bella, D.), 701.
 Thng Soon Tee (*see* Phang, S. E.), 108.
 Thomas, A. J. (*see* Hayman, D. F.), 451.
 Thomas, A. J. (*see* Petrow, V.), 16.
 Thomas, B. J., 17T.
 Thomas, I. L., 456.
 Thomas, I. L. (*see* Robins, D. C.), 128.
 Thomas, J. and Buckley, D., 225.
 Thomas, J. and Canty, J., 587.
 Thornton, M. J. (*see* Duncan, W. A. M.), 217.
 Tomich, E. G. (*see* Child, K. J.), 374.
 Tomich, E. G. (*see* Davis, B.), 249.
 Turner, T. D. and Russell, A. D., 395.

U

Uroič, B. (*see* Supek, Z.), 284.

V

van Deenen, L. L. M., Houtsmuller, U. M. T., de Haas, G. H. and Mulder, E., 429.

Valzelli, L. (*see* Garrattini, S.), 509.
 Vaškú, J. (*see* Strebek, R.), 658.
 Vijayvargiya, R. (*see* Bose, B. C.), 58.
 Verghese, J. (*see* Northover, B. J.), 615.

W

Wahba, S. K. (*see* Karawya, M. S.), 611.
 Walters, V. (*see* Chauhan, N. M.), 605, 130T.
 Watt, J. (*see* Anderson, W.), 119T.
 Wellendorf, M., 157.
 West, G. B., 618, 828.
 West, G. B. and Whittet, T. D., 93T.
 Whittaker, R., 177, 803.
 Whittet, T. D. (*see* West, G. B.), 93T.
 Wibberley, K., 87T.
 Wiberg, G. S., Devlin, W. F., Stephenson, N. R., Carter, J. R. and Bayne, A. J., 777.
 Wild, A. M. (*see* Jackman, G. B.), 679.
 Williamson, D. M. (*see* David, A.), 127.
 Wills, B. A. (*see* Heathcote, J.), 232.
 Wilson, A. (*see* Scott, C. A.), 31T.
 Wilson, A. B., 700.
 Wilson, H. (*see* Evans, E. R.), 347.
 Wood, G. F. (*see* Anderson, C. A.), 186.
 Woodward, E. J. (*see* David, A.), 127.
 Wright, S. E., 613.
 Wright, S. E. (*see* Golder, W. S.), 268.
 Wynne, N. A. (*see* Atkinson, R.), 794.

PRINTED BY
W. HEFFER & SONS LTD.,
CAMBRIDGE, ENGLAND.

JOURNAL OF PHARMACY AND PHARMACOLOGY

Editor:

G. Brownlee, D.Sc., Ph.D., F.P.S.

Assistant Editor:

J. R. Fowler, B.Pharm., F.P.S.

TRANSACTIONS OF THE BRITISH PHARMACEUTICAL CONFERENCE

Editor:

D. W. Mathieson, B.Sc., Ph.D., F.R.I.C.

Press Editor:

J. R. Fowler, B.Pharm., F.P.S.

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., F. HARTLEY, B.Sc., Ph.D., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., J. J. LEWIS, M.Sc., F.P.S., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.

SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 1

January, 1962

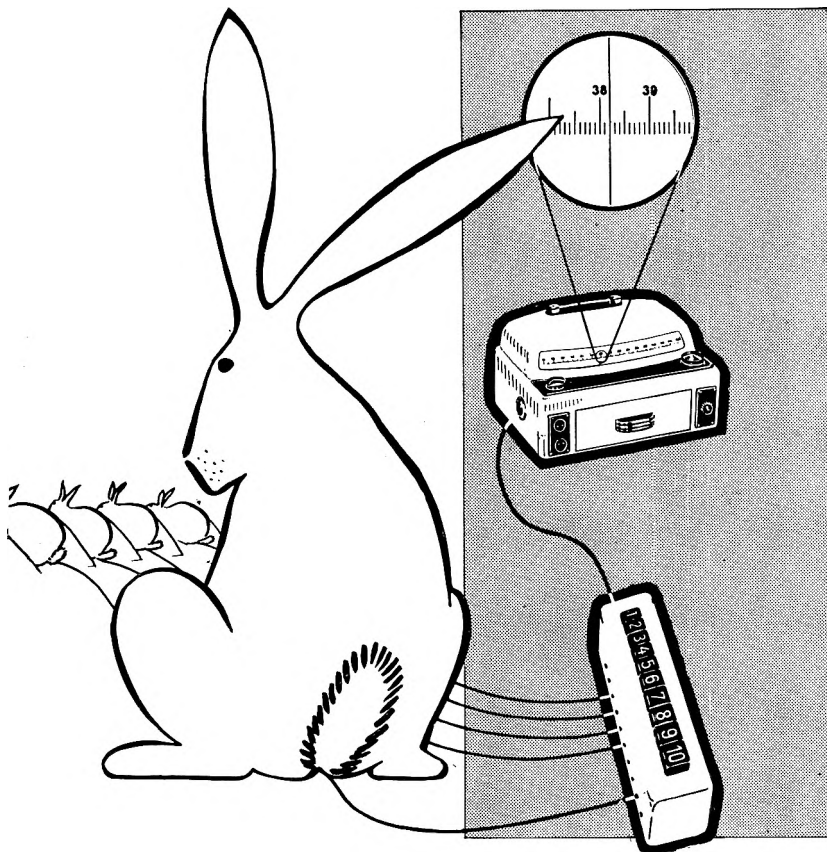
CONTENTS

Review Article	PAGES
THE MECHANISM OF CONTACT SENSITISATION. By H. O. Schild, M.D., D.Sc.	1-8
Research Papers	
THE INHIBITION OF THE L-HISTIDINE DECARBOXYLASES OF GUINEA-PIG KIDNEY AND RAT HEPATOMA. By B. Robinson and D. M. Shepherd	9-15
SOME <i>N</i> -SUBSTITUTED DERIVATIVES OF 4-PHENYL-1,2,3,6-TETRAHYDRO-PYRIDINES. By V. Petrow, O. Stephenson and A. J. Thomas . .	16-21
AFRICAN <i>RAUWOLFIA</i> SPECIES. PART II. THE STRUCTURE OF THE ROOT AND STEM OF <i>RAUWOLFIA MOMBASIANA</i> STAPE. By William E. Court	22-36
THE SYNTHESIS OF ACETYLCHOLINE BY ACETONE DRIED POWDERS FROM THE BRAINS OF NORMAL RATS AND OF THIAMINE-DEFICIENT RATS. By B. Bhagat and Mary F. Lockett	37-40
<i>WRIGHTIA TINCTORIA</i> BARK, AN ADULTERANT OF KURCHI. By C. K. Atal and P. D. Sethi	41-45
THE OXIDATION OF ALDEHYDES IN AQUEOUS SOLUTIONS OF CETO-MACROGOL. By J. E. Carless and A. G. Mitchell	46-55
A NOTE ON THE PAPER CHROMATOGRAPHIC SEPARATION OF CODEINE, MORPHINE AND NALORPHINE. By Harold V. Street	56-57
A SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PHENINDIONE. By B. C. Bose and R. Vijayvargiya	58-60
New Apparatus	
APPARATUS FOR TESTING THE RESISTANCE TO WET HEAT OF BACTERIAL SPORES ON PAPER CARRIERS. By A. M. Cook and M. R. W. Brown	61-62
Book Review	63
Letter to the Editor	
A NEW DEFLOCCULANT AND PROTECTIVE COLLOID FOR BARIUM SULPHATE. By W. Anderson.	64

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. McCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

PYROGEN TESTING with TYPE TE3 ELECTRIC THERMOMETER



Used for conducting pyrogen tests by serological institutes; pharmaceutical laboratories and hospitals in more than 30 countries, this precision electric thermometer affords distinct and important advantages over the ordinary mercury type.

- ★ Guaranteed accuracy to within ± 0.1 centigrade.
- ★ Easy-to-read spot illuminated graduated scale.
- ★ Automatic compensation for variations in room temperature.
- ★ Using connection boxes (illustrated) up to 30 animals can be tested simultaneously.
- ★ Correct temperature shown within 1 to 2 seconds of depressing push-button.
- ★ Instrument always ready for immediate use—no tiresome calibration necessary.

For fully descriptive literature and prices write to:

SIEREX LTD

15-18 Clipstone Street, London W.1
Tel: LANgham 2464

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 2

February, 1962

CONTENTS

Review Article	PAGES
A NEW GENERAL CONCEPT OF THE NEUROHUMORAL FUNCTIONS OF ACETYLCHOLINE AND ACETYLCHOLINESTERASE. By George B. Koelle, Ph.D., M.D.	65-90
Research Papers	
THE PRESSOR ACTION OF GUANETHIDENE IN THE SPINAL CAT. By A. L. Bartlet	91-95
ASSAY OF DIGITALIS IN PIGEONS BY INTRAPERITONEAL ADMINISTRATION. By Vladimir Kušević	96-102
THE FUNGISTATIC ACTIVITY OF METHYL AND PROPYL HYDROXYBENZOATES AND A MIXTURE OF THESE AGAINST <i>PENICILLIUM SPINULOSUM</i> . By H. N. Gerrard, M. S. Parker and K. Bullock	103-107
A COLOUR TEST TO DISTINGUISH BETWEEN THE FRUITS OF <i>ILICLIUM VERUM</i> AND <i>ILICLIUM ANISATUM L.</i> By S. E. Phang, M.C. Dutt and Thng Soon Tee	108-109
AN ANATOMICAL STUDY OF ETHIOPIAN KHAT (LEAF OF <i>CATHA EDULIS</i> FORSK). By Parirokh Shadan and E. J. Shellard . .	110-118
THE LOCAL ANAESTHETIC PROPERTIES OF ESTERS OF <i>N</i> -SUBSTITUTED α -AMINOPHENYLACETIC ACIDS. By M. Shapero and K. B. Edwards	119-122
A MODIFIED PROCEDURE FOR THE DETERMINATION OF ISONIAZID IN MIXTURES WITH SODIUM <i>p</i> -AMINOSALICYLATE. By Lee Kum-Tatt and Ho Yan-Hon	123-124
Letters to the Editor	
SYNTHESIS AND ANTICONVULSANT ACTIVITY OF SOME <i>N</i> -PHENETHYLACETAMIDES. By G. S. Sidhu, P. B. Sattur and (Mrs.) Salma Jaleel	125
THE EFFECT OF IMPRAMINE ON THE RESERPINE TOXICITY IN ADRENALECTOMISED RATS. By R. Montanafi and M. A. Stockham	126-127
6-METHYLCORTISONE ACETATE 3-ENOL ETHERS—A NEW GROUP OF ANTI-INFLAMMATORY AGENTS. By A. David, D. N. Kirk, V. Petrow, D. M. Williamson and (Miss) E. J. Woodward . .	127
SURFACE ACTIVITY OF LYSOPHOSPHATIDYLETHANOLAMINE. By D. C. Robins and I. L. Thomas	128

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

UNIVERSITY OF ST. ANDREWS

LECTURESHIP IN PHARMACOLOGY

Applications are invited for a Lectureship in Pharmacology in the Department of Pharmacology and Therapeutics, Queen's College, Dundee, with effect from a date to be arranged. Salary scale: medically qualified—£1050 × £100 to £2000 (efficiency bar £1450); non-medically qualified—£1050 × £50 to £1400 (efficiency bar) × £75 to £1850. Placing on appropriate scale according to qualifications and experience, but at a stage below the efficiency bar. Preference will be given to applicants possessing post-graduate science degrees in Pharmacology. F.S.S.U.; Family Allowance; Grant towards furniture removal expenses. Applications (6 copies) containing the names of three referees, should be lodged with the Secretary of the University, c/o Queen's College, Dundee, from whom further particulars may be obtained, not later than February 17, 1962.

Recent Developments in the Sterilisation of Surgical Materials

Report of a symposium organised by the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain and Smith & Nephew Research Limited at the School of Pharmacy, University of London, April 11-13, 1961.

Pages xii + 236 Price 30s. (postage 1s.)

31 illustrations, 11 diagrams, 33 graphs, 12 tables

Sterilisation by Ionising Radiations · Gaseous Methods of Sterilisation · Hospital Organisation in Relation to the Sterilisation of Surgical Materials · Sterility Tests · Review of the proceedings.

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

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 3

March, 1962

CONTENTS

Review Article	PAGES
QUATERNARY AMMONIUM COMPOUNDS IN MEDICINAL CHEMISTRY. I. By P. F. D'Arcy, B.Pharm., Ph.D., M.P.S. and E. P. Taylor, B.Pharm., B.Sc., Ph.D., F.R.I.C.	129-146
Research Papers	
THE ALKALOIDS OF THE GENUS <i>DATURA</i> , SECTION BRUGMANSIA. Part I. <i>D. CORNIGERA</i> Hook. By W. C. Evans and M. Pe Than	147-156

[Continued on page ii]

Recent Developments in the Sterilisation of Surgical Materials

Report of a symposium organised by the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain and Smith & Nephew Research Limited at the School of Pharmacy, University of London, April 11 - 13, 1961.

Pages xii + 236 Price 30s. (postage 1s.)
31 illustrations, 11 diagrams, 33 graphs, 12 tables

Sterilisation by Ionising Radiations · Gaseous Methods of Sterilisation · Hospital Organisation in Relation to the Sterilisation of Surgical Materials · Sterility Tests · Review of the proceedings.

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

CONTENTS

Research Papers—(continued)

	PAGES
THE MORPHOLOGY AND HISTOLOGY OF SEEDS OF <i>DATURA CORNIGERA</i> HOOK. By M. Wellendorf	157-160
THE EFFECT OF DEFICIENCY AND SMALL EXCESS OF THIAMINE ON THE RAT PHRENIC NERVE DIAPHRAGM PREPARATION. By B. Bhagat and Mary F. Lockett	161-168
THE BIOLOGICAL ASSAY OF OXYTOCIN IN THE PRESENCE OF ERGOMETRINE. By B. Berde and E. Stürmer	169-171
THE HYDROLYSIS OF ETHYL BENZOATE, DIETHYL PHTHALATE AND BENZOCAINE IN CETRIMIDE SOLUTIONS. By A. G. Mitchell ..	172-176
THE NEUROMUSCULAR BLOCKING ACTION OF SUXAMETHONIUM ON THE RAT DIAPHRAGM. By R. Whittaker	177-181
THE EFFECTS OF ANTI-INFLAMMATORY DRUGS ON SOME ASPECTS OF INTERMEDIARY METABOLISM. By C. Bryant and M. J. H. Smith	182-185
A NOTE ON THE PEROXIDE VALUE OF LANOLIN. By C. A. Anderson and G. F. Wood	186-187
Book Reviews	188-189

Letters to the Editor

EFFECT OF PYROGALLOL AND CATECHOL ON ISOLATED SMOOTH ORGANS. By Juan A. Izquierdo and Augusto V. Juorio ..	190
A MODIFIED DUAL UNIT ORGAN BATH FOR ISOLATED TISSUES. By R. T. Brittain and D. J. Rowe	191-192

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. McCoubrey, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
 SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 4

April, 1962

CONTENTS

Review Article	PAGES
QUATERNARY AMMONIUM COMPOUNDS IN MEDICINAL CHEMISTRY. II. By P. F. D'Arcy, B.Pharm., Ph.D., M.P.S., and E. P. Taylor, B. Pharm., B.Sc., Ph.D., F.R.I.C.	193-216
Research Papers	
SOME FACTORS INFLUENCING THE ABSORPTION OF GRISEOFULVIN FROM THE GASTROINTESTINAL TRACT. By W. A. M. Duncan, G. Macdonald and M. J. Thornton	217-224
THE EFFECT OF <i>ORTHO</i> SUBSTITUTION ON THE PHARMACOLOGY OF BENZOYLCHOLINE. By J. Thomas and D. Buckley	225-231
HYDROLYTIC DESTRUCTION OF THIAMINE, ESPECIALLY IN THE PRESENCE OF CYANOCOBALAMIN. By Jennifer Heathcote and B. A. Wills	232-236
ESTIMATION AND URINARY EXCRETION OF TETRAHYDROAMINO- ACRIDINE. By P. N. Kaul.	237-242
ENZYME INHIBITING ACTION OF TETRAHYDROAMINOACRIDINE AND ITS STRUCTURAL FRAGMENTS. By P. N. Kaul	243-248
SPECTROPHOTOFUOROMETRIC DETERMINATION OF EMETINE IN ANIMAL TISSUES. By B. Davis, M. G. Dodds and E. G. Tomich	249-252
Letters to the Editor	
ANTICONVULSANT ACTIVITY OF PROCAINE AND ITS FIVE CONGENERS AGAINST EXPERIMENTALLY INDUCED CONVULSIONS. By Kanti Kapila and R. B. Arora	253-254
ORALLY EFFECTIVE HYPOGLYCAEMIC AGENTS FROM PLANTS. By H. D. Brahmachari and K. T. Augusti	254-255
THE EFFECT OF pH ON THE STABILITY OF PENICILLIN-INDUCED SPHERO PLASTS. By W. B. Hugo and A. D. Russell	256

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.,
G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C.
HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C.,
A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C.,
L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F.
SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc.,
F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.



*microbiological
reagents and media*

THE ONLY COMPLETE LINE

DETECTION OF THE **SALMONELLA-SHIGELLA** GROUP

Bacto-SS Agar

is a selective medium especially designed for use in isolation of fastidious *Shigella* and *Salmonella* strains. The selective action of this medium restrains to a large extent the development of coliform bacteria with minimum restriction of fastidious strains of the pathogens. Because of the inhibitive action of the medium of coliform bacteria, it is possible to inoculate the medium heavily with faeces thereby greatly increasing the chance of positive isolations from samples containing very few pathogens.

Bacto-Bismuth Sulphite Agar

is a highly selective medium for isolation of *Salmonella typhosa*. The unusual selective properties of this medium permit the use of large inocula of faeces and other suspected material without overgrowth of extraneous intestinal bacteria.

Bacto-MacConkey Agar

is an excellent differential medium for use in conjunction with Bacto-SS Agar and Bacto-Bismuth Sulphite Agar. This medium supports rapid and luxuriant growth of even the most fastidious strains of the typhoid-dysentery group.

Although MacConkey Agar does not inhibit coliform bacteria it does afford excellent differentiation of colonies of pathogens from those of the lactose fermenting bacilli.

Bacto-Tetrathionate Broth Base

Bacto-Selenite Broth

enrichment media for isolation of intestinal pathogens. These are excellent aids in the detection of carriers and examination of other materials for members of the *Salmonella-Shigella* group.

*Please send for
the latest technical
information.*



BAIRD & TATLOCK (LONDON) LIMITED, CHADWELL HEATH, ESSEX, ENGLAND.

Branches in London, Manchester and Glasgow.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 5

May, 1962

CONTENTS

Research Papers	PAGES
THE EFFECTS IN RABBITS OF THYROIDECTOMY AND TREATMENT WITH TRIIODOTHYRONINE ON THE SENSITIVITY TO NORADRENALINE AND THE CONTENT OF NORADRENALINE IN AORTA AND SPLEEN. By W. H. Macmillan and M. J. Rand	257-267
THE URINARY EXCRETION OF TRITIATED BUTYLATED HYDROXYANISOLE AND BUTYLATED HYDROXYTOLUENE IN THE RAT. By W. S. Golder, A. J. Ryan and S. E. Wright	268-271
THE SPASMOLYTIC ACTIONS OF PYROGALLOL AND CATECHOL ON THE ISOLATED GUINEA-PIG ILEUM. By E. S. Johnson.	272-279
DIRECT COLORIMETRIC DETERMINATION OF SMALL QUANTITIES OF <i>m</i> -AMINOPHENOL IN SODIUM AMINOSALICYLATE. By Tatjana Bičan-Fister	280-283
THE EFFECT OF ADRENERGIC BLOCKING AGENTS AND OF CHLORPROMAZINE ON BLOOD PRESSURE INCREASE BY VASOPRESSIN AND ANGIOTENSIN. By Z. Supek, B. Uroić, V. Gjuriš and N. Marijan	284-287
DITERPENE ALKALOIDS. ISOLATION AND STUDY OF TWO NEW ALKALOIDS. By Nazar Singh and K. L. Chopra	288-293
THE EFFECTS OF PROLONGED ADMINISTRATION OF SOME ADRENOCORTICAL STEROIDS IN THE RAT. By P. F. D'Arcy and E. M. Howard	294-305
SOME DERIVATIVES OF 1,2,3,6-TETRAHYDROPYRIDINE. By V. Petrow and O. Stephenson	306-314
Letters to the Editor	
ECTOPIC VENTRICULAR ARRHYTHMIA AFTER CORONARY OCCLUSION IN THE INDIAN DOMESTIC PIG. By R. B. Arora and D. S. Sivappa	315-316
OBSERVATIONS ON CONDITIONED AND UNCONDITIONED ON- AND OFF-BEHAVIOURAL RESPONSES TO A BUZZER. By Ivan Izquierdo	316-318
PEROXIDE VALUE OF ANHYDROUS LANOLIN. By E. W. Clark and G. F. Kitchen	318-319
CARDIOTONIC ACTION OF HAMYCIN. By H. R. K. Arora	320
THE STABILITY OF AQUEOUS SOLUTIONS OF PICROTOXIN TO LIGHT. By P. W. Ramwell and J. E. Shaw	321-322
Book Reviews	322-324

DIFCO

microbiological reagents and media

THE ONLY COMPLETE LINE OFFERED IN U.K.

Requirements of the Bacteriologist, Biochemist, Biologist, Pathologist and Pharmacologist can be met very quickly either from our extensive stocks, or in the case of certain specialised products, within a few days.

*Over 60 years
experience ensure*

UNIFORMITY
STABILITY
ECONOMY

*Please send for
the latest technical
information.*



**complete
laboratory
service**

Culture Media
Microbiological Assay Media
Tissue Culture Media
Serological Reagents
Antisera
Diagnostic Reagents
Sensitivity Disks
Unidisks
Peptones
Hydrolysates
Amino Acids
Enzymes
Enrichments
Dyes
Indicators
Carbohydrates
Biochemicals

BAIRD & TATLOCK (LONDON) LIMITED, CHADWELL HEATH, ESSEX, ENGLAND.
Branches in London, Manchester and Glasgow.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 6

June, 1962

CONTENTS

Review Article	PAGES
BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS. PART I. SYNTHETIC NITROGENOUS STEROIDS. By M. Alauddin, B. Pharm. and M. Martin-Smith, M.Sc., Ph.D.	325-349
Research Papers	
ALKANOLAMIDES STERICALLY RELATED TO ERGOMETRINE. By J. Chilton and J. B. Stenlake	350-366

[Continued on page ii]

Recent Developments in the Sterilisation of Surgical Materials

Report of a symposium organised by the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain and Smith & Nephew Research Limited at the School of Pharmacy, University of London, April 11 - 13, 1961.

Pages xii + 236 Price 30s. (postage 1s.)
31 illustrations, 11 diagrams, 33 graphs, 12 tables

Sterilisation by Thermal Methods, Ionising Radiations, and by Gaseous Methods · Hospital Organisation in Relation to the Sterilisation of Surgical Materials · Sterility Tests · Review of the proceedings.

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

CONTENTS

Research Papers—continued

	PAGES
DISSOCIATION CONSTANTS OF SOME COMPOUNDS RELATED TO LYSERGIC ACID. PART II. ERGOMETRINE, ERGOMETRININE AND ALKANOLAMIDES OF 3-DIMETHYLAMINOPROPIONIC ACID, 1-METHYLHEXAHYDRONICOTINIC ACID AND ARECAIDINE. By J. Chilton and J. B. Stenlake	367-370
A NOTE ON THE ACTION OF GALLAMINE ON ISOLATED RABBIT AURICLES. By J. L. H. Laity and B. K. Garg	371-373
COMMON DRUGS THAT MAY INVALIDATE SPECTROPHOTOFUOROMETRIC ASSAYS OF BLOOD GRISEOFULVIN. By K. J. Child, Carole Bedford and E. G. Tomich	374-377
CHRONIC TOXICITY STUDIES ON FOOD COLOURS. V. OBSERVATIONS ON THE TOXICITY OF BRILLIANT BLUE FCF, GUINEA GREEN B AND BENZYL VIOLET 4B IN RATS. By W. A. Mannell, H. C. Grice and M. G. Allmark	378-384
THE EFFECTS OF BRETILIUM AND COCAINE ON NORADRENALINE DEPLETION. By B. A. Callingham and Rosemary Cass	385-389
A NOTE ON THE ACTIVITY OF SIX PENICILLINS AGAINST <i>Escherichia coli</i> . By A. D. Russell	390-392

Letters to the Editor

HEPARIN AND ANAPHYLAXIS. By H. L. Dhar and R. K. Sanyal	393
MECHANISM OF INCREASED CAPILLARY PERMEABILITY INDUCED BY <i>Echis carinatus</i> (SAW-SCALED VIPER) VENOM: A POSSIBLE NEW APPROACH TO THE TREATMENT OF VIPERINE SNAKE POISONING. By P. Somani and R. B. Arora	394-395
THE ACTIVITY OF AMPICILLIN AGAINST <i>Escherichia coli</i> . By T. D. Turner and A. D. Russell	395-396
NEW ORAL HYPOGLYCAEMIC AGENTS. By P. N. Luthra and J. N. Tayal	396

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. McCoubrey, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.

SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London, W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 7

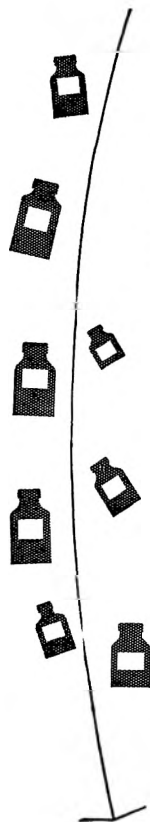
July, 1962

CONTENTS

	PAGES
Symposium on the Influence of Animal Strain Selection and Conditioning on Biological Experiments and Assays	
CHAIRMAN'S INTRODUCTION: W. Lane-Petter, M.A., M.B., B.Chir.	397-398
PRACTICAL ASPECTS OF STRAIN VARIATION IN RELATION TO PHARMACOLOGICAL TESTING. By D. M. Brown and B. O. Hughes ..	399-405
STRAIN VARIATION IN MICE. By Annie M. Brown	406-410
THE CONDITIONING OF EXPERIMENTAL ANIMALS. By P. F. D'Arcy	411-415
DISCUSSION	416-428
Research Papers	
MONOMOLECULAR LAYERS OF SYNTHETIC PHOSPHATIDES. By L. L. M. van Deenen, U. M. T. Houtsmuller, G. H. de Haas and E. Mulder	429-444
THE SOLUBILISING PROPERTIES OF LIQUORICE. By K. C. James and J. B. Stanford	445-450
HYPOGLYCAEMIC AGENTS. PART II. By D. F. Hayman, V. Petrow, O. Stephenson and A. J. Thomas	451-455
FLOCCULATION AND CONDUCTIVITY OF PHOSPHATIDE SOLS. By I. L. Thomas	456-463
New Apparatus	
A MODIFIED PLETHYSMOGRAPHIC APPARATUS FOR RECORDING VOLUME CHANGES IN THE RAT PAW. By J. M. Harris and P. S. J. Spencer	464-466
Letter to the Editor	
ABSENCE OF GENERAL ANAESTHETIC PROPERTIES IN A NUMBER OF TERPENOID HEMISUCCINATES. By K. Ahmad, F. MacL. Carey, T. Khatoom, J. J. Lewis and M. Martin-Smith	467-468

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.



DIFCO

*Microbiological
reagents
and media*

***delivered
to your bench quickly***

Hundreds of different products in the complete Difco range are kept in stock ready to be on your bench without delay. We shall always be pleased to obtain other items specially to order.

Speed, convenience, reliability . . . and remember that Difco offer the only *complete* line of culture media available in U.K. Please send for the latest literature concerning your special interests.



**complete
laboratory
service**



BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND.

Branches in London, Manchester and Glasgow.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 8

August, 1962

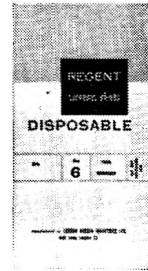
CONTENTS

	PAGES
Review Article	
BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS. PART II. STEROIDAL ALKALOIDS. By M. Alauddin, B.Pharm. and M. Martin-Smith, M.Sc., Ph.D.	469-495
Research Papers	
CARDIOTONIC SUBSTANCES FROM <i>BERSAMA ABYSSINICA</i> FRES. SUB. <i>SPECIES ABYSSINICA</i> . By J. A. Lock	496-502
THE RELATIONSHIP BETWEEN ELECTRICAL RESISTANCE AND DIS- PERSED PHASE CONCENTRATION IN OIL IN WATER EMULSIONS. By I. H. Harrison and K. C. James	503-508
EFFECT OF IMPRAMINE, AMITRIPTYLINE AND THEIR MONOMETHYL DERIVATIVES ON RESERPINE ACTIVITY. By S. Garattini, A. Giachetti, A. Jori, L. Pieri and L. Valzelli	509-514
THE ANTIVERATRINIC ACTION OF SOME LOCAL ANAESTHETICS. By V. N. Sharma and R. B. Arora	515-521
HYPOGLYCAEMIC AGENTS. PART III. By D. F. Hayman, V. Petrow and O. Stephenson	522-533
Letters to the Editor	
CALOPHYLLOLIDE, A COMPLEX COUMARIN ANTICOAGULANT FROM <i>CALOPHYLLUM INOPHYLLUM</i> LINN. By R. B. Arora, C. N. Mathur and S. D. S. Seth	534-535
EFFECT OF HYDROCORTISONE ON CAPILLARY PERMEABILITY CHANGES INDUCED BY <i>ECHIS CARINATUS</i> (SAW-SCALED VIPER) VENOM IN THE RAT. By P. Somani and R. B. Arora	535-537
THE EFFECTS OF ETHER ON POTASSIUM FLUX IN SKELETAL MUSCLE PREPARATIONS. By J. J. Lewis, N. J. Mir and T. C. Muir	537-539
Book Reviews	539-540

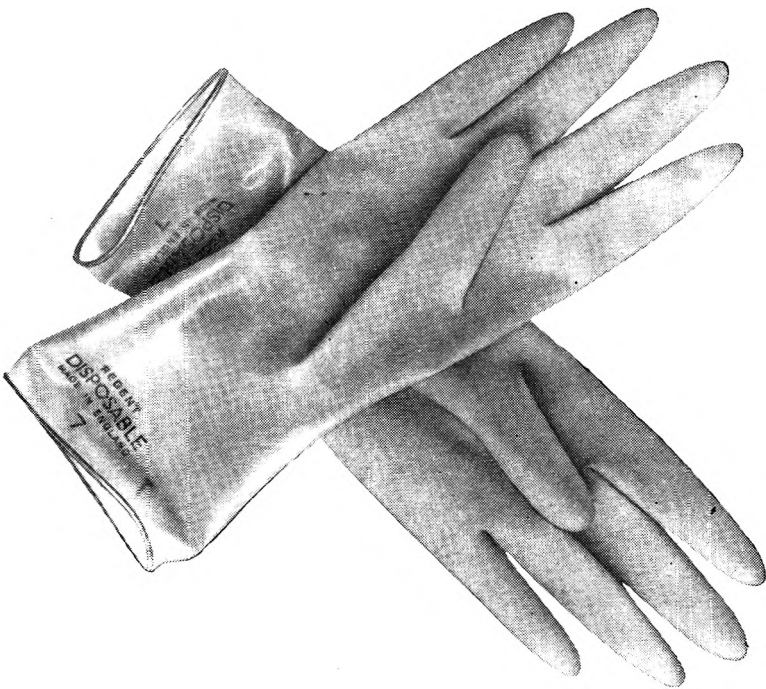
EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.,
G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C.
HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C.,
A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C.,
L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F.
SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc.,
F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

Save time and money with Regent Disposable Gloves by eliminating multi-stage reconditioning processes, thus simplifying sterilisation and liberating valuable skill for nursing. Because hands are assumed to be an important vehicle of cross-infection, the use of a new glove of guaranteed integrity each time also makes a contribution towards the elimination of this problem. Regent Disposable gloves reduce hand fatigue and provide 'bare hand' tactile sensitivity. They are supplied packed ready for instant sterilisation, complete with Ethicon Bio-sorb powder.



Regent Surgeons' Disposable Gloves



Available under Ministry of Health Central Contract

Manufactured by **LONDON RUBBER INDUSTRIES LIMITED**
HALL LANE • LONDON E4

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 9

September, 1962

CONTENTS

	PAGES
Research Papers	
ANTAGONISM OF GUANETHIDINE BY DEXAMPHETAMINE AND OTHER RELATED SYMPATHOMIMETIC AMINES. By M. D. Day and M. J. Rand	541-549
DRUG-PLASMA BINDING MEASURED BY SEPHADEX. By C. F. Barlow, H. Firemark and L. J. Roth	550-555
PHARMACOLOGICAL SCREENING OF SOME WEST INDIAN MEDICINAL PLANTS. By P. C. Feng, L. J. Haynes, K. E. Magnus, J. R. Plimmer and H. S. A. Sherratt	556-561

[Continued on page ii

Practical Pharmaceutical Chemistry Quantitative Analysis

A. H. BECKETT & J. B. STENLAKE

This new and authoritative textbook is primarily intended for the use of students reading for degrees in Pharmacy, for those studying for the Pharmaceutical Chemist Qualifying Examination of the Pharmaceutical Society of Great Britain as well as for those undertaking more advanced courses in this country and overseas; but the great number of practical exercises it contains together with its coverage of newer methods will also make it valuable to those professionally engaged in the practice of pharmaceutical analysis. A prospectus is available.

Royal 8vo, 406 pages, 63s net

UNIVERSITY OF LONDON · THE ATHLONE PRESS

CONTENTS

Research Papers—(continued)	PAGES
SIMPLE HYPOTENSIVE AND HYPERTENSIVE PRINCIPLES FROM SOME WEST INDIAN MEDICINAL PLANTS. By E. Durand, E. V. Ellington, P. C. Feng, L. J. Haynes, K. E. Magnus and N. Philip	562-566
ULTRASONIC IRRADIATION OF SOME PHOSPHOLIPID SOLS. By L. Saunders, J. Perrin and D. Gammack	567-572
THE MORPHOLOGY AND ANATOMY OF THE LEAF OF <i>Podophyllum hexandrum</i> ROYLE. By (Miss) S. Ellis and K. R. Fell	573-586
THE SYNTHESIS OF <i>ortho</i> -SUBSTITUTED 2-DIETHYLAMINOETHYL BENZOATES AS POTENTIAL LOCAL ANAESTHETICS. By J. Thomas and J. Canty	587-596
THE CHEMICAL DETERMINATION OF LIOTHYRONINE AND THYROXINE IN ENZYMIC HYDROLYSATES OF PORK THYROID. By W. F. Devlin and N. R. Stephenson	597-604
STUDIES ON THE KINETICS OF FUNGICIDAL ACTION. PART II. THE EFFECT OF TEMPERATURE ON THE VIABILITY OF <i>Penicillium notatum</i> SPORES IN WATER AND SOLUTIONS OF PHENOL. By N. M. Chauhan and V. Walters	605-610
A NOTE ON THE ANALYSIS OF OIL OF PEPPERMINT BY AN ALUMINIUM OXIDE-SILICIC ACID DOUBLE COLUMN. By M. S. Karawya and S. K. Wahba	611-612
 Letters to the Editor	
THE IDENTIFICATION OF DIGOXIN METABOLITE B (DIGITOXIN METABOLITE C) WITH DIGOXIGENIN DI-DIGITOXOSIDE. By S. E. Wright	613-614
ESTIMATION OF THIOTEPA IN URINE. By D. N. Raine	614-615
THE ACTION OF ARYLOXYALIPHATIC ACIDS ON THE PERMEABILITY OF BLOOD VESSELS. By B. J. Northover and J. Verghese	615-616
EFFECTS OF ORALLY EFFECTIVE HYPOGLYCAEMIC AGENTS FROM PLANTS ON ALLOXAN DIABETES. By H. D. Brahmachari and K. T. Augusti	617
FUNCTION OF MAST-CELLS. By G. B. West	618-619
Book Reviews	619-620

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., J. W. FAIRBAIRN, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C., G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., A. D. MACDONALD, M.D., M.A., M.Sc., A. McCoubrey, B.Sc., Ph.D., M.P.S., F.R.I.C., L. SAUNDERS, D.Sc., Ph.D., F.R.I.C., E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., G. SYKES, M.Sc., F.R.I.C., G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.
 SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 10

October, 1962

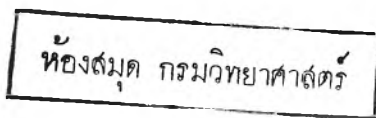
CONTENTS

Review Article	PAGES
RECENT ADVANCES IN FREEZE-DRYING. By R. I. N. Greaves, M.D.	621-640
Research Papers	
THE PHOSPHORYLATION OF ANTI-ADRENERGIC QUATERNARY AMMONIUM SALTS RELATED TO CHOLINE. By F. C. Copp, T. S. G. Jones and A. McCoubrey	641-646
THE ABSORPTION OF BRETILIUM AND RELATED QUATERNARY AMMONIUM SALTS FROM THE ALIMENTARY TRACT. By A. L. A. Boura and A. McCoubrey	647-657
COMPARATIVE STUDY OF THE CALCIPHYLACTIC CHALLENGING POTENCY OF VARIOUS IRON COMPOUNDS. By Ralph Strebel, Jaromir Vašků and Hans Selye	658-663
STUDIES ON <i>Datura leichhardtii</i> MUELL. EX BENTH. PART I. THE ANATOMY OF THE LEAF AND STEM. By W. C. Evans and N. A. Stevenson	664-678
STUDIES IN THE FIELD OF DIURETIC AGENTS. PART VI. SOME SULPHAMOYL BENZOIC ACIDS. By G. B. Jackman, V. Petrow, O. Stephenson and A. M. Wild	679-686
THE EFFECT OF THE ADMINISTRATION OF WATER OR ISOTONIC NaCl SOLUTION ON THE URINARY EXCRETION OF 5-HYDROXYINDOLE-ACETIC ACID IN THE RAT. By G. Bertaccini and V. Erspamer	687-697
Letters to the Editor	
A SEX DIFFERENCE IN SENSITIVITY OF GFF MICE TO AN ANAESTHETIC STEROID. By R. M. Atkinson, M. A. Pratt and E. G. Tomich	698
DILUTIONS OF SULPHURIC ACID. By T. J. Betts	698-699
AN ADRENERGIC NEURONE BLOCKING ACTION OF DIMETHYLPHENYL-PIPERAZINIUM. By A. B. Wilson	700

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., D. C. GARRATT, D.Sc. Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., F. HARTLEY, B.Sc., Ph.D., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., J. J. LEWIS, M.Sc., F.P.S., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C. G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.

SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.



the wider significance of

local decamethylene-bis-

(4-aminoquinaldinium chloride)

- dequalinium



By providing effective local antibacterial and antifungal therapy for infections of the skin and mucous membrane, Dequadin frequently spares the use of antibiotics.

Dequadin has a wider antimicrobial spectrum than penicillin and it is active against organisms resistant to antibiotics. Furthermore, no resistant strains have been reported following the use of Dequadin.

Recent laboratory work has shown that Dequadin is retained on tissue. To demonstrate this unusual property, a special laboratory test was devised involving the use of ^{14}C Dequadin. Visual recording of the retention of Dequadin on tissue was supplied by a technique involving the use of auto-radiographs.



DEQUADIN

*a product of Allen & Hanburys
research*

*in
LOZENGES CREAM
PAINT TULLE DRESSINGS*

ALLEN & HANBURY'S LTD · LONDON · E.2

S61/220/H

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 £5 0s. 0d. Single Copies 10s.

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London, W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 11

November, 1962

CONTENTS

Research Papers

	PAGES
CURARE-LIKE DRUGS AND VAGAL SYNAPSES: COMPARATIVE STUDY <i>IN VITRO</i> ON THE ISOLATED VAGUS-STOMACH PREPARATION OF THE RAT. By D. Della Bella, F. Rognoni and U. M. Teotino	701-706
THE RETENTION OF AQUEOUS SUSPENSIONS ON LEAF SURFACES. By S. B. Challen	707-714
OBSERVATIONS ON THE USE OF A MOUSE BIOASSAY METHOD FOR INVESTIGATING PURGATIVE ACTIVITY. By R. T. Brittain, P. F. D'Arcy and J. J. Grimshaw	715-721
THE DISTRIBUTION AND EXCRETION BY CATS OF A NEW HYPOTENSIVE DRUG, <i>N</i> -BENZYL- <i>N'</i> <i>N''</i> -DIMETHYLGUANIDINE. By A. L. A. Boura, W. G. Duncombe, R. D. Robson and A. McCoubrey	722-726
BIOCHEMICAL PROPERTIES OF BRETILIUM. By A. McCoubrey	727-734
A NOTE ON THE INFLUENCE OF CHLORPROMAZINE AND DIETHAZINE ON THE STORES OF CATECHOLAMINE IN THE ADRENAL GLANDS AND AORTIC WALLS OF RATS. By Ruth A. Davis, C. L. Kaul and Mary F. Lockett	735-738
THE EFFECTS OF HYDROCORTISONE ON THE CHANGES IN LIPID METABOLISM INDUCED IN GUINEA-PIG LUNG TISSUE BY ANAPHY- LAXIS <i>IN VIVO</i> . By P. Goadby and W. G. Smith	739-745
A NOTE ON THE USE OF CELLULOSE PHOSPHATE CATION-EXCHANGE PAPER FOR THE SEPARATION OF CATECHOLAMINES, AND SOME OTHER BIOGENIC AMINES. By Michael Roberts	746-749
THE INFLUENCE OF ENVIRONMENTAL CHANGES ON THE CARDIO- TOXICITY OF ISOPRENALINE IN RATS. By T. Balazs, J. B. Murphy and H. C. Grice	750-755
POTENTIAL RESERPINE ANALOGUES. PART III. By M. S. Chod- nekar, L. K. Sharp and W. H. Linnell	756-760
A NEW COMPLEXOMETRIC METHOD FOR THE DETERMINATION OF SOME SULPHONAMIDES. By H. Abdine and W. S. Abdel Sayed.	761-763

Letter to the Editor

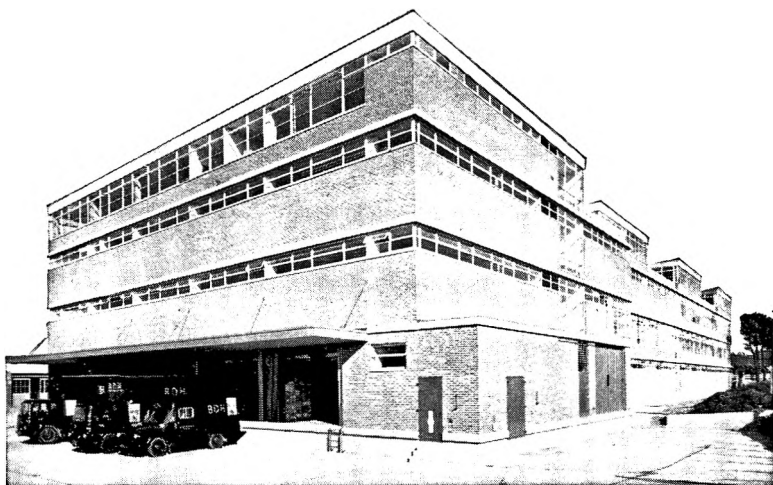
THE CONCENTRATION OF CATECHOLAMINES IN THE TURTLE HEART AND VAGAL ESCAPE. By A. H. Friedman and B. Bhagat	764
--	-----

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., D. C. GARRATT, D.Sc. Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., F. HARTLEY, B.Sc., Ph.D., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., J. J. LEWIS, M.Sc., F.P.S., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C. G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.

SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

WHAT'S NEW? FROM B.D.H.



NEW WAREHOUSES, NEW OFFICES

The new offices and warehouses of the B.D.H. Laboratory Chemicals Division are now in full operation. They cover some six acres, and provide exceptional facilities for the storage assembly and despatch of the Division's six or seven thousand products. The substantial areas consequently left free in the Poole factory will be used for increased production and for extended analytical, biochemical and development laboratories.

One of the world's main suppliers of laboratory chemicals, B.D.H. has associated companies handling these products in Australia, Canada, India, New Zealand and South Africa, and agents or distributors in many other countries. Sales centres for B.D.H. laboratory chemicals in England are established in London, Liverpool, and Bristol, supplementing the main distribution from Poole.



THE BRITISH DRUG HOUSES LTD
B.D.H. LABORATORY CHEMICALS DIVISION · POOLE · DORSET

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 **£5 0s. 0d.** Single Copies **10s.**

17 BLOOMSBURY SQUARE, LONDON, W.C.1

Cables: Pharmakon, London. W.C.1. Telephone: HOLborn 8967

Vol. XIV No. 12

December, 1962

Review Article	CONTENTS	PAGES
	SELENIUM ANALOGUES OF BIOLOGICALLY ACTIVE SULPHUR COMPOUNDS. By D. Dingwall, B.Sc., M.P.S.	765-776
Research Papers		
	A COMPARISON OF THE THYROXINE:TRI-IODOTHYRONINE CONTENT AND BIOLOGICAL ACTIVITY OF THYROID FROM VARIOUS SPECIES. By G. S. Wiberg, W. F. Devlin, N. R. Stephenson, J. R. Carter and A. J. Bayne	777-783
	THE NATURE OF RESISTANCE OF A PENICILLIN TO HYDROLYSIS BY PENICILLINASE. By N. Citri and N. Garber	784-793
	A METHOD FOR THE ESTIMATION OF ADRENALINE AND NORADRENALINE IN URINE. By R. Atkinson and N. A. Wynne	794-797
	THE ESTIMATION OF <i>N</i> -BENZYL- <i>N'</i> <i>N''</i> -DIMETHYLGUANIDINE (BW 467C60) IN URINE AND SOME OBSERVATIONS ON ITS REACTION WITH HYPOBROMITE. By A. McCoubrey	798-802
	THE EFFECT OF LOWERED TEMPERATURE ON THE NEUROMUSCULAR BLOCKING ACTION OF SUXAMETHONIUM ON THE RAT DIAPHRAGM. By R. Whittaker	803-807
	THE BACTERIOSTATIC ACTIONS OF TETRACYCLINE AND OXYTETRACYCLINE. By J. G. Jones and G. A. Morrison	808-824
New Apparatus		
	A SIMPLE MECHANICAL COMPUTER FOR RELATING THE CONTRACTILE RESPONSES OF TISSUES. By G. Paterson	825-827
Letters to the Editor		
	DRUGS AND RAT PREGNANCY. By G. B. West	828-830
	ANTI-INFLAMMATORY ACTIVITY OF MUSK. By R. K. Mishra, R. B. Arora and S. D. S. Seth	830-831
	EFFECTIVENESS OF 5-HYDROXYTRYPTAMINE IN ECTOPIC VENTRICULAR TACHYCARDIA RESULTING FROM ACUTE MYOCARDIAL INFARCTION IN THE DOG. By K. Kapila and R. B. Arora	831-832
Book Reviews		833-836

EDITORIAL BOARD

H. S. BEAN, B.Pharm., Ph.D., F.P.S., D. C. GARRATT, D.Sc. Ph.D., F.R.I.C., J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C., F. HARTLEY, B.Sc., Ph.D., F.P.S., F.R.I.C., E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., J. J. LEWIS, M.Sc., F.P.S., A. D. MACDONALD, M.D., M.A., M.Sc., A. MCCOUBREY, B.Sc., Ph.D., M.P.S., F.R.I.C., D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C., G. F. SOMERS, B.Sc., Ph.D., F.P.S., J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C. G. B. WEST, B.Pharm., D.Sc., Ph.D., F.P.S., R. T. WILLIAMS, D.Sc., Ph.D.

SECRETARY: F. W. ADAMS, B.Sc., F.P.S., F.R.I.C.

the wider significance of

local decamethylene-bis-

(4-aminoquinaldinium chloride)

- dequalinium



By providing effective local antibacterial and antifungal therapy for infections of the skin and mucous membrane, Dequadin frequently spares the use of antibiotics.

Dequadin has a wider antimicrobial spectrum than penicillin and it is active against organisms resistant to antibiotics. Furthermore, no resistant strains have been reported following the use of Dequadin.

Recent laboratory work has shown that Dequadin is retained on tissue. To demonstrate this unusual property, a special laboratory test was devised involving the use of ^{14}C Dequadin. Visual recording of the retention of Dequadin on tissue was supplied by a technique involving the use of auto-radiographs.



DEQUADIN

*a product of Allen & Hanburys
research*

*in
LOZENGES CREAM
PAINT TULLE DRESSINGS*

ALLEN & HANBURY'S LTD · LONDON · E.2

JOURNAL OF PHARMACY AND PHARMACOLOGY
TRANSACTIONS OF THE
BRITISH PHARMACEUTICAL CONFERENCE

EDITOR: D. W. MATHIESON, B.Sc., Ph.D., F.R.I.C.

PRESS EDITOR: J. R. FOWLER, B.Pharm., F.P.S.

17 BLOOMSBURY SQUARE, LONDON, W.C.1.

Vol. XIV Supplement

December 1962

CONTENTS

	PAGES
Report of Proceedings	1 <i>T-8 T</i>
Symposium on Drug Addiction	
FIRST INTRODUCTORY ADDRESS. By A. D. Macdonald, M.D., M.A., M.Sc.	9 <i>T-16 T</i>
SECOND INTRODUCTORY ADDRESS (SUMMARY). By J. M. John- ston, C.B.E., M.B., Ch.B., M.D., F.R.C.S.Ed., F.R.C.P.Ed.	16 <i>T-17 T</i>
THIRD INTRODUCTORY ADDRESS (SUMMARY). By B. J. Thomas, M.P.S.	17 <i>T-19 T</i>
Conference Lecture	
PHARMACOGENETICS—A STUDY OF INHERITED VARIABILITY IN THE RESPONSE TO DRUGS. By C. A. Clarke, M.A., M.D., F.R.C.P.	20 <i>T-30 T</i>
Science Papers	
AN INVESTIGATION OF THE METABOLISM OF NEOSTIGMINE IN PATIENTS WITH MYASTHENIA GRAVIS. By Carol A. Scott, P. T. Nowell and A. Wilson	31 <i>T-33 T</i>
SOME EFFECTS OF A HEMICHOLINIUM COMPOUND (HC-3) ON NEUROMUSCULAR TRANSMISSION IN THE CAT. By E. R. Evans and H. Wilson	34 <i>T-36 T</i>
MYASTHENIC-LIKE FEATURES OF THE NEUROMUSCULAR TRANS- MISSION FAILURE PRODUCED BY TRIETHYLCHOLINE. By W. C. Bowman, B. A. Hemsworth and M. J. Rand	37 <i>T-41 T</i>
THE RESPONSE OF THE PIG UTERUS TO OXYTOCIN AT DIFFERENT STAGES IN THE OESTRUS CYCLE. By A. Knifton	42 <i>T-43 T</i>
AN APPARATUS FOR TESTING ANTICONSULSANT DRUGS BY ELECTROSHOCK SEIZURES IN MICE. By C. H. Cashin and H. Jackson	44 <i>T-47 T</i>
LOCAL ANAESTHETIC ACTIVITY IN DIETHYLAMINOACETYL DERIVA- TIVES OF SUBSTITUTED BENZYLAMINES. By R. F. Collins and B. J. Large	48 <i>T-58 T</i>
THE DETERMINATION OF ERGOTAMINE IN PREPARATIONS CON- TAINING ERGOTAMINE TARTRATE AND CYCLIZINE HYDRO- CHLORIDE. By A. C. Caws and B. E. Lawrence	59 <i>T-62 T</i>

CONTENTS

Science Papers—(continued)	PAGES
THE DETERMINATION OF CALCIUM IN HEAVY MAGNESIUM CARBONATE USING GLYOXAL BIS(2-HYDROXYANIL). By M. A. Leonard	63 T-65 T
THE ANALYSIS OF POLDINE METHYL METHOSULPHATE BY INFRARED SPECTROSCOPY. By H. D. C. Rapson, K. W. Austin and E. A. Cutmore	66 T-72 T
THE COLORIMETRIC DETERMINATION OF PHENOLPHTHALEIN. By J. Allen, (Miss) B. Gartside and C. A. Johnson	73 T-76 T
THE USE OF TETRAPHENYLBORON FOR THE DETERMINATION AND CHARACTERISATION OF ORGANIC BASES IN PHARMACEUTICAL PREPARATIONS. By C. A. Johnson and R. E. King	77 T-82 T
WATER FOR INJECTION BY ION-EXCHANGE. By A. M. Cook and L. Saunders	83 T-86 T
SOME PHYSICAL PROPERTIES OF INTERFACIAL FILMS OF POTASSIUM ARABATE. By K. Wibberley	87 T-92 T
A NOTE ON THE STABILITY OF SOLUTIONS OF ISOPRENALINE. By G. B. West and T. D. Whittet	93 T-94 T
THE ASSAY OF PROTAMINE SULPHATE FOR ITS CAPACITY TO NEUTRALISE HEPARIN. By V. K. Birkinshaw and K. L. Smith	95 T-96 T
THE OXIDATION OF EMULSIFIED AND SOLUBILISED BENZALDEHYDE. By J. E. Carless and J. Swarbrick	97 T-99 T
SURFACE ACTIVITY OF A SERIES OF SYNTHETIC NON-IONIC DETERGENTS. By P. H. Elworthy and C. B. Macfarlane	100 T-102 T
THE CONTROLLED POTENTIAL REDUCTION OF CRYSTAL VIOLET AND BRILLIANT GREEN AT THE STIRRED MERCURY CATHODE. By C. G. Butler and (Mrs.) F. P. Martin	103 T-106 T
STUDIES ON <i>Datura leichhardtii</i> MUELLEX BENTH. PART II. ALKALOIDAL CONSTITUENTS. By W. C. Evans and N. A. Stevenson	107 T-110 T
THE PARTICLE SIZE DISTRIBUTION OF MARBLE ON WET BALL MILLING. By M. I. Barnett and K. C. James	111 T-115 T
DETERMINATION OF TRICHLOROETHYL PHOSPHATE IN PHARMACEUTICAL PREPARATIONS. By P. F. G. Boon	116 T-118 T
THE EFFECT OF A SULPHATED POLYSACCHARIDE ON THE ACIDITY AND VOLUME OF HISTAMINE-STIMULATED GASTRIC SECRETION IN THE GUINEA-PIG. By W. Anderson, R. Marcus and J. Watt	119 T-121 T
THE POLAROGRAPHIC ASSAY OF STREPTOMYCIN. By R. Goodey, T. E. Couling and (Miss) J. E. Hart	122 T-126 T
STUDIES ON THE POSTIRRADIATION OXYGEN EFFECT IN BACTERIAL SPORES. By A. Tallentire and N. A. Dickinson	127 T-129 T
THE EFFECT OF AGE ON THE VIABILITY OF <i>Penicillium notatum</i> SPORES IN WATER AND SOLUTIONS OF PHENOL. By N. M. Chauhan and V. Walters	130 T-131 T

REVIEW ARTICLE

THE MECHANISM OF CONTACT SENSITISATION

BY H. O. SCHILD, M.D., D.Sc.

Professor of Pharmacology, University College, London

It has long been known that human skin can be sensitised by contact with simple chemical substances and the patch test of Jadassohn was used as a clinical method to detect this kind of sensitisation. Experimental contact sensitisation in guinea-pigs was produced for the first time in 1928 by means of neosalvarsan (Frei, 1928) soon to be followed by similar experiments with *para*-phenylenediamine (Mayer, 1931), phenylhydrazine (Jadassohn, 1930) and primula extract (Bloch and Steiner-Wourlisch, 1930). In a remarkable investigation Bloch and other (1930) demonstrated that the local application of primula extract to guinea-pig skin was followed a few days later by sensitisation of the entire skin. They showed that repeated application of the extract did not produce desensitisation and that the sensitisation could not be transmitted by means of serum or wheal fluid. Earlier (Bloch and Steiner-Wourlisch, 1926) these same workers had shown that a sufficiently large dose of primula extract would sensitise almost 100 per cent of a group of human subjects, thus disposing of the idea that hypersensitivity could be achieved only in a small proportion of "idiosyncratic individuals".

Landsteiner and Jacobs (1935) used substituted benzene derivatives, for example, dinitrochlorobenzene (DNCB) and picryl chloride (PC) to induce contact sensitisation in guinea-pigs, and most of the subsequent work in this field has been carried out with this type of compound. Following the intracutaneous or epicutaneous application of DNCB or PC to guinea-pig skin, a generalised skin hypersensitivity develops on the 5th to the 9th day. If at this stage a second application is made elsewhere on the surface of the skin, a pinkish reaction on a slightly swollen background begins to arise at the second site after a few hours; this reaction becomes maximal after 24–48 hr. The individual susceptibility of guinea-pigs towards contact sensitisation varies. Some guinea-pigs cannot be sensitised at all, and strains of markedly different genetic susceptibility have been isolated (Chase, 1941).

The main site of the contact sensitisation reaction is in the basal layers of the epidermis. Although there is a superficial resemblance between the primary toxic effect of a large dose of PC and DNCB in a non-sensitised guinea-pig and the effect of a much smaller dose of the same compound in a sensitised guinea-pig the histological character of the lesion in the two instances is different (Jadassohn, Bujard and Brun, 1955; de Weck and Brun, 1956; Fisher and Cooke, 1958a). The primary toxic response is characterised by degeneration of epidermal cells with moderate leucocytosis, whilst the allergic response is characterised by a rapid massive extravasation of mononuclear cells migrating in trails directly into the

epidermis. The cell extravasation may be followed by vacuolisation and vesiculation leading to a disruption of the epidermis and to cellular death and exfoliation.

Delayed Hypersensitivity and Anaphylactic Hypersensitivity

Besides producing delayed sensitisation simple chemical substances can also produce a typical anaphylactic sensitisation characterised by Dale-Schultz reactions, "immediate" wheal and flare and circulating antibody. It depends largely on the route and manner of administration of the antigen which type of sensitisation prevails.

Anaphylactic sensitisation tends to occur after the intraperitoneal injection of a simple chemical substance (hapten, proantigen) or of a conjugate produced by the reaction of a hapten with protein *in vitro*. Delayed skin reactivity occurs after the application of haptens to the skin—either to its surface or intradermally—but not usually after intraperitoneal injections (Chase, 1954). However, simple haptens can produce delayed skin reactivity when they are injected intraperitoneally together with killed tubercle bacilli (Landsteiner and Chase, 1941) or their purified wax fraction (Raffel and Forney, 1948). The mode of action of tubercle bacilli in favouring skin sensitisation by intraperitoneal injection of low molecular chemical substances is not clear. Mayer (1956) has suggested that the effect of mycobacteria in promoting delayed reactivity may be due to accumulation in tubercles of collagen (Rich, 1951) with which the hapten combines. In support of this view he showed (Mayer, 1957) that a pro-collagen injected with PC produced a similar adjuvant effect to tubercle bacilli in promoting delayed skin reactivity.

Most workers have found that conjugates made by allowing simple haptens to react with protein *in vitro* do not produce delayed reactivity in guinea-pig skin even when administered by intracutaneous injection (Gell, 1944; Chase, 1954; Eisen, Kern, Newton and Helmreich, 1959). Injection of picryl conjugates may, however, be followed by the appearance of delayed hypersensitivity to the protein carrier in the absence of hypersensitivity against the haptenic group (Benacerraf and Gell, 1959a). Only exceptionally have delayed sensitisations by protein conjugates been reported through the administration of large doses of picryl proteins (Benacerraf and Gell, 1959b) or of conjugates made by combining PC with homologous erythrocyte stromata (Landsteiner and Chase, 1941). It is, however, difficult in these experiments to exclude entirely the possibility that traces of unconjugated PC may have been responsible for the sensitisation.

Although conjugates generally fail to produce delayed sensitisation of the skin they are often highly effective in producing serum antibodies capable of inducing anaphylactic sensitivity, as shown by a positive Dale-Schultz reaction, generalised anaphylaxis in the guinea-pig or Prausnitz-Kustner reactions after the serum is transmitted to normal guinea-pigs. Highly reactive compounds such as acyl chlorides, which presumably combine with proteins as soon as they are injected, are also highly effective

THE MECHANISM OF CONTACT SENSITISATION

in producing anaphylactic sensitisation. When an animal has been sensitised by means of acyl chloride intraperitoneally, the injection of an acyl protein into the skin produces an immediate flare and wheal reaction (Landsteiner and Jacobs, 1936). Gell, Harington and Michel (1948) have tested the antigenicity of certain highly reactive compounds and compared this with their hydrolysis rate and their reactivity with amino groups. They concluded that factors favouring antigenicity of the immediate type are, a relatively slow rate of reaction and a high conjugation to hydrolysis ratio. Thus compounds which are hydrolysed with extreme ease would be expected to be relatively ineffective in forming antigens *in vivo* whereas more stable compounds will not only be more likely to react with amino groups before hydrolysis occurs but they may also in part survive unchanged till they are taken up by cells in which they may find an environment more suitable for conjugation.

The same low molecular weight substances may induce anaphylactic sensitisation and delayed skin contact sensitisation. Nevertheless the two types of sensitisation are independent and separable when they occur together. Thus animals showing both anaphylactic and contact sensitisation can be completely desensitised to the anaphylactic type of sensitisation either spontaneously or experimentally without in any way losing their delayed cutaneous reactivity (Landsteiner and Chase, 1937; Raffel and Forney, 1948). Animals which have been passively sensitised by injection of circulating antibody reactive to picryl protein show typical Arthus reactions when treated with PC intradermally, but no delayed reactions (Benacerraf and Gell, 1959b). Furthermore the presence of precipitating antibodies confers no protection against delayed reactivity (Gell, 1944).

Sensitising Activity and Chemical Reactivity

Landsteiner and Jacobs (1935) investigated a number of chloro- and nitro-derivatives of benzene for their skin sensitising effects and concluded that a close connection existed between skin sensitising capacity and the possession of labile Cl or NO₂ groups. They considered that active compounds carried out substitution reactions and attached themselves to the basic groups of proteins. Brownlie and Cumming (1946) later confirmed that aromatic skin-sensitising nitro-compounds formed condensation products with amino-acids *in vitro*. Some active skin sensitisers are themselves unreactive with proteins but they may be metabolised to reactive derivatives. Examples are picric acid which possesses nitro groups which are not readily detached, and also *para*-phenylenediamine and the polyhydric phenols contained in poison ivy. The latter are probably oxidised in the body to quinones which then react with proteins. Eisen, Orris and Belman (1952), have pointed out that mere adsorption on proteins is insufficient for contact sensitisation and that formation of a covalent link is probably necessary.

Protein binding appears to be necessary both for the induction of sensitisation and for eliciting a reaction. Eisen and others (1952) investigated eight dinitrophenyl derivatives for their capacity to elicit delayed

skin reactions in guinea-pigs sensitised by dinitrofluorobenzene. The four derivatives which produced skin reactions were all capable of combining with proteins whilst the remaining four compounds which produced no skin reactions also failed to combine with proteins. Protein binding was demonstrated in two ways: firstly, by allowing γ -globulin to react with the haptens *in vitro* and measuring protein-binding spectroscopically; secondly, by treating guinea-pig skin with the haptens *in vivo* and identifying the formed dinitrophenyl amino-acids chromatographically after excision of the skin and acid hydrolysis. It was found that each of the active compounds had combined with the ϵ -NH₂ group of lysine to form dinitrophenyllysine. Another group of active skin sensitising compounds were shown to react with the —SH and —S—S— groups of cysteine and cystine in hair and epidermis (Eisen and Belman, 1953). Conjugation of hapten with proteins in the basal layer of the epidermis is considered by Eisen and Tabachnik (1958) to be an essential step in the process of eliciting a contact sensitisation reaction.

If the formation of protein conjugates *in vivo* underlies both "immediate" and "delayed" sensitisation some kind of explanation is required to account for the relative ineffectiveness of pre-formed protein conjugates in causing contact sensitisation; and also, the failure of intraperitoneal injections of simple chemical substances (haptens) to induce contact sensitisation unless they are combined with an adjuvant such as tubercle bacilli.

An interesting explanation of these anomalies has been put forward by Mayer (1956) who suggested that the sensitising properties of haptens were closely related to their tanning properties, that is their ability to form cross links with adjacent protein macromolecules. Haptens might form cross links with different types of protein according to their site of injection: with fibrous proteins of the keratin and collagen groups when in contact with the epidermis, and with globular proteins of the albumin and globulin groups when injected intraperitoneally. In this way different complete antigens may be produced: in the epidermis, rigid, oriented, difficultly soluble or insoluble antigens which could act as templates for equally insoluble sessile antibodies; in the peritoneum, soluble antigens possessing globular carrier proteins on which the humoral, soluble antibodies are moulded. Mayer attributes the effect of mycobacteria in promoting delayed reactivity to the high collagen content of tubercles as already discussed.

An entirely different explanation of the low effectiveness of protein conjugates in contact sensitisation is suggested by some work of Eisen and others (1959). These authors found protein conjugates consistently ineffective in producing delayed sensitisation even when the protein was derived from hair or epidermis. They then incubated haptens and their corresponding protein conjugates with lymph nodes *in vitro* and measured uptake. The simple haptens were concentrated 30 to 300 times inside the lymph node cells whilst the protein conjugates were not concentrated at all. This suggests that contact sensitisation may depend on an initial uptake of hapten by lymph node cells followed by intracellular conjugation of the hapten with protein.

THE MECHANISM OF CONTACT SENSITISATION

Development of Contact Sensitisation

When DNCB is applied to the skin of a guinea-pig some of it combines with skin protein. If the skin is extirpated 24 hr. later about half the material still present is in a combined form and of this 99 per cent is present in the epidermis mostly combined with the NH_2 groups of lysine residues (Eisen and Tabachnik, 1958). Some of the DNCB is absorbed into the circulation and excreted in the urine, but the strategic site for the induction of contact sensitisation is the local lymphatic system.

Seeberg (1951) has shown that the skin can be sensitised to DNCB by injecting the compound directly into an exposed lymph gland under complete avoidance of the skin. Frey and Wenk (1957) carried out a series of interesting experiments with skin stumps connected with the body by blood vessels and nerves. The lymphatic system of the skin stumps was either left intact or removed. DNCB produced initial sensitisation of the rest of the skin only when applied to a stump in which the lymphatic system was left intact. On the other hand the local lymphatic system was not required for the further maintenance of the sensitisation. If the regional lymph nodes were extirpated within 48 hr. of primary contact no sensitisation at all occurred but if the extirpation was carried out later there was an increasing incidence of sensitisation. If the extirpation took place 9 days after the primary contact all the experimental animals became and remained sensitised, suggesting that at this stage antibody production occurred also in lymph glands removed from the site of application.

The subsequent generalising of sensitisation most probably takes place through the blood stream as indicated by the following findings: firstly, skin sensitisation to DNCB can be transmitted by parabiosis (Haxthausen, 1943b); secondly, in cross-transplantation experiments with uniovular human twins of which one was sensitised to DNCB and the other unsensitised, a skin transplant from the unsensitised to the sensitised twin became itself sensitised whilst a transplant from the sensitised to the unsensitised twin lost its sensitisation (Haxthausen, 1943a); thirdly, in Frey and Wenk's (1957) experiments the application of DNCB to a remote part of the skin produced sensitisation of an isolated skin flap even when the stump had its lymphatic system removed.

Cellular Transfer of Contact Sensitisation

Contact sensitisation cannot be transferred by even very large quantities of plasma (Haxthausen, 1951), but it can be transferred by the cellular elements of blood as was first shown by Landsteiner and Chase (1942). These workers sensitised guinea-pigs by the intraperitoneal injection of PC bound to stromata of guinea-pig erythrocytes mixed with a suspension of dead tubercle bacilli (this treatment resulted in a strong hypersensitivity of the skin to PC). Repeated intraperitoneal injections of killed tubercle bacilli produced a peritoneal exudate containing leucocytes and lymphocytes. Exudate cells were collected from several donors and after centrifuging and washing they were injected into normal guinea-pigs.

Two days later the application of PC to the skin of the recipients induced a typical delayed erythematous reaction. A few days later the hypersensitivity subsided. The clear supernatant from the exudate failed to transmit the hypersensitivity.

Successful transfer of contact sensitisation has also been achieved with cells from spleen and lymph nodes (Chase, 1946), thymus (Haxthausen 1947), thoracic lymph duct (Skog, 1956), and blood (Haxthausen, 1951). The number of cells required for a successful transfer is about 5×10^8 . Active peritoneal exudates produced by the intraperitoneal injection of paraffin contain mainly mononuclear cells. Exudates produced by the injection of saline, containing predominantly polymorph-nuclear leucocytes, are inactive (Haxthausen, 1951). Earlier work seemed to indicate that the transfer factor was a cell-fixed antibody which could not be extracted and was present only in freshly prepared cells. Thus cells damaged by freezing and heating (Chase, 1941) prolonged standing (Nilzen, 1952) and haemolysis (Skog, 1956) were found inactive. More recently, however, Jeter, Tremaine and Seebohm (1954) have reported that peritoneal exudate cells disrupted by sonic oscillations are capable of transferring contact sensitisation to DNCB. These observations have been confirmed (Turk, 1961). Jeter, Laurence and Seebohm (1957) reported that the active extracts contained a component resembling an α -1-globulin which was absent in similarly prepared extracts from normal cells.

Mechanism of Delayed Skin Reaction

The role of cells of the mononuclear series in contact sensitisation seems clearly established by transfer experiments and it is also shown by the massive extravasation of lymphocytes after the application of allergen to sensitised skin. The delay in the response can be explained, in part at least, by the time required for accumulation of cells at the site of administration of the antigen. However, this is probably not the whole explanation of the delay if the tuberculin reaction can be taken as a guide. Thus Metaxas and Metaxas (1955) found that when tuberculin sensitised cells were injected intradermally together with tuberculin the characteristic delay of the tuberculin reaction was still present. The delay is thus probably in the reaction itself.

Very little is known of the pharmacological and biochemical events which underlie the delayed skin reaction. It has been suggested that the sensitised cells act simply as carriers of antibody which is subsequently transferred to tissue cells. In that case the antigen would presumably be reacting with tissue cells which in turn would be releasing pharmacologically active substances responsible for the delayed reaction. Another suggestion is that sensitised mononuclear cells metamorphose into sensitised epithelial cells (Andrew and Andrew, 1949). Perhaps the most probable assumption is that the antigen reacts with sensitised mononuclear cells which are attracted to the skin but the mechanism of this reaction is unknown. Indeed any reaction scheme between cell-bound antibody and protein-bound hapten presents formidable theoretical difficulties which so far have not been resolved.

THE MECHANISM OF CONTACT SENSITISATION

Another probable assumption is that as a further step in the reaction sequence pharmacologically active substances are released which cause the delayed vasodilator response. Contrary to earlier views that histamine is implicated only in "immediate" anaphylactic reactions (Mongar and Schild, 1962) evidence has recently been forthcoming which suggests that histamine may also play a part in delayed hypersensitivity. This evidence is rather indirect and derives from two sources. The first: in a typical "delayed" reaction such as the tuberculin reaction the histidine decarboxylase activity of the skin is increased (Schayer and Ganley, 1961). Schayer (1959) has suggested that a protracted release of newly formed histamine may be responsible for the vasodilatation. The second: the histamine content of guinea-pig skin rises during "delayed" skin reactions.

The increase begins about 3 hr. after administration of the antigen and is maximal after 24 to 72 hr. The histamine increase is correlated with the infiltration of mononuclear cells but it cannot be explained simply by the importation of histamine by these cells since the increase of histamine considerably exceeds the amount present in the infiltrating cells. The increased histamine content may thus be due partly to increased histamine formation (Inderbitzin, 1961). Fisher and Cooke (1958b) found that the histamine content of the skin increased in a primary toxic reaction due to DNCB as well as in an allergic reaction due to this same substance but it was much greater in the allergic reaction. These authors are of opinion that histamine functions as an accelerator of repair processes in the skin rather than as a cause of the dermatitis. Other pharmacologically active substances, for example polypeptides, may also be involved in the vascular reaction of delayed hypersensitivity, but so far their presence has not been demonstrated, possibly due to a lack of suitable experimental procedures for detecting them. Such substances could be present in a preformed state in the infiltrating cells or they could be formed as a consequence of the reaction of sensitised cells with antigen.

Lymph node cells from guinea-pigs sensitised with DNCB exhibit changes in their metabolic pattern, for example, they incorporate methionine and orthophosphate at an increased rate. These metabolic changes are not directly correlated with cell proliferation and it has been suggested that they may be related to the formation of an intracellular phosphoprotein antibody (Kern and Eisen, 1959).

REFERENCES

- Andrew, W. and Andrew, N. V. (1949). *Anat. Rec.*, **104**, 217-241.
Benacerraf, B. and Gell, P. G. H. (1959a). *Immunology*, **2**, 52-63.
Benacerraf, B. and Gell, P. G. H. (1959b). *Ibid.*, **2**, 219-229.
Bloch, B. and Steiner-Wourlich, A. (1926). *Arch. f. Dermatol. Syphil.*, **152**, 283-303.
Bloch, B. and Steiner-Wourlich, A. (1930). *Ibid.*, **162**, 349-378.
Brownlie, I. A. and Cumming, W. M. (1946). *Biochem. J.*, **40**, 640-644.
Chase, M. W. (1941). *J. exp. Med.*, **73**, 711-726.
Chase, M. W. (1946). *J. Bact.*, **51**, 643.
Chase, M. W. (1954). *Int. Arch. Allergy*, **5**, 163-191.
de Weck, A. and Brun, R. (1956). *Dermatologica*, **113**, 335-368.
Eisen, H. N. and Belman, S. (1953). *J. exp. Med.*, **98**, 533-549.
Eisen, H. N., Kern, M., Newton, W. T. and Helmreich, E. (1959). *Ibid.*, **110**, 187-206.

H. O. SCHILD

- Eisen, H. N., Orris, L. and Belman, S. (1952). *Ibid.*, **95**, 473-487.
- Eisen, H. N. and Tabachnick, M. (1958). *Ibid.*, **108**, 773-796.
- Fisher, J. P. and Cooke, R. A. (1958a). *J. Allergy*, **29**, 411-428.
- Fisher, J. P. and Cooke, R. A. (1958b). *Ibid.*, **29**, 396-410.
- Frei, W. (1928). *Klin. Wschr.*, **7**, 1026-1031.
- Frey, J. R. and Wenk, P. (1957). *Int. Arch. Allergy*, **11**, 81-100.
- Gell, P. G. H. (1944). *Brit. J. exp. Path.*, **25**, 174-192.
- Gell, P. G. H., Harington, C. R. and Michel, R. (1948). *Ibid.*, **29**, 578-589.
- Haxthausen, H. (1943a). *Acta dermato-ven.*, **23**, 438-454.
- Haxthausen, H. (1943b). *Ibid.*, **24**, 286-297.
- Haxthausen, H. (1947). *Ibid.*, **27**, 275-285.
- Haxthausen, H. (1951). *Ibid.*, **31**, 659-665.
- Inderbitzin, T. (1956). *Int. Arch. Allergy*, **8**, 150-159.
- Inderbitzin, T. (1961). *Ibid.*, **18**, 85-99.
- Jadassohn, W. (1930). *Klin. Wschr.*, **9**, 551.
- Jadassohn, W., Bujard, E. and Brun, R. (1955). *J. invest. Dermatol.*, **24**, 247-253.
- Jeter, W. S., Laurence, K. A. and Seebohm, P. M. (1957). *J. Bact.*, **74**, 680-683.
- Jeter, W. S., Tremaine, M. M. and Seebohm, P. M. (1954). *Proc. Soc. exp. Biol. N.Y.*, **86**, 251-253.
- Kern, M. and Eisen, H. N. (1959). *J. exp. Med.*, **110**, 207-219.
- Landsteiner, K. and Chase, W. M. (1937). *Ibid.*, **66**, 337-351.
- Landsteiner, K. and Chase, W. M. (1941). *Ibid.*, **73**, 431-438.
- Landsteiner, K. and Chase, W. M. (1942). *Proc. Soc. exp. Biol., N.Y.*, **49**, 688-690.
- Landsteiner, K. and Jacobs, J. (1935). *J. exp. Med.*, **61**, 643-656.
- Landsteiner, K. and Jacobs, J. (1936). *Ibid.*, **64**, 625-639.
- Mayer, R. L. (1931). *Arch. f. Dermatol. Syphil.*, **163**, 223-244.
- Mayer, R. L. (1956). *Progr. Allergy*, **4**, 79-172.
- Mayer, R. L. (1957). *Int. Arch. Allergy*, **10**, 13-22.
- Metaxas, M. N. and Metaxas-Buehler, M. (1955). *J. Immunol.*, **75**, 333-347.
- Mongar, J. L. and Schild, H. O. (1962). *Physiol. Rev.* (in the press).
- Nilzen, A. (1952). *Acta dermato-ven., Suppl.* **29**, 231-239.
- Raffel, S. and Forney, J. E. (1948). *J. exp. Med.*, **88**, 485-502.
- Rich, A. R. (1951). *The Pathogenesis of Tuberculosis*. 2nd ed. Thomas; Blackwell.
- Schayer, R. W. (1959). *Mechanisms of Hypersensitivity*. Boston: Little, Brown and Co.
- Schayer, R. W. and Ganley, O. H. (1959). *Amer. J. Physiol.*, **197**, 721-724.
- Seeberg, G. (1951). *Acta dermato-ven.*, **31**, 592-598.
- Skog, E. (1956). *Ibid.*, **36**, 1-10.
- Turk, J. L. (1961). Communication to British Society for Immunology. June meeting.

RESEARCH PAPERS

THE INHIBITION OF THE L-HISTIDINE DECARBOXYLASES OF GUINEA-PIG KIDNEY AND RAT HEPATOMA

BY B. ROBINSON AND D. M. SHEPHERD

*From the Department of Pharmacology and Therapeutics,
University of St. Andrews, Queen's College, Dundee*

Received October 26, 1961

The preparation of some potential inhibitors of L-histidine decarboxylase is described. These, and certain commercially available compounds, have been compared for their ability to inhibit *in vitro* the histidine decarboxylases of guinea-pig kidney and of the transplantable rat hepatoma (F-Hep). Structure-activity relationships of these inhibitors are discussed.

IN a recent paper (Mackay and Shepherd, 1960) several compounds were shown to be inhibitors of guinea-pig kidney L-histidine decarboxylase (GPHD), and it was suggested that such compounds might provide useful pharmacological tools. Later it was found that the transplantable rat hepatoma, F-Hep, contained an L-histidine decarboxylase (F-HepHD) which differed in its properties from GPHD (Mackay, Riley and Shepherd, 1961). Comparative studies on the inhibition of these two enzymes *in vitro* have now been made using several new inhibitors.

Enzyme Inhibition Studies

Enzyme activity was determined by assaying the histamine formed, using the isolated ileum of the guinea-pig. The amounts of histamine present initially in the extracts were very small compared with those formed during the incubations. The concentration, C50, of inhibitor required to reduce the initial rate of the uninhibited reaction by half was used as an index of inhibitory potency. The procedure with guinea-pig kidney extracts was as previously described (Mackay and Shepherd, 1960). With F-Hep extracts, incubations were at pH 6.8 and 36°, 1 μ g. of pyridoxal-5'-phosphate was added per ml. of extract, and the L-histidine concentration was 6.4×10^{-4} M.

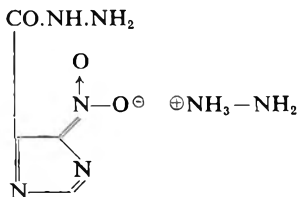
Chemical Studies

While the chemical preparations are described in the experimental section, the properties of 4(5)-nitroimidazole-5(4)-carboxyhydrazide require further comment.

Owing to the influence of the two electron-attracting groups in ethyl 4(5)-nitroimidazole-5(4)-carboxylate, the *N*-hydrogen atom of the imidazole ring is more liable to be lost as a proton than in ethyl imidazole-4(5)-carboxylate, which contains only one electron-attracting group on the ring. Thus although ethyl imidazole-4(5)-carboxylate reacted with

B. ROBINSON AND D. M. SHEPHERD

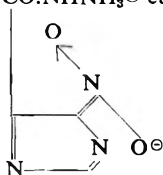
hydrazine hydrate to give the corresponding hydrazide, ethyl 4(5)-nitroimidazole-5(4)-carboxylate and hydrazine hydrate gave the yellow hydrazone salt, I, of the hydrazide.



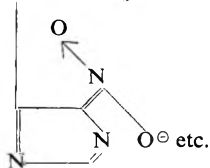
(I)

While this salt crystallised unchanged from concentrated aqueous solution, recrystallisation from dilute aqueous solution gave the free hydrazide as orange needles. The orange colour of this hydrazide in the solid phase and in concentrated aqueous solution is attributed to formation of an intermolecular salt, II, owing to the basic properties of the hydrazide grouping and the strong acidic properties of the imidazole ring containing two electron-attracting substituents. In dilute aqueous solution, however, the hydrazide exists in the unimolecular state, III, as is shown by examination of the ultra-violet spectrum of such solutions.

CO.NHNH₃[⊕] etc.

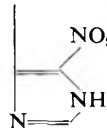


CO.NHNH₃[⊕]



(II)

CO.NHNH₂



(III)

DISCUSSION

The C50 values given below and in Table I have been multiplied by the factor 10⁴ to facilitate comparisons. It should be noted that C50 values for GPHD are not directly comparable with those for F-HepHD, as the measurements on the two enzymes are necessarily made at different pH values and substrate concentrations.

It is known (Mackay and Shepherd, 1960) that 4(5)-methyl-5(4)-nitroimidazole (C50 = 34) is a better inhibitor of GPHD than is 4(5)-methylimidazole (C50 = 440). This may be due to the electron-attracting properties of the nitro-group increasing the electrostatic

INHIBITION OF L-HISTIDINE DECARBOXYLASES

attraction between the imidazole ring and the apo-enzyme. Compounds 1 and 2 (Table I), which contain electron-attracting groups, were therefore tested as inhibitors. As expected, compound 1 had approximately the same C50 value ($C_{50} = 30$) as 4(5)-methyl-5(4)-nitroimidazole on GP_HD. Compound 2 ($C_{50} = 65$), though containing two electron-attracting groups, was a weaker inhibitor of the enzyme, possibly because in this instance the further increase in electrostatic attraction between the apo-enzyme and the imidazole ring of the inhibitor may be less than the accompanying decrease in hydrogen bonding between these entities. In the inhibition of F-Hep_HD also, compound 1 was more effective than compound 2.

TABLE I
CONCENTRATIONS OF VARIOUS COMPOUNDS REQUIRED TO PRODUCE
50 PER CENT INHIBITION (C_{50}) OF L-HISTIDINE DECARBOXYLASES

No.	Compound	$C_{50} \times 10^4 M$	
		GP _H D	F-Hep _H D
1	Imidazole-4(5)-carboxylic acid	30	150
2	4(5)-Nitroimidazole-5(4)-carboxylic acid	65	300
3	Imidazole-4(5)-carboxyhydrazide	20	15
4	4(5)-Nitroimidazole-4(5)-carboxyhydrazide	6.5	0.75
5	L-Histidine hydrazide $1\frac{1}{2}H_2SO_4$	0.85	2
6	Hydrazine salt of 4	0.2	0.15
7	Hydrazine hydrate	0.35	0.1
8	DL-5-HTP	0.65	75
9	DL- α -Methyl-5-HTP	0.075	1
10	DL- α -Methylhistidine dihydrochloride	150	15
11	L-DOPA	0.2*	7.5
12	DL-DOPA	0.2	4.5
13	DL- α -Methyl-DOPA	0.01*	70
14	Catechol	1.8*	65
15	Salicylic acid	35	30

* Quoted from the results of Mackay and Shepherd, 1960

The inhibitory effect of a series of hydrazides (compounds 3–6) bearing some structural relation to L-histidine was then studied. The inhibition of GP_HD by compound 3 differed only slightly from its inhibition by compound 1, although F-Hep_HD was more sensitive to compound 3 ($C_{50} = 15$) than to compound 1 ($C_{50} = 150$). Compound 4 ($C_{50} = 6.5$) is more powerful as an inhibitor of GP_HD than is compound 1 ($C_{50} = 30$), the nitro-group on the ring presumably increasing the interaction between the inhibitor and apo-enzyme; as an inhibitor of F-Hep_HD compound 4 ($C_{50} = 0.75$) is much more powerful than compound 1 ($C_{50} = 150$). Compound 5 is a good inhibitor of both enzymes ($C_{50} = 0.85$ and 2 for GP_HD and F-Hep_HD respectively). The different effects of these inhibitors on the two enzymes may reflect a greater affinity and specificity of the F-Hep_HD for the imidazole ring. Support for this view is found in the fact that F-Hep_HD has no detectable DOPA- or 5-HTP-decarboxylase activity (Mackay, Riley and Shepherd, 1961); GP_HD on the other hand is claimed to be non-specific, and to decarboxylate DOPA and 5-HTP more rapidly than L-histidine (Udenfriend, Lovenberg and Weissbach, 1960). The high inhibitory potency of compound 6, as compared with compound 4, against both enzymes, is consistent

with its free hydrazine content since hydrazine hydrate, compound 7, has C50 values similar to those of compound 6, and acts by direct combination with the co-enzyme, pyridoxal 5'-phosphate. Compounds 3-5 may not only react directly with the co-enzyme, but they are also capable of sorption to the apo-enzyme by means of their imidazole nucleus. The relative C50 values for compounds 4, 6 and 7 in both series are in agreement with the structures I and III assigned to compounds 4 and 6 on the basis of chemical evidence.

It has recently been shown (Weissbach, Lovenberg and Udenfriend, 1960) that in their rate of decarboxylation by a guinea-pig kidney preparation and in their ability to inhibit the decarboxylation of natural aromatic amino-acids, the α -methylamino-acids fall in the order α -methylDOPA > α -methyl-5-HTP > α -methyl-TP. The relative rates of decarboxylation of the natural amino-acids DOPA, 5-HTP, TP and histidine by this preparation are in the sequence DOPA > 5-HTP > TP > histidine. In accordance with these observations we have found that α -methyl-histidine (compound 10), is a much weaker inhibitor of GPHD than are α -methylDOPA (compound 13) or α -methyl-5-HTP (compound 9).

While DL- α -methylDOPA is a more potent inhibitor of GPHD than is L-DOPA (C50 = 0.01 and 0.2 respectively), their relative inhibitory powers are reversed in the inhibition of F-HepHD (C50 = 50-150 and 6-12 respectively, Mackay and Shepherd, (1962)), DL- α -methylDOPA becoming a rather poor inhibitor; the C50 values of these two compounds with F-HepHD have been redetermined and confirmed. The apparent discrepancy is not due to the use of racemic α -methylDOPA in comparison with the L-isomer of DOPA, since DL-DOPA has similar C50 values to L-DOPA for both enzymes (Table I). No preferential destruction of the DL- α -methylDOPA by other enzymes in the F-HepHD extract was detected by paper chromatography of aliquots taken at various times as the incubation proceeded, and no preferential binding of the DL- α -methylDOPA by foreign protein present in the enzyme extract was found by equilibrium dialysis.

Catechol was a relatively poor inhibitor of F-HepHD (C50 = 65) whereas for GPHD it was very effective (C50 = 1.8, Mackay and Shepherd, 1960). Salicylic acid, however, had comparable C50 values for both enzymes (Table I). This further illustrates the difference between the apo-enzyme moieties of the two enzymes.

The reproducibility of the method for the measurement of C50 values was examined by making several determinations of the C50 values of imidazole-4(5)-carboxyhydrazide for GPHD: five incubations gave 17, 16, 24, 26 and 23, with a standard deviation of 4.5. The C50 values of imidazole-4(5)-carboxylic acid for F-HepHD were, for four incubations 151, 169, 142 and 188 with a standard deviation of 20.4.

EXPERIMENTAL

Ethyl 4(5)-nitroimidazole-5(4)-carboxylate. A solution of 5.1 g. 4(5)-nitroimidazole-5(4)-carboxylic acid (Windaus and Langenbeck, 1923) in 100 ml. dry ethanol, was protected from atmospheric moisture and

INHIBITION OF L-HISTIDINE DECARBOXYLASES

saturated with dry hydrogen chloride. After refluxing 1 hr., the solution was re-saturated with dry hydrogen chloride and refluxed a further 2 hr. Evaporation of the ethanol gave a pale-yellow solid which recrystallised from ethanol as white plates (4.88 g., 80 per cent), m.p. 200–203°. A further crop (0.5 g., 8 per cent), m.p. 195–201° was obtained by concentration of the mother liquors. After two recrystallisations from ethanol the ester formed white plates, m.p. 205–207° (Found: C, 38.85; H, 3.7. $C_6H_7N_3O_4$ requires C, 38.9; H, 3.8 per cent) λ_{\max} 279–280 $m\mu$ (ϵ 4,540, in ethanol), unchanged on addition of hydrochloric acid, but changed on addition of aqueous sodium hydroxide to λ_{\max} 345 $m\mu$ (ϵ 7850). The infra-red spectrum (in Nujol) showed a strong band at 1720 cm^{-1} ($C = O$).

Reaction of ethyl 4(5)-nitroimidazole-5(4)-carboxylate with hydrazine hydrate. When a solution of the ester (3.0 g.) in 99–100 per cent hydrazine hydrate (10 ml.) was warmed on a steam bath for 2 hr., progressive darkening of the reaction mixture occurred; longer heating led to extensive decomposition. Ethanol (200 ml.) was added to the cooled dark-red solution and, after $\frac{1}{2}$ hr. at room temperature, the deposit was filtered off and washed with ethanol to give I as yellow crystals (2.9 g., 88 per cent), m.p. 186–188° (with decomposition). Evaporation of the ethanol from the filtrate left a red glass (0.36 g.) which did not crystallise and was not examined further.

Recrystallisation of the yellow solid from water (25 ml.) gave orange needles II (0.60 g., 22 per cent), m.p. 247–249° (with decomposition), unchanged on further recrystallisation from water. (Found: C, 27.75; H, 3.0. $C_4H_5N_5O_3$ requires C, 28.1; H, 3.1 per cent.) λ_{\max} 304–305 $m\mu$ (ϵ 4890, in water), unchanged on addition of hydrochloric acid, but changed to λ_{\max} 354–355 $m\mu$ (ϵ 8630) on addition of aqueous sodium hydroxide. The infra-red spectrum (in Nujol) showed strong bands at 1,660 cm^{-1} and 3,400 cm^{-1} . ($C = O$ and $N-H$ respectively.) Concentrated aqueous solutions of the product were orange, but more dilute solutions were colourless.

Evaporation of the mother liquors from the above recrystallisation to about 6 ml., followed by rapid cooling gave yellow needles, I (1.8 g., 55 per cent), m.p. 188–190° (with decomposition). (Found: C, 23.6; H, 4.55; N, 47.4. $C_4H_6N_7O_3$ requires C, 23.6; H, 4.4; N, 47.4 per cent.) λ_{\max} 345 $m\mu$ (ϵ 6310, in water), changed to λ_{\max} 302–303 $m\mu$ (ϵ 5,690) on addition of hydrochloric acid, and to λ_{\max} 353–355 $m\mu$ (ϵ 8,900) on addition of aqueous sodium hydroxide. The infra-red spectrum (in Nujol) showed medium bands at 1,690 cm^{-1} and 3,300 cm^{-1} ($C = O$ and $N-H$ respectively) and was identical with that of the initial total reaction product. Mixed m.p. of the two compounds showed no depression.

A solution of II (121.5 mg.) in 99–100 per cent hydrazine hydrate (2 ml.) was heated on a steam bath for 1 hr., cooled, and treated with ethanol (100 ml.). The yellow crystalline precipitate, when filtered off and washed with ethanol, was identical with I (m.p., mixed m.p., ultra-violet and infra-red spectra).

I recrystallised from a dilute aqueous solution to give orange needles identical with II (m.p., mixed m.p., ultra-violet and infra-red spectra). Concentration of the mother liquors followed by rapid cooling gave yellow needles, which by m.p., mixed m.p., ultra-violet and infra-red spectral comparisons were shown to be unchanged I.

L-Histidine hydrazide. Although this preparation has been briefly described elsewhere (Horii, Murakami, Tamura, Uchida, Yamamura, Miki and Kato, 1956) full details are recorded below as this paper is in Japanese.

To a solution of L-histidine methyl ester dihydrochloride (Fischer and Cone, 1908) (4.5 g.) in warm, dry methanol (40 ml.) was added a solution of sodium (2.0 g.) in dry methanol (35 ml.). The mixture, protected from atmospheric moisture, was stood at room temperature for 1 hr. with occasional shaking; dry ether (200 ml.) was then added. After a further $\frac{1}{2}$ hr. at room temperature with occasional shaking, the sodium chloride was filtered off and washed with dry ether. Removal of the solvent from the combined filtrate gave a pale-yellow oil (3.2 g.) which did not crystallise, but which was free of chloride ions.

A solution of the above oil in 99–100 per cent hydrazine hydrate (8 ml.), after refluxing for $2\frac{1}{2}$ hr., gave on evaporation a gum (3.5 g.) which did not crystallise. To a solution of this gum in 2N-sulphuric acid (20 ml.) ethanol (25–30 ml.) was gradually added, the oil initially precipitated soon crystallising. The white solid (3.95 g.) was broken up, filtered, and washed with ethanol; after drying *in vacuo* over P_2O_5 it had m.p. 236–239° (with slight decomposition). Repetition of this purification procedure gave histidine hydrazide $1\frac{1}{2}H_2SO_4$ as white prisms (3.8 g., 61 per cent), m.p. 238–240° (with slight decomposition) [Horii, Murakami, Tamura, Uchida, Yamamura, Miki and Kato, 1956, give m.p. 240° (with decomposition)]. (Calc. for $C_6H_{11}N_4O \cdot 1\frac{1}{2}H_2SO_4$: C, 22.4; H, 3.9. Found: C, 22.1; H, 3.75 per cent.)

The following compounds were prepared as described in the literature: imidazole-4(5)-carboxylic acid (Pyman, 1916); imidazole-4(5)-carboxyhydrazide (Balaban, 1930); 4(5)-nitroimidazole-5(4)-carboxylic acid (Windaus and Langenbeck, 1923); DL- α -methylhistidine dihydrochloride (Robinson and Shepherd, 1961a).

Acknowledgements. DL- α -Methyl-5-HTP was kindly supplied by the Upjohn Company, Kalamazoo, Michigan, U.S.A. All other compounds used were obtained commercially.

We are grateful to Dr. P. B. Marshall for helpful advice and discussion. One of us (B.R.) acknowledges the award of a Post-doctoral Research Fellowship by the Wellcome Foundation.

REFERENCES

- Balaban, I. E. (1930). *J. chem. Soc.*, 268–273.
 Fischer, E. and Cone, L. H. (1908). *Liebigs. Ann.*, **363**, 107–117.
 Horii, Z., Murakami, Y., Tamura, Y., Uchida, H., Yamamura, Y., Miki, K. and Kato, M. (1956). *J. pharm. Soc. Japan*, **76**, 1319–1321.
 Mackay, D., Riley, J. F. and Shepherd, D. M. (1961). *J. Pharm. Pharmacol.*, **13**, 257–261.

INHIBITION OF L-HISTIDINE DECARBOXYLASES

- Mackay, D. and Shepherd, D. M. (1960). *Brit. J. Pharmacol.*, **15**, 552-556.
Mackay, D. and Shepherd, D. M. (1962). *Biochim. Biophys. Acta*, in the press.
Pyma, F. L. (1916). *J. chem. Soc.*, **109**, 186-202.
Robinson, B. and Shepherd, D. M. (1961a). *J. chem. Soc.*, 5037-5038.
Robinson, B. and Shepherd, D. M. (1961b). *Biochim. Biophys. Acta*, **53**, 431-433.
Udenfriend, S., Lovenberg, W. M. and Weissbach, H. (1960). *Fed. Proc.*, **19**, 7.
Weissbach, H., Lovenberg, W. and Udenfriend, S. (1960). *Biochem. Biophys. Res. Comms.*, **3**, 225-227.
Windaus, A. and Langenbeck, W. (1923). *Chem. Ber.*, **56**, 683-686.

SOME *N*-SUBSTITUTED DERIVATIVES OF-1,2,3,6-TETRAHYDRO-4-PHENYLPYRIDINES

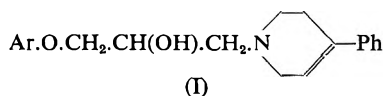
BY V. PETROW, O. STEPHENSON AND A. J. THOMAS

From The British Drug Houses Ltd., Graham Street, London, N.1

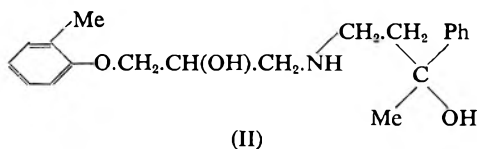
Received October 19, 1961

The relationship between structure and hypotensive activity in the title compounds has been investigated.

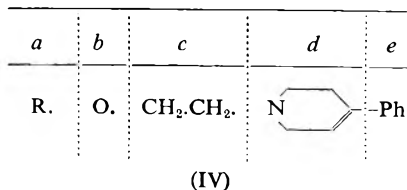
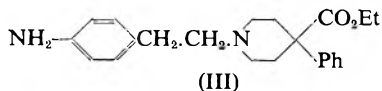
FOLLOWING the discovery by Beasley, Petrow and Stephenson (1958), that certain 3-aryloxy-1-(1,2,3,6-tetrahydropyrid-1-yl)propan-2-ols possessed appreciable analgesic activity, the preparation of some related derivatives (I) of 1,2,3,6-tetrahydro-4-phenylpyridine (Schmidle and Mansfield, 1956) for pharmacological study was undertaken.



Four such compounds (I; where Ar = *o*-tolyl, *o*-allylphenyl, *p*-acetamidophenyl and *p*-aminophenyl) and additionally, one related "open chain" analogue (II) derived from 4-amino-2-phenylbutan-2-ol (Mansfield and Schmidle, 1956) were prepared, but none of the compounds possessed analgesic activity.



Next, following the report of Weijlard and others (1956) and Orahovats, Lehman and Chapin (1957) on the analgesic activity of ethyl 1-(4-aminophenethyl)-4-phenylisonipecotatate (III), some formally related 1-aryloxyalkyl-1,2,3,6-tetrahydro-4-phenylpyridines (IV) were prepared. Though these, too, were found to be devoid of analgesic potency, some of the first compounds prepared for routine screening were found to have anti-adrenaline and hypotensive properties when given intravenously to cats.



1,2,3,6-TETRAHYDRO-4-PHENYLPYRIDINES

In view of this somewhat unexpected result it seemed worthwhile to attempt to delineate the structural requirements for hypotensive potency. To this end a series of compounds was prepared in which the structural features marked (a) . . . (e) in formula (IV) were varied in turn systematically. Their biological study led to the following conclusions on the relationship between structure and activity:

(a) The aryl group R is not absolutely essential for activity, as compounds in which R = H or a lower alkyl group, are still potent. When R is a substituted aryl group however, the position of the substituent in the aromatic nucleus has a definite effect upon potency. Thus, for example, in the tolyl derivatives listed in the Table, *o*- > *m*- > *p*- in hypotensive properties.

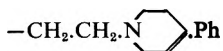
(b) The ether linkage in (IV) is not necessary for activity as phenethyl derivatives of 1,2,3,6-tetrahydro-4-phenylpyridine were invariably as active as the corresponding aryloxyalkyl compounds.

(c) Limited variation in the length of the methylene chain had but little effect upon potency. Thus, the ethylene compound was only slightly more active than the corresponding tri- or tetra-methylene derivatives.

(d) The double bond in the tetrahydropyridine nucleus was not essential for hypotensive activity. The corresponding piperidine derivatives were found to be potent hypotensive agents.

(e) The 4-phenyl group represents an essential structural feature. Its removal leads to almost complete loss of activity. Replacement of the 1,2,3,6-tetrahydro-4-phenylpyridine residue (*d*, *e*, IV) by open-chain structures derived from allylethylamine or cinnamylethylamine likewise leads to loss of potency as does substitution of 1,2,3,4-tetrahydroisoquinoline for 1,2,3,6-tetrahydro-4-phenylpyridine.

Hypotensive potency is thus retained by structures differing significantly from (IV), providing such structures involve the unit:



EXPERIMENTAL

1,2,3,6-Tetrahydro-1-(2-hydroxy-3-o-tolyloxypropyl)-4-phenylpyridine. A mixture of 1,2-epoxy-3-*o*-tolylloxypropane (5.5 g.) and 1,2,3,6-tetrahydro-4-phenylpyridine (5.8 g.) in benzene (5 ml.) was heated under reflux for 2 hr. and was then diluted with light petroleum (b.p. 40–60°). The *product* (8 g.) which separated on cooling had m.p. 84–86° after crystallisation from benzene-light petroleum (b.p. 60–80°). Found: C, 77.6; H, 7.8; N, 4.6. C₂₁H₂₅NO₂ requires C, 78.0; H, 7.8; N, 4.3 per cent. The *hydrochloride* had m.p. 149–151° after crystallisation from ethyl acetate. Found: Cl, 10.2; N, 4.3. C₂₁H₂₆ClNO₂ requires Cl, 9.9; N, 3.9 per cent.

1-(3-o-Allylphenoxy-2-hydroxypropyl)-1,2,3,6-tetrahydro-4-phenylpyridine prepared by reaction of 3-*o*-allylphenoxy-1,2-epoxypropane with 1,2,3,6-tetrahydro-4-phenylpyridine in benzene solution was obtained

V. PETROW, O. STEPHENSON AND A. J. THOMAS

in 67 per cent yield, as a straw coloured oil, b.p. 231° at 0.3 mm. Found: N, 3.7. C₂₃H₂₇NO₂ requires N, 4.0 per cent. The hydrochloride had m.p. 142° after crystallisation from ethyl acetate-methanol. Found: C, 71.7; H, 7.2; Cl, 9.2; N, 3.8. C₂₃H₂₈ClNO₂ requires C, 71.4; H, 7.3; Cl, 9.2; N, 3.6 per cent.

TABLE I

$$\text{R.O.}(\text{CH}_2)_2\text{NH}^+\text{C}_6\text{H}_4\text{Ph} \text{Cl}^-$$

R	m.p. °C	Formula	Found				Required			
			C	H	Cl	N	C	H	Cl	N
H	177-179	C ₁₃ H ₁₆ ClNO	65.7	7.6	15.0	5.6	65.1	7.6	14.8	5.8
Me	186-188	C ₁₄ H ₁₈ ClNO	66.1	8.0	—	5.5	66.2	7.9	—	5.5
Et	154-156	C ₁₅ H ₂₀ ClNO	67.4	8.4	12.9	5.2	67.3	8.3	13.2	5.2
Ph	195-198	C ₁₉ H ₂₄ ClNO	72.2	7.0	10.9	4.7	72.3	7.0	11.2	4.4
<i>o</i> -Tolyl	199-200	C ₂₀ H ₂₆ ClNO	73.2	6.9	10.8	4.1	72.8	7.2	10.7	4.2
<i>m</i> -Tolyl	172-174	C ₂₀ H ₂₆ ClNO	72.3	7.2	—	4.3	72.8	7.2	—	4.2
<i>p</i> -Tolyl	174	C ₂₀ H ₂₆ ClNO	—	—	11.2	3.9	—	—	10.7	4.2
<i>o</i> -MeO.C ₆ H ₄	174-176	C ₂₀ H ₂₆ ClNO ₂	69.0	6.8	10.6	4.3	69.5	7.0	10.3	4.1
<i>m</i> -MeO.C ₆ H ₄	168-170	C ₂₀ H ₂₆ ClNO ₂	69.7	7.0	10.1	3.9	69.5	7.0	10.3	4.1
<i>p</i> -MeO.C ₆ H ₄	174-177	C ₂₀ H ₂₆ ClNO ₂	69.4	6.8	10.3	4.0	69.5	7.0	10.3	4.1
<i>o</i> -Cl.C ₆ H ₄	173-175	C ₁₉ H ₂₁ Cl ₂ NO	65.3	5.9	19.8	3.7	65.1	6.0	20.2	4.0
<i>p</i> -Cl.C ₆ H ₄	193-195	C ₁₉ H ₂₁ Cl ₂ NO	65.3	5.9	20.6	3.8	65.1	6.0	20.2	4.0
<i>p</i> -Br.C ₆ H ₄	180-182	C ₁₉ H ₂₁ BrClNO	58.1	5.3	29.8*	3.4	57.8	5.4	29.2*	3.5
<i>p</i> -AcNH.C ₆ H ₄	232-235	C ₂₁ H ₂₅ ClN ₂ O ₃	—	—	—	7.5	—	—	—	7.5
<i>p</i> -C ₆ H ₄ .C ₆ H ₄	194-198	C ₁₈ H ₂₀ ClNO	76.6	6.7	9.4	3.5	76.6	6.7	9.0	3.6

* Total Halogen

1-(3-Acetamidophenoxy-2-hydroxypropyl)-1,2,3,6-tetrahydro-4-phenylpyridine had m.p. 164-166° after crystallisation from ethanol. Found: C, 71.9; H, 6.7; N, 7.8. C₂₂H₂₆N₂O₃ requires C, 72.1; H, 7.2; N, 7.7 per cent. The hydrochloride had m.p. 239-240° after crystallisation from methanol-ethyl acetate. Found: C, 65.7; H, 6.6; Cl, 9.1; N, 7.0. C₂₂H₂₇ClN₂O₃ requires C, 65.6; H, 6.8; Cl, 8.8; N, 7.0 per cent.

1-(3-*p*-Aminophenoxy-2-hydroxypropyl)-1,2,3,6-tetrahydro-4-phenylpyridine dihydrochloride. The foregoing *p*-acetamido-compound (11.0 g.) was suspended in 6N hydrochloric acid (50 ml.) and the mixture heated under reflux for 2 hr. The resultant solution was evaporated to dryness at reduced pressure and the residual solid crystallised from methanol-ether to yield the product (10.2 g.) m.p. 268-270° (decomp). Found: C, 60.0; H, 6.9; Cl, 18.1; N, 6.9. C₂₀H₂₆Cl₂N₂O₂ requires C, 60.5; H, 6.6; Cl, 17.9; N, 7.0 per cent.

(3-Hydroxy-3-phenylbutyl)(2-hydroxy-3-*o*-tolylxypropyl)amine. A mixture of 1,2-epoxy-3-*o*-tolylxypropane (4.9 g.) and 4-amino-2-phenylbutan-2-ol (5.4 g.) was heated under reflux for 6 hr. when excess of solvent was boiled off. The product (5.5 g.) was isolated as an oil, b.p. 220-222° at 0.3 mm. Found: C, 72.9; H, 9.8; N, 4.0. C₂₀H₂₇NO₃ requires C, 72.9; H, 9.6; N, 4.3 per cent.

1,2,3,6-Tetrahydro-4-phenyl-1-(2-*o*-tolylxyethyl)pyridine. A mixture of 2-*o*-tolylxyethyl bromide (21.5 g.) and 1,2,3,6-tetrahydro-4-phenylpyridine (17.5 g.) in methanol (50 ml.) was treated with a solution of potassium hydroxide (5.6 g.) in methanol (30 ml.) and the mixture heated under reflux for 2 hr., when excess of methanol was boiled off.

1,2,3,6-TETRAHYDRO-4-PHENYLPYRIDINES

The residue was diluted with water and the base isolated with ethyl acetate. It (17.5 g.) had b.p. 197–198° at 0.3 mm. Found: N, 5.0. $C_{20}H_{23}NO$ requires N, 4.8 per cent. The *hydrochloride* had m.p. 199–200° after crystallisation from ethanol-ethyl acetate.

1-(2-*Ethoxyethyl*)-4-*phenylpiperidine*. A solution of 2-bromoethyl ethyl ether (7.6 g.) and 4-phenylpiperidine (8.0 g.) in ethanol (60 ml.) containing anhydrous sodium carbonate (2.7 g.) was heated under reflux for 3 hr. The mixture was cooled, diluted with water and the *base* isolated with chloroform. It (9.3 g.) had b.p. 98–102° at 0.05 mm. ($n_D^{21} = 1.5154$). Found: C, 77.4; H, 9.61; N, 6.2. $C_{15}H_{23}NO$ requires C, 77.2; H, 9.9; N, 6.0 per cent. The *hydrochloride* was very hygroscopic and was not purified.

1-(2-*m-Methoxyphenoxyethyl*)-4-*phenylpiperidine*. A mixture of 2-*m*-methoxyphenoxyethyl bromide (4.8 g.), 4-phenylpiperidine (4.1 g.) and anhydrous sodium carbonate (1.4 g.) in ethanol (80 ml.) was heated under reflux for 9 hr. The *base* was isolated with chloroform as described in the preceding example and converted directly to the *hydrochloride* in ethanol-ether. It (5.2 g.) had m.p. 179–181° after crystallisation from the same solvent mixture. Found: C, 68.8; H, 7.6; N, 4.3. $C_{20}H_{26}ClNO_2$ requires C, 69.1; H, 7.5; N, 4.0 per cent.

1,2,3,6-*Tetrahydro-4-phenyl-1-(2-o-tolyloethyl)pyridine*. A mixture of 2-*o*-tolylethyl bromide (10.0 g.), 1,2,3,6-tetrahydro-4-phenylpyridine (8.0 g.) and sodium carbonate (2.7 g.) in ethanol (80 ml.) was heated under reflux for 8 hr. The *product* (8.2 g.) had b.p. 160–166° at 0.2 mm. ($n_D^{22} = 1.5900$). Found: C, 86.8; H, 8.2; N, 5.0. $C_{20}H_{23}N$ requires C, 86.6; H, 8.4; N, 5.1 per cent. The *hydrochloride* had m.p. 224–228° after crystallisation from anhydrous ethanol. Found: C, 76.7; H, 7.5; Cl, 11.5; N, 4.5. $C_{20}H_{24}ClN$ requires C, 76.5; H, 7.7; Cl, 11.3; N, 4.5 per cent.

4-*Phenyl-1-(2-o-tolyloethyl)piperidine* was obtained (a) by reaction of 2-*o*-tolylethyl bromide with 4-phenylpiperidine in ethanol containing anhydrous sodium carbonate. It had b.p. 150–154° at 0.1 mm. ($n_D^{22} = 1.5639$). Found: C, 86.2; H, 9.0; N, 5.2. $C_{20}H_{25}N$ requires C, 86.0; H, 9.0; N, 5.0 per cent.

(b) A solution of 1,2,3,6-tetrahydro-4-phenyl-1-(2-*o*-tolylethyl)pyridine (8.0 g.) in ethanol (50 ml.) was hydrogenated at room temperature in the presence of a 5 per cent palladium-barium sulphate catalyst (1.0 g.). After filtration and concentration the oil was distilled at reduced pressure to yield the *product* (b.p. 150–154° at 0.1 mm.). The *hydrochloride* separated from ethanol in nodules m.p. 253–256°. Found: C, 76.2; H, 8.0; Cl, 11.0; N, 4.6. $C_{20}H_{26}ClN$ requires C, 76.1; H, 8.3; Cl, 11.2; N, 4.4 per cent.

1,2,3,6-*Tetrahydro-4-phenyl-1-(3-o-tolyloxypropyl)pyridine*, was prepared in 55 per cent yield by reaction of 3-*o*-tolylethyl bromide and 1,2,3,6-tetrahydro-4-phenylpyridine as described earlier. The crude *base* was converted directly into the *hydrochloride* which had m.p. 183–184° after crystallisation from ethanol. Found: C, 73.0; H, 7.5; N, 4.2. $C_{21}H_{26}ClNO$ requires C, 73.3; H, 7.6; N, 4.1 per cent.

1,2,3,6-Tetrahydro-4-phenyl-1-(4-*o*-tolylxybutyl)pyridine hydrochloride had m.p. 117–120° (from ethanol-ether). Found: Cl, 10.2; N, 4.2. $C_{22}H_{28}ClNO$ requires Cl, 9.9; N, 3.9 per cent.

1-(3-Ethoxypropyl)-1,2,3,6-tetrahydro-4-phenylpyridine had b.p. 48–53° at 0.1 mm. Found: C, 77.9; H, 9.3; N, 5.7. $C_{16}H_{23}NO$ requires C, 78.3; H, 9.4; N, 5.7 per cent.

1,2,3,6-Tetrahydro-1-(3-*m*-methoxyphenoxypropyl)-4-phenylpyridine hydrochloride had m.p. 163–165° (from ethanol-ether). Found: C, 70.3; H, 7.2; Cl, 10.2; N, 4.0. $C_{21}H_{26}ClNO_2$ requires C, 70.1; H, 7.3; Cl, 9.9; N, 3.9 per cent.

1-(2-Ethoxyethyl)-1,2,3,6-tetrahydropyridine, had b.p. 74–75° at 7 mm. Found: C, 69.6; H, 11.0; N, 9.4. $C_9H_{17}NO$ requires C, 69.7; H, 11.0; N, 9.0 per cent.

1,2,3,6-Tetrahydro-1-(2-*o*-tolylxyethyl)pyridine hydrochloride had m.p. 145–148° (from ethanol-ether). Found: C, 66.0; H, 7.8; Cl, 14.1; N, 5.6. $C_{14}H_{20}ClNO$ requires C, 66.2; H, 7.9; Cl, 14.0; N, 5.5 per cent.

1,2,3,6-Tetrahydro-1-(2-*m*-methoxyphenoxyethyl)pyridine hydrochloride had m.p. 136–138° (from ethanol-ether). Found: C, 62.1; H, 7.5; Cl, 13.3; N, 5.4. $C_{14}H_{20}ClNO_2$ requires C, 62.3; H, 7.5; Cl, 13.1; N, 5.2 per cent.

1,2,3,6-Tetrahydro-1-(2-tolylethyl)pyridine hydrochloride had m.p. 224–226° (from ethanol-ether). Found: C, 70.8; H, 8.3; Cl, 15.0; N, 6.0. $C_{14}H_{20}ClN$ requires C, 70.7; H, 8.5; Cl, 14.9; N, 5.9 per cent.

Allylethyl(2-*o*-tolylxyethyl)amine was prepared by reaction of 2-*o*-tolylxyethylamine with allyl bromide in ethanol in the presence of anhydrous sodium carbonate. It had b.p. 142–143° at 10 mm. Found: C, 76.5; H, 9.6; N, 6.5. $C_{14}H_{21}NO$ requires C, 76.6; H, 9.6; N, 6.4 per cent.

Cinnamylethyl(2-*o*-tolylxyethyl)amine. (a) Reaction of cinnamyl chloride with 2-*o*-tolylxyethylamine yielded the *product* as an oil, b.p. 150–152° at 0.05 mm., ($n_D^{19} = 1.5671$).

(b) Reaction of cinnamylethylamine (b.p. 121–124° at 10 mm. Found: C, 81.6; H, 9.7; N, 9.1. $C_{11}H_{15}N$ requires C, 81.9; H, 9.4; N, 8.7 per cent), with 2-*o*-tolylxyethyl bromide in ethanol in the presence of anhydrous sodium carbonate yielded the same *product* described in (a). Found: C, 81.7; H, 8.7; N, 4.7. $C_{20}H_{25}NO$ requires C, 81.3; H, 8.5; N, 4.7 per cent.

Dimethyl(2-*o*-tolylxyethyl)amine hydrochloride, had m.p. 175–177° (from ethanol-ether). Found: C, 61.4; H, 8.4; N, 6.7. $C_{11}H_{18}ClNO$ requires C, 61.2; H, 8.4; N, 6.5 per cent.

Diethyl(2-*o*-tolylxyethyl)amine hydrochloride, had m.p. 139–141° (from ethanol-ether). Found: C, 63.6; H, 9.1; Cl, 14.9; N, 5.9. $C_{13}H_{22}ClNO$ requires C, 64.0; H, 9.1; Cl, 14.5; N, 5.7 per cent.

1,2,3,4-Tetrahydro-2-(2-*m*-methoxyphenoxyethyl)isoquinoline hydrochloride. A mixture of *m*-methoxyphenoxyethyl bromide (9.2 g.), 1,2,3,4-tetrahydroisoquinoline (5.3 g.) and anhydrous sodium carbonate (2.2 g.) in ethanol (100 ml.) was heated under reflux for 8 hr. when excess of ethanol was boiled off. The residue was diluted with water

1,2,3,6-TETRAHYDRO-4-PHENYLPYRIDINES

and the *base* isolated with chloroform. Distillation of the chloroformic solution furnished the crude base (10.4 g.) which was converted to the *hydrochloride* (8.5 g.) in ethanol-ether. Crystallisation from the same solvent mixture yielded the *product*, m.p. 150–152°. Found: C, 67.3; H, 6.9; Cl, 11.5; N, 4.6. $C_{18}H_{22}ClNO_2$ requires C, 67.6; H, 6.9; Cl, 11.1; N, 4.4 per cent.

Tetrahydro-6-methyl-3-(2-phenoxyethyl)-6-phenyl-1,3-oxazine. (a) A mixture of 2-phenoxyethyl bromide (20.1 g.) and tetrahydro-6-methyl-6-phenyl-1,3-oxazine (17.7 g.) in ethanol (130 ml.) containing sodium carbonate (5.4 g.) was heated under reflux for 10 hr. After concentration to remove most of the ethanol, the mixture was diluted with water and the *base* isolated with chloroform. It (13.7 g.) had b.p. 165–170° at 0.05 mm. Found: C, 77.0; H, 7.8; N, 4.6. $C_{19}H_{23}NO_2$ requires C, 76.7; H, 7.8; N, 4.7 per cent.

(b) A mixture of 2-phenoxyethylamine hydrochloride (17.4 g.), 40 per cent formaldehyde solution (20 ml.) and α -methylstyrene (11.8 g.) was warmed with stirring. An exothermic reaction occurred at about 60° and this was controlled by cooling. Finally the mixture was heated at about 80° for 5 hr. and was then cooled and diluted with water. It was basified with 50 per cent sodium hydroxide solution and the *base* isolated with benzene. It (3.7 g.) had b.p. 175–185° at 0.4 mm. The *hydrochloride* had m.p. 220–222° (from ethanol). Found: N, 4.2. $C_{19}H_{24}ClNO_2$ requires N, 4.2 per cent. It (3 g.) was recovered unchanged after heating under reflux with concentrated hydrochloric acid (10 ml.) for 4 hr.

Acknowledgement. The authors thank Dr. A. David and his colleagues for the biological data.

REFERENCES

- Beasley, Y. M., Petrow, V. and Stephenson, O. (1958). *J. Pharm. Pharmacol.*, **10**, 103–111.
Mansfield, R. C. and Schmidle, C. J. (1956). *J. org. Chem.*, **21**, 699–700.
Orahovats, P. D., Lehman, E. G. and Chapin, E. W. (1957). *J. Pharmacol.*, **119**, 26–34.
Schmidle, C. J. and Mansfield, R. C. (1956). *J. Amer. chem. Soc.*, **78**, 1702–1705.
Weijlard, J., Orahovats, P. D., Sullivan, A. P., Purdue, G., Heath, F. K. and Pfister, K. (1956). *Ibid.*, **78**, 2342–2343.

AFRICAN RAUWOLFIA SPECIES

PART II. THE STRUCTURE OF THE ROOT AND STEM OF *Rauwolfia mombasiana* STAPF

BY WILLIAM E. COURT

From the Department of Pharmacy, City of Liverpool College of Technology

Received September 18, 1961

A substitute or adulterant for the roots of *R. vomitoria* Afz. is *R. mombasiana* Stapf, an East African shrub with a high reserpine yield. The anatomy of the root and stem is described and illustrated, and compared with published data about other African species.

DURING the last decade the root of the African tree *R. vomitoria* has become an important source of reserpine. Its widespread use has prompted the investigation of other African species which have occurred or may occur as substitutes or adulterants. One such species which has occurred in commerce as a substitute for *R. vomitoria* roots is *R. mombasiana* (Trease, private communication). The two species are closely related and Pichon (1947), in his classification of the genus *Rauwolfia*, has grouped them together with *R. cumminsii* Stapf in the section *Endolobus*. This section is characterised by a curious aestivation and apocarpous gynaecia.

Raymond-Hamet (1940) reported the hypotensive and adrenaline antagonistic activity of *R. mombasiana* extracts. Reserpine was isolated from the root in 1956, the published yields varying from 0.05 to 0.116 per cent (McAler, Weston and Howe, 1956; Korzun, St. André and Ulshafer, 1957). The highest yield of weakly basic alkaloids occurs in the root bark (Court, Evans and Trease, 1958).

R. mombasiana was first described by Stapf (1894) and recorded in the Kew Index, Supplement I (1886-95) together with *R. monoplyrena*, a species described by Schumann (1895) and now regarded as synonymous. The plant was briefly described by Delourme-Houdé (1944) as a false iboga, a substitute for *Tabernanthe iboga* Baill. Few diagrams and no numerical data were presented and, therefore, a detailed description of a range of specimens is given below and compared with the published anatomy of some other African species.

Habitat and Indigenous Use

A shrub growing to a height of 2 m., *R. mombasiana* occurs in coastal swamp forests. It is found in the Mombasa region of Kenya, on the East and West Usambaras and Pugu Hills of Tanganyika, in Zanzibar and in Mozambique (Feuell, 1955; Greenway, private communication).

The East African tribesmen use a preparation of the roots, ground with coconut oil, for the treatment of pimples. A mixed decoction is taken orally as a cure for gonorrhoea (Feuell, 1955).

Plant Material

The following material was used in this investigation :

1. *R. mombasiana* roots supplied by Dr. P. J. Greenway, East African Herbarium, Nairobi, Kenya, 1956.
2. *R. mombasiana* roots; commercial samples supplied by Professor G. E. Trease, Nottingham University, 1958.
3. *R. mombasiana* roots and stems supplied by Dr. P. J. Greenway, Nairobi, 1960.
4. *R. mombasiana* roots and stems collected near the mouth of the Tana River, north of Malindi, Kenya and supplied by the Department of Scientific and Industrial Research, 1960.

MACROSCOPY

Root

The roots occur as cylindrical or flattened, occasionally branched segments of varying lengths and up to about 6 cm. diameter. Narrower segments 0.5–2 cm. in diameter comprise the bulk of the samples examined. Externally the soft, pale yellowish brown cork shows irregular longitudinal furrowing and irregular buff or greyish patches of exposed cortical tissue. Frequently pieces of bark have broken away revealing the longitudinally furrowed, pale yellowish or reddish brown wood. Some segments bear the remains of side roots either as protuberances or stumps, or pale rootlet scars.

Smoothed transverse surfaces of the roots show a narrow bark seldom exceeding 3 mm. in thickness and an inner pale buff or yellowish, finely radiate wood possessing a few distinct growth rings.

The larger roots are tough and difficult to break but smaller roots break easily, the fracture being short in the bark and splintery in the wood.

Stem

The stems occur as cylindrical branched segments up to 5 cm. diameter. The external greyish-brown cork shows irregular longitudinal ridging and bears buff or pale brown, rounded or tangentially elongated lenticels. Semicircular leaf scars, occurring in whorls of 4 or occasionally 3 or 5, are frequently apparent on smaller stem segments. Smoothed transverse surfaces of the stems exhibit a narrow bark up to 1.5 mm. in thickness, a cylinder of secondary xylem with up to 10 growth rings and a small central pith, or cavity due to contraction of the pith, which may be up to 5 mm. diameter.

The fracture of the stems is fibrous in the bark, bark of small diameter segments being more fibrous than that of larger segments, and the fracture of the wood is splintery.

Sensory Characters

Dried roots and stems are almost odourless, the cork and the wood of root or stem is almost tasteless but the cortical tissue and phloem of each is intensely bitter. Powdered samples and exposed fractured surfaces of

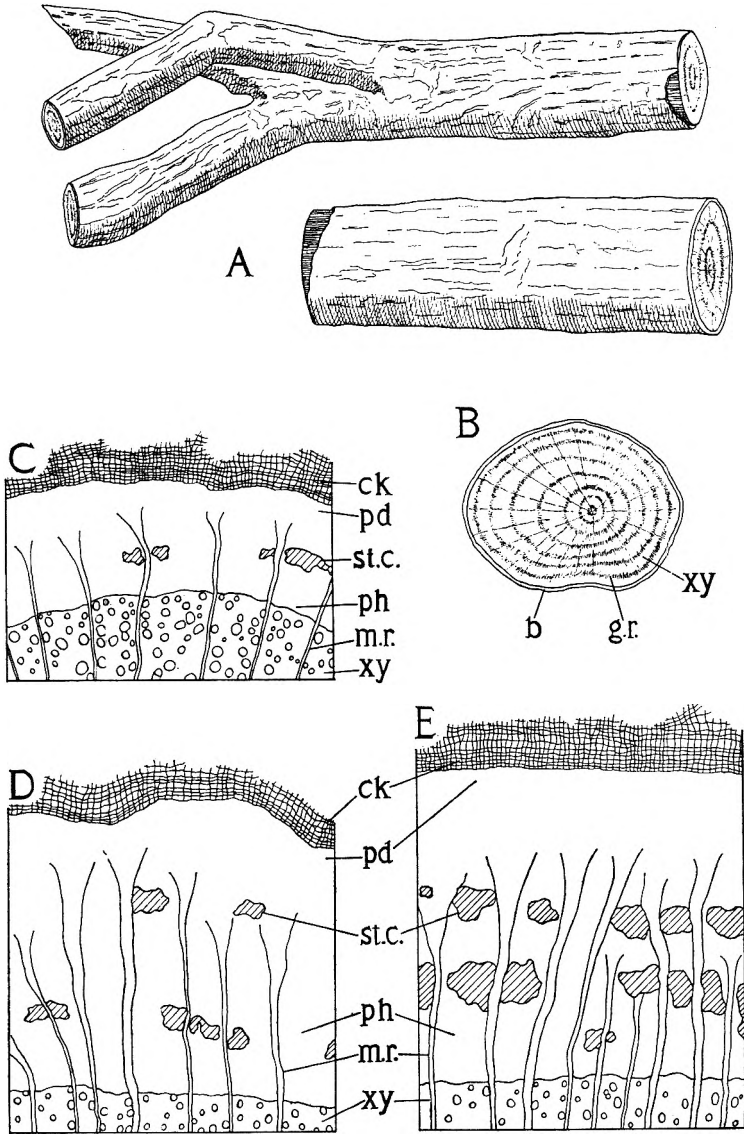


FIG. 1. *Rauwolfia mombasiana* Stapf. Root. A, external appearance, $\times 1$; B, smoothed transverse surface of root, $\times 3$; C, transverse section, root diameter 10 mm., $\times 15$; D, transverse section, root diameter 28 mm., $\times 15$; E, transverse section, root diameter 50 mm., $\times 15$. b, bark; ck, cork; g.r., growth ring; m.r., medullary ray; pd, phelloderm; ph, phloem; st.c., sclereid group; xy, xylem.

AFRICAN *RAUWOLFIA* SPECIES. PART II

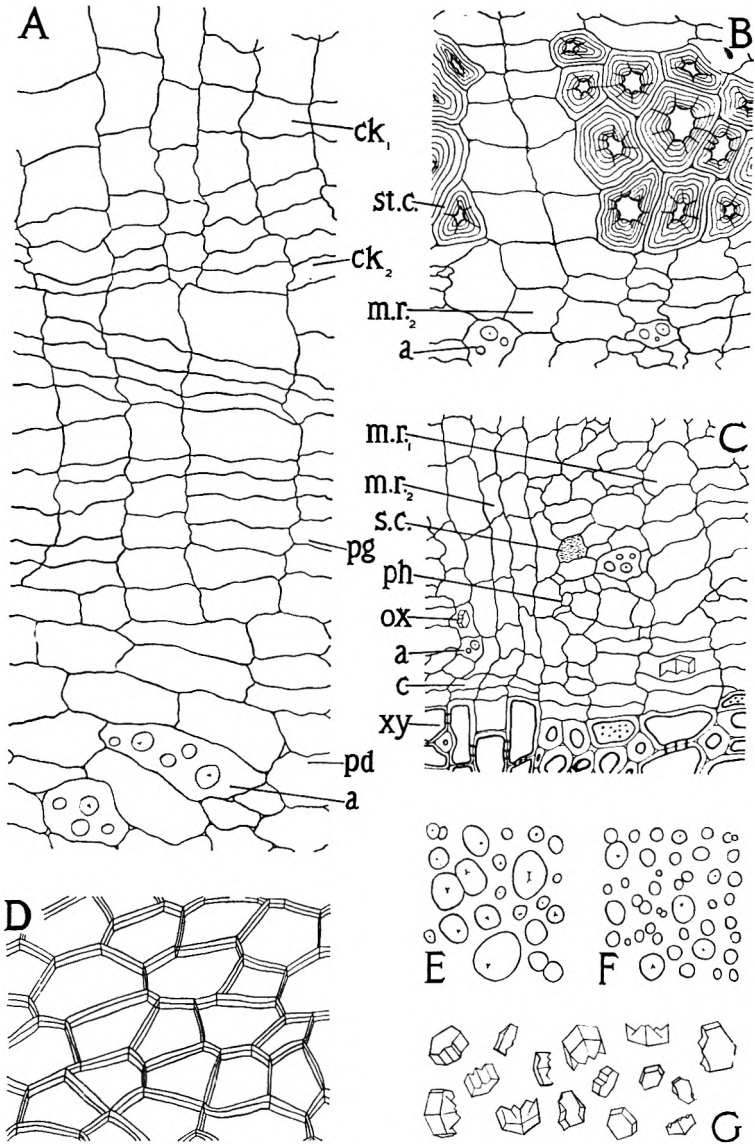


FIG. 2. *Rauwolfia mombasiana* Stapf. Root. A, transverse section of the outer tissues, root diameter 9 mm.; B, transverse section of the middle phloem, root diameter 10 mm.; C, transverse section of the inner phloem, root diameter 50 mm.; D, cork cells in surface view; E, starch grains from the wood; F, starch grains from the bark; G, calcium oxalate crystals from the bark. All $\times 200$. a, starch; c, cambium; ck₁, large lignified cork cells; ck₂, small unligified cork cells; m.r.₁, uniseriate medullary ray of upright cells; m.r.₂, multiseriate medullary ray of procumbent cells; ox, calcium oxalate crystal; pd, phelloderm; pg, phellogen; ph, phloem elements; s.c., secretion cell; st.c., sclereid; xy, xylem.

root or stem exhibit a bluish-green fluorescence in screened ultra-violet light; aqueous extracts fluoresce similarly.

MICROSCOPY

In the following description the symbols R, T and L refer to measurements made in the radial, tangential and longitudinal directions respectively of material mounted usually in Berlese mountant.

Root

The radially arranged cork cells occur as alternating zones of flattened, unligified, suberised cells, 3 to 8 cells in radial depth, and larger, lignified, suberised cells from 1 to 14 cells in radial depth. For the smaller cork cells, R = 8 to 16 to 24 to 35 μ , T = 20 to 39 to 55 to 94 μ and L = 23 to 35 to 55 to 86 μ ; and for the larger cells, R = 19 to 51 to 74 to 116 μ , T = 31 to 43 to 63 to 79 μ and L = 27 to 39 to 55 to 75 μ (Fig. 2,A). In surface view, the cork cells appear polygonal (Fig. 2,D).

The phellogen, a layer of thin-walled, radially flattened cells, is followed by the phellogerm which consists of 5 to 15 layers of cells. The phellogerm cells adjacent to the phellogen are arranged in regular radial rows whilst the innermost cells are oval in shape with intercellular spaces. The cell walls are cellulosic and sclereids are absent. For the phellogerm cells R = 16 to 24 to 35 to 47 μ , T = 35 to 51 to 74 to 141 μ and L = 23 to 39 to 59 to 86 μ . Starch and scattered twinned prisms of calcium oxalate occur in the phellogerm. The starch consists chiefly of single rounded grains 2 to 4 to 10 to 38 μ diameter. 2 to 4 compound grains also occur and may split into individual plano-convex or angular grains. The hilum usually appears as a central point or star-shaped cleft and many grains show a Maltese cross effect when examined in polarised light (Fig. 2,F).

The phloem is a relatively wide zone internal to the phellogerm and characterised by up to 3 interrupted bands of sclereids dependent on the diameter of the specimen (Fig. 1,C,D,E). The phloem contains secretion cells and is traversed by conspicuous rays (Fig. 2,B,C). The heterogeneous rays consist of groups of small procumbent cells often with wavy walls, 2 to 5 cells wide tangentially and up to 26 cells high with uniseriate upper and lower extensions consisting of 1 to 5 larger cells (Fig. 3,C). For the smaller cells R = 19 to 27 to 39 to 63 μ , T = 15 to 19 to 30 to 78 μ and L = 15 to 19 to 26 to 51 μ , and for the larger cells R = 12 to 20 to 27 to 40 μ , T = 31 to 55 to 75 to 110 μ and L = 23 to 39 to 63 to 99 μ .

The irregular sclereid groups in the outer phloem are up to about 10 cells in radial thickness, 20 cells tangentially and 40 cells in depth. Individual sclereids vary greatly from isodiametric to irregularly elongated fibre-like structures (Figs. 3,C; 5,D) and measure R = 12 to 27 to 51 to 118 μ , T = 16 to 31 to 55 to 130 μ and L = 37 to 68 to 97 to 251 μ . Sclereids isolated by maceration using chromic-nitric acid reagent measured 30 to 52 to 158 to 326 μ in length and 19 to 30 to 56 to 97 μ in breadth. The sclereids are lignified and possess stratified walls with funnel-shaped pits (Fig. 2,B). In the largest diameter roots the sclereids

form an almost continuous layer broken only by the passage of medullary rays.

In radial and tangential longitudinal sections of the secondary phloem, long rows of calcium oxalate crystals are evident in the phloem parenchyma cells, 2-4 crystals occurring in each cell (Fig. 3,C). These crystals consist of monoclinic prisms, usually twinned on one of the hemipyramid faces and exhibit, in polarised light, a bicolouration effect. Length of prisms = 15 to 18 to 26 to 34 μ ; breadth = 6 to 7 to 11 to 15 μ (Fig. 3,B,C).

Starch grains are distributed uniformly, although not abundantly, in the outer phloem and are usually less frequent in the inner functional phloem; they resemble those of the phelloderm.

Secretion cells are not numerous and are found occasionally in the phelloderm and, more frequently, in the inner phloem region. The amorphous contents of these cells stain with iodine solution, Sudan III and Tincture of Alkanna.

The primary xylem is indicated by four to six small groups of vessels near the centre of the root. The completely lignified secondary xylem consists of vessels, fibres and wood parenchyma and is traversed by medullary rays. In transverse sections the rounded or rather oval vessels occur solitary or in pairs. R = 27 to 50 to 98 to 165 μ and T = 24 to 49 to 90 to 131 μ . Numerous alternately arranged, bordered pits occur in the relatively thin, lignified vessel walls. Vessel segments isolated by chromic-nitric acid maceration show transverse and oblique perforation plates and peg-like prolongations (Fig. 5,G). For the isolated segments, length = 145 to 435 to 667 to 913 μ . A few nonfunctioning vessels may be occluded by brown amorphous material.

In transverse section the apotracheal wood parenchyma appears in short uniseriate rows connecting the vessels and medullary rays (Fig. 4,A). The cells appear, in longitudinal section, in vertical rows of up to 14 cells and the walls bear simple or half-bordered pits dependent on the nature of the adjacent cell structure (Fig. 4,B,C). R = 16 to 23 to 31 to 47 μ , T = 15 to 19 to 27 to 43 μ and L = 39 to 59 to 90 to 137 μ .

The heterogeneous medullary rays resemble those in the bark but are completely lignified and consist of a core of procumbent cells 2 to 5 cells in tangential width and up to 20 cells high with upper and lower uniseriate extensions of 1 to 6 larger upright cells. For the smaller cells R = 31 to 47 to 86 to 133 μ , T = 11 to 15 to 19 to 31 μ and L = 8 to 15 to 23 to 43 μ ; and for the larger cells R = 15 to 24 to 43 to 67 μ , T = 20 to 27 to 39 to 55 μ and L = 31 to 47 to 67 to 106 μ . The procumbent cells are, when viewed in longitudinal section, often nearly circular in outline with small intercellular spaces and heavily pitted walls (Fig. 4,B,C), and in transverse sections the uniseriate rays predominate (Fig. 4,A).

The numerous xylem fibres appear in transverse section as rounded or polygonal structures with thick lignified walls. The length of the fibres is 903 to 1,129 to 1,677 to 2,096 μ and the breadth is 16 to 20 to 31 to 47 μ . Most of the fibres are spindle-shaped with tapering apices and bases and the walls bear spirally arranged slit-like pits (Fig. 5,H).

Starch grains, 3 to 6 to 14 to 46 μ in diameter and similar to those in the

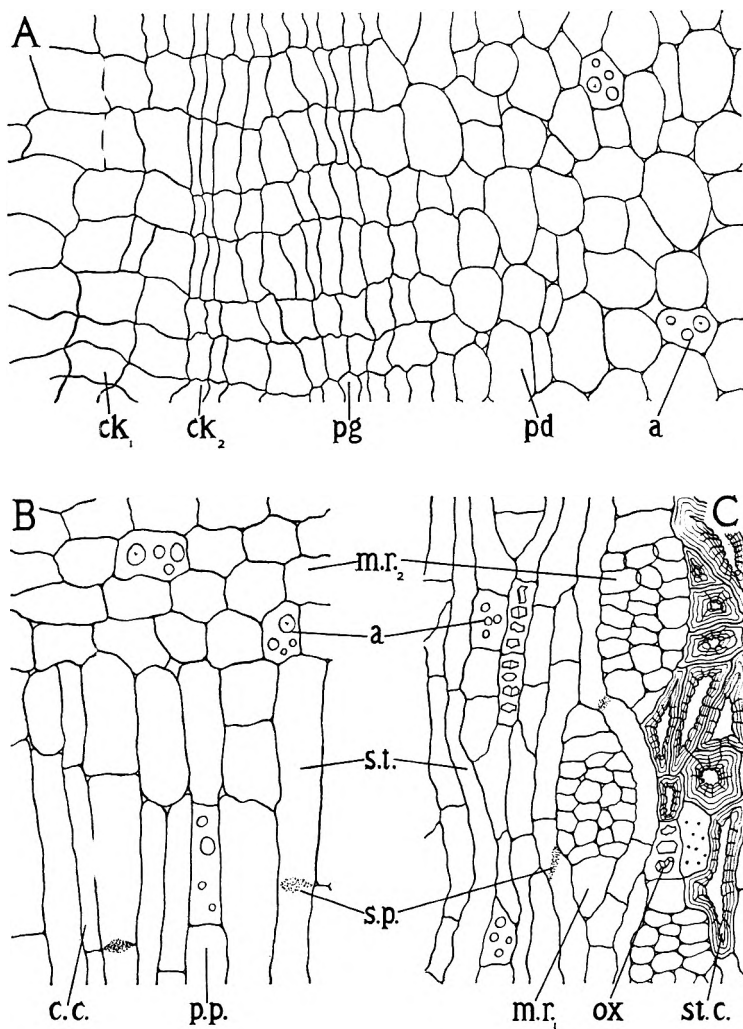


FIG. 3. *Rauwolfia mombasiana* Stapf. Root. A, radial longitudinal section of outer tissues, root diameter 23 mm., $\times 200$; B, radial longitudinal section of inner phloem, root diameter 23 mm., $\times 200$; C, tangential longitudinal section of inner phloem, root diameter 35 mm., $\times 100$. a, starch; c.c., companion cell; ck_1 , large lignified cork cells; ck_2 , small unlignified cork cells; $m.r_1$, uniseriate medullary ray of upright cells; $m.r_2$, multiseriate medullary ray of procumbent cells; ox, calcium oxalate crystal; pd, phelloderm; pg, phellogen; p.p., phloem parenchyma; s.p., sieve plate; s.t., sieve tube; st.c., sclereid.

AFRICAN *RAUWOLFIA* SPECIES. PART II

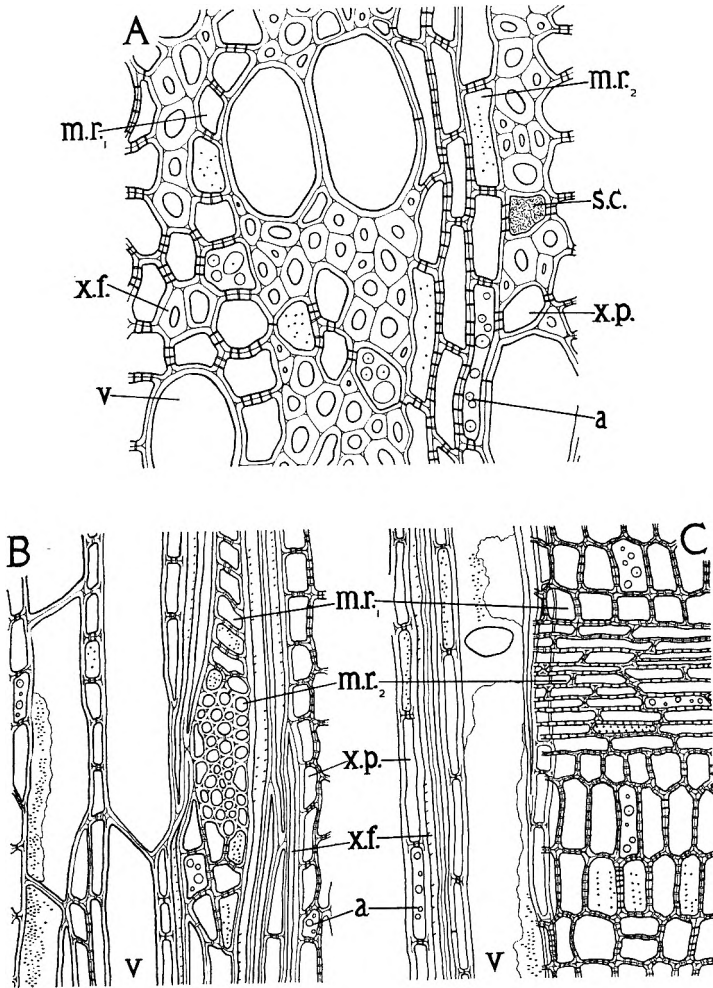


FIG. 4. *Rauwolfia mombasiana* Stapf. Root. Secondary Wood. A, transverse section, root diameter 10 mm., $\times 200$; B, tangential longitudinal section, root diameter 10 mm., $\times 100$; C, radial longitudinal section, root diameter 23 mm., $\times 100$. a, starch; m.r.₁, upright medullary ray cells; m.r.₂, procumbent medullary ray cells; s.c., secretion cell; v, vessel; x.f., xylem fibre; x.p., xylem parenchyma.

bark, occur freely in the wood parenchyma and medullary ray cells (Fig. 4,A,B,C). Occasional secretion cells containing material staining with iodine and Sudan III and a few calcium oxalate prisms are usually found in the wood.

Stem

The general arrangement of the tissues and the cell dimensions resemble those of the root. The soft outer cork layer is not as extensive as that of the root and the stratification is less obvious. Internal to the phelloderm and cortex, a narrow layer of about 12 rows of cells which are thicker-walled than the corresponding cells in the root, is a zone of highly refractive, unligified fibres. In specimens of small diameter the fibres form an almost continuous layer of up to 10 fibres in radial thickness and appear uniformly circular in shape, measuring 11 to 26 to 45 to 83 μ diameter (Fig. 6,C). The fibres are more widely scattered in the older and larger specimens and, after isolation by alkaline maceration, many fibres show pronounced swellings 26 to 45 to 64 to 113 μ in diameter (Fig. 7,A,C); hence their appearance in transverse section is variable. The length of these fibres exceeds 12 mm.

The outermost phloem is characterised by one or two interrupted rows of sclereids resembling those in the root bark. The inner secondary phloem is traversed by rays which are usually 2 to 5 cells wide and up to 20 small cells high with uniseriate upper and lower extensions of 2 to 5 larger cells. Typical phloem fibres are absent.

Calcium oxalate prisms and starch grains of the stem bark are similar in dimensions and distribution to those in the root bark.

The stem wood resembles the root wood although the vessels are somewhat smaller. R = 26 to 38 to 75 to 94 μ and T = 26 to 45 to 60 to 75 μ .

The parenchymatous central pith shows a peripheral ring of small-celled groups of perimedullary phloem tissue separated by rays of large-celled parenchyma (Fig. 8,A,B). The central tissue of the pith comprises a large-celled cellulosic parenchyma, individual cells containing starch grains and typical calcium oxalate prisms similar to those in the bark. Isolated sclereids or small groups of about 6 sclereids, resembling those in the bark, occur occasionally (Fig. 8,C).

Laticiferous Tissue

The presence of laticiferous tubes is generally regarded as an important feature of the Apocynaceae and Delourme-Houdé (1944) reported the occurrence of such tubes in the roots of *R. mombasiana*. A careful search for these structures was therefore undertaken.

Most specimens of root and stem showed secretion cells, parenchymatous cells containing granular material staining with iodine solution, Sudan III and Tincture of Alkanna. Such cells are distributed in the phloem and, to a lesser extent, in the phelloderm and wood.

Detailed examination of a wide range of tangential longitudinal sections revealed the presence of narrow, thin-walled, non-articulated laticiferous

AFRICAN *RAUWOLFIA* SPECIES. PART II

tubes in some root specimens. These tubes, which measure 15 to 52 μ diameter and generally occur in the outer phloem, contain granular matter and refractive globules and can be stained rose-pink using aqueous iodine solution followed by aqueous eosin solution and subsequent mounting in 2 per cent aqueous acetic acid (Fig. 8,D).

Similar, but more prominent, laticiferous tubes 19 to 32 to 60 to 90 μ diameter were observed in the stem bark, usually in close association with the unligified fibres, and also in the pith (Fig. 7,A,C; 8,A,B,C).

The Powdered Root

The principal features of the powdered root are:

1. Thin-walled yellow cork cells of two types—lignified cells and radially compressed unligified cells, the former being more frequent in occurrence.

2. Thin-walled cellulosic elements of the phelloderm and phloem containing starch grains, occasional calcium oxalate crystals and sometimes yellowish granular material.

3. Rounded, ovoid or plano-convex starch grains 2 to 4 to 14 to 46 μ diameter; occasional 2 to 4 compound grains.

4. Single or twinned monoclinic prisms and irregular crystalline masses of calcium oxalate.

5. Fragments of narrow, thin-walled laticiferous tubes containing granular matter or refractive globules.

6. Isodiametric, elongated or irregularly shaped lignified sclereids, either singly or in small groups.

7. Abundant fragments of lignified xylem elements derived from thin-walled vessels with alternately arranged bordered pits, xylem fibres and elongated xylem parenchyma and medullary ray cells usually containing starch grains.

8. Amorphous matter staining with iodine solution being the contents of ruptured laticiferous tissue.

DISCUSSION

The histological structure of *R. mombasiana* exhibits the characteristic features of the family Apocynaceae, typical elements being the unligified fibres in the pericyclic region of the stem and laticiferous canals and vessel segments with large communication pores and peg-like prolongations in the root and stem. Characteristic of the genus *Rauwolfia* is the occurrence of phloem sclereids, non-articulated laticiferous tubes, non-septate fibres and heterogeneous rays. The presence of unligified fibres in the pericyclic region and a well-defined central pith clearly differentiates the stem from the root.

The relatively small vessel diameters, the pronounced radial development of phloem and xylem and the intermediate sclereid development can be related with the shrub-like habit of the species (Woodson, 1957).

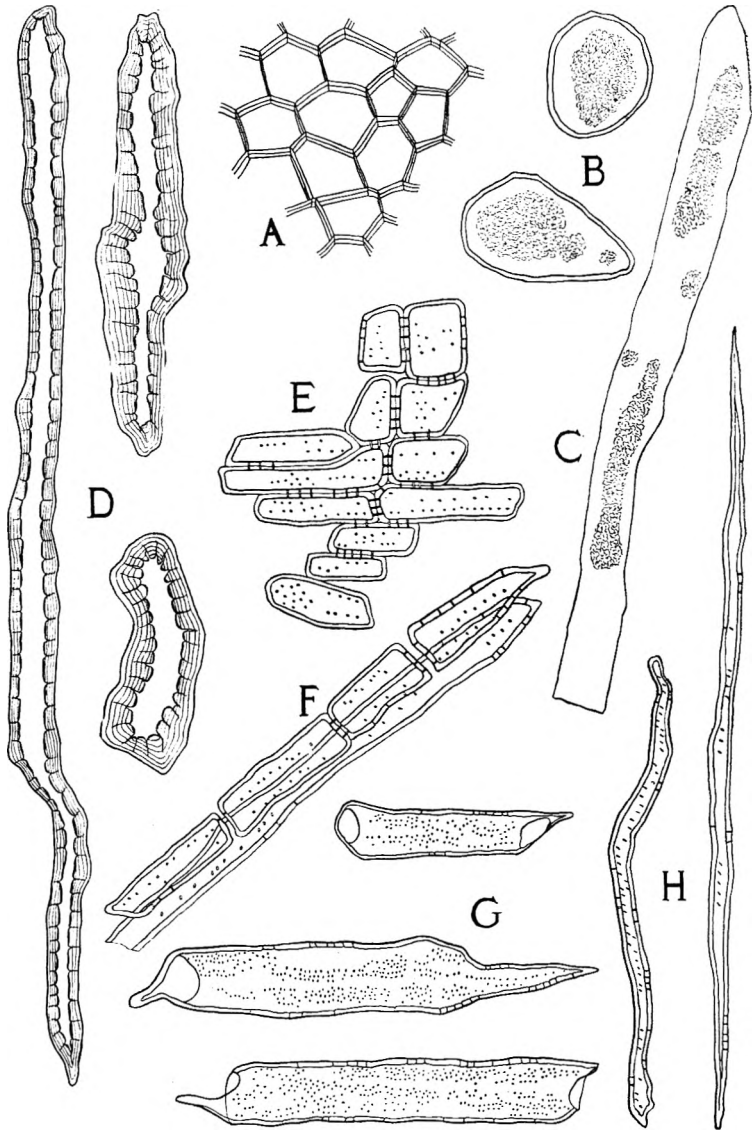


FIG. 5. *Rauwolfia mombasiana* Stapf. Isolated elements of the root. A, cork cells; B, secretion cells; C, laticiferous tube; D, sclereids; E, xylem medullary ray cells; F, xylem parenchyma cells; G, vessel segments; H, xylem fibres. A-F, $\times 200$; G, H, $\times 100$.

AFRICAN *RAUWOLFIA* SPECIES. PART II

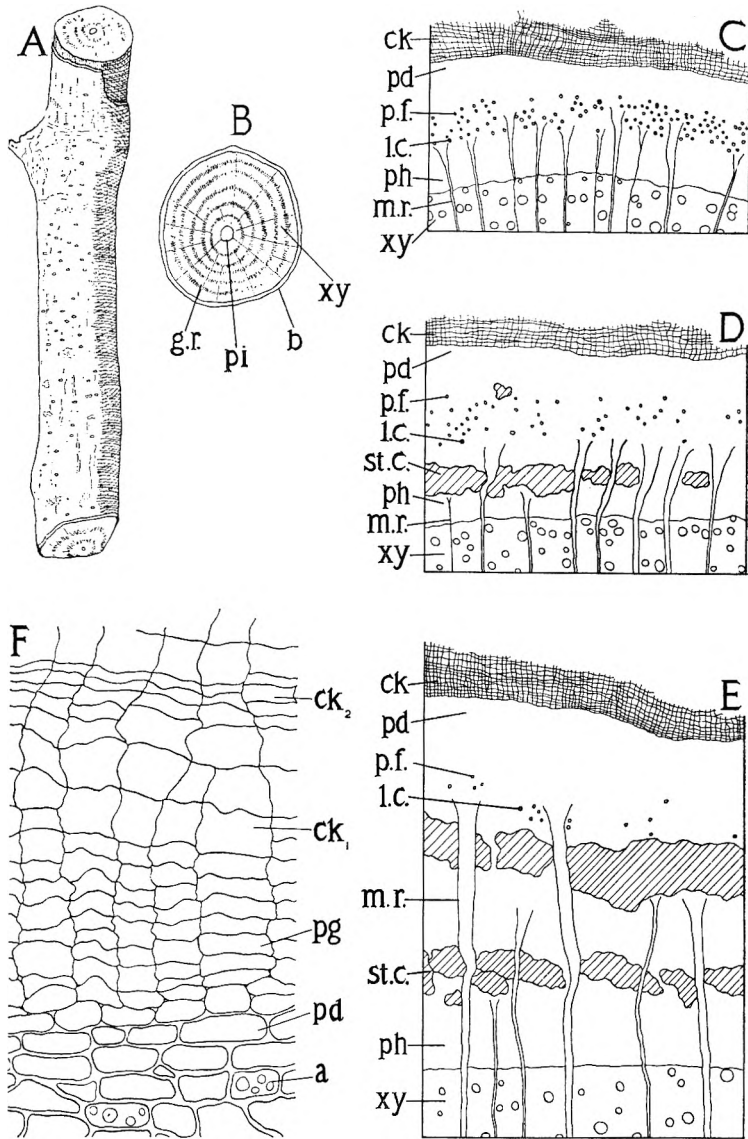


FIG. 6. *Rauwolfia mombasiana* Stapf. Stem. A, external appearance, $\times \frac{1}{3}$; B, smoothed transverse surface of stem, $\times \frac{2}{3}$; C, transverse section, stem diameter 9 mm., $\times 25$; D, transverse section, stem diameter 18 mm., $\times 25$; E, transverse section, stem diameter 36 mm., $\times 25$; F, transverse section of outer tissues, stem diameter 9 mm., $\times 200$. a, starch; b, bark; ck, cork; ck₁, large lignified cork cells; ck₂, small unligified cork cells; g.r., growth ring; l.c., laticiferous canal; m.r., medullary ray; pd, phelloderm; p.f., unlignified fibre; ph, phloem elements; pi, pith; st.c., sclereid group; xy, xylem.

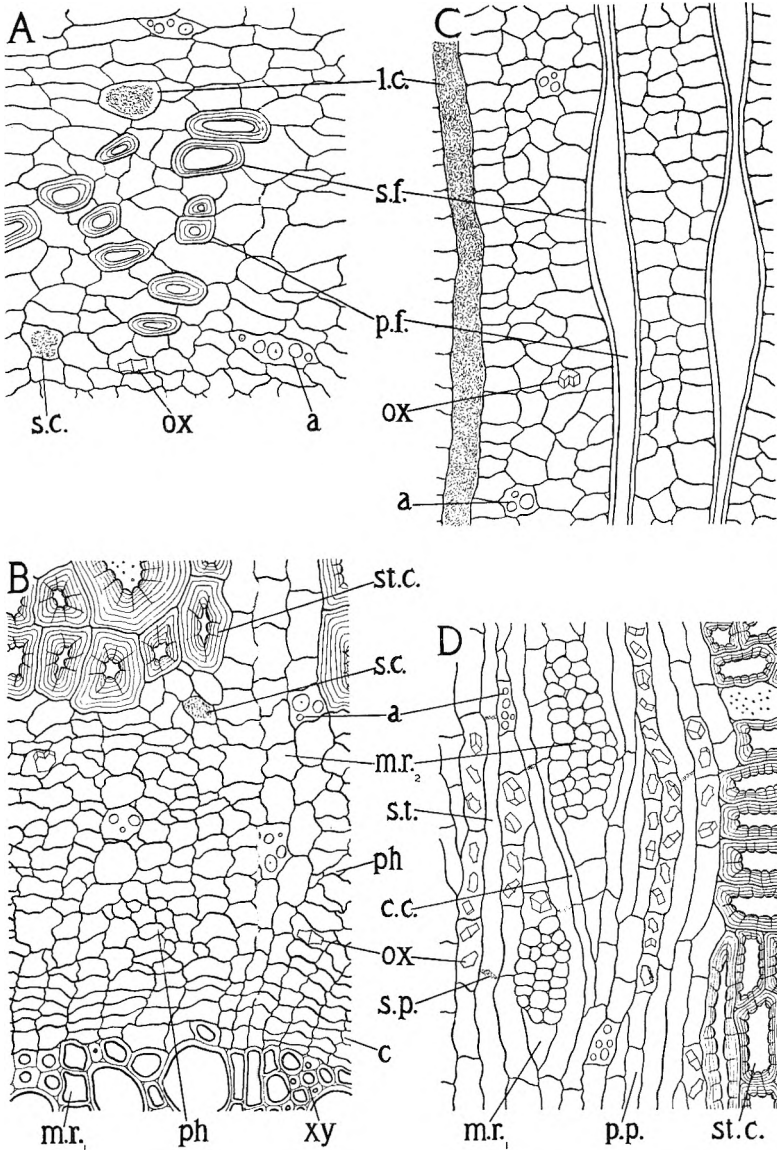


FIG. 7. *Rauwolfia mombasiana* Stapf. Stem. A, transverse section of pericyclic region, stem diameter 19 mm., $\times 200$; B, transverse section of inner phloem, stem diameter 18 mm., $\times 200$; C, longitudinal section of pericyclic region, stem diameter 19 mm., $\times 100$; D, tangential longitudinal section of phloem, stem diameter 19 mm., $\times 100$. a, starch; c, cambium; c.c., companion cell; l.c., laticiferous canal; m.r.₁, upright medullary ray cells; m.r.₂, procumbent medullary ray cells; ox, calcium oxalate crystal; p.f., unlignified fibre; ph, phloem elements; p.p., phloem parenchyma; s.c., secretion cell; s.f., swollen fibre; s.p., sieve plate; s.t., sieve tube; st.c., sclereid; xy, xylem.

AFRICAN *RAUWOLFIA* SPECIES. PART II

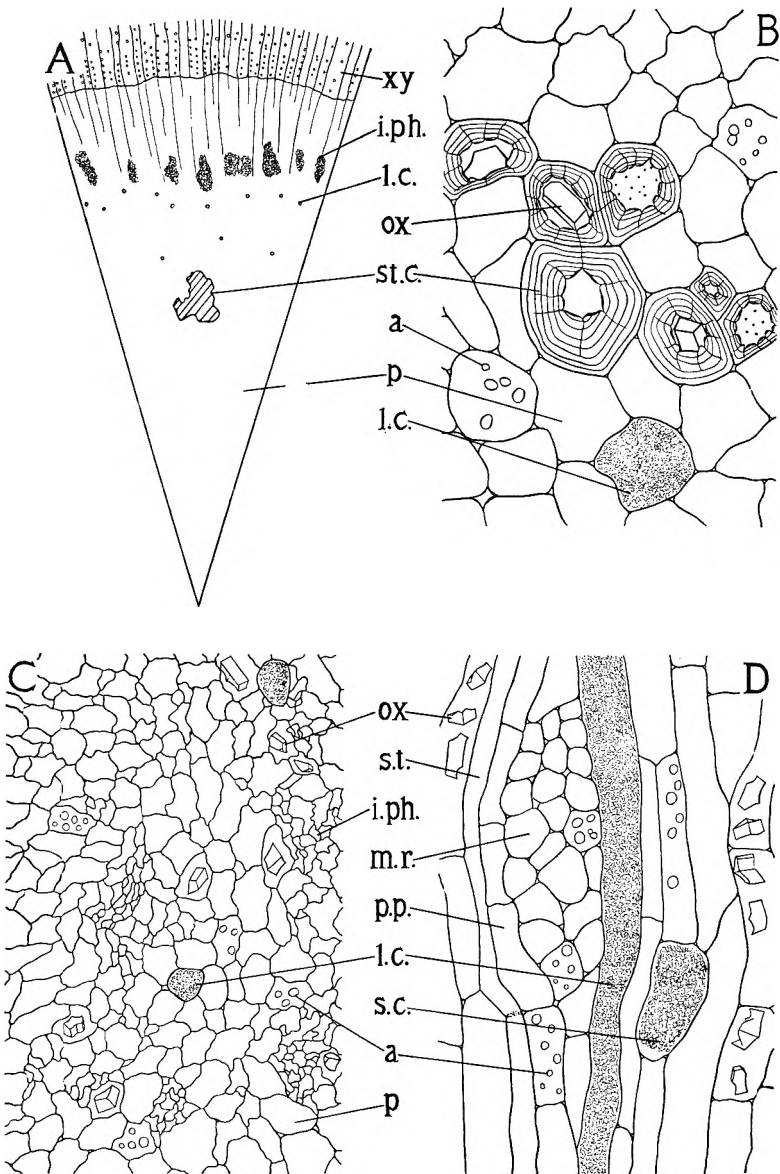


FIG. 8. *Rauwolfia mombasiana* Stapf. Stem and Root. A, transverse section of pith, stem diameter 42 mm., $\times 25$; B, transverse section of central pith, stem diameter 42 mm., $\times 200$; C, transverse section of outer pith, stem diameter 42 mm., $\times 200$; D, tangential longitudinal section of root showing laticiferous tissue, root diameter 40 mm., $\times 200$. a, starch; i.ph., perimedullary phloem; l.c., laticiferous canal; m.r., medullary ray; ox, calcium oxalate crystal; p, large-celled parenchyma; p.p., phloem parenchyma; s.c., secretion cell; s.t., sieve tube; st.c., sclereid group; xy, xylem.

R. mombasiana roots cannot readily be distinguished from other African *Rauwolfia* species by macroscopical examination. External colour is not a reliable criterion, colour variations often being dependent on the type of soil in which the plant has grown. Such variations have already been observed in samples of *R. tetraphylla* L. (Woodson, 1957) and *R. caffra* Sond. (Court, 1958).

Transverse sections of *R. mombasiana* can easily be distinguished from those of *R. caffra* (Court, Evans and Trease, 1957) and *R. macrophylla* Stapf (Paris, Dillemann and Chaumelle, 1957) as these latter two African species exhibit prominent sclereid groups in the phelloderm, extensive sclereid development in the phloem and larger vessel diameters, features associated with their arboreal form.

Sections of the roots of the shrubby species *R. obscura* K. Schum. (Paris and Dillemann, 1956) and *R. volkensii* Stapf (Court, 1961) reveal small diameter vessels and seldom exhibit sclereid groups, facts which differentiate them from the foregoing African species but not from each other.

R. vomitoria root is more difficult to distinguish from *R. mombasiana* root but, by comparison of sections from specimens of a similar diameter, the more extensive sclereid development and greater vessel sizes in *R. vomitoria* (Evans, 1956) become apparent.

Although *R. mombasiana* roots in the entire condition can be differentiated from the roots of the 5 African species about which data is available, their detection in the comminuted form as a substitute or adulterant for *R. vomitoria* roots presents a complex problem requiring further investigation.

REFERENCES

- Court, W. E. (1958). M. Pharm. Thesis, Nottingham University.
 Court, W. E. (1961). *J. Pharm. Pharmacol.*, **13**, 422-434.
 Court, W. E., Evans, W. C. and Trease, G. E. (1957). *Ibid.*, **9**, 237-250; (1958). *Ibid.*, **10**, 380-383.
 Delourme-Houdé, J. (1944). Doct. Pharm. Thesis, Paris University.
 Evans, W. C. (1956). *J. Pharm. Pharmacol.*, **8**, 120-133.
 Feuill, A. J. (1955). *Colon. Pl. Anim. Prod.*, **5**, 1-33.
 Korzun, B. P., St André, A. F. and Ulshafer, P. R. (1957). *J. Amer. pharm. Ass., Sci. Ed.*, **46**, 720-723.
 McAleer, W. J., Weston, R. G. and Howe, E. E. (1956). *Chem. Ind.*, 1387.
 Paris, R. and Dillemann, G. (1956). *Ann. Pharm. franc.*, **14**, 505-518.
 Paris, R., Dillemann, G. and Chaumelle, P. (1957). *Ibid.*, **15**, 360-367.
 Pichon, M. (1947). *Bull. Soc. bot. Fr.*, **94**, 31-39.
 Raymond-Hamet, M. (1940). *C.R. Acad. Sci. Paris*, **210**, 789-791.
 Schumann, K. (1895). *Engl. Pflanz. Ost. Afr. C.*, 318.
 Stapf, O. (1894). *Kew Bull.*, 21.
 Woodson, R. E. (1957). *Rauwolfia*, Boston: Little, Brown and Co.

THE SYNTHESIS OF ACETYLCHOLINE BY ACETONE DRIED POWDERS FROM THE BRAINS OF NORMAL RATS AND OF THIAMINE-DEFICIENT RATS

BY B. BHAGAT AND MARY F. LOCKETT

From the Department of Physiology and Pharmacology, Chelsea College of Science and Technology, London, S.W.3

Received September 28, 1961

The rate of synthesis of acetylcholine by rat brains was reduced by thiamine deficiency. There was a reduction in available coenzyme A but not in choline acetylase activity.

SOME years ago, Mann and Quastel (1939) compared the rates at which acetylcholine was synthesised by the brains of normal and polyneuritic pigeons. They found lower rates than normal when the concentration of potassium ions in the medium was high. Added thiamine restored the rate of synthesis in the polyneuritic tissue, but failed to influence the normal.

In recent years the measurement of activity attributable to choline acetylase in tissues (Hebb, 1955; Hebb and Smallman, 1956) has not only been greatly improved but means have been provided by which the quantity of coenzyme A present may be determined. It therefore seemed desirable to reinvestigate the influence of thiamine deficiency on the rate of synthesis of acetylcholine in brain using modern techniques for measuring both the enzymic activity and the co-enzyme A available in the tissue. We have used rats for this purpose.

METHODS

Female rats of a single Wistar strain, weighing 150 to 200 g. were used. They were housed in a room maintained at $21 \pm 0.5^\circ$, drank tap water and were fed the basic diet described by Fitzhugh, Knudsen and Nelson (1946). It consisted (per cent) of corn starch 60, casein 18, corn oil 6, dessicated whole liver powder 5, dried yeast 5 and U.S.P. salt mixture (XII, No. 2) 4, but the 2 per cent cod liver oil supplement was omitted. Instead, each rat received 0.5 ml. cod liver oil, orally by pipette each week. The thiamine content of this diet, assayed by the thiochrome method, was 138 $\mu\text{g.}$ per 100 g.

A diet deficient in thiamine was prepared from the basic diet by addition of 0.6 per cent sodium metabisulphite. It was used within 7 weeks of preparation. This treatment reduced the thiamine in the diet to less than 1 $\mu\text{g.}/100$ g. within 2 weeks of the sulphiting process. Rats fed the sulphited diet began to lose weight by the third or fourth week, and finally developed polyneuritis accompanied by bradycardia in the fifth or sixth week when they were ready for use, in parallel with control animals fed the basic unsulphited diet.

Assay of Choline Acetylase and Coenzyme A in Brain

The preparation of acetone dried powder from brains. The rats were killed by a single blow at the base of the neck, and decapitated. The whole brain was removed and ground in a cold mortar with 50 to 100 vol. of dry acetone at -4° . The sediment was collected by filtration using a No. 54 Whatman filter paper on a Buchner funnel. The resulting powder was kept over phosphorus pentoxide in a vacuum dessicator at -4° for 4–5 hr. before use. A separate powder was prepared from the brain of each rat.

Preparation of enzyme. The powder (10 mg./ml.) was suspended in normal saline containing 6 mg. l-cysteine hydrochloride per ml. The supernatant fluid was collected after centrifuging at 10,000 g at 1° for 3 hr.

Estimation of enzyme activity. The tubes prepared for incubation each contained enzyme derived from 25 mg. of acetone dried brain powder; l-cysteine hydrochloride, 15 mg.; sodium fluoride, 2 mg.; potassium chloride, 6 mg.; magnesium chloride, 4 mg.; eserine sulphate, 0.5 mg.; 0.3 ml. phosphate buffer, M/15, pH 7.0; choline chloride, 4 mg.; sodium citrate, 16.4 mg.; the disodium salt of adenosine triphosphate (ATP), 4 mg.; and coenzyme A, 100 μ g. (equivalent to 30 Lipmann units). Each tube was plugged with cotton wool and was incubated for 1 hr. in a water bath at 37° . Enzyme activity was then arrested by the addition of 0.5 ml. 0.3N HCl followed by rapid boiling and cooling. The tubes were stored at -10° overnight, and were neutralised to litmus as external indicator with 0.3N NaOH and brought to a volume of 7 ml. immediately before biological assay for acetylcholine content.

Estimation of coenzyme A content of brains. Estimates of the coenzyme in the individual rat brains differed in method from estimates of choline acetylase only in the following points. First, the enzyme used throughout was provided by a single, well mixed sample of acetone dried powder obtained from the brains of a number of normal rats. Secondly, coenzyme A was omitted from the incubation mixture and was replaced by 2 ml. of boiled extract of acetone dried powder (12 mg./ml.) from individual rat brains.

Assay of acetylcholine. The eserinated frog rectus preparation of Chang and Gaddum (1933) was used taking the precautions advised by Feldberg (1945), Feldberg and Mann (1945, 1946), and Feldberg and Hebb (1947) to avoid errors due to substances in the extracts which may potentiate the effects of acetylcholine. Throughout, 2×2 assays of Latin square design have been used for comparison of the quantities of acetylcholine formed by the enzyme or coenzyme A in the brain of a thiamine deficient rat with that synthesised by the brain of a normal rat. In addition, the sensitivity of each rectus preparation toward acetylcholine was assessed in order that a rough estimate of concentration should accompany the more accurate knowledge of relative potency.

Investigation of the optimum conditions for the synthesis of acetylcholine in extracts of acetone dried powders made from normal rat brain. Acetone dried powders prepared from normal rat brains were used to establish conditions needed for the high rates of synthesis of acetylcholine recorded

SYNTHESIS OF ACETYLCHOLINE AND THIAMINE DEFICIENCY

by former workers. Previous investigators have employed either citrate (Feldberg and Mann, 1946; Barker, 1951) or acetate (Hebb, 1955) as substrates for the acetylation of coenzyme A. Citrate was used hence the reaction medium contained ATP and coenzyme A. The enzyme used initially was prepared from the acetone dried powders as described by Feldberg and Mann (1946). Without 1-cysteine the yield was 922.5 ± 26.6 (4) $\mu\text{g./g.}$ dried powder/hr.

This finding is in good accord with the early observation of Feldberg and Mann. Purification of the enzyme by high speed centrifugation, introduced by Lipton (1946), and the addition of cysteine as stabiliser gave a rate of synthesis of acetylcholine of 1925 ± 14.3 $\mu\text{g./g./hr.}$ This compared satisfactorily with reported figures. Though reserpine was added to the reaction medium throughout this work to prevent breakdown of acetylcholine by cholinesterases, this precaution may have been needless. Nachmansohn and Berman (1946) have shown that acetone-dried brain yields powders almost devoid of cholinesterase activity.

RESULTS

Two series of experiments were made in which the acetylcholine synthesised by centrifuged extracts of the acetone dried powders from the brains of normal rats was compared with that made in corresponding extracts from the brains of animals deficient in thiamine. In the first series the reaction mixture contained added coenzyme A: in the second series it did not. The results of these experiments are shown in Table I.

TABLE I

A COMPARISON OF THE QUANTITIES OF ACETYLCHOLINE SYNTHESISED BY EXTRACTS OF ACETONE-DRIED POWDERS FROM THE BRAIN OF NORMAL AND THIAMINE-DEFICIENT RATS

Condition of test	Acetylcholine synthesised $\mu\text{g./g.}$ powder/hr.			
	Normal	Deficient in thiamine		
		Normal per cent	Significance of difference	
			<i>t</i> calc.	P
No added coenzyme A	700-1100	$75.8 \pm 9.3(8)$	2.48	<0.05
Coenzyme A added	1700-2100	$110.0 \pm 9.8(7)$	1.95	<0.01

There was no reduction in the choline acetylase activity of brains from thiamine-deficient animals: this is clearly shown by the results of experiments made in the presence of excess coenzyme. Thus the reduced rate of synthesis of acetylcholine by extracts of thiamine-deficient brains to which no coenzyme A has been added is attributed to reduced coenzyme A content.

This conclusion was examined in a third series of eleven experiments. The available coenzyme A in the brains of normal and of thiamine-deficient rats was compared by measurement of acetylcholine synthesised by aliquots of a single enzyme preparation when standardised boiled extracts of these brains replaced coenzyme A in the reaction mixture. In these experiments

B. BHAGAT AND MARY F. LOCKETT

the quantity of acetylcholine synthesised when boiled extracts of the brains from thiamine-deficient rats provided the coenzyme was 73.7 ± 9.3 (11) per cent of that found when boiled extracts of normal brains were used. The difference was significant ($t = 2.82$; $P = <0.05$).

DISCUSSION

There was no reduction in choline acetylase activity in the brains of rats made deficient in thiamine (Table I), but the rate of synthesis of acetylcholine in extracts of these brains is subnormal until coenzyme A is added. This fact indicates that a reduction in the coenzyme A present in the brain is responsible for the subnormal rate of synthesis. The reduced rate of synthesis of acetylcholine which we have observed in the brains of thiamine-deficient rats can explain the lowered concentrations of acetylcholine found by Lissák, Kovács and Nagy (1943) in the brain and cord of thiamine-deficient animals.

REFERENCES

- Barker, H. A. (1951) in *Phosphorus Metabolism*, edited by McElroy, W. D. and Glass, B., 1, 204. John Hopkins Press.
- Chang, H. C. and Gaddum, J. H. (1933). *J. Physiol.*, **79**, 255–285.
- Feldberg, W. (1945). *Ibid.*, **103**, 367–402.
- Feldberg, W. and Mann, T. (1945). *Ibid.*, **104**, 8–20.
- Feldberg, W. and Mann, T. (1946). *Ibid.*, **104**, 411–425.
- Feldberg, W. and Hebb, C. O. (1947). *Ibid.*, **106**, 8–17.
- Fitzhugh, O. S., Knudsen, L. F. and Nelson, A. A. (1946). *J. Pharmacol.*, **86**, 37–48.
- Hebb, C. O. (1955). *Quart. J. Physiol.*, **40**, 176–186.
- Hebb, C. O. and Smallman, B. N. (1956). *J. Physiol.*, **134**, 385–392.
- Lipton, M. A. (1946). *Fed. Proc.*, **5**, 145.
- Lissák, K., Kovács, T. and Nagy, E. K. (1943). *Pflugers Arch. ges. Physiol.*, **247**, 124–131.
- Mann, P. J. G. and Quastel, J. H. (1940). *Nature, Lond.*, **145**, 856–857.
- Nachmansohn, D. and Berman, M. (1946). *J. biol. Chem.*, **165**, 551–563.

WRIGHTIA TINCTORIA BARK, AN ADULTERANT OF KURCHI

BY C. K. ATAL AND P. D. SETHI

*From the Pharmacognosy Laboratory, Pharmacy Department,
Panjab University, Chandigarh-3, India*

Received September 18, 1961

The pharmacognostic features of the bark of *Wrightia tinctoria*, an adulterant of Kurchi, have been described and illustrated. Points which differentiate this adulterant from true Kurchi bark, are outlined.

KURCHI bark, *Holarrhena antidysenterica*, is an important anti-dysenteric drug and is official in the Indian Pharmacopoeia (1955). However, adulteration of this drug is so common that invariably all commercial samples are found adulterated. Prasad and Kaul (1956) have described in detail the pharmacognosy of Kurchi and one of its adulterants, *Wrightia tomentosa*. However, no work on *Wrightia tinctoria*, which is the more common adulterant and which possesses no antidysenteric principle (Chopra 1958), has been reported.

Wrightia tinctoria (family Apocyanaceae) is a small deciduous tree, commonly distributed in Rajasthan, Ceylon, Madras and Burma (Kirtikar and Basu, 1953).

MATERIAL AND METHODS

A fresh sample of the bark was collected from Sohna, district Gurgaon, near Delhi and preserved in 70 per cent ethanol, acetic acid and formaline mixture (90:5:5). It was authenticated by courtesy of Shri K. C. Sahni of the Forest Research Institute, Dehradun. Usual methods of sectioning and staining were employed.

Macroscopy

The bark (Fig. 1B) occurs in the form of channeled or quilled pieces, 1-2.5 cm. wide, 2-3 cm. long and 1-2 mm. thick. The outer convex surface is light grey in colour showing longitudinal wrinkles and furrows and numerous small whitish circular lenticels. The inner surface is smooth and pale brown in colour. The fracture is tough and brittle. There is no characteristic odour or taste.

Microscopy

The bark shows a distinct cork, a poorly developed cork cambium, a narrow secondary cortex and a wide phloem (Fig. 1A).

The cork (Fig. 1C) is composed of 3-8 layers of suberised cells which are squarish or tangentially elongated. Their tangential walls are thicker than the undulating radial walls. These cells measure T, 34-46-61 μ ; R, 20-30-38 μ .

Cork cambium is represented by one or two layers of indistinct cells.

Secondary cortex is roughly divisible into outer 3-5 layers of rectangular cells measuring T, 30-35-44 μ ; R, 7-14-20 μ and a few layers of larger irregular polygonal or rounded cells representing the inner cortex. The number of layers constituting the inner cortex vary because of the formation of cork cambium at different levels in the cortex in different bark samples.

Some of the cortex cells contain prism crystals of calcium oxalate which are characteristically rhomboid with their obtuse angles truncated or projecting (Fig. 1C).

The younger bark shows no sclerenchyma in the cortex. In older thicker barks, stone cells may be seen singly or in isolated groups (Fig. 1D). In still thicker pieces of bark, the number and the size of the stone cells constituting each group is considerably increased. Their walls are much thickened and show distinct pores and striated lignification. Prism crystals may be seen occasionally in the lumen of some of the stone cells and more often in the parenchyma cells which immediately surround the stone cell groups. These stone cells measure T, 13-40-51 μ ; R, 24-36-41 μ . The innermost layer of secondary cortex merges imperceptibly into the outermost layers of phloem tissue.

The phloem can be roughly divided into an inner, middle and outer phloem. The inner phloem shows uniformly 1-2 cell wide straight medullary rays composed of somewhat radially elongated cells which measure T, 17-28-34 μ , R, 17-37-41 μ . The rays are 10-18 cell high in a tangential section and their number varies from 15-17 per mm. arc. The sieve tube tissue also occurs as straight radial strands 2-4 cell wide. It shows irregular parenchyma in which are scattered sieve tubes with clearly defined transparent sieve plates, companion cells, isolated poorly lignified fibres and latex vessels showing dense granular contents. The fibres in longitudinal section or in macerated preparations (Fig. 1F), show obtuse or blunt ends and a non-uniform thickness due to bulging and constriction of the wall at several places along the entire length. These fibres are also septate and have a length of 14,900-16,500-19,900 μ and a breadth of 21-37-51 μ . Some of the fibres show one or more lateral branches. The latex vessels are best seen in a macerated preparation (Fig. 1F) where they appear as long septate tubular structures filled with a dense granular mass. They are 18-24-27 μ in breadth.

The middle portion of the phloem (Fig. 1E) shows a sudden broadening of medullary rays giving a funnel shaped appearance. This causes the adjacent medullary rays and phloem strands to run obliquely. The broadened medullary ray cells also become irregular in outline compared to regular radially elongated cells of the inner portion of the medullary rays. The phloem elements in this region are similar to those described earlier.

The outer phloem presents a highly irregular appearance because the cells constituting the phloem and medullary rays become indistinguishable from each other and also from the cells of the secondary cortex. Some of the medullary rays which had broadened in the mid-phloem again

WRIGHTIA TINCTORIA BARK

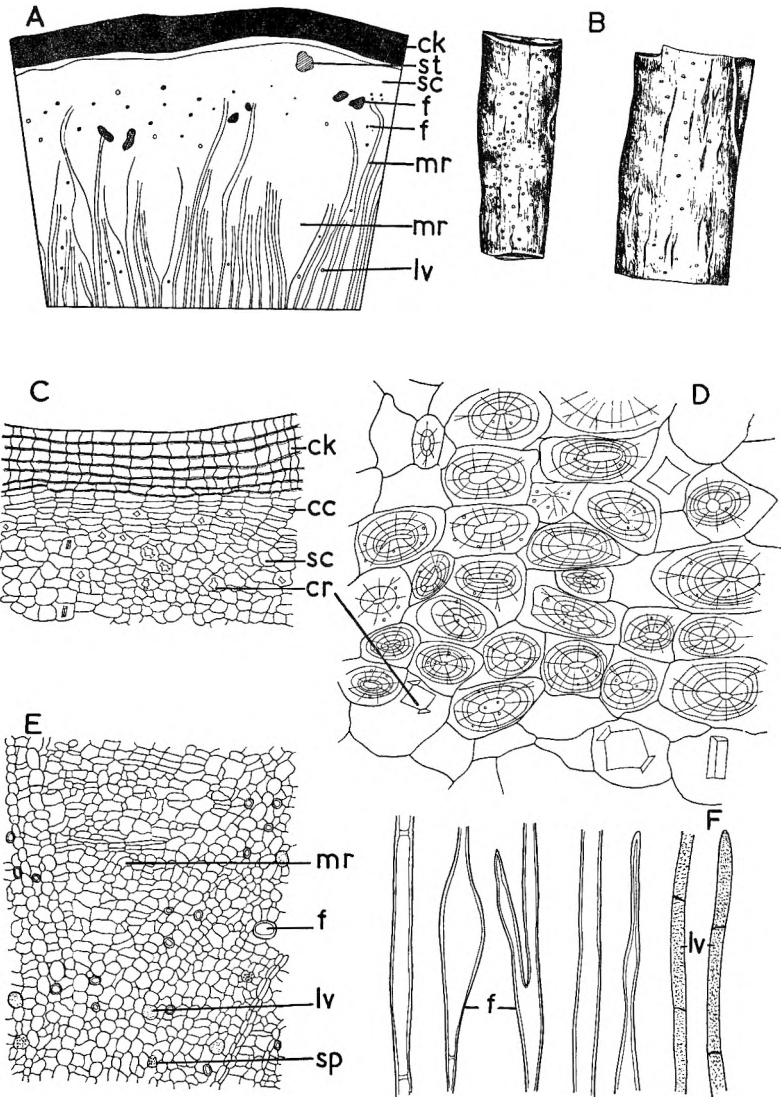


FIG. 1. A, General diagram of transverse section of bark of *Wrightia tinctoria*, $\times 9$, ck, cork; st, stone cell; f, fibre; mr, medullary ray; lv, latex vessel. B, Bark $\frac{1}{2}$ natural size. C, Transverse section showing cork and phelloderm $\times 28$, ck, cork; cc, cork cambium; sc, secondary cortex; cr, crystal. D, Transverse section showing group of stone cells $\times 216$. E, Transverse section showing mid-phloem $\times 62$, mr, medullary ray; f, fibre; lv, latex vessel; sp, sieve plate. F, Macerated preparation showing latex vessels and fibres, f, fibre; lv, latex vessel.

C. K. ATAL AND P. D. SETHI

converge, while others continue to broaden as they proceed outward. The rays also terminate at different levels in the outer phloem. Most of the phloem parenchyma in this region contains solitary prism crystals of calcium oxalate similar to those found in secondary cortex. The

TABLE I
FEATURES DISTINGUISHING *Wrightia tinctoria* FROM KURCHI

	<i>Holarrhona antidysenterica</i>	<i>Wrightia tinctoria</i>
<i>Macroscopy</i>		
Shape	Transverse or obliquely transverse raspings	Longitudinal channeled pieces
Size		
length	1—1.5 cm.	2—3 cm.
breadth	2—5 cm.	1—2.5 cm.
thickness	2—4 mm.	1—2 mm.
Outer surface colour and lenticels ..	Buff or reddish brown; prominent, circular or transversely elongated	Light grey small whitish circular
Inner surface	Rough and brown, often pieces of wood attached	Smooth and pale brown
Fracture	Short and granular	Tough and brittle
Taste	Extremely bitter and acrid	Bland
<i>Microscopy</i>		
Stone cells	Arranged in concentric tangential bands, only in phloem region, often show calcium oxalate crystals inside the cell, no striations and pores in the cells	Lesser in number, hardly any crystals inside the cell, only present in cortical region, being absent in the phloem. Distinct pores and striations can be seen in their walls
Pericyclic fibres	Non-lignified and present in early stages, getting peeled off in the mature bark	Absent in both young and mature bark
Phloem fibres	Absent	Present, showing constriction and bulging, varying from 14,900–19,900 μ in length
Phloem parenchyma	Polyhedral to more or less isodiametric	Irregular
Medullary rays	Mostly bi- or tri-seriate, becoming multiseriate up to 6 cell wide; some of the cells of medullary rays become thickened and lignified; 6–7 per mm. arc in the inner region	Mostly uniseriate, a few biseriate and 15–17 per mm. arc in the inner phloem
Calcium oxalate	Present in rosettes and prisms	Present as large prisms of characteristic shape
Latex	Present in cells of non-articulate type, the contents are cream coloured and somewhat transparent	Present in ducts of septate type, mostly in phloem region, the contents are granular and darker in colour
<i>Chemical</i>		
Alkaloid test with Mayer's reagent ..	Positive	Negative

sieve tubes and sieve plates in this region are not distinct. Latex vessels are scattered throughout this zone. The fibres occur both singly or in a group of 4–8 fibres in contrast to the inner and middle phloem, where they mostly occur singly.

WRIGHTIA TINCTORIA BARK

Differentiation from Kurchi Bark

The description of *W. tinctoria* bark reveals a number of differences from the authentic bark of *H. antidysenterica* (Prasad and Kaul, 1956). These points of distinction are outlined in Table I.

Acknowledgement. The authors are indebted to Dr. K. N. Gaid, Head of the Department for his encouragement during the course of this investigation.

REFERENCES

- Chopra, R. N. (1958). *Indigenous Drugs of India*, 2nd ed., p. 342, Calcutta; N. U. Dhir and Sons.
- Kirtikar, K. R. and Basu, B. D. (1953). *Indian Medicinal Plants*, 2nd ed.; Vol. 2, p. 1582, Allahabad, L. M. Basu.
- Pharmacopoeia of India* (1955). 1st ed., p. 358, Dehli; Manager of Publication.
- Prasad, S. and Kaul, R. N. (1956). *Indian J. Pharm.*, **18**, 426-430.

THE OXIDATION OF ALDEHYDES IN AQUEOUS SOLUTIONS OF CETOMACROGOL

BY J. E. CARLESS AND A. G. MITCHELL*

From the Chelsea School of Pharmacy, Chelsea College of Science and Technology, London, S.W.3

Received November 2, 1961

The oxidation of emulsions and solutions of five paraffinic aldehydes in aqueous solutions of cetomacrogol was measured manometrically at 25°. The rate of oxidation depends on the saturation of the dispersion and not on the concentrations of aldehyde and cetomacrogol except in so far as these control saturation. A method of expressing saturation, applicable to both solutions and emulsions is proposed. Differences between the oxidation rates of emulsions containing aldehydes of different chain length are shown to depend mainly on the proportion of aldehyde in the disperse phase.

THE oxidation of oil-soluble vitamins solubilised by non-ionic surface-active agents was studied by Coles and Thomas (1952), Kern and Antoshkiw (1950) and Patel, Kumpta and Radhakrishna (1955). The reports on the stability of solubilised vitamins are conflicting. Carless and Nixon (1957, 1960) have shown that emulsions of methyl linoleate and benzaldehyde oxidise more readily than solutions; the surface-active agents used were cetomacrogol and potassium laurate. Essential oils are readily solubilised by non-ionic surface-active agents but there is little published information on their stability to atmospheric oxidation. Natural oils are complex materials and in the present work aldehydes of different chain length were used as simple reference compounds. Aldehydes are particularly suitable since their oxidation is conveniently fast and relatively uncomplicated by side reactions. Oxidation of aldehydes is known to proceed by a chain reaction similar to that of olefinic materials (Bawn and Williamson, 1951; Cooper and Melville, 1951; Ingles and Melville, 1953).

EXPERIMENTAL

Materials

Aldehydes. Aliphatic aldehydes in the series from n-hexanal to n-decanal were fractionally distilled under oxygen-free nitrogen at reduced pressure using an all-glass still of 18-20 theoretical plates, and the distillate protected from light. They were stored protected from light under nitrogen in flasks, from which samples could be removed under a stream of nitrogen. The purity of the aldehydes was checked by gas chromatography using a stationary phase of 30 per cent vaseline on celite. The C₇ to C₁₀ aldehydes produced single peaks but the hexanal distillate contained an impurity which corresponded to about

* Present address: Pharmaceutics Department, University of Malaya in Singapore, Singapore 3.

OXIDATION OF ALDEHYDES

10 per cent of 2-methylpentanal. The physical characters of the aldehydes are given in Table I.

TABLE I
PHYSICAL DATA

Aldehyde	Boiling point	Refractive index 20°
n-Hexanal	30-31° at 19 mm. 129° at 758 mm.	1.407 ₀
n-Heptanal	52-53° at 17 mm. 153° at 759 mm.	1.413 ₈
n-Octanal	69-70° at 18 mm. 170° at 758 mm.	1.419 ₃
n-Nonanal	60° at 2 mm. 185° at 758 mm.	1.423 ₃
n-Decanal	60-61° at 0.7 mm. 208° at 755 mm.	1.428 ₃

Cetomacrogol 1000 B.P.C. A commercial product "Texofor AIP" was a creamy white amorphous solid m.p. 44.5-46° and refractive index of 1.451₃ at 60°. The hydroxyl number (B.P.C. 1959 method) was 41.1. From elemental analysis the ratio of C:H:O was 59.1:10.4:30.5. Assuming a molecular weight of 1,300, stock solutions were prepared, stored in the dark and diluted as required. The critical micelle concentration determined from surface tension measurements was found to be 0.0006 per cent w/v.

Methods

Measurement of solubility of aldehydes in water. Excess 2,4-dinitrophenylhydrazine reagent was added to a saturated solution of aldehyde in water at 25°, and the precipitate of 2,4-dinitrophenylhydrazone collected and assayed by the method of Monty (1958).

Measurement of solubility of aldehydes in cetomacrogol. Known amounts of aldehyde were weighed into a series of ampoules containing the required concentration of cetomacrogol. The ampoules were sealed and rotated at 25° overnight. The end point was estimated visually and taken as the mean between an oversaturated and an undersaturated dispersion.

Measurement of oxidation. A Warburg apparatus was used as described elsewhere (Carless and Nixon, 1957). Measurements of oxygen uptake were made at a temperature of 25° under conditions of uniform illumination and a shaking rate of 109 strokes/min. At shaking rates above 73 strokes/min. the oxygen uptake was independent of the agitation for 1, 2 or 4 ml. samples. Dispersions of aldehyde in cetomacrogol were made under standard conditions and 1, 2 or 4 ml. samples used in the reaction flasks. 1×10^{-4} M cupric sulphate was included to "swamp" any catalytic impurities. Under these conditions, rates of oxidation were reproducible within ± 6 per cent. After each determination the reaction flasks were washed in hot water, rinsed with acetone, ether, acetone and dried, heated in concentrated sulphuric acid for 1 or 2 hr., and washed 20 times in tap water, twice in distilled water and dried in an oven.

RESULTS

Solubilities of the aldehydes. The solubility of the aldehydes in water and in solutions of cetomacrogol is shown in Table II. Solubility curves are shown in Fig. 1.

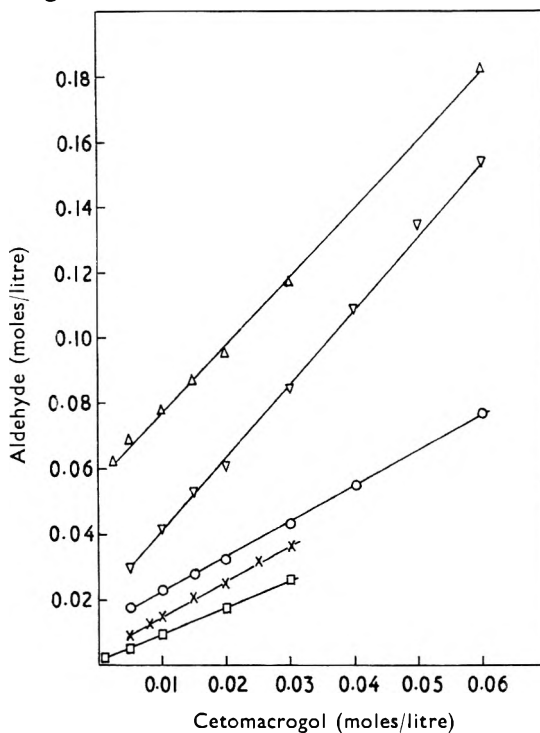


FIG. 1. Solubility curves of aliphatic aldehydes in cetomacrogol solution at 25°. Δ hexanal; ∇ heptanal; \circ octanal; \times nonanal; \square decanal.

Oxidation of the aldehydes in water. No measurable amount of oxygen uptake was detected even by the most water-soluble aldehyde unless present in excess of its solubility as a suspension. The oxidation

TABLE II

SOLUBILITIES OF NORMAL ALIPHATIC ALDEHYDES IN SOLUTIONS OF CETOMACROGOL AND WATER AT 25°

Cetomacrogol molar	Molar concentration of aldehyde				
	n-Hexanal	n-Heptanal	n-Octanal	n-Nonanal	n-Decanal
0	0.009	0.002	0.001	0.0002	0.00009
0.0010	0.059	—	—	—	0.0026
0.0020	—	—	—	0.0049	0.0030
0.0025	0.062	—	—	—	—
0.005	0.069	0.030	0.018	0.0089	0.0051
0.008	—	—	—	0.013	0.0079
0.010	0.078	0.042	0.023	0.015	0.0093
0.015	0.087	0.053	0.028	0.021	—
0.020	0.096	0.061	0.032	0.025	0.017
0.03	0.117	0.085	0.045	0.037	0.026
0.04	—	0.109	0.055	—	—
0.06	0.183	0.154	0.073	—	—

OXIDATION OF ALDEHYDES

rate of suspended aldehyde increased with concentration although it was difficult to obtain concordant results. The variation arises because the aldehyde forms pools on the surface of the water instead of remaining in discrete droplets.

Oxidation of aldehydes in organic solvents. The oxidation rates of the aldehydes dissolved in n-butyl laurate and isopropyl myristate, respectively are shown in Fig. 2. In any one solvent the rates of oxidation of the aldehydes could be fitted to a common rate curve, indicating that there was no fundamental difference between the individual aldehydes.

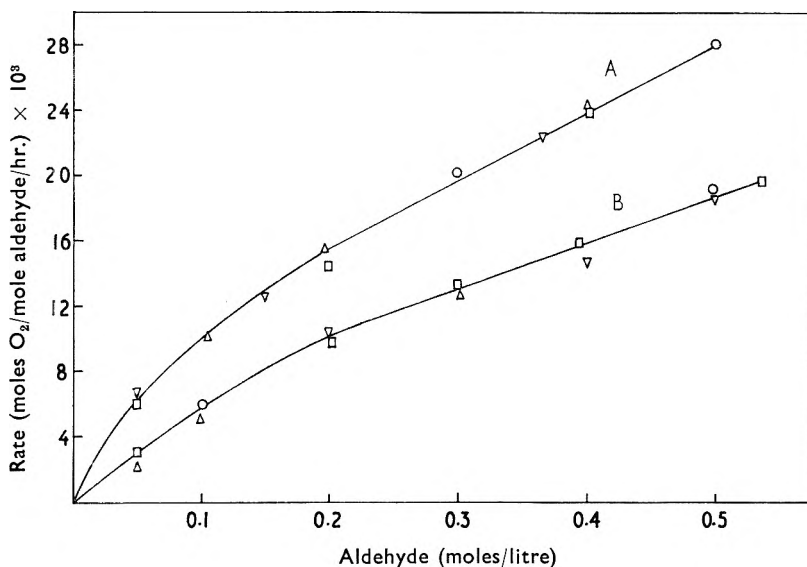


FIG. 2. Oxidation of aldehydes in organic solvents. A, isopropyl myristate. B, butyl laurate. Catalyst: 1×10^{-4} M cupric stearate. Δ hexanal; ∇ heptanal; \circ octanal; \square decanal.

Oxidation of aldehydes in cetomacrogol solutions. The rate of oxidation was dependent on both aldehyde and cetomacrogol concentrations. No induction period was observed but depending on chain length and concentration of aldehyde; there was a variable initial period during which the oxidation uptake progressively increased until a steady rate was reached. All rates of oxidation were measured under steady rate conditions.

Effect of aldehyde concentration on oxidation rate. By keeping the cetomacrol concentration constant and adding increasing amounts of aldehyde it was possible to produce dispersions ranging from solutions to emulsions. The oxidation rates, calculated as moles oxygen per litre of dispersion, are shown in Fig. 3. A change in the slope of the rate curve occurs when the aldehyde is increased beyond its solubility limit and emulsion droplets separate. The oxidation rate of emulsions was

directly proportional to the concentration of aldehyde. The proportionality coefficient was the same for emulsions of hexanal, octanal and decanal but different for nonanal and heptanal.

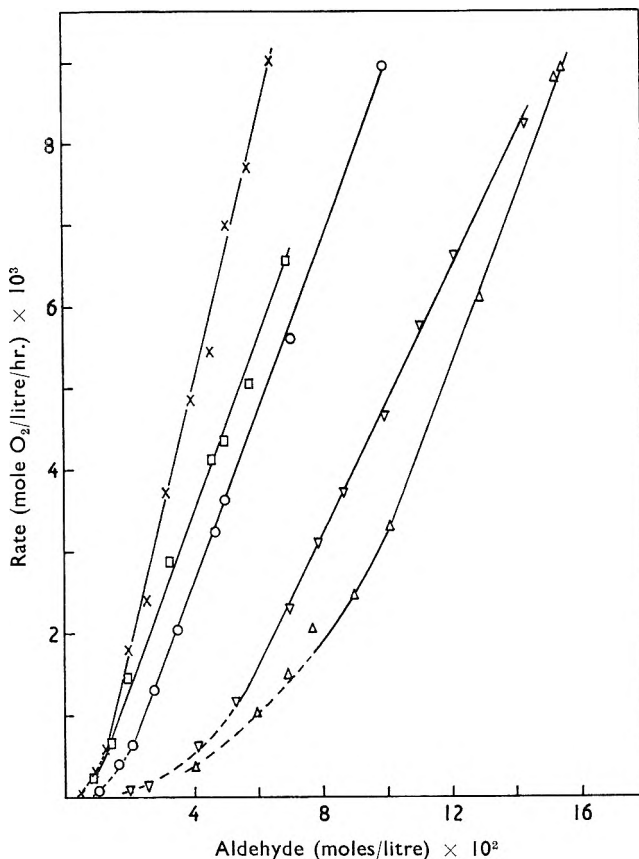


FIG. 3. Oxidation rate of aldehydes dispersed in cetomacrogol solutions showing the effect of variation of aldehyde concentration. Cetomacrogol concentration 0.01 M, temperature 25°. Δ hexanal; ∇ heptanal; \circ octanal; \times nonanal; \square decanal. - - - Solution; — emulsion.

The effect of cetomacrogol concentration on oxidation rate. By keeping the aldehyde concentration constant and altering the concentration of cetomacrogol, dispersions were produced ranging from emulsions at low concentrations of cetomacrogol, to solutions at higher concentrations. The oxidation rates of these dispersions is shown in Fig. 4. The oxidation rate of emulsions was inversely proportional to the cetomacrogol concentration at low concentrations and was further reduced as the solubilised state was approached. At any given concentration of cetomacrogol, the oxidation rate increased with chain length.

OXIDATION OF ALDEHYDES

The relationship between oxidation and "Saturation Ratio." Saturated solutions of the aldehydes in different concentrations of cetomacrogol in water were prepared. In spite of widely different concentrations of aldehyde and cetomacrogol, the rates of oxidation per mole of aldehyde

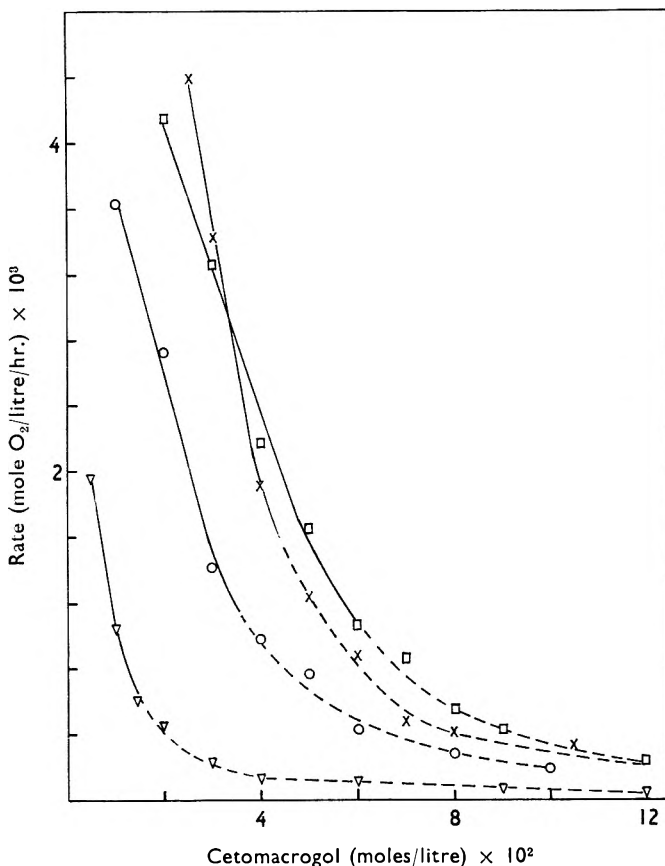


FIG. 4. The influence of cetomacrogol concentration on the rate of oxidation of aldehydes at 25°. Aldehyde concentration 0.05 M. Δ heptanal; \circ octanal; \times nonanal; \square decanal. - - - Solution; — emulsion.

were the same. Similarly, half saturated solutions oxidised at a constant rate. The degree of saturation of the dispersion was expressed as a saturation ratio R , in which

$$R = \frac{X}{\bar{Y}}$$

where X is the concentration of aldehyde present and Y is the concentration of aldehyde in a saturated solution. For a saturated solution $R = 1$, while for an emulsion $R > 1$ and for a solution $R < 1$. Oxidation

rates of dispersions at different degrees of saturation are shown in Table III and Fig. 5. At any given saturation ratio the oxidation rate of each aldehyde is almost constant and the even chain length aldehydes show closely similar oxidation rates when compared at the same value of R. The data presented in Table IV were derived from Fig. 4 in conjunction with the solubility curves of octanal and decanal in cetomacrogol. The oxidation rates of different aldehydes at the same

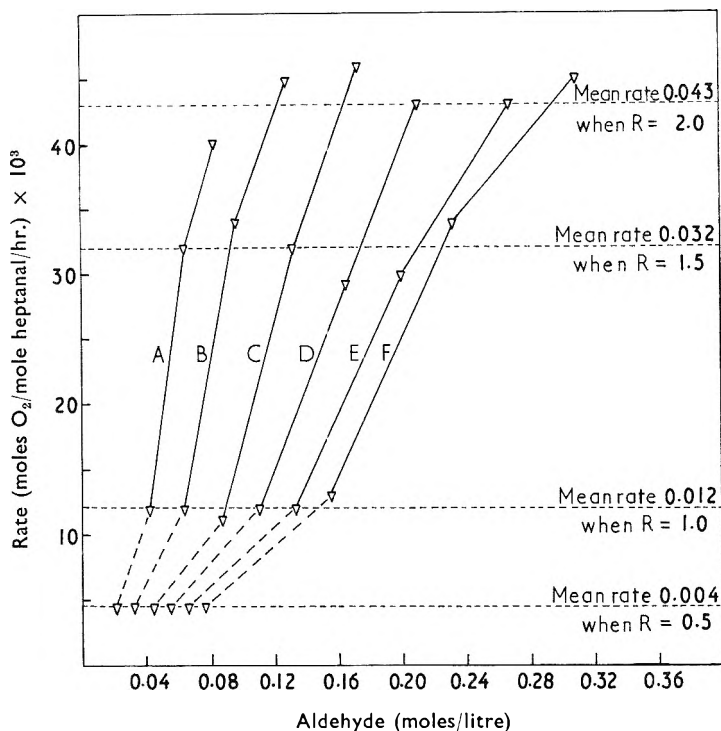


FIG. 5. Dependence of oxidation rate of heptanal in cetomacrogol solutions on the Saturation Ratio. Cetomacrogol solutions, A 0.01 M; B 0.02 M; C 0.03 M; D 0.04 M; E 0.05 M; F 0.06 M. ----- solution; ——— emulsion.

saturation ratio are again equal. Where necessary for calculation purposes, the solubility curves were extrapolated above the experimentally determined points.

DISCUSSION

The mechanism of oxidation of aldehydes in aqueous solutions of cetomacrogol is complex since reactions may occur at several different sites in the system. The possible sites of reaction are the true aqueous phase, the emulsion droplets, the micellar "pseudo-phase," or the emulsion droplet-water interface. The oxidation rates of equimolar amounts of suspended hexanal and decanal in water were approximately

OXIDATION OF ALDEHYDES

TABLE III

DEPENDENCE OF OXIDATION RATE ON THE SATURATION RATIO (R) FOR DISPERSIONS OF THE NORMAL ALIPHATIC ALDEHYDES IN CETOMACROGOL

Cetomacrogol molar	R	Aldehyde molar				Rate (moles O ₂ /mole aldehyde/hr.) × 10 ³			
		C ₆	C ₇	C ₈	C ₁₀	C ₆	C ₇	C ₈	C ₁₀
0.01	0.5	—	0.021	0.011	—	—	4	6	—
0.02	0.5	—	0.032	0.017	—	—	4	6	—
0.03	0.5	—	0.044	0.022	—	—	4	7	—
0.04	0.5	—	0.055	0.026	—	—	4	7	—
0.05	0.5	—	0.066	0.032	—	—	4	6	—
0.06	0.5	—	0.077	0.037	—	—	4	6	—
0.01	1.0	0.077	0.042	0.023	0.009	26	12	28	24
0.02	1.0	0.095	0.064	0.034	0.017	26	12	24	19
0.03	1.0	0.119	0.087	0.044	0.025	23	11	26	23
0.04	1.0	0.141	0.110	0.053	0.033	26	12	24	19
0.05	1.0	0.161	0.133	0.065	0.042	23	12	23	22
0.06	1.0	0.183	0.155	0.073	0.051	23	13	22	21
0.01	1.5	0.116	0.064	0.035	0.014	41	32	54	48
0.02	1.5	0.145	0.096	0.050	0.027	50	34	51	53
0.03	1.5	0.174	0.131	0.065	0.039	51	32	52	54
0.04	1.5	0.197	0.164	0.081	0.051	48	29	51	53
0.05	1.5	0.232	0.199	0.096	0.065	46	30	48	50
0.06	1.5	0.264	0.231	0.111	0.077	46	34	43	42

the same. Hexanal suspensions will contain about 100 times more aldehyde in solution than decanal suspensions, Table II, and it is therefore unlikely that the amount of aldehyde in the true aqueous phase influences the reaction.

It is evident that emulsion droplets provide "units" of high aldehyde concentration in which oxidation can proceed rapidly. The fall in rate associated with the increase in cetomacrogol concentration (Fig. 4), is simply the result of transfer of aldehyde from emulsion droplets to micelles. The subdivision of aldehyde into smaller "units," that is, micelles, reduces the local concentration of aldehyde.

For any given concentration of cetomacrogol, the oxidation rate of solutions and emulsions increases on ascending the homologous series (Figs. 3 and 4). For emulsions this can be explained on the basis of the increased aldehyde in the emulsion droplets. The following calculation illustrates this point: the oxidation rates of 0.05M decanal and 0.05M

TABLE IV

RELATION BETWEEN OXIDATION RATE AND SATURATION RATIO FOR 0.05M SOLUTIONS OF OCTANAL AND DECANAL IN CETOMACROGOL SOLUTION

R	Concentration <i>c</i> of cetomacrogol necessary to produce stated R		Rate* (moles O ₂ /mole aldehyde/hr.) × 10 ³ at concentration <i>c</i>	
	Octanal	Decanal	Octanal	Decanal
0.5	0.086	0.12	5	5
0.6	0.069	0.10	7	6
0.8	0.049	0.0735	14	14
0.9	0.042	0.0655	18	18
1.0	0.0365	0.590	22	23
1.2	0.0280	0.049	34	33
1.5	0.020	0.039	51	50
2.0	0.0115	0.029	69	68

* Determined from data in Fig. 4

octanal in 0.02M cetomacrogol are 4.15×10^{-3} and 2.7×10^{-3} moles O_2 /litre/hr. respectively (Fig. 4), i.e., a difference of 1.45×10^{-3} . From the solubility data, 0.032M decanal and 0.017M octanal will be present in the respective emulsion phases, i.e., a difference of 0.015M. From Fig. 3, the addition of 0.015M octanal or decanal to an emulsion will increase the rate by 1.5×10^{-3} , which agrees closely with the observed difference of 1.45×10^{-3} .

The oxidation rates of equimolar amounts of solubilised aldehyde increases with chain length of the aldehyde (Fig. 4). Such dispersions are uncomplicated by the presence of emulsion droplets and it is, therefore, possible to consider the role of the micelle in oxidation. At a constant cetomacrogol concentration it is reasonable to expect that a constant number of micelles are present and thus each will contain the same number of aldehyde molecules. The amount of aldehyde in the "true aqueous phase" is small compared with that in the micellar phase and will contribute little to the overall rate of reaction. It is generally accepted that the polarity of a solubilisate affects the site of solubilisation; polar compounds are solubilised in the outer hydrophilic region of the micelle while non-polar compounds are solubilised in the hydrocarbon-like interior (Alexander and Johnson, 1949). On this basis one would expect the longer chain aldehydes to be concentrated towards the centre of the micelle. The oxidation rate increases as collision between reactive species will become more frequent.

The change from emulsion to solution is accompanied by an enormous increase in the interfacial area of "exposed" aldehyde. However, the rate of reaction decreases on passing from the emulsified to the solubilised states, Figs. 3 and 4. Moreover the change in rate over this range is a gradual one. Hence it is unlikely that the reaction at the emulsion droplet interface is a controlling factor. This aspect has been discussed by Carless and Nixon (1957).

Although the results obtained in this present work do not provide evidence for the mechanism of reaction, they show that the oxidation rate can be related with the degree of saturation of the dispersion and indicate the reaction site. The Saturation Ratio concept is a measure of the chemical potential of the dispersion and provides a convenient means of defining its physical state. The extent of saturation may be altered by varying either the concentration of aldehyde or concentration of cetomacrogol, but at any one Saturation Ratio, the rate of oxidation per mole of aldehyde, remains constant. Moreover the oxidation rates of the C_6 , C_8 and C_{10} aldehydes are about the same when measured at the same Saturation Ratio. From Tables III and IV it is evident that the relation between saturation and oxidation holds for emulsions as well as for solutions.

The oxidative behaviour of aldehydes dispersed in cetomacrogol solutions differ greatly from their behaviour in inert organic solvents. The concentration of aldehyde in cetomacrogol solutions can be increased without increasing the rate of oxidation per mole, provided that the Saturation Ratio is unchanged. This contrasts with the oxidation

OXIDATION OF ALDEHYDES

of aldehydes molecularly dispersed in n-butyl laurate or isopropyl myristate (Fig. 3), where the rate shows the expected increase with the concentration.

REFERENCES

- Alexander, A. E. and Johnson, P. (1949). *Colloid Science*, p. 686, Oxford University Press.
- Bawn, C. E. H. and Williamson, J. B. (1951). *Trans. Faraday Soc.*, **47**, 721-734.
- Carless, J. E. and Nixon, J. R. (1957). *J. Pharm. Pharmacol.*, **9**, 963-972.
- Carless, J. E. and Nixon, J. R. (1960). *Ibid.*, **12**, 348-359.
- Coles, C. L. J. and Thomas, D. F. W. (1952). *Ibid.*, **4**, 898-901.
- Cooper, H. R. and Melville, H. W. (1951). *J. chem. Soc.*, 1984-2002.
- Ingles, T. A. and Melville, H. W. (1953). *Proc. Roy. Soc., A*, **218**, 175-189.
- Kern, C. J. and Antoshikiw, T. (1950). *Industr. Engn Chem.*, **42**, 709-713.
- Monty, K. J. (1958). *Analyt. Chem.*, **30**, 1350-1352.
- Patel, F. S. M., Kumpta, U. S. and Radhakrishna, M. V. (1955). *J. Sci. Industr. Research (India)*, **14C**, 17-21.

A NOTE ON THE PAPER CHROMATOGRAPHIC SEPARATION OF CODEINE, MORPHINE AND NALORPHINE

BY HAROLD V. STREET

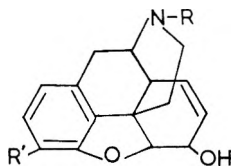
From the Department of Forensic Medicine, University of Edinburgh

Received August 29, 1961

Codeine has been separated from a mixture of codeine, morphine and nalorphine on Whatman ET20 ion-exchange paper, in 5 min. A mixture of morphine and nalorphine has been separated in 90 min. by horizontal circular reversed phase paper chromatography. Using ascending chromatography at 86° on tributyrin-treated paper, an unequivocal resolution of a mixture of morphine, nalorphine and codeine has been achieved within 20 min.

IN the treatment of acute morphine poisoning, nalorphine is often used as a specific antidote. Curry (1959) isolated morphine and nalorphine from the intestinal contents of an 18 months child who died after the ingestion of 10 mg. of morphine sulphate. A total of 20 mg. of nalorphine was given during treatment.

Furthermore, according to Stewart and Stolman (1960) some 5-17 per cent of a 30 mg. injected dose of codeine is excreted in the urine of man as morphine. It follows that the toxicologist may occasionally be faced with the problem of differentiating these three closely related compounds.



Morphine	R = Me; R' = OH
Codeine	R = Me; R' = OCH ₃
Nalorphine	R = CH ₂ CH:CH ₂ ; R' = OH

In an excellent chapter on Alkaloids, Farmilo and Genest (1961) give a list of twelve solvent systems for the paper chromatographic separation of morphine and codeine. Nalorphine is mentioned in two of these systems, but in neither instance is it separated from codeine. This I have set out to do, in the first instance using the pure alkaloids in admixture.

With ascending paper chromatography on modified cellulose anion-exchange paper, development with ammonia solution and the three pure crystalline alkaloids separately and in admixture I have been able to separate codeine from the mixture in 5 min.

Morphine and nalorphine are not resolved by this procedure but by horizontal circular paper chromatography on paper treated with glycerol monoricinoleate and dried, and development with a phosphate buffer, nalorphine could be separated from codeine or morphine within 90 min.

A clear resolution of all three compounds in admixture was effected

SEPARATION OF CODEINE, MORPHINE AND NALORPHINE

using the ascending technique, and paper treated with tributyrin, dried, and developed with the phosphate buffer at a temperature of 86° for 20 min. Individual alkaloids were run on the same paper for comparison.

An iodoplatinate reagent was used to identify the alkaloids in the latter two procedures, but could not be used with anion exchange paper with which it reacted. Therefore the spots in the ion-exchange paper were examined under light of a selected wavelength.

EXPERIMENTAL

Ascending ion-exchange chromatography. Whatman ECTEOLA (ET20) modified cellulose anion-exchange paper sheets were cut into 4 in. × 4½ in. rectangles. The alkaloids were applied in 100 µg. amounts and the sheets were made into a cylinder and chromatographed in a suitable cylindrical glass jar using freshly prepared ammonia solution (0.2N). After 5 min. the paper was examined in light of wavelength 254 mµ in which the alkaloids show up as dark areas on a white faintly fluorescent background. Light blue fluorescent spots may also be seen, but these should be ignored as they are due to decomposition products of the alkaloids. The R_F value for codeine was 0.84. The other alkaloids remained at the origin.

Horizontal circular paper chromatography. Whatman No. 1 slotted (26.5 cm. diam.) papers were used with the apparatus described by Kawerau (1956). The papers were treated with glycerol monoricinoleate (10 per cent in acetone) and air dried. The alkaloids were applied in 100 µg. amounts and the papers developed with M/15 phosphate buffer, pH 7.4, for 90 min. The papers were dried in warm air and then dipped into iodoplatinate reagent prepared by mixing together 10 per cent platinum chloride (1 ml.) and 4 per cent potassium iodide (25 ml.) and diluting to 50 ml. with distilled water. The alkaloids appear as purplish black spots on a brown background. R_F values were: codeine 0.58, morphine 0.57, nalorphine 0.41.

Ascending reversed phase paper chromatography. Whatman No. 1 papers were treated with tributyrin (10 per cent in acetone) and air dried. The alkaloids separately and in admixture in amounts of 100 µg. were applied to the paper, which was then chromatographed in a suitable cylindrical jar in an incubator at 86° using the M/15 phosphate buffer for development. Resolution of the three alkaloids was complete in 15 min. but an even better separation is obtained after a further 5 min. The paper was dipped in the iodoplatinate reagent and the alkaloids identified as described in the previous paragraph. R_F values were: codeine 0.62, morphine 0.80, nalorphine 0.38.

REFERENCES

- Curry, A. S. (1959). *Methods of Biochemical Analysis*, vol. 7, edited by Glick, London: Interscience.
- Farmilo, C. G. and Genest, K. (1961). *Toxicology*, vol. 2, edited by Stewart, C. P. and Stolman, A. London: Academic Press.
- Kawerau, E. (1956). *Chromatog. Methods*, 1, No. 2, October.
- Stewart, C. P. and Stolman, A. (1960). *Toxicology*, vol. 1, London: Academic Press.

A SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF PHENINDIONE

BY B. C. BOSE AND R. VIJAYVARGIYA

From the Department of Pharmacology, M.G.M. Medical College, Indore, India, M.P.

Received August 4, 1961

A spectrophotometric method for the estimation of phenindione in pharmaceutical preparations and body fluids and tissues is described. It has a greater sensitivity than the existing British Pharmacopoeia method. Advantage has been taken of the solubility of the drug in toluene and the sensitivity of measurement has been found to be enhanced by the addition of alcoholic potassium hydroxide solution. This method accurately estimates 1–2 mg. of the substance as opposed to the 300 mg. required for the B.P. method. Further, as little as 2.5 $\mu\text{g./ml.}$ of the substance can be detected in biological fluids after eliminating interfering substances.

FEW methods are available for the estimation of phenindione in pharmaceutical preparations. The B.P. method, developed by Sharp (1955), involves bromination followed by iodometric titration. But this requires at least 150 mg. of the substance. A spectrophotometric method, in which the substance is dissolved in aqueous potassium hydroxide solution and the extinction measured at 279 $m\mu$, has also been described (Council on Pharmacy and Chemistry of the American Medical Association, 1953). This again is not very sensitive, and cannot be used for the estimation of the drug in biological fluids.

EXPERIMENTAL

A Beckman Spectrophotometer Model Du with 1 cm. standard silica cells was used. Of several solvents initially investigated, toluene was considered to be the most suitable, as although it was found to be less sensitive than some of the other solvents spectrophotometrically, it extracted phenindione from aqueous medium after acidification.

The sensitivity of measurement was found to be greatly increased if a mixture of toluene and 0.05N alcoholic potassium hydroxide was used. This gives a maximum extinction at 288 $m\mu$. Taking known concentrations of the compound in 2 ml. of toluene and adding 3 ml. of 0.05N alcoholic potassium hydroxide solution and measuring the extinction at 288 $m\mu$ gave a linear relation.

Conc./ $\mu\text{g./ml.}$	1	2	4	6	8	10	15	20
Extinction	0.032	0.060	0.104	0.155	0.202	0.256	0.365	0.480

Estimation in Commercial Samples

Tablets of phenindione were weighed, powdered, and extracted in a mortar with toluene and the extract filtered, and diluted to a final strength of 10 to 20 $\mu\text{g./ml.}$ Phenindione as powder, was dissolved directly in toluene and the concentration adjusted to the same level.

The results of the estimations are : 10 mg. of powder gave a recovery of 10.2 mg. ; three 50 mg. samples from tablets gave recoveries of 51.2, 51.5 and 51.5.

It can be seen that binding material present in the tablets did not interfere with the analysis which showed an error of 2-3 per cent.

The method was extended to the estimation of phenindione in biological materials.

Estimation of Phenindione in Tissues and Body Fluids

Phenindione can be extracted from aqueous medium with toluene after acidification. The optimum pH at which quantitative recovery can be obtained was found to lie between pH 1 and 2.

Proteins are removed with trichloroacetic acid at this optimum pH, but as interfering substances cannot be completely eliminated by the above treatment, the toluene extract must be further extracted with aqueous potassium hydroxide which removes phenindione quantitatively. After acidification of the alkaline solution the drug was re-extracted with toluene for final estimation (Table I).

TABLE I

SHOWING RECOVERY PER CENT OF PHENINDIONE AFTER TREATMENT WITH TRICHLOROACETIC ACID, TOLUENE AND AQUEOUS POTASSIUM HYDROXIDE

Phenindione content μg.	Phenindione detected μg.	Deviation per cent
200	205	+ 2.5
400	385	- 3.5
500	510	+ 2.0
750	720	- 4.0
1000	950	- 5.0

From these observations it will be seen that treatment with acid and alkali followed by toluene after each did not interfere with the estimation, which had an error of ± 5 per cent.

Estimation of Phenindione in Liver Homogenates and Blood

The homogenised liver from freshly killed rats, or human serum, was used. Known quantities of the drug were added to samples and the final concentration adjusted to 10 μg./ml.

3-5 ml. of the samples were taken in a centrifuge tube and diluted to 10 ml. with water. 2.0 ml. of 10 per cent trichloroacetic acid was added and mixed thoroughly to precipitate the proteins. 5 ml. of toluene was then added, and the tube shaken for 2 min., then centrifuged. From the toluene layer 2.5 ml. was transferred to a second centrifuge tube containing 2.5 ml. of 0.1N aqueous potassium hydroxide solution. This tube was shaken thoroughly for 1 min. and centrifuged. The toluene layer was discarded and 2 ml. of alkaline extract were transferred to a third centrifuge tube, to which 0.5 ml. of 1 per cent hydrochloric acid was added. The pH of the solution was then adjusted to 1-2. To the mixture, 5 ml. of toluene was added and the tube shaken for 1 min. After centrifugation 2 ml. of the toluene layer solution were transferred to a test tube.

ESTIMATION OF PHENINDIONE

3 ml. of 0.05N alcoholic potassium hydroxide was added and mixed, and the estimation was made at 288 m μ , keeping the slit width at 0.88 mm. The findings are shown in Table II.

TABLE II
RECOVERY PER CENT OF KNOWN QUANTITIES OF PHENINDIONE FROM LIVER HOMOGENATE AND BLOOD SERUM

Liver homogenate			Serum		
Phenindione added μ g.	Phenindione detected μ g.	Deviation per cent	Phenindione added μ g.	Phenindione detected μ g.	Deviation per cent
100	102	+ 2.0	100	97	- 3.0
200	210	+ 5.0	200	210	+ 5.0
300	290	- 3.3	300	315	+ 5.0
400	380	- 5.0	400	385	- 3.75

REFERENCES

- British Pharmacopoeia* (1958). 479.
 Council on Pharmacy and Chemistry of the American Medical Association (1953).
J. Amer. med. Ass., **152**, 142.
 Sharp, L. K. (1955). *J. Pharm. Pharmacol.*, **7**, 177-182.

NEW APPARATUS

APPARATUS FOR TESTING THE RESISTANCE TO WET HEAT OF BACTERIAL SPORES IN PAPER CARRIERS

BY A. M. COOK AND M. R. W. BROWN

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

Received November 30, 1961

THE purpose of this report is to give details of an apparatus mentioned in a previous communication from this Department (Cook and Brown, 1960).

Construction

The apparatus consists essentially of an autoclave which has been modified so that spore impregnated paper discs may be introduced into the heated autoclave without causing loss of pressure.

Six holes were drilled into the lid and into each was fitted a cylindrical brass plunger $7\frac{1}{2}$ in. long and $6/10$ in. in diameter, together with sleeve,

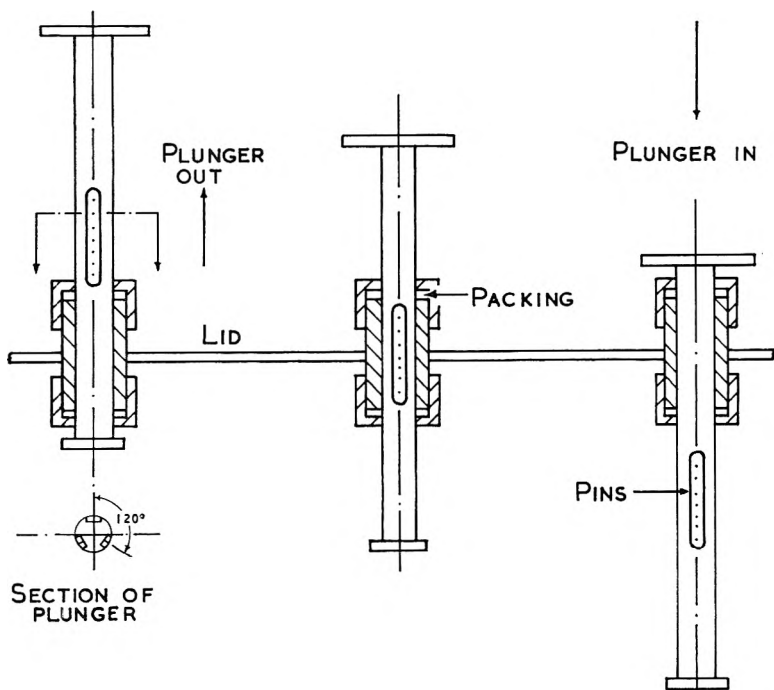


FIG. 1. Modified lid of autoclave with plungers.

locking and gland nuts. Three equidistant channels were cut longitudinally into the barrels and down the centre of each channel was embedded a row of seven stainless steel pins. These pins were designed to support

the paper discs so that they do not project beyond the surface of the barrel. The length of the channel is less than that of the sleeve; this prevents escape of steam up the channel on entry into the autoclave. Graphited packing material, provided with the gland nut, prevents pressure loss while the barrel is inside the apparatus (see Fig. 1).

A "T" piece screwed into the top of the barrel acts as a handle to facilitate rapid entry and withdrawal. A circular brass disc, of greater diameter than that of the barrel, was screwed on to the bottom of each plunger to prevent the plunger being completely withdrawn from the autoclave. A thermocouple was included by drilling a groove longitudinally down the surface of one of the plungers to carry the wires which terminate in a bimetallic strip which replaces one of the pins. The groove was then packed with a thermostable filler. As well as this thermocouple, the apparatus is fitted with a thermometer and a pressure gauge.

Use of Apparatus

The apparatus is intended for use with "Antibiotic Assay paper discs"* but other kinds of carriers may be used. The spore impregnated paper carriers, previously perforated, are fitted separately to the pins with forceps. When the plunger is loaded the discs are brought into instantaneous contact with steam at constant temperature by pushing the barrel down into the heated autoclave. After a time interval measured by a stop watch the plunger is withdrawn from the steam. Immediately afterwards the paper carriers are aseptically transferred to the recovery medium and incubated.

Acknowledgment. We wish to acknowledge the excellent technical assistance given by Mr. J. Deer.

REFERENCE

Cook, A. M. and Brown, M. R. W. (1960). *J. Pharm. Pharmacol., Suppl.*, **12**, 116T-118T.

* Whatman Antibiotic Assay Discs, W. and R. Balston, Ltd., obtained from H. Reeve Angel & Co. Ltd., 9, Bridewell Place, London, E.C.4.

BOOK REVIEW

AN INTRODUCTION TO THE MATHEMATICS OF MEDICINE AND BIOLOGY. By J. G. Defares and I. N. Sneddon. Pp. xii + 663 (including Index). North-Holland Publishing Company, Amsterdam, 1960. 85s.

This book has been produced primarily for the research worker in the biological sciences and for the research minded clinician, it is written on the assumption that most readers have ceased the active study of mathematics. The theory of statistics is not treated although the mathematics required has been included.

The book opens with a chapter on algebraic preliminaries, which should not cause any reader too much difficulty; this deals with number systems, indices, logarithms, series, binomial theorem, approximations and partial fractions. Problems to which answers are given are contained in all chapters.

The second chapter concerns functions of a single variable; the relationship between algebraic functions and graphs is discussed fully. The trigonometrical functions are introduced together with the graphs of these functions. Many examples are drawn from physiological and medical research.

Chapter three is entitled 'limits and derivatives' and provides the foundation for differential calculus. The treatment is mainly formal but a few biological examples are given. Chapter four develops the rules for algebraic integration. Chapter five introduces integration as a geometrical concept of area and then proceeds to algebraic integration. The treatment is clearly set out and is thorough, perhaps a little too thorough for the purpose for which the book is written. Chapter six deals with logarithms and exponential functions and has an interesting section on the applications of these functions in biology.

Chapter seven on techniques of integration is long and comprehensive, dealing with such methods as successive reduction of integrals and the use of gamma functions. The integrals associated with the theory of statistics are discussed, and there are sections on the Laplace transform and on the use of tables of integrals.

Chapter eight deals with functions of more than one variable. It is mainly concerned with partial derivatives and illustrates their application to the calculation of small errors arising from several sources and to the theory of thermodynamics. There is a section on double integration and also a discussion on the meaning of entropy as employed in cybernetics.

Chapter nine is on differential equations and is again comprehensive and advanced. Higher degree equations and symbolic operators are treated and there are sections on partial differential equations such as the wave and diffusion equations. The use of Fourier series and other methods for solving these equations, are described.

In the final chapter which is entitled "further applications to medicine and biology", the differential equations arising in the consideration of topics such as the form of the arterial pulse, the uptake of K^{42} by human erythrocytes, the growth of isolated populations, the oxygen debt, forced and damped oscillations, the time course of pupil contractions during illumination and theories of nervous excitation, are considered.

There is an appendix on determinants which defines them and develops some of their properties without, however, illustrating their application.

Altogether this is a lucidly written advanced textbook in mathematics and it is copiously illustrated with biological and medical examples. The reader who works his way through this book will be well prepared to interpret experimental results in quantitative biology. The book helps to show the way in which courses in mathematics for biology should be developed so as to become a more important part of the training of students in biology and medicine.

L. SAUNDERS.

LETTER TO THE EDITOR

A New Deflocculant and Protective Colloid for Barium Sulphate

SIR,—For use as a radiographic contrast medium in the gastrointestinal tract barium sulphate is usually presented as a concentrated suspension containing 100 per cent w/v or more of barium sulphate, which may or may not be diluted before use. In water and in solutions of many hydrophilic colloids such concentrations produce preparations lacking in pourability and other characteristics which allow easy manipulation and administration.

Satisfactory fluidity of concentrated suspensions may be achieved by deflocculating, and with barium sulphate it is well known that pastes can be made fluid by the addition of small quantities of suitable salts, for example, citrate. With the addition of suitable hydrocolloids, this procedure may allow the formation of concentrated suspensions satisfactory in flocculation, sedimentation rate and absence of claying on storage, but not necessarily satisfying other desirable criteria, such as easy dispersion in acid gastric juice containing mucin and absence of flocculation after dispersion in the gastric juice and during subsequent passage through the gut. With deflocculants like citrate used with the usual hydrocolloids to aid suspension, flocculation normally occurs immediately on pouring the suspension into an excess of dilute hydrochloric acid, dilute sodium chloride solutions or into acid gastric juice. Flocculation and gross clumping of the suspension is even more apparent when gastric mucin is present.

The efficiency of the preparation as an X-ray contrast medium will depend on the evenness and thickness of coverage afforded to the mucosa by the barium sulphate and the greater the flocculation which has occurred the less regular and less satisfactory the coverage is likely to be.

The very low solution viscosity of the sulphated polysaccharide, degraded carrageenan (Ebimar, Evans Medical Ltd.) (Anderson, 1961) and its negative charge, and the electrochemical properties of barium sulphate in aqueous suspension, suggested that degraded carrageenan should function as a useful deflocculant and protective colloid for barium sulphate and allow desirable fluidity in concentrated suspension. A suspension containing a small quantity of degraded carrageenan (for example, barium sulphate: degraded carrageenan ratio, 100:1) can be shown by microscopic and sedimentation volume methods not to flocculate on addition to dilute acid. Also, in conjunction with ghatti gum mucilage the degraded carrageenan appears to be capable of protecting the particles of barium sulphate from the much more potent flocculating effects of human acid gastric juice containing mucin. Although degraded carrageenan reacts with mucoprotein under certain conditions, it is not yet clear to what extent other factors are involved in the mechanism of the enhanced resistance to flocculation of the barium sulphate particles, and this is being studied further. Preliminary studies indicate that these properties are shared by certain other polyanions.

Studies of the effectiveness of suspensions of differing degrees of deflocculation *in vitro* by X-ray examination of the suspensions, in cells of narrow width (0.75 mm.) made from microscope cover glasses, have been unconvincing except where flocculation was so marked as to make complete coverage impossible. To evaluate fully such a deflocculating agent as degraded carrageenan appears to require clinical study as the preparation is of a complex nature and is involved in physiological conditions which are impossible to reproduce exactly *in vitro*.

The Evans Medical Research Laboratories,
Liverpool, 24.
December 7, 1961.

W. ANDERSON.

REFERENCE

Anderson, W. (1961) *J. Pharm. Pharmacol.*, **13**, 139-147.