REVIEW ARTICLE

BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS.

PART I. SYNTHETIC NITROGENOUS STEROIDS

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THE broad spectrum of biological activity found within the group and the multiplicity of actions displayed by certain individual members, make the steroids one of the most intriguing classes of biologically-active compounds. Structural modification studies, whose extent is unequalled in any other area of medicinal chemistry, have not only furnished so many steroidal derivatives that structure-action relationship studies are possible to a degree undreamt of in other fields, but they have also led to the introduction of several cheaper, safer, more specific and more potent therapeutic agents. Among the many steroidal derivatives now known, are a number of compounds which incorporate nitrogen atoms in their molecular structure, and it is the purpose of the present review to indicate the importance of these nitrogenous steroids and to evaluate, where possible, the influence of the nitrogen atoms upon the biological activity displayed. Certain aspects of the subject have been reviewed previously, particularly the pharmacology of the veratrum alkaloids—for more recent reviews see Abreu (1959), Hoobler and Dontas (1953), Krayer (1958), Stoll (1954). The earlier work is exhaustively reviewed by Krayer and Acheson (1946) who refer to previous review articles. A brief survey of the biological properties of nitrogenous steroids in general has also been published (Voigt and Kallistratos, 1957).

To set the field in perspective the present review will consider nitrogenous steroids in several contexts. Suitable examples, chosen mainly from the synthetic derivatives, will be used to illustrate how the group fits in with modern concepts of drug action and a brief survey will then be given of the biological properties of the rapidly expanding group of known steroidal alkaloids. With the large number of nitrogenous steroids now known, it is quite impossible to achieve a complete coverage of the pertinent literature, but a serious attempt has been made to make the survey as representative as possible.

Clinical Implications

At the present day clinical application of steroids possessing nitrogen atoms is very limited, although there are several indications that the full potentialities have not yet been realised. This is particularly true in the field of synthetic derivatives, as it is only within the past few years that

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the intensive search for modified steroidal hormones, showing high specificity or accentuating a minor or secondary biological characteristic of the natural analogue, has been extended to include more than a handful of nitrogenous derivatives. It does not therefore seem unreasonable to anticipate that among new nitrogenous steroids will be found compounds exhibiting clinically desirable carcinolytic properties, improved anabolic to androgenic ratios or superior lipodiatic to oestrogenic indices. Indeed certain steroidal [3, 2-c]-pyrazoles (Clinton and others, 1959, 1961b) and [2, 3-d]-isoxazoles (Clinton and others, 1961a) are already known to possess very favourable anabolic to androgenic ratios. Moreover, as these derivatives are active by the oral route and one representative, 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I), has shown

I

II

promise on preliminary clinical trial (Howard and others, 1959), such compounds may well find a permanent place as therapeutic agents. Anabolic properties are also present in other nitrogenous steroids including 17β-hydroxy-17α,2′-dimethylandrostano-[3,2-b]-thiazole, certain *N*-substituted 2-aminomethylene-17α-methyldihydrotestosterones (Zderic and others, 1960) and various substituted 16α-aminopregnenes (Rhone-Poulenc, 1960).

The possibility of securing new therapeutic agents amongst nitrogenous steroids is by no means confined to synthetic derivatives, however, and naturally-occurring compounds may also have their role to play. Thus the recently characterised bisquaternary steroidal alkaloid, malouetine (II), which occurs in *Malouetia bequaertiana* (Janot, Lainé and Goutarel, 1960), has been shown in preliminary experiments to possess competitive neuromuscular-blocking potency equal to that of (+)-tubocurarine whilst being only one third as toxic (Quévauviller and Lainé, 1960) and so this compound or related drugs could conceivably offer alternatives to tubocurarine as an adjunct to surgery. The three possible isomers of malouetine involving the configurations of the nitrogen atoms, namely the diquaternary bases in which the nitrogen atoms are in the 3β-20β-, 3α-20α- and 3α-20β- configurations have been prepared synthetically (Goutarel, 1961) and it will be of some interest to learn of their relative potencies. Again, the steroidal alkaloid funtumidine, on the basis of motility tests in rats, has been claimed to possess tranquillizing properties comparable to those of reserpine (La Barre and Desmarez, 1959) and so alkaloids of this type may have a role to play in this area of clinical medicine.

Certain clinically acceptable nitrogenous steroids are found in nature but are not used because cheaper or superior agents are available. Examples are the suberylarginine derivatives of the bufadienolides, such as bufotoxin and gamabufotoxin (III), which are constituents of the poison

III

secreted by the parotid glands of the toad, and which have similar activity to the plant cardenolide and scilladienolide glycosides employed clinically in the treatment of congestive heart failure. It is of some interest that the toxic principles secreted by the salamander are also nitrogenous steroid derivatives (Schöpf, 1961) although these compounds are of a different chemical type and are without potential clinical application.

Nitrogenous derivatives of cardenolides are also found in plants. Examples are uscharine (IV) (Hesse and Mix, 1959) and its dihydro-derivative voruscharine (Hesse and Ludwig, 1960) which occur in the latex

IV

of *Calotropis procera*. These compounds do not appear to have seen clinical trial but uscharine has been shown to have a potency 58 per cent of that of ouabain in the etherised cat (Chen, Bliss and Robbins, 1942).

The nitrogenous steroids which have seen the most extensive clinical application are the steroidal alkaloids of the protoveratrine type which produce a reflex fall in blood pressure through a generalised vasodilatation and fall in heart rate. Crude and complex mixtures of these alkaloids saw a certain amount of clinical use around the turn of the century, but their toxicity and unpleasant side effects brought them into disfavour. The use of the purified principles saw a resurgence of popularity several

years ago with the heightened interest in the problem of hypertension (see for example Meilman and Krayer, 1952; Meilman, 1959; Robson and Keele, 1956), especially as they produce vasodilatation in all peripheral circuits including the brain and kidneys, and are free from the disturbance of the postural reflexes produced by ganglion-blocking agents. Unfortunately these highly desirable physiological properties are more than offset by the narrow margin between the therapeutic and toxic doses and the fact that emesis nearly always occurs with therapeutic doses. Hence these alkaloids have been virtually eliminated from clinical use, although they may still find occasional application in the treatment of certain toxaemias of pregnancy (see for example Finnerty and Fuchs, 1953; Meilman, 1953; Krupp and others, 1956).

The fact that the protoveratrine group of alkaloids afford such a clinically desirable integrated response, and yet cannot be employed because of their side effects, has presented a most tantalising challenge to the medicinal chemist and numerous attempts have been made to prepare synthetic nitrogenous steroids retaining the hypotensive properties, but devoid of the side effects. So far these efforts have met with little success. Among the compounds prepared are numbered several cholylamine esters (Fieser and Wei-Yuan Huang, 1953), certain ternorcholanylthiazoles (Dodson, 1955a), various 16α-aminopregnenolones (Rhone-Poulenc, 1960; Gould and others, 1956), some aminoalkanol esters of 17α-hydroxy-3-ketoandrost-5-en-17β-carboxylic acid (Bloom, 1956) and two 17-imidazolylandrostenes (Sturtevant, 1958).

Other steroidal alkaloids have seen limited clinical trial in the treatment of conditions other than hypertension. For example the "Kurchi" alkaloids from various *Holarrhena* spp. have been employed in the treatment of amoebic dysentery, both free and in the form of bismuth iodide complexes (see for example Acton and Chopra, 1933; Tanguy, Robin and Raoult, 1948; Lavier, Crosnier and Merle, 1948) and *Solanum* alkaloids were once used in the treatment of asthma and neuralgia (Leclerc, 1938), but neither class is of any great value.

SYNTHETIC NITROGENOUS STEROIDS AND THEIR PLACE IN MODERN THEORY

The lack of knowledge of the principles by which biological activity is related to chemical structure has necessitated numerous tedious structural modification studies in which the medicinal chemist has sought to improve upon known drugs of proven efficacy, and many steroids possessing nitrogen atoms have played their role in this work. Usually the preparation of such compounds has been conducted on purely empirical grounds but occasionally it has followed from the application of theoretical concepts such as the receptor theory of drug action, the theory of metabolite displacement, the concept of bioisosterism or the supporting moiety theory. In the following sections examples will be given of nitrogenous steroids which have either been prepared from consideration of these concepts or which can be discussed in retrospect in terms of these concepts. It is felt that this treatment will place the compounds in their correct perspective and at the same time afford a comprehensive survey without giving rise

merely to a catalogue of the various synthetic nitrogenous steroids which have been studied biologically.

Receptor Theory

Although very little is known of the intimate nature of the hypothetical "drug receptors" in the tissues, the receptor theory has proved an extremely useful aid to the medicinal chemist in his attempts to rationalise drug action in so far as it stresses the importance of the 3-dimensional geometrical shape and electronic distribution of the drug molecule. It was consideration of the receptor theory which led to the planned synthesis of the anabolic steroidal [3,2-c]-pyrazoles and [2,3-d]-isoxazoles, where it was assumed that the receptors concerned with the androgenic and anabolic properties of the natural male hormones differed in their nature (Clinton and others, 1961b). Attention was concentrated on securing an alteration of the intergroup distance between the substituents at C(3) and C(17), with an accompanying change in the nucleophilicity of the C(3) substituent, and satisfaction of these requirements led logically to the preparation of the pyrazole and isoxazole derivatives.

After oral administration 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I) proved to be some 30 times as potent as 17α-methyltestosterone in the rat nitrogen retention test, whilst it was only one quarter as androgenic in the ventral prostate weight gain test (Arnold, Beyler and Potts, 1959). In the levator ani muscle test in immature castrated male rats it proved to be twice as myotrophic when given by the oral route as 17α-methyltestosterone (Potts, Beyler and Burnham, 1960), and it also proved effective in reversing the catabolic actions of cortisone acetate in the same animals (Beyler, Potts and Burnham, 1960). Surprisingly, acylation of the pyrazole ring imparted some oestrogenicity to the pyrazole series and a 6α-methyl group decreased both the androgenic and anabolic properties (Clinton and others, 1961b).

The isoxazole derivatives show broadly similar activity to the pyrazole compounds (Clinton and others, 1961a; Zderic and others, 1960) although one, 17β-hydroxy-17α-methyl-19-norandrost-4-eno-[2,3-d]-isoxazole (V), showed a conspicuous lack of specificity. Thus it exhibited progestational

OH
H
N
O

V

activity equal to progesterone on intramuscular administration and to ethisterone on oral administration, as well as showing anabolic, myotrophic, androgenic and oestrogenic properties (Clinton and others, 1961a).

In their visualisation of drug-receptor interaction Van Rossum and Ariens (1957) suggest that the "drug-receptor complex" is basically an interaction of fields of force originating in the drug molecule and in the tissue. Electrostatic and van der Waals forces play the dominant role, and it is postulated that certain specific interactions within the general field determine the intrinsic activity or ability of the drug to evoke the biological response. If this representation is correct then maintenance of the general interaction with concurrent variation in electron density at certain specific areas might be expected to produce large changes in intrinsic activity without appreciable changes in the affinity for the receptor. In the glucocorticoid field such a situation would appear to arise from the introduction of an electron-withdrawing substituent in the form of a 9α-fluorine atom which greatly enhances potency. The spectacular success of this introduction of a 9α-fluorine atom has logically led to investigations of the effect of introducing other electron-withdrawing groups at various positions in the steroid nucleus and among the compounds so prepared are several with groups containing nitrogen. Thus several 5α-, 7α-, 9α- and 11β-thiocyanato-steroid hormone derivatives have been synthesised (Kawasaki and Mosettig, 1959; Schaub and Weiss, 1961; Takeda, Kubota and Kawanami, 1960) and it was discovered that 3,20-dioxo-5α-thiocyanatopregnane (VI) and 17α-ethynyl-17β-hydroxy-3-oxo-5α-thiocyanato-19-norandrostane (VII) were approximately equal in

VI VII

progestational activity to their parent compounds progesterone and 19-norethynyltestosterone respectively. Similarly the 4,5-dihydro-5α-thiocyanato-analogue of cortisone acetate showed comparable activity to cortisone acetate (Takeda, Kubota and Kawanami, 1960) although the 4,5-dihydro-5α-thiocyanato-derivative of hydrocortisone acetate showed little or no activity. Doubt is expressed, however, whether the thiocyanato-derivatives were themselves active in view of their ready reconversion into the parent hormone.

Other steroid hormone analogues with electron-withdrawing groups in the molecule include various 5- and 6-cyano-derivatives (Bowers, 1961; Bowers and others, 1959) and several 6-nitro compounds (Bowers, Ibáñez and Ringold, 1959; Bowers, Sánchez and Ringold, 1959). Of these, 6α-nitro-17α-acetoxyprogesterone was found in the Clauberg assay, oral route, to be 3–4 times as active as 17α-acetoxyprogesterone as a progestational agent (Bowers, Ibáñez and Ringold, 1959). On the other

hand both 6α- and 6β-nitrotestosterone were inactive as myotrophic, androgenic or gonadotrophin-suppressing agents in the parabiotic rat (Bowers, Sánchez and Ringold, 1959). The isomeric 6α- and 6β-nitroprogesterones exhibited less than one eighth the progestational activity of progesterone in the guinea-pig copulatory assay (Bowers, Sánchez and Ringold, 1959) and 21-nitroprogesterone was inactive (Bowers and Ringold, 1959).

The 2-nitro-, 4-nitro- and 2,4-dinitro-derivatives of oestrone (Werbin and Holoway, 1956) and oestradiol (Patton, 1959b) are known, but like the 16-isonitroso-derivative of oestrone-3-methyl ether (Litvan and Robinson, 1938) they do not appear to have been tested biologically.

Various nitrogenous steroids, where the nitrogen forms part of an electron-donating group, have also been studied. In particular considerable attention has been paid to derivatives of oestrone and oestradiol. Amongst such compounds may be mentioned 2-amino-4-methyloestra-1,3,5(10)-trien-17β-ol (Dannenberg and others, 1960), 3-amino-4-methyloestra-1,3,5(10)-trien-17β-ol (VIII) (Dannenberg and others, 1959) and

OH

H_2N

VIII

certain 2-dialkylaminomethyl derivatives (Patton, 1959a; 1960) which all proved devoid of activity. It is claimed however that a derivative of oestrone thought to possess the 17-spiro-oxazolidine structure IX exhibits an oral activity ten times that of oestrone (Hebo, 1951).

O

NH

HO

IX

Several synthetic steroids possessing the provitamin D 5,7-diene system with a terminal tertiary amino-function in the side chain have been prepared but on irradiation, only slight anti-rachitic activity was observable in the most favourable substance (Louw, Strating and Backer, 1955). The amides from which these amines were prepared were also inactive on irradiation.

Other examples of the introduction of an electron-donating nitrogen function into the steroid side chain are afforded by a number of *N*-substituted 21-amino-11β,17α-dihydroxypregna-1,4-diene-3,20-dione compounds of type X, which have been shown to retain the glucocorticoid

CH_2NRR' ... HO ... OH ... O

X

activity of prednisolone from which they are derived, as evidenced by the results of liver glycogen accumulation and rat foot oedema tests (Tóth, Tuba and Szporny, 1961).

More complex structural modification studies involving nitrogen have been reported in the cardenolide field. Thus strophanthidin reacts with primary and secondary amines to form nitrogenous derivatives (Bembry, Elderfield and Krueger, 1960), one of which, tryptamine-strophanthidin, not only retains a typical digitalis-like action on the isolated papillary muscle of the cat (Greiner and Reilly, 1952), but unlike the glycosides of the strophanthus series, it is active by the oral route in man (Otto and others, 1953). Unfortunately, however, it often produces emesis. The structure originally assigned to this compound (Otto and others, 1953) has since been retracted (Bembry and others, 1960) and it is now believed to be that shown in XI. Other cardenolide derivatives which have been

O ... $N{\cdot}CH_2CH_2$ ... N H ... CHO ... OH ... HO ... OH

XI

studied biologically include the 3-diethylaminoacetate and the 3-nicotinate of strophanthidin (Küssner, 1939; Steldt, Anderson and Chen, 1944). In the cat the diethylaminoacetate proved more potent than the parent aglycone, but the nicotinate was less active (Steldt and others, 1944).

Two *p*-dimethylaminophenylnitrones related to cortisone and hydrocortisone, viz. 17α-hydroxy-3,11,20-trioxopregn-4-en-21-*p*-dimethylaminophenylnitrone (XII) and the corresponding 11β-hydroxy compound,

XII

have been shown to retain glucocorticoid activity as demonstrated in the liver glycogen deposition assay (Leanza and others, 1954), but several 21-pyridinium salts derived from cortisone and hydrocortisone were inactive, as was the iminolactone XIII.

XIII

There are a number of references in the literature to the screening of various nitrogenous steroids only remotely related structurally to steroid hormones, and as might be expected, in most tests these compounds proved inactive, as, for example, the series of 24-amino-derivatives prepared from bile acids which showed no antirheumatic activity (Wessely and Swoboda, 1951). It is therefore a little surprising that certain substituted amino-alkanol esters of bile acids appear to exhibit some anti-inflammatory activity based on claims of their efficacy in the treatment of fibrositis and certain types of arthritis (Burtner, 1951).

Considerable attention has been devoted to structural modifications of the steroid nucleus itself and a number of these studies have been concerned with the introduction of nitrogen atoms into various ring positions. At the present time, in addition to several homoaza-steroids, aza-steroids are known in which each secondary carbon atom of the steroid nucleus, with the exceptions of C(1) and C(11), has been replaced by nitrogen (see for example Doorenbos and Mu Tsu Wu, 1961; Gut and Uskokovic, 1961; Knof, 1961; Kutney and Johnston, 1961; Jacobs and Brownfield, 1960; Shoppee and Krueger, 1961). Little work appears to have been published on the biological properties of these compounds so far, but they can be expected to provide interesting information in terms of the receptor theory with their regions of high electron density actually incorporated in the nucleus. Certain oxygenated 12a-aza-C-homo-steroids

have been reported (Mazur, 1957a,b) to inhibit the harmful deposition of liver glycogen occurring as an untoward effect in cortisone therapy and 4-aza-pregn-5-en-3,20-dione is claimed to exhibit marked anti-inflammatory activity in rats (Wildi, 1959). Weak androgenic properties and anti-oestrogenic activity are present in certain lactams belonging to the 4-aza-androstane series (Doorenbos and Huang, 1961). Several bis-dehydrodoisynolic acid analogues possessing the 1,2,3,4-tetrahydro-benz-[f]-isoquinoline nucleus were inactive as oestrogens and also failed to exhibit any androgenic or anti-inflammatory activity (Nelson and Hsi, 1961).

Nitrogenous Steroids as Antimetabolites

Where the affinity for a receptor fitted by a normal metabolite of a living organism is also present in a synthetic analogue of the metabolite, but the analogue exhibits a greatly reduced intrinsic activity, then the analogue is likely to function as an antagonist of the metabolite. Recently there has been an intense interest in the planned synthesis of antimetabolites, and this has been referred to as "the revolution in pharmacology" (Woolley, 1960). In the steroid field, several antimetabolites, such as the spirolactone antagonists of aldosterone (Atwater and others, 1961; Barter, 1960) are well established whilst other compounds, such as the halogenated analogues of the corticoid steroids (Fried, 1957) and the dihydrocardenolides (Cosmides, Muja and Carr, 1956) are believed to competitively antagonise their parent compounds. Yet nitrogen-containing steroids seem to have been little investigated as metabolite-displacing agents. Cholesterylamine, however, has been demonstrated to be a weak inhibitor of the use of cholesterol by the cockroach (Noland, 1954) and there are claims in the patent literature (Dodson, 1955b; Rorig, 1953) that certain nitrogenous steroids possess antihormonal activity. It is also possible that the antimicrobial activity displayed by various nitrogenous steroids may be due to antagonism towards steroidal metabolites of the organism, although it is clear that many of these compounds act by virtue of their surface-active properties (Stacey and Webb, 1947a). Such surface activity is widespread within the steroid field, and is particularly pronounced in the saponins, cardiac glycosides, various steroidal alkaloids, and the bile acids. In addition to conferring antimicrobial properties, the surface activity also confers haemolytic properties and it is therefore not unexpected that 3,6-diaminocholestane is a haemolytic agent (Stückradt, 1939).

A large number of nitrogenous steroids have been tested for antibacterial and antifungal activity. These compounds cover a wide range of chemical complexity and include both synthetic compounds and alkaloids. Among the simpler synthetic compounds may be mentioned the epimeric 7-aminocholesterols (Barnett, Ryman and Smith, 1946a), various mono- and diaminocholestane derivatives with the nitrogen functions in the 3-, 6- or 7-positions (Barnett, Ryman and Smith, 1946b) and several hydroxylated 23-aminonorcholane derivatives, including 23-guanido-3,7,12- trihydroxynorcholane (James and others, 1946). The monoaminocholestane

derivatives showed some activity against Gram-positive organisms (Barnett and others, 1946a,b) and the potency is increased in the diamino-compounds which in addition, showed some activity against Gram-negative organisms (Barnett and others, 1946b).

Amino-steroids prepared from bile acids in which the nitrogen atom is attached directly to the steroid nucleus exhibit but weak antibacterial properties (Hilton, Jones and Westwood, 1955; Jones, Webb and Smith, 1949; Redel and others, 1951) but where the amino-group is in the side chain, the potency is higher (Hilton and Webb, 1951; Stacey and Webb, 1947b).

Among the more complex synthetic nitrogenous steroids which have been shown to exhibit antibacterial properties are a number of *N*-substituted 16-amino-derivatives (Schering, 1955) and 3,3-di(*N*-acetyl-*p*-aminophenylmercapto)-7,12-diketocholanic acid (Jones, Smith and Webb, 1948). Several *N*-substituted 22-aminobisnorcholanes and their derived quaternary methiodides were found to possess antifungal activity in tests with *Candida albicans* (Herzog, Payne and Hershberg, 1955), but the amides from which they were derived showed little or no activity. Various aminopregnane derivatives of varying chemical complexity also possess antimicrobial activity (Kull, Castellano and Mayer, 1953; Micheli and Bradsher, 1955). The anti-amoebal properties of the steroidal alkaloid conessine inspired the screening of several synthetic nitrogenous steroids (Dodgson and Haworth, 1952), but none was more active than conessine itself. Certain compounds of this type showed antibacterial activity of about one twentieth that of streptomycin (Joska, Černý and Šorm, 1954).

Of a series of quinolino-, indolo-, pyrrolo-, thiazolo- and triazafluoreno-steroids prepared as potential antimicrobial agents only one, XIV, was

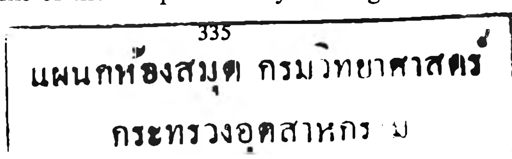
NH_2 N H H

XIV

sufficiently soluble to be tested and it proved inactive (Antaki and Petrow, 1951). *N*-Phenyl-3β-cholestanamine and *N*-*p*-tolyl-3β-cholestanamine, which were prepared during a search for drugs effective in leprosy and tuberculosis, also proved to be inactive (Buu-Hoi and Cagniant, 1944).

Bioisosterism

Certain nitrogenous steroidal derivatives are of particular interest in terms of the concept of bioisosterism (Schatz, 1960; Friedman, 1951) which postulates that compounds in isosteric relationship should possess either similar or opposite biological activity. This behaviour can be rationalised in terms of the receptor theory of drug action since the great



similarity in chemical and physical properties shown by isosteres should ensure similar affinities for the same receptors, mimicry or antagonism then being determined by the intrinsic acitivities of the individual isosteres. In the extended definition of isosterism (Erlenmeyer, 1948), functional groups which are related by the Hydride Displacement Law of Grimm (1934 and earlier refs.) are considered to be isosteric and so such a relationship will pertain for steroids related by the substitution of the -NH-group for the -O- function. Unfortunately, however, this particular isosteric relationship is complicated by the greater willingness of the nitrogen atom to enter salt formation and so strictly comparable biological activities are not necessarily to be expected. At physiological pH the amino-steroids will be ionised and so may have difficulty in penetrating permeability barriers (Brodie and Hogben, 1957) and may not reach the receptor. Although most of the amino-isosteres of steroidal hormones which have so far been tested are inactive and lack the ability to antagonise their natural analogues, potent oestrogenic activity is present in 17β-acetamido-3-acetoxyoestra-1,3,5(10)-triene (XV) (Dannenberg, Scheurlen

XV

and Simmer-Rühle, 1956) in which salt formation at the nitrogen atom is prevented by the amide function. The activity of this compound is also of interest in connection with the suggestion that oestrogenic activity is associated with the presence in the molecule of two groups capable of entering into hydrogen-bonding and which are held in a certain steric relationship (see for example Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). It has been further suggested that the distance between the two groups is a multiple of the "identity distance" (Long and Schueler, 1954) which is the distance between peptide links in a polypeptide chain.

The inactive oestrogen isosteres which have been reported are the 3-amino-isostere of equilenin (Bachmann and Dreiding, 1950) the 3-amino-, 17β-amino- and the 3,17β-diamino-isosteres of oestradiol and the 3-amino-isostere of oestrone (Hecker and Walk, 1960; Gold and Schwenk, 1959).

In the androgen series 3,17β-diaminoandrost-4-ene, 3-amino-17β-hydroxyandrost-4-ene and 3-oxo-17β-aminoandrost-4-ene have been reported to be devoid of male hormone activity (Joska and Šorm, 1956), but the analogue of testosterone, 17β-amino-3-oxo-androst-4-ene (XVI), although showing no activity in male rats, produced a pronounced increase in kidney weight and lesser increases in the liver and adrenal weights of female rats (Gaunt and others, 1954).

NH2

O

XVI

Replacement of the oxygen atom in the lactone ring of scillaren A by the-NH-group gave rise to a very sparingly soluble isostere which showed no cardiotonic activity when tested on the isolated guinea-pig auricle at a concentration of 10^{-6}M (Uhle and Schröter, 1961).

Drug Latentiation

Nitrogenous steroids have played a small but nevertheless significant role in drug latentiation (Harper, 1959) where a chemical derivative of an active drug is administered to overcome unfavourable rates of biotransformation or unfavourable solubility, distribution, transport and absorption characteristics—the active drug being regenerated *in vivo*. The steroidal moiety has sometimes functioned as the latentiating agent and at other times an active steroid has been latentiated.

Examples of the use of steroids as latentiating agents include the preparation of insoluble steroidal amine salts of penicillin capable of maintaining prolonged therapeutic concentrations of the antibiotic in the bloodstream (Coghill, Weston and MacCorquodale, 1950; Madinaveitia, 1955; Vaidya and Boyce, 1959) and the application of the cholesterol-6-sulphonate anion to yield an insoluble thiamine salt with which to enrich cereal flour (Mima, 1955). Another example is afforded by the choline salts of cholic acid and desoxycholic acid which exert actions typical of both moieties on the guinea-pig intestine (Meyer and McEwen, 1948). Attempted latentiation of 3-indolylacetic acid by the formation of steroid esters failed, however, to enhance parthenocarpic fruit induction in the tomato (Hofert and Sell, 1960).

Examples of nitrogenous moieties being used to latentiate biologically-active steroids are far more numerous. For instance much attention has been devoted to the preparation of amine salts of steroid hormone sulphate esters to increase the water solubility of the parent hormone. Among such compounds can be listed the ethylenediamine salts of the sulphate esters of oestrone, oestradiol, equilenin, androsterone and pregnenolone (Abbot, 1954a,b); the piperazine salts of the sulphate esters of oestradiol and equilenin (Hasbrouck, 1953); and the procaine (Deans and Scarrow, 1951), amphetamine (Grant, Glen and Barker, 1950) and 2-aminopyridine (Beall and Grant, 1952) salts of the sulphate esters of various steroidal oestrogens. Of these compounds piperazine oestradiol sulphate (XVII) has been used clinically. Dehydroandrosterone and androstenediol lose their biological activity if administered as dialkylamino sulphuric ester derivatives (Goisis and Polvani, 1955). Various quaternary ammonium

XVII

derivatives of hydrocortisone, prednisolone and dexamethasone retain activity (Mori and Nakagawa, 1961).

Latentiation of steroids may also be a natural phenomenon since amino-acid conjugates of steroidal hormones have been discovered in aqueous adrenal cortical extracts (Voigt and Schroeder, 1956 and earlier refs.) in urine (Eades, Pollack and King, 1954; Schneider and Frahm, 1955) in blood (Hudson and Lombardo, 1955) and in liver (Butenandt, 1956) and chorion-gonadotrophic extracts (Schneider and Frahm, 1956; Schneider and Birtel, 1956). These discoveries prompted Schroeder and Voigt (1958) to investigate the efficacy of glycyltestosterone in the survival test on adrenalectomised golden hamsters, but the compound was inactive and also devoid of androgenic properties (Overbeck, 1957). Nevertheless certain steroids in the form of amino-acid esters do appear to retain their activity (Organon, 1960), and latentiation of hydrocortisone by conversion to the more soluble diethylaminoacetate hydrochloride has been used in dermatology (Welsh, 1956; Kuhn, 1959). Prednisolone and dexamethasone have also been administered as their diethylaminoacetates (Dorner and Hohlweg, 1961; Zicha and others, 1960). The β-diethylaminoethyl ester of dehydrodesoxycholic acid was found to have one-quarter of the potency of dehydrocholic acid as a choleretic agent on a molar basis (Gunter and others, 1950).

Recently interest has been aroused in the preparation of steroidal nucleotides like XVIII (Oertel and Agashe, 1960; Riess and Ourisson, 1961) and in the preparation of the nicotinic esters of male, female and adrenocorticoid hormones (Weichsel and Zirm, 1961), the synthesis of

XVIII

the latter being inspired by the fact that favourable analgesic activity was retained in the bisnicotinic ester of morphine (Skursky, 1957).

An important example of drug latentiation in the steroid field is provided by the general anaesthetic hydroxydione which is the sodium salt of the hemisuccinate of 21-hydroxypregnane-3,20-dione. In this compound the hemisuccinate moiety confers increased water solubility on a predominantly lipid soluble molecule and rapid hydrolysis by the non-specific esterase of the serum regenerates the parent compound which then crosses the blood-brain barrier and quickly builds up an anaesthetically active concentration in the brain (Figdor and others, 1957; Jakoby and Tomkins, 1956). There would seem to be no *a priori* reason why other readily hydrolysed water-solublising groups could not be employed in place of the sodium hemisuccinate moiety and indeed a series of substituted aminoacetates, tertiary amine hemisuccinate salts and a substituted ammonium phosphate salt of 21-hydroxy-pregnane-3,20-dione have been tested for anaesthetic potency (Fidgor and others, 1957). Also included in the study were the hemisuccinates of two 3-spirothiazolidine derivatives of 21-hydroxy-20-oxo-5α-pregnane. The results showed that the nature of the solubilizing group could markedly influence the activity displayed. The aminoacetates exhibited high potency and high toxicity. Within the series there was much variation in the time of onset of anaesthesia.

Although anaesthetic properties are associated with many steroids (Figdor and others, 1957; Selye, 1942) the phenomenon appears a structurally specific one as nuclear substitution profoundly alters activity (Figdor and others, 1957). Moreover if the action was simply an extension of the properties of the gaseous general anaesthetics whose activity can be related with their thermodynamic activities and hence with their concentration in the central nervous system (Ferguson, 1939), one would expect other predominantly lipid soluble molecules of intermediate molecular weight to form a connecting bridge between the simple anaesthetics on the one hand and the steroids on the other. The sodium hemisuccinates of representative mono- and di-terpenoids, however, proved inactive (Ahmad and others, 1961).

Little attention would appear to have been given to the possibility of improving the solubility, absorption and transport properties of nitrogenous drugs by forming inclusion compounds with desoxycholic acid, although it has long been recognised that various alkaloids do form choleic acids (Wieland and Sorge, 1916)—that is, inclusion compounds with desoxycholic acid. Inclusion compounds in which steroid hormone molecules are the entrapped species have, however, excited some interest and recently investigations of this kind of compound have been made with phenylurethane and hippuric acid as models for the study of the interaction of proteins with steroids (Dirscherl and Gerhards, 1961).

Supporting Moiety Theory

Nitrogenous steroids played an important part in the development of the supporting moiety theory which contends that the molecules of pharmacologically active substances consist of a radical moiety determining the

type of activity displayed and a supporting moiety conferring affinity for the site of action. Cavallini and his colleagues who were the first to formally state this theory (Cavallini, 1955; Cavallini and Massarani, 1959) employed such compounds in their early experiments. The actual compounds included the β-diethylaminoethyl ethers of oestrone, testosterone and 3α-hydroxy-17-oxoandrost-5-ene (XIX), and the bis β-diethyl-

$Et_2NCH_2CH_2O$

XIX

aminoethyl ethers of oestradiol and 3,17-dihydroxyandrost-5-ene (Cavallini and Massarani, 1951b). The combination of "stripped down" drug molecule (Gero and Withrow, 1957) or radical moiety (diethylaminoethanol) and the steroidal supporting moiety produced drugs with potent coronary vasodilatator properties (Cavallini and Massarani, 1951b, 1959) whilst the bisquaternary salts derived from the two di-ether compounds showed curare-like properties (Cavallini and Massarani, 1959; Cavallini and others, 1951). These quaternary salts also showed *in vitro* anticholinesterase activity (Cogni and Salvaneschi, 1951). Quaternary salts derived from the mono-diethylaminoethyl ethers exhibited ganglion-blocking activity (Cavallini and Massarani, 1959).

Coupling to the predominantly lipid soluble steroid nucleus would be expected to confer upon a radical moiety not only different solubility characteristics but perhaps also more favourable adsorption properties. Since the plasma proteins are of such a nature as to readily bind cholesterol, it is conceivable that such compounds could use an existing transport mechanism.

Application of these ideas was independently made by several groups of workers in the sulphonamide field, who attempted to overcome the unfavourable lipid solubility characteristics of this group of antibacterials by preparing sulphonamido derivatives of cholesterol (Lieb, 1947; Kwartler, 1948) or the bile acids (Redel and others, 1951; Haslewood, 1941). Sulphacholazine (XX,R = $NH{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4$-*p*-$NH_2$) was found

OH COR HO OH H

XX

to possess *in vitro* activity against streptococci and moreover on intravenous administration to rabbits it was demonstrated to gain access to the bile (Barber, Dible and Haslewood, 1943). It was unfortunate that the compound showed little activity against the coliform group of organisms. More recently steroidal 4-amino-2-methoxyphenyl ethers have been prepared with the object of using the bile acid transport system to bring schistosomicidal amines in contact with the adult schistosomes residing in the portal veins (Davis, 1962).

The N^1-hydroxycholanyl-*p*-aminophenylsulphonamides (e.g. XX,R = $NH{\cdot}SO_2{\cdot}C_6H_4$-*p*-$NH_2$) are claimed to exhibit antibacterial and antiviral properties (Berczeller, 1948) but where the sulphonamide moiety is attached directly to the steroid nucleus as, for example, at position 7, the compounds show little or no activity (Redel and others, 1951).

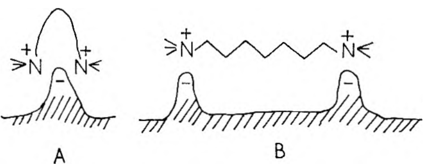
The rigid nucleus of steroids which possess a benzenoid ring A or a trans A/B ring junction is of interest as a supporting moiety from another point of view, since it can function as a skeletal framework upon which two or more radicals can be held in fixed spatial relationship to one another. Such a function for the steroid nucleus has been suggested to be involved in the activity displayed by the natural oestrogens where the oxygen functions at C(3) and C(17) are held at a rigid intergroup distance (see for example: Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). Other supporting moieties would be expected to be able to fulfil this role and in this may lie at least a part explanation for the potent oestrogenic properties displayed by such chemically diverse molecules as certain isoflavones, chlorotrianisene, the oestrogenolic acids and the oestrogenic stilbenes. Relatively little success has been achieved in securing alternative supporting moieties to replace the steroid nucleus in the androgenic and corticosteroid fields, although several attempts have been made (e.g. Clarke and Martini, 1959).

The hypothesis, first advanced by Paton and Zaimis (1949, 1951), that bisquaternary ammonium neuromuscular blocking agents interact by a two point attachment with anionic sites, normally involved in the physiological functioning of acetylcholine, has led to a number of attempts to define closely the actual interonium distance at the time of drug-receptor complex-formation. Unfortunately most compounds tested are conformationally non-rigid and so incapable of affording the desired information, as there is no reason to suppose that the thermodynamically most stable conformation of the isolated molecule is that actually adopted at the receptor. Whether various rigid bisquaternary ammonium salts in which the steroid nucleus functions as the supporting moiety will provide further information remains to be discovered. Although the receptor itself could conceivably be non-rigid, demonstration of activity in one rigid bisquaternary salt and absence of activity in another with a different interonium distance would represent a great advance.

Such steroidal bisquaternary ammonium salts will also be of interest in terms of Gill's hypothesis (1959) that completely rigid molecules should prove to be inactive due to variability in the receptors. This generalisation, which rests on the absence of ganglion-blocking activity in a limited

number of compounds, such as the completely rigid *NNN′N′*-tetramethyl-*p*-phenylenediamine dimethiodide (Wien and Mason, 1953) and certain virtually rigid furan derivatives still retaining a limited degree of rotational flexibility (Gill and Ing, 1958), certainly requires further substantiation. That there is a strong possibility that the molecules chosen do not possess a suitable interonium distance is indicated by the fact that other rigid molecules do indeed exhibit pronounced pharmacological activity. Examples are afforded by the natural oestrogens, testosterone, and the virtually rigid *cis* 1-hydroxy-2-trimethylammoniumcyclopentane, which shows marked depolarizing properties on the kitten phrenic nerve diaphragm (Standaert and Freiss, 1960).

Moreover, the two-point attachment theory is by no means universally accepted. Loewe and Harvey (1952) have postulated a one-point attachment theory in which the bulk of the molecule shields the receptor—the so-called "adumbration theory"—and their ideas have been extended by Fakstorp and others (1957). Again, conductimetric experiments have shown the extreme stability of the ion pair involving a single anion and a bisquaternary ammonium cation (Brody and Fuoss, 1956) which raises the possibility that the receptor-complex could be of type A, rather than type B (Cavallito and Gray, 1960).



In view of all these facts, steroidal bisquaternary ammonium salts may well prove to be of great importance in distinguishing between the various possibilities.

It is to be noted that malouetine (II) is not fully rigid, due to rotation about the C(17)—C(20) bond and to the possibility of chair to boat conformational isomerism in ring A, and these effects permit some variation in the interonium distance.

The discovery that marked antituberculous properties were present in various 4,4′-diaminodiphenylsulphone derivatives, thiosemicarbazones and hydrazones, resulted in the synthesis of many related compounds, including several steroidal derivatives in which the steroidal portion can be regarded as a supporting moiety. Some of these compounds proved to have a high activity, especially the bile acid amide derivatives of diaminodiphenylsulphone (Berczeller, 1949; 1958). One compound in this series, 4-(3-hemisuccinyldesoxycholylamino)-4′-hemisuccinylamino-diphenyl-sulphone (XXI) also proved to be an active inhibitor of the multiplication of the PR8 strain of influenza virus A in the chick embryo (Berczeller, 1958–59).

The hydrazones and isonicotinylhydrazones of testosterone, oestrone and dehydroandrosterone all proved active against the tubercle bacillus *in vitro* (Cavallini and others, 1952; Mantegazza and Tommasini, 1952)

OH

CONH·⬡·SO_2·⬡·NH·CO·$CH_2CH_2CO_2H$

$HO_2CCH_2CH_2CO_2$

H

XXI

as did the thiosemicarbazones of testosterone, progesterone and dehydroandrosterone (Mantegazza and Tommasini, 1951). These derivatives are virtually devoid of the physiological properties of the parent steroids (Cavallini and Massarani, 1951a; Ercoli, Koller and de Ruggièri, 1951). Dehydroisoandrosteryl-thiolpyrazinoate which was prepared as an analogue of the antituberculous ethylthiolpyrazinoate proved inactive (Kushner and others, 1955) as did the benzoylhydrazone of cholestenone (Offe, Siefken and Domagk, 1952). Cholesteryl *p*-nitrobenzoate, unlike certain other esters of *p*-nitrobenzoic acid was inactive against pneumococci (Mayer and Oechslin, 1939).

The promise shown by the nitrogen mustards as potential anti-cancer agents inspired the utilisation of the steroid nucleus as a supporting moiety in this field as well, and several mono- and bis-(2-chloroethyl)aminosteroids have been synthesised (Burstein and Ringold, 1961; Gensler and Sherman, 1958; Havranek and Doorenbos, 1960). Only three of these compounds appear to have been tested for anti-tumour activity, however, and these proved inactive (Havranek and Doorenbos, 1960). *Added in proof:* A more recent investigation has shown that antitumour activity is present in certain steroidal nitrogen mustards (Rao and Price, 1962).

The potent positive inotropic cardiac activity present in both the steroidal cardiac glycosides and the erythrophleum alkaloids led two groups of workers to prepare steroidal analogues of the latter, in which bile acids were used to replace the diterpenoid acids as supporting moieties (Ruzicka, Plattner and Engel, 1944; Uhle, Mitman and Krayer, 1956), but the new compounds were virtually inactive. It will be interesting to see whether steroidal esters of pyrrole -α-carboxylic acid will be prepared as analogues of the diterpenoid alkaloid ryanodine (Valenta and others, 1962) which exhibits such a remarkable pharmacological action on muscle (Hillyard and Procita, 1959 and refs. cited).

Several groups of naturally-occurring nitrogenous steroids can probably be quoted as exemplifying the supporting moiety theory, although in some cases it is difficult to distinguish a supporting moiety function from a latentiation function. A good illustration of this situation is afforded by the taurine and glycine conjugates of the bile acids whose anions are the true bile salt anions. Another example is afforded by the readily hydrolysed veratrum ester alkaloids, such as protoveratrine, which is some 6,000 times as toxic on a molar basis as is its alkamine, protoverine (Krayer, Moe and Mendez, 1944).

It is also possible to consider the suberylarginine radical of the toad poisons as a supporting moiety, but the position with the cardiotonic steroids is particularly complex. It is generally held that the strong positive inotropic action is intimately linked with the unsaturated lactone function (Chen and Elderfield, 1940; Goodman and Gillman, 1955) but to regard this group as the active moiety is a gross oversimplification, as varying degrees of positive inotropic action are shown by the dihydrocardenolides (e.g. Jacobs and Hoffmann, 1927; Vick, Kahn and Acheson, 1957), the bile acids (e.g. Wakim, Essex and Mann, 1939), certain steroidal alkaloids (Benforado, 1957; Krayer, Moe and Mendez, 1944; Quévauviller and Blanpin, 1958), cortisone (Fleischhacker, 1956) and various other steroids (e.g. Abrams and Harris, 1951; Hajdu and Szent-Gyorgyi, 1952; Tanz and Kerby, 1961) none of which possess an unsaturated lactone. Indeed it is tempting to regard the lactone as a supporting moiety intensifying an activity associated with a hydroxylated steroid nucleus (Craig and Jacobs, 1943). A supporting moiety role can probably be assigned to the sugar residues of the cardiac glycosides since they have a marked influence on distribution and solubility properties and so affect the time of onset and duration of action (Chen, Henderson and Anderson, 1951; Keyl and Dragstedt, 1954). Although some scilladienolide genins show some potency in bioassays, the duration of action is transitory and so it is concluded that the sugar moieties play an indispensable role in determining the activity of the glycosides (Stoll, 1956). The effect of varying the number and nature of the sugar residues on the activity displayed has been summarised in several places (Chen, 1945; Fieser and Fieser, 1959; Oettel, 1947; Tamm, 1957).

The pharmacological actions of the suberylarginine conjugates of the bufadienolides were studied by Gessner (1926) and his work was followed by an elegant series of papers by K. K. Chen and his colleagues (e.g. Chen and Chen, 1934). The results of these and other studies (Arora, 1953; Chen, Anderson and Rose, 1952) would indicate that the compounds possess broadly similar pharmacological properties to the cardiac glycosides, but that the suberylarginine moiety does affect the rate of penetration into and removal from the myocardial tissue, thus producing differences of a quantitative nature.

Other Theoretical Interests of Nitrogenous Steroids

Several isolated examples are known in which nitrogenous steroids have played minor roles in studies designed to throw more light on the intimate nature of biological processes. One such instance involves the application of complex steroid derivatives in the study of artificial antigens (Grob and Goldberg, 1949).

The interesting hypothesis has put forward that certain steroids may interact with enzymes and other proteins by formation of spirothiazolidines since 3-oxo-steroids lacking a 4,5-double bond were shown to form such compounds under a variety of conditions with cysteine (Lieberman, 1946), but this suggestion is in need of further substantiation.

References

Abbot (1954a). Brit. Patent 708541 in *Chem. Abstr.* (1955), **49**, 6326.
Abbot (1954b). U.S. Patent 2,666,066 in *Chem. Abstr.* (1954), **48**, 12816.
Abrams, W. B. and Harris, T. N. (1951). *Amer. Heart J.*, **42**, 867–883.
Abreu, B. E. (1959) in *Hypertension*, editor J. Moyer, pp. 327–332, Philadelphia: Saunders.
Acton, H. W. and Chopra, R. N. (1933). *Indian med. Gaz.*, **68**, 6.
Ahmad, K., Khatoom, T., Lewis, J. J. and Martin-Smith, M. (1961). Unpublished work.
Antaki, H. and Petrow, V. (1951). *J. chem. Soc.*, 901–904.
Arnold, A., Beyler, A. L. and Potts, G. O. (1959). *Proc. Soc. exp. Biol. N.Y.*, **102**, 184–187.
Arora, R. B. (1953). *J. Pharmacol.*, **108**, 26–32.
Atwater, N. W., Bible, R. H., Brown E. A., Burtner, R. H., Mihina, J. S., Nysted, L. N. and Sollman, P. B. (1961). *J. org. Chem.*, **26**, 3077–3083.
Bachmann, W. E. and Dreiding, A. S. (1950). *J. Amer. chem. Soc.*, **72**, 1,329–1,331.
Barber, M., Dible, J. H. and Halsewood, G. A. D. (1943). *Biochem. J.*, **37**, p. vi.
Barnett, J., Ryman, B. E. and Smith, F. (1946a). *J. chem. Soc.*, 524–526.
Barnett, J., Ryman, B. E. and Smith, F. (1946b). *Ibid.*, 528–530.
Barter, F. C. (1960). *The Clinical Use of Aldosterone Antagonists*, Springfield, Illinois; Thomas.
Beall, D. and Grant, G. A. (1952). U.S. Patent 2,581,350 in *Chem. Abstr.*, (1952), **46**, 7596.
Bembry, T. H., Elderfield, R. C. and Krueger, G. L. (1960). *J. org. Chem.*, **25**, 1,175–1,179.
Benforado, J. M. (1957). *J. Pharmacol.*, **120**, 412–425.
Berczeller, A. (1948). U.S. Patent 2,441,129 in *Chem. Abstr.*, (1948), **42**, 5,622.
Berczeller, A. (1949). U.S. Patent 2,485,253 in *Chem. Abstr.*, (1950), **44**, 2,575.
Berczeller, A. (1958). *Dis. of Chest*, **33**, 475–481.
Berczeller, A. (1958–59). *Antibiot. Ann.*, 88–94.
Beyler, A. L., Potts, G. O. and Burnham, D. F. (1960). *Abstracts 1st International Congress of Endocrinology*, 829–830.
Bloom, B. M. (1956). U.S. Patent 2,763,645 in *Chem. Abstr.*, (1957), **51**, 3,678.
Bowers, A. (1961). *J. org. Chem.*, **26**, 2043–2047.
Bowers, A., Denot, E., Sánchez, M. B., Sánchez-Hidalgo, L. M. and Ringold, H. J. (1959). *J. Amer. chem. Soc.*, **81**, 5233–5242.
Bowers, A., Ibáñez, L. C. and Ringold, H. J. (1959). *Ibid.*, **81**, 3707–3710.
Bowers, A. and Ringold, H. J. (1959). *Ibid.*, **81**, 3710–3712.
Bowers, A., Sánchez, M. B. and Ringold, H. J. (1959). *Ibid.*, **81**, 3702–3706.
Brodie, B. B. and Hogben, C. A. M. (1957). *J. Pharm. Pharmacol.*, **9**, 345–380.
Brody, O. V. and Fuoss, R. M. (1956). *J. phys. Chem.*, **60**, 156–160.
Burstein, S. H. and Ringold, H. J. (1961). *J. org. Chem.*, **26**, 3084–3086.
Burtner, R. B. (1951). U.S. Patent 2,562,351 in *Chem. Abstr* (1951), **45**, 9812.
Butenandt, A. (1956). Cited by Voigt and Kallistratos (1957).
Buu-Hoi, Ng. Ph. and Cagniant, P. (1944). *Ber. dtsch. chem. Ges.*, **77**B, 761–766.
Cavallini, G. (1955). *Farmaco*, **10**, 644.
Cavallini, G. and Massarani, E. (1951a). *Boll. Soc. ital. Biol. sper.*, **27**, 629–630.
Cavallini, G. and Massarani, E. (1951b). *Farm. sci. e tec. (Pavia)*, **6**, 291–299.
Cavallini, G. and Massarani, E. (1959). *J. med. pharm. Chem.*, **1**, 365–370.
Cavallini, G., Ferrari, W., Mantegazza, P. and Massarani, E. (1951). *Farm. sci. e tec. (Pavia)*, **6**, 815–825.
Cavallini, G., Massarani, E., Mazzuchi, F. and Ravenna, F. (1952). *Ibid.*, **7**, 397–404.
Cavallito, C. J. and Gray, A. P. (1960). In *Fortschritte der Arzneimittelforschung*, editor E. Jucker, Vol. 2, p. 135. Basel: Birkhauser.
Chen, K. K. (1945). *Ann. Rev. Physiol.*, **7**, 681.
Chen, K. K., Anderson, R. C. and Rose, C. L. (1952). *J. Pharmacol.*, **106**, 314–318.
Chen, K. K., Bliss, C. I. and Robbins, E. B. (1942). *Ibid.*, **74**, 223–234.
Chen, K. K. and Chen, A. L. (1934). *Arch. int. Pharmacodyn*, **47**, 297–317.
Chen, K. K. and Elderfield, R. C. (1940). *J. Pharmacol.*, **70**, 338–346.
Chen, K. K., Henderson, F. C. and Anderson, R. C. (1951). *Ibid.*, **103**, 420–430.
Clarke, R. L. and Martini, C. M. (1959). *J. Amer. chem. Soc.*, **81**, 5716–5724.
Clinton, R. O., Manson, A. J., Stonner, F. W., Beyler, A. L., Potts, G. O. and Arnold, A. (1959). *Ibid.*, **81**, 1513–1514.
Clinton, R. O., Manson, A. J., Stonner, F. W., Christiansen, R. G., Beyler, A. L., Potts, G. O. and Arnold, A. (1961a). *J. org. Chem.*, **26**, 279.

Clinton, R. O., Manson, A. J., Stonner, F. W., Neumann, H. C., Christiansen, R. G., Clarke, R. L., Ackerman, J. H., Page, D. F., Dean, J. W., Dickinson, W. B. and Carabateas, C. (1961b). *J. Amer. chem. Soc.*, **83**, 1478–1491.
Coghill, R. D., Weston, A. W. and MacCorquodale, D. W. (1950). U.S. Patent 2,519,112 in *Chem. Abstr.* (1951), **45**, 666.
Cogni, G. and Salvaneschi, S. (1951). *Atti. soc. lombarda. sci. med. e biol.*, 7, 60–64, in *Chem. Abstr.* (1953), **47**, 6459.
Cosmides, G. J., Muja, T. S. and Carr, C. J. (1956). *J. Pharmacol.*, **118**, 286–295.
Craig, L. C. and Jacobs, W. A. (1943). *Science*, **97**, 122.
Dannenberg, H., Dannenberg-von Dresler, D. and Köhler, T. (1960). *Chem. Ber.*, **93**, 1989–1998.
Dannenberg, H., Doering, C. H. and Dannenberg-von Dresler, D. (1959). *Hoppe Seyl. Z.*, **317**, 174–181.
Dannenberg, H., Scheurlen, H. and Simmer-Rühle, I. (1956). *Liebigs Ann.*, **600**, 68–80.
Davis, M. (1962). *J. chem. Soc.*, 178–181.
Deans, S. A. V. and Scarrow, J. A. (1951). U.S. Patent 2,555,579, in *Chem. Abstr.* (1952), **46**, 1054.
Dirscherl, W. and Gerhards, E. (1961). *Liebigs Ann.*, **639**, 181–194.
Dodgson, D. P. and Haworth, R. D. (1952). *J. chem. Soc.*, 67–71.
Dodson, R. M. (1955a). U.S. Patent 2,705,232 in *Chem. Abstr.* (1956), **50**, 5793.
Dodson, R. M. (1955b). U.S. Patent 2,709,701 in *Chem. Abstr.* (1956), **50**, 5782.
Doorenbos, N. J. and Huang, C. L. (1961). *J. org. Chem.*, 26, 4106–4108.
Doorenbos, N. J. and Mu Tsu Wu (1961). *Ibid.*, **26**, 2548–2549.
Dorner, G. and Hohlweg, W. (1961). *Z. exp. Med.*, **134**, 162.
Eades, C. H., Pollack, R. L. and King, J. S. (1954). *Fed. Proc.*, **13**, 201.
Ercoli, A., Koller, M. and de Ruggieri, P. (1951). *Farm. sci. e tec. (Pavia)*, **6**, 471–472, in *Chem. Abstr.* (1952), **46**, 8142.
Erlenmeyer, H. (1948). *Bull. Soc. Chim. biol., Paris*, **30**, 792–805.
Fakstorp, J., Pedersen, J. G. A., Poulsen, E. and Schilling, M. (1957). *Acta pharm. tox. Kbh.*, **13**, 52–58.
Ferguson, J. (1939). *Proc. roy. Soc.*, B **127**, 387–404.
Fieser, L. F. and Fieser, M. (1959). *Steroids*, pp. 800–808, New York: Reinhold.
Fieser, L. F. and Wei-Yuan Huang (1953). *J. Amer. chem. Soc.*, **75**, 6306–6307.
Figdor, S. K., Kodet, M. J., Bloom, B. M., Agnello, E. J., P'an, S. Y. and Laubach, G. D. (1957). *J. Pharmacol.*, **119**, 299–309.
Finnerty, F. A. and Fuchs, C. J. (1953). *Amer. J. Obst. Gynec.*, **66**, 830–841.
Fisher, A. L., Keasling, H. H. and Schueler, F. W. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 439–441.
Fleischhacker, H. (1956). *Wien. klin. Wschr.*, **68**, 989–992.
Fried, J. (1957). *Cancer*, **10**, 752–756.
Friedman, H. L. (1951). *Nat. res. Counc. Wash. Pub.*, **206**.
Gaunt, R., Leathem, J. H., Tuthill, C. H., Antonchak, N., Gilman, M. and Renzi, A. A. (1954). *Endocrinology*, **54**, 272–283.
Gensler, W. J. and Sherman, G. M. (1958). *J. org. Chem.*, **23**, 1227–1228.
Gero, A. and Withrow, C. L. (1957). *Nature, Lond.*, **180**, 1354–1355.
Gessner, O. (1926). *Arch. exp. Path. Pharmak.*, **118**, 325–357.
Gill, E. W. (1959). *Proc. roy. Soc.*, **B150**, 381–402.
Gill, E. W. and Ing, H. R. (1958). *J. chem. Soc.*, 4728–4731.
Goisis, M. and Polvani, F. (1955). *Biol. Latina*, **8**, 86–106.
Gold, A. M. and Schwenk, E. (1959). *J. Amer. chem. Soc.*, **81**, 2198–2200.
Goodman, L. S. and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 672, New York: Macmillan.
Gould, D., Shapiro, E. L., Finckenor, L. E., Gruen, F. and Hershberg, E. B. (1956). *J. Amer. chem. Soc.*, **78**, 3158–3163.
Goutarel, R. (1961). *Tetrahedron*, **14**, 126–137.
Grant, G. A., Glen, W. L. and Barber, R. J. (1950). U.S. Patent 2,534,121 in *Chem. Abstr.* (1951), **45**, 3878.
Greiner, T. and Reilly, J. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 141–144.
Grimm, H. G. (1934). *Angew. Chem.*, **47**, 594–601.
Grob, C. A. and Goldberg, W. A. (1949). *Helv. chim. Acta*, **32**, 191–197.
Gunter, M. J., Kim, K. S., Magee, D. F., Ralston, H. and Ivy, A. C. (1950), *J. Pharmacol.*, **99**, 465–478.
Gut, M. and Uskoković, M. (1961). *J. org. Chem.*, **26**, 1943–1944.
Hajdu, S. and Szent-Gyorgyi, A. (1952). *Amer. J. Physiol.*, **168**, 171–175.
Harper, N. J. (1959). *J. med. pharm. Chem.*, **1**, 467–500.

Hasbrouck, R. B. (1953). U.S. Patent 2,642,427 in *Chem. Abstr.* (1954), **48**, 6474.
Haslewood, G. A. D. (1941). *Biochem. J.*, **35**, 1307–1310.
Havranek, R. E. and Doorenbos, N. J. (1960). *J. Amer. pharm. Ass. Sci. Ed.*, **49**, 328–329.
Hebo, H. (1951). U.S. Patent 2,557,655 in *Chem. Abstr.* (1952), **46**, 3094.
Hecker, E. and Walk, E. (1960). *Chem. Ber.*, **93**, 2928–2937.
Herzog, H. L., Payne, C. C. and Hershberg, E. B. (1955). *J. Amer. chem. Soc.*, **77**, 5324–5327.
Hesse, G. and Ludwig, G. (1960). *Liebigs Ann.*, **632**, 158–171.
Hesse, G. and Mix, K. (1959). *Ibid.*, **625**, 146–156.
Hillyard, I. W. and Procita, L. (1959). *J. Pharmacol.*, **127**, 22–28.
Hilton, M. L., Jones, A. S. and Westwood, J. R. B. (1955). *J. chem. Soc.*, 3449–3453.
Hilton, M. L. and Webb, M. (1951). *Ibid.*, 2767–2768.
Hofert, J. F. and Sell, H. M. (1960). *J. org. Chem.*, **25**, 1831–1833.
Hoobler, S. W. and Dontas, A. (1953). *Pharmacol. Rev.*, **5**, 151–157.
Howard, R. P., Norcia, L. N., Peter, J. A. and Furman, R. H. (1959). Cited by Clinton and others (1961b).
Hudson, P. B. and Lombardo, M. E. (1955). *J. clin. Endocr.*, **15**, 324–330.
Jacobs, T. L. and Brownfield, R. B. (1960). *J. Amer. chem. Soc.*, **82**, 4033–4039.
Jacobs, W. A. and Hoffmann, A. (1927). *J. biol. Chem.*, **74**, 787–794.
Jakoby, W. B. and Tomkins, G. (1956). *Science*, **123**, 940–941.
James, S. P., Smith, F., Stacey, M. and Webb, M. (1946). *J. chem. Soc.*, 665–670.
Janot, M. M., Lainé, F. and Goutarel, R. (1960). *Ann. pharm. franc.*, **18**, 673–677.
Jones, A. S., Smith, F. and Webb, M. (1948). *Nature, Lond.*, **162**, 857–858.
Jones, A. S., Webb, M. and Smith, F. (1949). *J. chem. Soc.*, 2164–2168.
Joska, J., Černý, V. and Šorm, F. (1954). *Coll. Trav. chim. Tchécosl.*, **19**, 551–558.
Joska, J. and Šorm, F. (1956). *Ibid.*, **21**, 754–760.
Kawasaki, T. and Mosettig, E. (1959). *J. org. Chem.*, **24**, 2071–2072.
Keyl, A. C. and Dragstedt, C. A. (1954). *J. Pharmacol.*, **110**, 411–414.
Knof, L. (1961). *Liebigs Ann.*, **642**, 194–199.
Krayer, O. (1958) in *Pharmacology in Medicine*, 2nd ed., editor V. A. Drill, pp. 515–524, New York: McGraw-Hill.
Krayer, O. and Acheson, G. H. (1946). *Physiol. Rev.*, **26**, 383–446.
Krayer, O., Moe, G. K. and Mendez, R. (1944). *J. Pharmacol.*, **82**, 167–186.
Krupp, P. J., Farris, C., Pierce, C. and Jacobs, A. (1956). *Amer. J. Obst. Gynec.*, **71**, 247–254.
Kuhn, B. H. (1959). *J. Amer. med. Women's Ass.*, **14**, 54–55.
Kull, F. C., Castellano, G. A. and Mayer, R. L. (1953). *J. invest. Dermatol.*, **21**, 227–228.
Kushner, S., Dalalian, H., Bach, F. L., Centola, D., Sanjurjo, J. L. and Williams, J. H. (1955). *J. Amer. chem. Soc.*, **77**, 1152–1155.
Küssner, W. E. (1939). *Merck's Jahrb.*, **53**, 45–51.
Kutney, J. P. and Johnston, R. A. (1961). *Chem. and Ind.*, 1713–1714.
Kwartler, C. E. (1948). Cited in Northey "*The Sulphonamides and Allied Compounds*" (1948) ref. No. 1503, New York: Reinhold.
La Barre, J. and Desmarez, J. J. (1959). *Arch. int. Pharmacodyn.*, **119**, 514–516.
Lavier, G., Crosnier, R. and Merle, F. (1948). *Bull. Soc. Path. exot.*, **41**, 548.
Leanza, W. J., Conbere, J. P., Rogers, E. F. and Pfister, K. (1954). *J. Amer. chem. Soc.*, **76**, 1691–1694.
Leclerc, H. (1938). *Presse méd.*, **46**, 480.
Lieb, H. (1947). *Monatshefte*, 77, 324–332.
Lieberman, S. (1946). *Experientia*, **2**, 411–412.
Litvan, F. and Robinson, R. (1938). *J. chem. Soc.*, 1997–2001.
Loewe, S. and Harvey, S. C. (1952). *Arch. exp. Path. Pharmak.*, **214**, 214–226.
Long, J. P. and Schueler, F. W. (1954). *J. Amer. pharm. Ass., Sci. Ed.*, **43**, 79–86.
Louw, D. F., Strating, J. and Backer, H. J. (1955). *Rec. Trav. chim. Pays-Bas*, **74**, 1540–1554.
Macovski, E. and Georescu, J. (1946). *Bull. Sect. Sci. Acad. roumaine*, **28**, 645–667.
Madinaveitia, J. L. (1955). Brit. Patent 729,160 in *Chem. Abstr.* (1956), **50**, 2128.
Mantegazza, P. and Tommasini, R. (1951). *Boll. Soc. ital. Biol. sper.*, **27**, 631–633.
Mantegazza, P. and Tommasini, R. (1952). *Atti. soc. lombarda sci. med. e biol.*, **7**, 496–503.
Mayer, R. L. and Oechslin, C. (1939). *Arch. int. Pharmacodyn.*, **62**, 211–230.
Mazur, R. H. (1957a). U.S. Patent 2,806,028 in *Chem. Abstr.* (1958), **52**, 2102.
Mazur, R. H. (1957b). U.S. Patent 2,806,029 in *Chem. Abstr.* (1958), **52**, 2102.
Meilman, E. (1953). *J. clin. Invest.*, **32**, 80–89.

Meilman, E. (1959). in *Hypertension*, editor J. Moyer, pp. 395–399, Philidelphia: Saunders.
Meilman, E. and Krayer, O. (1952). *Circulation*, 6, 212–221.
Meyer, A. E. and McEwan, J. P. (1948). *Amer. J. Physiol.*, **153**, 386–392.
Micheli, R. A. and Bradsher, C. K. (1955). *J. Amer. chem. Soc.*, **77**, 4788–4793.
Mima, H. (1955). Japanese Patent 8125('55) in *Chem. Abstr.*, (1957), **51**, 18387.
Mori, Y. and Nakagawa, T. (1961). *J. pharm. Soc., Japan*, **81**, 972–975.
Nelson, N. A. and Hsi, R. S. P. (1961). *J. org. Chem.*, **26**, 3086–3090.
Noland, J. L. (1954). *Arch. Biochem.*, **52**, 323–330.
Oertel, G. W. and Agashe, B. D. (1960). *Biochem. Biophys. Acta*, **45**, 1–8.
Oettel, H. (1947). *Pharmazie*, **2**, 385–388.
Offe, H. A., Siefken, W. and Domagk, G. (1952). *Z. Naturforsch.*, 7B, 446–462.
Oki, M. (1952). *J. chem. Soc., Japan*, **73**, 252–254. *in Chem. Abstr.* (1953), 47, 3522.
Organon (1960). *Derwent Fine Chemicals Patent J.*, **188** [5], 3A No. 833,582.
Otto, H. L., Greiner, T., Gold, H., Palumbo, F., Warshaw, L., Kwit, N. T. and Chen, K. K. (1953). *J. Pharmacol.*, **107**, 225–231.
Overbeck, G. A. (1957). Cited by Voigt and Kallistratos (1957).
Paton, W. D. M. and Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **4**, 381–400.
Paton, W. D. M. and Zaimis, E. J. (1951), *Ibid.*, **6**, 155–168.
Patton, T. L. (1959a). *Chem. and Ind.*, 923–924.
Patton, T. L. (1959b). *J. org. Chem.*, **24**, 1795–1796.
Patton, T. L. (1960). *Ibid.*, **25**, 2148–2152.
Potts, G. O., Beyler, A. L. and Burnham, D. F. (1960). *Proc. Soc. exp. Biol., N.Y.*, **103**, 383–384.
Quévauviller, A. and Blanpin, O. (1958). *J. de Physiol.*, **50**, 1123–1127.
Quévauviller, A. and Lainé, F. (1960). *Ann. pharm. franç.*, **18**, 678–680.
Rao, G. V. and Price, C. C. (1962). *J. org. Chem.*, **27**, 205–210.
Redel, J., Bouteville, A., Gauthier, B. and Nguyen-Huu Quy, (1951). *Bull. Soc. chim. France*, 524–526.
Rhone-Poulenc (1960). *Derwent Fine Chemicals Patent J.*, **207**, [6], 3A, No. 847,445.
Riess, J. and Ourisson, G. (1961). *Bull. Soc. chim. France*, 1243–1244.
Robson, J. M. and Keele, C. A. (1956). *Recent Advances in Pharmacology*, pp. 82–86, 2nd ed., London: Churchill.
Rorig, K. (1953). U.S. Patent 2,664,423 in *Chem. Abstr.* (1955), **49**, 7608.
Ruzicka, L., Plattner, P. A. and Engel, B. G. (1944). *Helv. chim. Acta*, **27**, 1553–1560.
Schatz, V. B. (1960). In *Medicinal Chemistry*, editor A. Burger, 2nd ed., pp. 72–78, New York: Interscience.
Schaub, R. E. and Weiss, M. J. (1961). *J. org. Chem.*, **26**, 3915–3925.
Schering (1955). Brit. Patent 735,568 in *Chem. Abstr.* (1956), **50**, 7872.
Schneider, W. G. and Birtel, A. (1956). *Klin. Wschr.*, **34**, 1175–1178.
Schneider, W. G. and Frahm, H. (1955). *Acta endocr.*, **20**, 279–285.
Schneider, W. G. and Frahm, H. (1956). *Naturwiss.*, **43**, 61.
Schöpf, C. (1961). *Experientia*, **17**, 285–295.
Schroeder, W. and Voigt, K. D. (1958). *Acta endocr.*, **27**, 110–117.
Selye, H. (1942). *Endocrinology*, **30**, 437–453.
Shoppee, C. W. and Krueger, G. (1961). *J. chem. Soc.*, 3641–3655.
Skursky, J. (1957). *Wien. med. Wschr.*, **107**, 678–679.
Stacey, M. and Webb, M. (1947a). *Proc. roy. Soc.*, B **134**, 522–537.
Stacey, M. and Webb, M. (1947b). *Ibid.*, B**134**, 538–543.
Standaert, F. G. and Friess, S. L. (1960). *J. Pharmacol.*, **128**, 55–64.
Steldt, F. A., Anderson, R. C. and Chen, K. K. (1944). *Ibid.*, **82**, 98–102.
Stoll, A. (1954). *Gazz. chim. ital.*, **84**, 1190–1209.
Stoll, A. (1956). in *Medicinal Chemistry*, Vol. 2, editors, F. F. Blicke and C. M. Suter, p. 24, New York: Wiley.
Stückradt, H. (1939). *Arch. exp. Path. Pharmak.*, **191**, 362–368.
Sturtevant, F. M. (1958). *Proc. Soc. exp. Biol., N.Y.*, **97**, 619–621.
Takeda, K., Kubota, T. and Kawanami, J. (1960). *Pharm. Bull., Tokyo*, **8**, 615–620.
Tamm, C. (1957). *Fortschr. Chem. org. Naturstoffe*, **14**, 71–140.
Tanguy, F., Robin, C. and Raoult, A. (1948). *Méd. trop.*, **8**, 12.
Tanz, R. D. and Kerby, C. F. (1961). *J. Pharmacol.*, **131**, 56–64.
Tóth, J., Tuba, Z. and Szporny, L. (1961). *Nature, Lond.*, **191**, 607.
Uhle, F. C., Mitman, B. A. and Krayer, O. (1956). *J. Pharmacol.*, **116**, 444–449.
Uhle, F. C. and Schröter, H. (1961). *J. org. Chem.*, **26**, 4169–4171.
Vaidya, S. S. and Boyce, S. F. (1959). *Antibiot. Ann.*, **9**, 364–368.

Valenta, Z., Wiesner, K., Babin, D. R., Bögri, T., Forrest, T. P., Fried, F. and Reinshagen, H. (1962). *Experientia*, **18**, 111–113.
Van Rossum, J. M. and Ariens, E. J. (1957). *Ibid.*, **13**, 161–163.
Vick, R. L., Kahn, J.B. and Acheson, G. H. (1957). *J. Pharmacol.*, **121**, 330–339.
Voigt, K. D. and Kallistratos, G. (1957). *Endokrinologie*, **35**, 56–64.
Voigt, K. D. and Schroeder, W. (1956). *Acta endocr.*, **21**, 343–358.
Wakim, K. G., Essex, H. E. and Mann, F. C. (1939). *Amer. Heart J.*, **18**, 171–175.
Weichsel, H. and Zirm, K. L. (1961). *Monatshefte*, **92**, 667–671.
Welsh, A. L. (1956). *Int. Rec. Med. gen. Pract. Clinics.*, **169**, 775–777.
Werbin, H. and Holoway, C. (1956). *J. biol. Chem.*, **223**, 651–660.
Wessely, F. and Swoboda, W. (1951). *Monatshefte*, **82**, 437–442.
Wieland, H. and Sorge, H. (1916). *Hoppe-Seyl, Z.*, **97**, 1–27.
Wien, R. and Mason, D. F. J. (1953). *Brit. J. Pharmacol.*, **8**, 306–314.
Wildi, B. S. (1959). U.S. Patent 2,897,202 in *Chem. Abstr.* (1960), **54**, 646.
Woolley, D. W. (1960). In *Fortschritte der Arzneimittelforschung*, Vol. II, (ed. E. Jucker), pp. 613–636, Basel: Birkhäuser.
Zderic, J. A., Halpern, O., Carpio, N., Ruiz, A., Limon, D. C., Magaña, L., Jiménez, H., Bowers, A. and Ringold, H. J. (1960). *Chem. and Ind.*, 1625–1626.
Zicha, L., Schmid, E., Scheiffarth, F., Graf, N. and Koschera, H. (1960). *Arzneimitt.-Forsch.*, **10**, 831–834.

RESEARCH PAPERS

ALKANOLAMIDES STERICALLY RELATED TO ERGOMETRINE

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The ethanolamides of 3-dimethylaminopropionic acid, 1-methyl-1,2,5,6-tetrahydronicotinic acid (arecaidine) and 1-methylhexahydronicotinic acid, and propanolamides of 3-dimethylaminopropionic acid and arecaidine have been synthesised. None of these compounds had demonstrable oxytocic activity on the isolated oestrous rat uterus in concentrations up to 1 mg./ml. Arecaidine propanolamide inhibited acetylcholine-induced contractions in concentrations of 0·075 to 0·3 mg./ml. The 2-styryl derivatives of 3-dimethylaminopropionic and 4-dimethylaminobutyric acids were found to be unstable.

No entirely synthetic compound related to the ergot alkaloids has found general clinical acceptance as an oxytocic, nor has any structural feature or combination of features been shown to be consistently associated with oxytocic activity. A major difficulty, however, lies in the assessment of oxytocic activity, since results may vary widely depending on the choice of technique, of experimental animal or on the endocrine balance of the same animal on different occasions (Rosen, Blumenthal, Townsend, Tislow and Seifter, 1956). In addition, the relative potencies of oxytocics on the human uterus have been shown to bear little relation to those observed in experimental animals (De Jongh, 1956; Garrett and Embrey, 1958); indeed clinically useful oxytocics may well have been overlooked because of their low potency in animal experiments.

Almost all structural features which have been shown to be associated with the oxytocic activity of the ergot alkaloids are associated with ring D of the lysergic acid molecule (I, R=OH) (unsaturation in the C(9)–C(10) position, Rothlin and Cerletti, 1950, the configuration at C(5), and the nature and configuration of the substituent at C(8), Stoll and Hofmann, 1943). It is not surprising, therefore, that most synthetic oxytocics modelled on lysergic acid have included structures related to this ring or to its open-chain analogues.

Derivatives of ω-amino-acids (Table I) may be considered as open-chain analogues of ring D of lysergic acid. Direct comparison of their oxytocic activities is impossible because of the different methods of assessment used, but it appears that similarity in chemical structure to the ergot

TABLE I

DERIVATIVES OF ALIPHATIC ω-AMINO-ACIDS SHOWING OXYTOCIC ACTIVITY

$$\begin{matrix} R_1 \\ R_2 \end{matrix} \rangle N.(CH_2)n\ COR_3$$

Cpd. No.	n	R_1	R_2	R_3	References	Oxytocic action on animals
II	2	Me	—OMe, —$CH_2.CH_2$—, OMe	NH·CH_2CH_2OH; OEt	Baltzly, Dvorkovitz and Phillips (1949)	Slight
III	2	Me	—CH_2CH_2—, MeO, OMe	OEt		Greater than II
IV	3 to 6	Me			Baltzly and Phillips (1949)	Similar to III
V	2	Me	$CH_2.CHMe$—, OMe	OEt	Plieninger (1953)	Greater in (−) isomer
VI	2	Me		OEt or NEt_2	Gearien and Liska (1954)	
VII	2	Me	—CH_2CH_2—, N	OMe or NEt_2	Norris and Blicke (1952)	Slight
VIII	2	Me		—NHCH(Me)CH_2OH	Marini-Bettolo, Chiavarelli; and Bovet-Nitti (1951, 1952)	Comparable with Ergometrine
IX	2	Me	,,	NEt_2	Marini-Bettolo, Chiavarelli and Bovet (1950)	Similar to VIII
X	1	Me	,,	,,	Bovet-Nitti (1952)	Similar to VIII
XI	1	Et	Et	NEt_2	Bovet-Nitti (1952); Marini-Bettolo and Cavalla (1954)	

alkaloids does not tend to be associated with high activity, and Rosen and co-workers (1956) have reported the marked potency of simple glycine derivatives (XI) which bear only a remote resemblance to lysergic acid derivatives. Steric relationships between these compounds and lysergic acid were not generally considered by the authors, although the optical isomers of compound (V) were isolated and found to differ in their oxytocic action (Plieninger, 1953).

Amongst piperidine derivatives which have been prepared as potential oxytocics (Table II) the lack of apparent relation between structural similarity to lysergic acid and oxytocic acitivity is even more marked; *N*-(3-indolylmethyl)piperidine derivatives (XIV) are more active than 2-(3-indolylmethyl)piperidines (XII), and similar compounds with a carboxyl substituent corresponding to the essential C(8) carboxyl in lysergic acid are of negligible activity compared with the same compounds lacking such a carboxyl substituent (De Jongh and van Proosdij-Hartzema, 1952). On the other hand, compounds (XVI) and (XVII) with little resemblance to lysergic acid are quite powerful oxytocics.

TABLE II

PIPERIDINE DERIVATIVES SHOWING OXYTOCIC ACTIVITY

Cpd. No.	Substituent on N	Substituent at C_2	Other substituents	References	Oxytocic activity on animals
XII	Me	N —CH_2—	H	Akkerman and Veldstra (1954)	Active but less so than XIV and XV
XIII	N —CH_2—	Me	H	Hoffman and Schellenberg (1954)	
XIV	N —CH_2—	Me	4,6—Me 3,5—H	Akkerman, De Jongh and Veldstra (1951)	More active than XII, XIII and XV, but unstable
XV	N —CH_2—	Me	6—Me 3,4,5—H	Akkerman, De Jongh and Veldstra (1951)	More active than XII and XIII, less than XIV
XVI	—CH_2—	H	H	Schindler and Voegtli (1949)	
XVII	CH_2— MeO OMe	H	H	Votava and Podvalava (1956)	High activity
XVIII	H	Me	3— OMe; 5—COOMe; 4,6—H	Plieninger (1953)	Low activity

Substitution of a carboxyl group in the 3-position of 1,2,3,4-tetrahydroquinoline (XIX) yields compounds with some structural resemblance to rings C and D of lysergic acid and oxytocic activity has been demonstrated in a compound of this type (XIX; R=NHEt; R′=Me) (Cain, Plampin and Sam, 1955; Koelle and Kamijo, 1954).

Lack of any evident structure-action relation in oxytocics has made the choice of compounds for synthesis and testing necessarily an empirical one. A new approach may however be offered by recent detailed studies of the configuration at C(8) and the conformation of lysergic acid, isolysergic acid and their derivatives (Cookson, 1953; Stenlake, 1953;

Stoll Petrzilka, Rutschmann, Hofmann and Günthard, 1954; Stenlake, 1955). Since isolysergic acid, with an axial carboxyl substituent at C(8) produces derivatives of negligible oxytocic activity compared with those of lysergic acid in which the carboxyl substituent is equatorial (Stoll and Hofmann, 1943), it appears that the spacial relation between the carboxyl group and neighbouring atoms is of importance in determining the activity of the ergot alkaloids. The synthesis of the model compounds of similar molecular shape described below seemed therefore to be worthy of investigation. Stereochemical considerations are discussed by Chilton and Stenlake (1962).

XIX XX XXI

XXII XXIII XXIV

$NMe_2 \cdot CH_2 \cdot CH_2 \cdot CONH \cdot CH_2 \cdot CH_2 \cdot O \cdot CO \cdot CH_2 \cdot CH_2 \cdot NMe_2$

XXV

$NMe_2 \cdot CH_2 \cdot CH_2 \cdot CO \cdot O \cdot CH_2 \cdot CH_2 \cdot NH_2$ XXVI

$NMe_2 \cdot CH_2 \cdot CH_2 \cdot CONH \cdot CH_2 \cdot CH_2 \cdot OH$ XXVII

DISCUSSION

Styryl-substituted Aliphatic ω-Amino-acids

Formula XX indicates how 2-styryl-3-dimethylaminopropionic acid may be considered as an open-chain analogue of lysergic acid, the double bond of the styryl substituent corresponding to that in the C(9)–C(10) position of lysergic acid and the phenyl group to Ring A. The only related compounds reported in the literature had benzyl or naphthylmethyl substituents in the 2-position and were inactive as oxytocics (Norris and Blicke, 1952).

Unsuccessful attempts to prepare compound (XX) by a Mannich reaction of styrylacetic acid and its ethyl ester with formaldehyde and dimethylamine indicated that the activity of the 2-methylene group in

styrylacetic acid is not sufficient to permit such a reaction. Potentiation of this activity by the use of styrylmalonic acid in place of styrylacetic acid produced the required reaction with spontaneous decarboxylation. Unfortunately a marked tendency to deamination in aqueous solution, even at room temperature, led to poor recovery of the required product and prevented the preparation of further derivatives. Such deamination of 2-substituted dialkylaminopropionic acids is well known (Mannich and Ganz, 1922) and occurred with the benzyl and naphthylmethyl compounds mentioned previously, but only on prolonged heating above 100°.

The unexpectedly low stability of 2-styryl-3-dimethylaminopropionic acid led to the preparation of the homologous ethyl 2-styryl-4-dimethylaminobutyrate (XXI), since this would be expected to show less tendency to deaminate, while the oxytocic activity of related amino-acid derivatives had been shown to be largely unaffected by increase in the number of methylene groups separating the amino and carboxyl functions (Baltzly and Phillips, 1949). Preparation of this compound by condensation of the sodio derivative of ethyl styrylacetate with dimethylaminoethyl chloride gave a small yield of the required amino-ester, the major product being a dimer of ethyl styrylacetate. The amino-ester was unstable, darkening and resinifying even in a sealed container stored in the dark. Because of the apparent inherent instability of 2-styryl-ω-dialkylamino acids, further routes to these compounds were not explored.

Alkanolamides of Amino-acids

The ethanolamides and propanolamides of 3-dimethylaminopropionic acid (XXII), 1-methylhexahydronicotinic acid (XXIII) and 1-methyl-1,2,5,6-tetrahydronicotinic acid (arecaidine, XXIV) were prepared because of the structural relation between these acids and ring D of lysergic acid and hence between their propanolamides and the corresponding fragment of ergometrine. This relation was considered sufficient to make these compounds of potential value as oxytocics, especially since other amides of simple *N*-substituted 3-aminopropionic acid derivatives had been shown by Rosen and co-workers (1956) to have oxytocic activity. It has also been shown by Chilton and Stenlake (1962) that the propanolamides of (XXII) and (XXIV) and the ethanolamides of (XXII), (XXIII) and (XXIV) have their amino- and carboxyl groups in a steric configuration related to that of ergometrine and different from that of the pharmacologically-inert ergometrinine.

Preparation of these alkanolamides by aminolysis of esters, a standard method for the commercial synthesis of alkanolamides, was successful only for the previously known (Phillips and Baltzly, 1947) ethanolamide of 1-methylhexahydronicotinic acid. The methyl ester of 1-methyl-1,2,5,6-tetrahydronicotinic acid (arecoline) formed an addition compound on heating with ethanolamine, while ethyl 3-dimethylaminopropionate decomposed at the temperature required for reaction.

Synthesis of alkanolamides by reaction of acid chlorides and amino-alcohols does not appear to have been widely investigated, and published

work (Knorr and Rössler, 1903; Brinziger and Koddebusch, 1949) has been largely concerned with reactions of aromatic acid chlorides. We have found that the acid chloride of 3-dimethylaminopropionic acid in chloroform reacts with ethanolamine to produce almost exclusively the unwanted *O*,*N*-bis-(3′-dimethylaminopropionyl)-2-aminoethanol (XXV), with a small yield of 2-aminoethyl 3-dimethylaminopropionate (XXVI). Since *O*-acylation could not be suppressed by variation of the reaction conditions, whereas *N*-acylation was markedly reduced under acidic conditions, the *O*-acyl compound (XXVI) was prepared by reaction of the hydrochlorides and converted to the required 2-(3′-dimethylaminopropionamido)ethanol (XXVII) by alkali-induced acyl migration as described by Phillips and Baltzly (1947) and others. Preparation of (±)-2-aminopropyl 3-dimethylaminopropionate and 2-aminoethyl 1-methylhexahydronicotinate in chloroform by this method resulted in poor yields; reaction of the acid chloride hydrochloride of arecaidine with ethanolamine hydrochloride in chloroform gave no crystalline product.

The difference in behaviour between 3-dimethylaminopropionyl chloride hydrochloride and 1-methylhexahydronicotinyl chloride hydrochloride on reaction with ethanolamine hydrochloride was considered to be due to their differing solubilities in the chloroform used as solvent, since the reactivities of the two compounds would otherwise be expected to be of the same order. No suitably inert solvent was found for 1-methylhexahydronicotinyl chloride hydrochloride and reaction was accordingly carried out in the absence of solvent. A mixture of the acid chloride hydrochloride and ethanolamine hydrochloride in fine powder formed a eutectic system melting at about 120° and reacting immediately to form almost exclusively the required 2-aminoethyl 1-methylhexahydronicotinate dihydrochloride, which was readily converted to the corresponding alkanolamide by treatment with aqueous alkali as before.

Analogous reactions of 1-methyl-1,2,5,6-tetrahydronicotinyl chloride hydrochloride with ethanolamine hydrochloride and (±)-2-aminopropanol hydrochloride in the absence of solvent gave good yields of the amino-ester dihydrochlorides. Treatment of the latter with aqueous alkali induced complete $O \rightarrow N$ acyl migration as evidenced by a fall in the potentiometric titre of precisely 50 per cent and a change in the neutralisation curve from that of a diacidic base to that of a monoacidic base. The resultant alkanolamides were however, exceedingly water-soluble, rendering extraction wasteful and tedious, while the isolated compounds were syrupy, very hygroscopic compounds which yielded no crystalline derivatives suitable for characterisation. These alkanolamides were accordingly used in the solution in which they were prepared, excess alkali being neutralised by hydrochloric acid.

1-Methylhexahydronicotinyl chloride hydrochloride and (±)-2-aminopropanol hydrochloride reacted in the absence of solvent with evolution of hydrogen chloride but failed to yield any crystallisable product. Similar failure to obtain a crystalline product on catalytic reduction of (±)-2-aminopropyl 1,2,5,6-tetrahydronicotinate dihydrochloride indicated that the required product may well have been formed

from the acid chloride reaction but is probably inherently non-crystallisable. No other crystallisable derivatives of this amino-ester could be found, and treatment with aqueous alkali gave a gummy hygroscopic product which again failed to form any crystalline derivatives.

The acid chloride hydrochloride of 3-dimethylaminopropionic acid decomposed below the melting point of any mixture of it with ethanolamine or (±)-2-aminopropanol hydrochloride, preventing reaction in the absence of solvent. It was found that the alkanolamides of this acid could be prepared in good yield by reaction of 3-dimethylaminopropionyl chloride with the ethyl esters of glycine and (±)-alanine to form peptide esters, which could be selectively reduced by careful treatment with lithium aluminium hydride to produce the required alkanolamides in satisfactory yield. Similar reductions of ester groups in the presence of amides have been previously described (Felkin, 1950; Berlinguet, 1954). This method offered no advantage for preparation of the ethanolamide but was the best available route to the propanolamide. It would appear to offer a suitable method for the preparation of optically-active alkanolamides, since active amino-acids are available and the mild conditions of reaction should not induce racemisation.

Attempts to prepare the acid azide of arecaidine as an intermediate in the synthesis of alkanolamides as described by Stoll and Hofmann (1943) were not successful.

Pharmacological Action

Pharmacological investigation of the alkanolamides described in this section (for which the authors are indebted to Dr. S. Nanjappa of the Department of Experimental Pharmacology, University of Glasgow) showed that none of these compounds had demonstrable oxytocic activity on the isolated oestrous rat uterus in concentrations up to 1 mg./ml.

The propanolamide of arecaidine inhibited acetylcholine-induced contractions of the oestrous rat uterus in concentrations of 0·075–0·3 mg./ml., an effect also shown by higher concentrations of the propanolamide of dimethylaminopropionic acid (0·3–1·0 mg./ml.) and by the ethanolamides of *N*-methylhexahydronicotinic acid (1 mg./ml.) and 3-dimethylaminopropionic acid (0·7–1 mg./ml.). Whilst these results on the rat do not entirely rule out the possibility of activity in higher animals, it seems unlikely that compounds showing such a low level of activity in animal experiments would prove useful in humans.

No effect on cat blood-pressure was shown by any of the compounds in doses up to 5 mg./kg., nor did they have any inhibitory effect on the action of adrenaline or noradrenaline on cat blood-pressure.

The lack of oxytocic action shown by these alkanolamides reflects the findings of earlier workers who reported that the alkanolamides of derivatives of ω-amino-aliphatic acids, piperidine-carboxylic acids and tetrahydroquinoline were either inert as oxytocics or else showed much less activity than did corresponding esters or simple alkylamides. Amongst purely synthetic compounds, only the propanolamide of *N*-tetrahydronaphthyl-*N*-methyl-3-aminopropionic acid (VIII) is reported to have

marked oxytocic activity ("comparable with ergometrine on the isolated uterus of the guinea pig and rabbit").

It does not seem possible in the light of present evidence to speculate on the nature of the contribution made by the carboxylamido group to the oxytocic activity of the ergot alkaloids and semi-synthetic lysergic acid amides, but it appears that the alkanolamido group is specific in potentiating oxytocic action in only a very small range of compounds.

EXPERIMENTAL

3-Dimethylamino-2-styrylpropionic acid. Styrylmalonic acid (1·03 g., 0·05 mole), prepared by the method of Ivanov and Pschenichnii (1937), was dissolved in 60 per cent aqueous solution of dimethylamine (0·38 ml.) and treated with 40 per cent solution of formaldehyde (0·4 ml.) at 0°. There was an immediate transient effervescence. The mixture was left overnight at 0°, acidified with dilute hydrochloric acid, and the amorphous solid which separated was extracted with ether. Evaporation of the ethereal extract *in vacuo* yielded a solid (0·49 g.) which could not be crystallised, but which gave a crystalline derivative with *p*-bromophenacyl bromide, m.p. 142° (from ethanol), probably *p*-bromophenacyl-2-styrylacrylate. Found: C, 60·5; H, 4·0; Br, 20·8. $C_{19}H_{15}BrO_3$ requires C, 61·4; H, 4·0; Br, 21·3 per cent.

The aqueous fraction remaining after extraction of the acid was treated with a slight excess of sodium bicarbonate and extracted with ether to remove any unchanged dimethylamine, then a slight excess of sodium picrate solution was added. On the addition of a few drops of dilute hydrochloric acid, crystals (23 mg., 0·9 per cent), m.p. 146° (from ethanol) of the *picrate* of *3-dimethylamino-2-styrylpropionic acid* were obtained. Found: C, 51·5; H, 4·5; N, 12·3. $C_{19}H_{20}N_4O_9$ requires C, 50·9; H, 4·5; N, 12·5 per cent.

4-Dimethylamino-2-styrylbutyric acid. Sodium (0·575 g., 0·025 mole) was dissolved in dry ethanol (20 ml.) and ethyl styrylacetate (4·75 g., 0·025 mole) added with stirring and cooling. After 60 hr. at room temperature, the mixture was treated with a solution of dimethylaminoethyl chloride (obtained from dimethylaminoethyl chloride hydrochloride, 3·58 g., 0·025 mole) in xylene (7 ml.) added dropwise with continuous stirring. On heating under reflux for 2 hr. a white precipitate was obtained. The mixture was cooled, acidified with dilute hydrochloric acid, extracted with ether and the ethereal extract reserved. The aqueous fraction was basified with solution of ammonia and again extracted with ether. The ether extract was dried (Na_2SO_4), evaporated *in vacuo* and the oily residue fractionally distilled *in vacuo* to yield a colourless viscous oil (0·92 g., 14 per cent), b.p. 100–110°/0·4 mm., $n_D^{18°}$, 1·515, probably ethyl 4-dimethylamino-2-styrylbutyrate. Equiv. (potentiometric titration) 258. $C_{16}H_{23}NO_2$ requires equiv. 261. The product resinified and darkened rapidly on storage in a sealed vessel in the dark. The picrate of this base was initially oily, but slowly formed crystals, m.p. 106° (from aqueous ethanol) of the *picrate* of *4-dimethylamino-2-styrylbutyric acid.* Found:

C, 51·4; H, 4·7; N, 12·2. $C_{20}H_{22}N_4O_9$ requires C, 51·95; H, 4·8; N, 12·1 per cent.

The reaction was repeated, heating the sodium ethoxide and ethyl styrylacetate under reflux for 2 hr. initially. The non-basic ether-soluble fraction was again reserved. The yield of base, identical with that from the previous reaction was 0·88 g. (13 per cent).

The non-basic ethereal fractions from the previous reactions were combined and fractionally distilled *in vacuo* to yield ethyl styrylacetate, and a very viscous yellow liquid, b.p. 206–208°/0·8 mm. The latter was hydrolysed by heating under reflux for 30 min. with ethanolic potassium hydroxide. Removal of the alcohol by evaporation *in vacuo*, acidification with hydrochloric acid, and extraction with ether produced an acid, m.p. 153° (from benzene and light petroleum), probably a dimer of styrylacetic acid. Found: C, 74·2; H, 6·6; equiv. (potentiometric titration) 164; m.w. (freezing-point depression of camphor) ca. 294. $C_{20}H_{20}O_4$ requires C, 74·1; H, 6·2 per cent; equiv. 162; m.w. 324). Styrylacetic acid has m.p. 54°.

Ethyl styrylacetate (4·75 g., 0·025 mole) was added to "molecular" sodium (0·575 g., 0·025 mole) suspended in xylene (30 ml.). No reaction was evident in the cold; on warming, a red resin was formed without evolution of hydrogen.

Reaction of ethyl 3-*dimethylaminopropionate and ethanolamine.* (*a*) Equal volumes of ethyl 3-dimethylaminopropionate prepared by the method of Adamson (1949) (14·5 g., 0·1 mole) and ethanolamine were heated together in a still fitted with a short fractionating column so that the fraction distilling below 90° was continually removed. The distillate (6·12 g.) gave a positive iodoform reaction (theoretical yield of ethanol, 4·6 g.) and on addition of ethanolic solution of picric acid gave crystals of dimethylamine picrate, m.p. 150° undepressed on mixture with authentic dimethylamine picrate.

The residue in the still fractionally distilled at 1 mm. gave a fore-run of ethanolamine and a few drops of oily liquid distilling at 140–190°. The residue was brown and resinous and could not be distilled. Neither it nor the distillate formed crystalline hydrochlorides, picrates or oxalates.

(*b*) A repeat reaction in which the ester and ethanolamine were heated together at 100° for 4 hr. yielded only unchanged starting material.

2-*Aminoethyl* 3′-*dimethylaminopropionate dihydrochloride.* (*a*) A suspension of 3-dimethylaminopropionic acid hydrochloride (6·12 g., 0·04 mole) in thionyl chloride (20 ml.) was heated in a water bath at 65° until effervescence ceased and the mixture became clear. Heating above this temperature caused resinification. Excess thionyl chloride was removed by evaporation *in vacuo* and the white crystalline residue washed with light petroleum and again dried *in vacuo*. The acid chloride hydrochloride so obtained was suspended in dry chloroform (20 ml.) and ethanolamine (2·44 g., 0·04 mole) in dry chloroform (10 ml.) added in one quantity at room temperature with stirring. After standing overnight at room temperature, the chloroform was removed by evaporation *in vacuo* and the syrupy residue dried *in vacuo* over potassium hydroxide.

Ethanol (30 ml.) was added and set aside overnight at 0°. The crystalline residue was filtered off and recrystallised from aqueous ethanol to give 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride (1·1 g., 12 per cent) m.p. 181°. Found: C, 35·9; H, 7·7; Cl, 30·6; N, 12·0. $C_7H_{18}Cl_2N_2O_2$ requires C, 36·0; H, 7·7; Cl, 30·5; N, 12·0 per cent.

The ethanolic mother-liquors left after removal of the amino-ester dihydrochloride were concentrated *in vacuo* and treated with dry ether to yield a deliquescent hydrochloride m.p. 154° (4·1 g. 50 per cent) which on the addition of sodium picrate solution formed the dipicrate of O,N-*bis*-(*3-dimethylaminopropionyl*)*ethanolamine*. Found C, 41·05; H, 4·5; N, 17·3. $C_{24}H_{31}N_9O_{17}$ requires C, 40·2; H, 4·3; N, 17·6 per cent.

(*b*) A suspension of 3-dimethylaminopropionyl chloride hydrochloride (0·05 mole) in dry chloroform (30 ml.) was treated with ethanolamine hydrochloride (4·85 g., 0·05 mole) which had been finely powdered by levigation under dry light-petroleum. No immediate reaction was evident, but, on heating under reflux, hydrogen chloride was evolved and the amount of insoluble matter increased. After 2 hr., evolution of hydrogen chloride ceased, the mixture was allowed to cool and the chloroform removed by evaporation *in vacuo*. The residue was dissolved in a minimum quantity of boiling ethanol, filtered and allowed to cool, yielding 2-aminoethyl 3'-dimethylaminopropionate dihydrochloride (7·5 g.) m.p. 181°, identical with that obtained previously. Evaporation of the mother liquors yielded a further 1·0 g. of product (total yield 8·5 g.; 73 per cent).

2-(*3'-Dimethylaminopropionamido*)*ethanol*. 2-Aminoethyl 3'-dimethylaminopropionate dihydrochloride (7·5 g., 0·033 mole) in a minimum volume of water was treated under cooling with a slight excess of potassium hydroxide in a minimum of water and extracted with chloroform (3 × 30 ml.). The combined chloroform extracts were dried ($MgSO_4$) and the chloroform removed by evaporation *in vacuo* to leave an oil (3·0 g., 57 per cent) $n_D^{18°}$, 1·477 probably slightly impure 2-(3'-dimethylaminopropionamido)ethanol. Found equiv. (potentiometric) 164, 165, $C_7H_{16}N_2O_2$ requires equiv. 160.

Short-path distillation of the oil at 0·8 mm. yielded a colourless oily distillate b.p. 155–160°. Some loss of vacuum occurred during heating and the small residue of volatile base recovered from the solid carbon dioxide-acetone cooled vapour trap, gave a picrate m.p. 150° undepressed on mixture with authentic dimethylamine picrate.

A portion of the distillate fraction (b.p. 155–160°, $n_D^{18°}$, 1·489, equiv. 193) was mixed with excess dimethylamine and left at room temperature for 48 hr. Excess dimethylamine was removed at 100° *in vacuo* to yield a residue of equiv. 166. Equimolecular proportions of the base (equiv. 166) and oxalic acid were separately dissolved in minimum amounts of ethanol and mixed. Ethanol was removed by distillation *in vacuo* and the resultant syrup treated with boiling acetone to give, on cooling, crystals m.p. 85° (from acetone) of 2-(*3'-dimethylaminopropionamido*)-*ethanol acid oxalate*. Found: C, 43·1; H, 7·4; N, 11·0. $C_9H_{18}N_2O_6$ requires C, 43·2; H, 7·2; N, 11·2 per cent.

The succinate, prepared as described for the oxalate, using 2 moles of base to each mole of succinic acid consisted of hygroscopic crystals, m.p. 108° (from acetone). Found: C, 49·5; H, 8·8; N, 12·9. $C_{18}H_{38}N_4O_8$ requires C, 49·3; H, 8·7; N, 12·8 per cent.

Attempts to prepare crystalline picrates, picrolonates, hydrochlorides and naphthylisocyanates of the base were unsuccessful.

Ethyl 2-(3'-*dimethylaminopropionamido*)*acetate*. A suspension of 3-dimethylaminopropionyl chloride hydrochloride (0·05 mole) in dry chloroform (30 ml.) was treated with a solution of ethyl aminoacetate (5·15 g., 0·05 mole) in chloroform (20 ml.) added in one quantity. The mixture was heated under reflux until no further hydrogen chloride was evolved (about 2 hr.) and the chloroform removed by evaporation *in vacuo* to leave a gummy solid (9·8 g., 82 per cent) which crystallised on kneading under dry ether. Recrystallisation from ethanol/ether gave highly deliquescent crystals m.p. 124° probably slightly impure ethyl 2-(3'-dimethylaminopropionamido)acetate hydrochloride. Found: Cl, 16·11. $C_9H_{19}ClN_2O_3$ requires Cl, 14·9 per cent. Repeated recrystallisation did not improve the analysis.

The hydrochloride was suspended in dry ether (30 ml.) and dry ammonia passed through it for 10 min. with continual stirring. The product was filtered, the solid re-suspended in ether and the process repeated. After a third repetition the ethereal fractions were combined and the ether removed by evaporation *in vacuo*. The oily residue was left overnight in a desiccator at 0·5 mm. pressure in the presence of potassium hydroxide and paraffin wax to yield *ethyl* 2-(3'-*dimethylaminopropionamido*)*acetate*, $n_D^{18°}$, 1·4621. Found: C, 53·6; H, 8·6; N, 13·6. $C_9H_{18}N_2O_3$ requires C, 53·5; H, 8·9; N, 13·9 per cent.

2-(3'-*Aminopropionamido*)*ethanol by reduction of ethyl* 2-(3'-*amino-propionamido*)*acetate*. Ethyl 2-(3'-dimethylaminopropionamido)acetate (3 g., 0·0015 mole) was dissolved in dry ether (30 ml.) and treated with lithium aluminium hydride (0·68 g., 0·002 mole) added in small portions with continuous stirring and ice-cooling. The mixture was allowed to attain room-temperature with continuing constant stirring, the excess of lithium aluminium hydride was decomposed by the addition of moist ether followed by ice, and the product was filtered. The solid residue, on extraction with chloroform and evaporation of the extract *in vacuo*, yielded an oily base (1·1 g., 46 per cent) which formed an oxalate identical with the acid oxalate of 2-(3'-dimethylaminopropionamido)ethanol obtained previously.

(±)-2-*Aminopropyl* 3'-*dimethylaminopropionate dihydrochloride*. Finely powdered (±)-2-aminopropanol hydrochloride (0·76 g., 0·02 mole) prepared by the method of Vogl and Pöhm was added in one quantity to a suspension of 3-dimethylaminopropionyl chloride hydrochloride (0·005 mole) in dry chloroform and heated under reflux until no more hydrogen chloride was evolved (about 45 min.). The chloroform was removed by evaporation *in vacuo* and the solid residue recrystallised from aqueous ethanol to yield (±)-2-*aminopropyl* 3'-*dimethylaminopropionate dihydrochloride* (0·47 g.) m.p. 189°. Found: C, 38·6; H, 7·8; N, 11·4.

$C_8H_{20}Cl_2N_2O_2$ requires C, 38·9; H, 8·1; N, 11·3 per cent. Concentration of mother liquors yielded a further 0·11 g. of product (total yield 0·58 g. 46 per cent). The residual fraction in the mother liquors (0·49 g.) could not be crystallised and did not give a crystallisable picrate on treatment with sodium picrate solution.

Numerous repetitions of the reaction gave yields varying from 5 to 46 per cent. Increase in scale generally diminished the percentage yield.

(±)-2-(3′-*Dimethylaminopropionamido*)*propanol*. The method was identical with that described for 2-(3′-dimethylaminopropionamido)ethanol. An oily base was obtained which formed on treatment with oxalic acid the *acid oxalate* of (±)-2-(3′-*dimethylaminopropionamido*)-*propanol*, m.p. 114° (from acetone/ethanol). Found: C, 45·8; H, 7·9; N, 10·5. $C_{10}H_{20}N_2O_6$ requires C, 45·5; H, 7·6; N, 10·6 per cent. The succinate was crystalline but excessively deliquescent.

(±)-*Ethyl* 2-(3′-*dimethylaminopropionamido*)*propionate*. 3-Dimethylaminopropionyl chloride hydrochloride (0·05 mole) was treated with (±)-ethyl 2-aminopropionate to yield an extremely deliquescent hydrochloride, m.p. 96° (from ethanol/ether). Found: Cl, 14·3. $C_{10}H_{21}ClN_2O_3$ requires Cl, 14·1 per cent. Treatment of a suspension of this hydrochloride in dry ether with excess dry ammonia as described previously gave (±)-*ethyl* 2-(3′-*dimethylaminopropionamido*)*propionate* as a colourless oil, $n_D^{18°}$, 1·4558 (8·78 g., 81 per cent). Found: C, 55·7; H, 9·3; N, 12·9. $C_{10}H_{20}N_2O_3$ requires C, 55·6; H, 9·3; N, 13·0 per cent.

(±)-2-(3′-*dimethylaminopropionamido*)*propanol by lithium aluminium hydride reduction of ethyl* (±)-2-(3′-*dimethylaminopropionamido*)*propionate*. The total product from the previous reaction was reduced as described for the corresponding acetate. Excess lithium aluminium hydride was decomposed by the cautious addition of ice and the product filtered. The solid residue was extracted twice with 50 ml. portions of boiling ethanol, which were combined and evaporated *in vacuo* to a syrupy residue. This was extracted with 25 ml. of boiling ethanol, and the extract was treated with ether to form an opalescent solution. After filtration, this was again evaporated *in vacuo* to yield 4·95 g. (70 per cent) of a viscous oil. Oxalic acid (4 g.) was added as an ethanolic solution and the resulting precipitate, consisting mostly of inorganic salts of oxalic acid, was removed by filtration. The filtrate on evaporation to dryness and extraction with boiling acetone gave crystals, m.p. 114° identical with those of the acid oxalate of (±)-2-(3′-dimethylaminopropionamido)propanol obtained previously.

2-(1′-*Methylhexahydronicotinamido*)*ethanol acid oxalate*. The method of Phillips and Baltzly (1947) was modified as follows: a solution of 1-methylhexahydronicotinic acid hydrochloride, prepared by the method of Preobrazhenskii and Fisher (1941), (3 g., 0·0166 mole) in dry ethanol (30 ml.) was saturated with hydrogen chloride then heated under reflux for 1 hr. The mixture was evaporated *in vacuo* to a syrup which was treated with a slight excess of 20 per cent aqueous sodium hydroxide and at once extracted with chloroform. The chloroform extract was dried (Na_2CO_3) and the chloroform removed by evaporation *in vacuo* to yield

ethyl 1-methylhexahydronicotinate (2·2 g.). The base was mixed with excess ethanolamine (2 ml.) and heated to 180° in a distillation flask fitted with a short fractionating column. Ethanol, b.p. 78° was evolved for about 20 min., after which no distillate boiling below 160° could be obtained and heating was stopped. Excess ethanolamine was removed by evaporation *in vacuo*, the viscous oil (2·1 g.) dissolved in a little ethanol, mixed with a slight excess of ethanolic oxalic acid, then evaporated to a syrup *in vacuo*. On extraction with boiling acetone and cooling, 2-(1'-*methylhexahydronicotinamido*)*ethanol acid oxalate* (1·8 g., 46 per cent), m.p. 124° (from acetone) was obtained. Found: C, 48·8; H, 7·4; N, 10·0. $C_{11}H_{20}N_2O_6$ requires C, 47·8; H, 7·25; N, 10·15 per cent.

Treatment of the product of this reaction with a large excess of ethanolic hydrogen chloride gave crystals of 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride, m.p. 216° (from ethanol). Phillips and Baltzly (1947) gave m.p. 214°.

2-*Aminoethyl* 1'-*methylhexahydronicotinate dihydrochloride*. (*a*) A suspension of 1-methylhexahydronicotinic acid hydrochloride (4·49 g., 0·025 mole) in thionyl chloride (25 ml.) was heated under reflux for 15–20 min. until evolution of hydrogen chloride ceased and the mixture became homogeneous. Excess thionyl chloride was removed by evaporation *in vacuo* and the crystalline residue washed with dry light petroleum until the washings were colourless. Light petroleum was removed by decantation followed by drying *in vacuo* and the product suspended in dry chloroform (60 ml.). Finely powdered ethanolamine hydrochloride (2·85 g., 0·03 mole) was added, and the mixture heated under reflux until hydrogen chloride ceased to be evolved (5–6 hr.). The chloroform was evaporated *in vacuo* and the residue dissolved in a minimum volume of boiling ethanol. On cooling, 2-aminoethyl 1'-methylhexahydronicotinate dihydrochloride (315 mg., 5 per cent), m.p. 216° (from ethanol) was obtained.

Evaporation of the alcoholic mother liquors gave 4·1 g. of an uncrystallisable residue which was almost completely soluble in chloroform. The small portion of chloroform-insoluble material was identical with the aminoester dihydrochloride m.p. 216° obtained initially. The chloroform-soluble portion could still not be crystallised, but yielded on treatment with sodium picrate, *O,N-bis-*(1'-*methylhexahydronicotinyl*)-2-*aminoethanol dipicrate* m.p. 227° (decomp.) (from aqueous ethanol). Found: C, 43·4; H, 4·4; N, 16·5. $C_{28}H_{35}N_9O_{17}$ requires C, 43·7; H, 4·6; N, 16·4 per cent.

(*b*) The previous preparation was repeated, replacing the dry chloroform by dry tetrahydrofuran. The yield of amino-ester dihydrochloride was as before.

(*c*) The acid chloride hydrochloride was prepared as before from 1-methylhexahydronicotinic acid hydrochloride (0·18 g., 0·001 mole). The washed and dried product was then mixed intimately with dry and finely powdered ethanolamine hydrochloride (0·12 g., 0·0012 mole) and heated slowly on an oil bath. The mixture melted at a bath temperature of about 120° and evolved hydrogen chloride, whereupon heating was continued at 120–140° until no more gas was evolved (about 20 min.). It was then allowed to cool and dissolved in a minimum quantity of boiling

ethanol, which on cooling yielded 177 mg. (68 per cent) of crystals m.p. 216° (from ethanol) identical with the 2-aminoethyl 1′-methylhexahydronicotinate dihydrochloride obtained previously.

Reaction of methyl 1-*methyl*-1,2,5,6-*tetrahydronicotinate and ethanolamine*. Methyl 1-methyl-1,2,5,6-tetrahydronicotinate hydrobromide (Arecoline Hydrobromide B.P.C., 1·18 g., 0·005 mole) in water (0·8 ml.) was treated with potassium hydroxide (0·4 g.) in water (0·5 ml.) and the mixture extracted with three successive portions of chloroform (20 ml.) which were combined, dried (Na_2CO_3), and evaporated to a syrup *in vacuo*. An equal volume of ethanolamine was added and the mixture heated as described for the previous two reactions to yield a viscous oil (0·774 g.) on evaporation of excess ethanolamine. Treatment with excess ethanolic oxalic acid gave a gummy residue which crystallised from aqueous methanol, m.p. 205–206° (decomp.). Found: C, 49·0; H, 7·6; N, 11·0, 11·3 per cent. This is not in agreement with the required values for 2′-(1-methyl-1,2,5,6-tetrahydronicotinamido)ethanol acid oxalate ($C_{11}H_{18}N_2O_6$ requires C, 48·2; H, 6·6; N, 10·2).

The product may have been *NN*-bis-4-(1-methyl-3-(2′-hydroxyethyl)-carbonamidopiperidyl)ethanolamine dioxalate ($C_{24}H_{43}N_5O_{13}$ requires C, 47·3; H, 7·1; N, 11·5) since a sample of hydrochloride (prepared by treatment of an aqueous solution of the oxalate with an equivalent amount of calcium chloride solution and filtering) showed no absorption maximum between 200 and 250 mμ, compared with arecoline hydrobromide which had E (1 per cent, 1 cm.) = 563 at 205 mμ.

2′-*Aminoethyl* 1-*methyl*-1,2,5,6-*tetrahydronicotinate dihydrochloride*. The acid chloride hydrochloride was prepared from 1-methyl-1,2,5,6-tetrahydronicotinic acid hydrochloride (0·89 g., 0·005 mole) as described for the corresponding hexahydronicotinic acid derivative and mixed intimately with finely powdered ethanolamine hydrochloride (1 g.) by trituration under light petroleum. The solvent was removed by decantation, the solid residue dried *in vacuo* and heated on an oil bath. Frothing and evolution of hydrogen chloride occurred at about 140°, whereupon the temperature was raised slowly to 180° and maintained at that temperature until no further reaction was visible (about 20 min.). After cooling, the product was dissolved in a minimum volume of boiling ethanol which yielded on cooling 0·84 g. (65 per cent) of brownish platelets. Recrystallisation from ethanol (charcoal) gave 2′-*aminoethyl* 1-*methyl*-1,2,5,6-*tetrahydronicotinate dihydrochloride* m.p. 258° (decomp.). Found: C, 41·8; H, 6·9; Cl, 27·1; N, 10·7. $C_9H_{18}Cl_2N_2O_2$ requires C, 42·0; H, 7·0; Cl, 27·6; N, 10·9 per cent.

2′-(1-*Methyl*-1,2,5,6-*tetrahydronicotinamido*)*ethanol*. (*a*) A saturated aqueous solution of 2′-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (1·28 g., 0·005 mole) was treated with a slight excess of 40 per cent aqueous potassium hydroxide and extracted with three portions of chloroform (30 ml.). The combined chloroform extracts were dried (Na_2SO_4) and evaporated *in vacuo* to a very viscous syrup. Treatment with a slight excess of ethanolic oxalic acid gave a product (1·02 g.) m.p. 92° (from aqueous ethanol). Recrystallisation from methanol and

acetone gave a product m.p. 158° (decomp.) but in poor yield. This material on further recrystallisation from aqueous acetone had m.p. 92°, undepressed on mixture with the original product. Found for oxalate: m.p. 92°; C, 40·6; H, 7·7; N, 8·8. $C_{11}H_{18}N_2O_6 3H_2O$ requires C, 40·2; H, 7·3; N, 8·5 per cent.

An aqueous solution of the oxalate, treated with a slight excess of calcium chloride, filtered and titrated potentiometrically showed a neutralisation curve typical for the dihydrochloride of a diacid base. The oxalate was therefore *2-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate oxalate.*

(*b*) 2′-Aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (25·7 mg., 0·1 millimole) was dissolved in electrolyte-free water (10 ml.) and an aliquot portion (2 ml., 0·02 millimole) titrated potentiometrically with carbonate-free potassium hydroxide solution. The volume required for complete neutralisation was 1·10 ml. and the titration curve was characteristic of a diacidic base. The remainder of the solution was adjusted to a pH value of 13 by the addition of solution of potassium hydroxide and allowed to stand overnight. It was then brought to pH 3 by the addition of hydrochloric acid, made up to 10 ml. with electrolyte-free water, and a further aliquot portion (2·5 ml., 0·02 millimole) was again titrated under identical conditions. The volume required for complete neutralisation was now 0·512 ml. and the neutralisation curve was typical of that for a monoacidic base as shown by comparison of the pH values at $\frac{1}{2}$, $\frac{2}{3}$ and $\frac{1}{3}$ neutrality. This indicated that O → N acyl-migration had occurred and that the solution contained the required *2′-(1-methyl-1,2,5,6-tetrahydronicotinamido)ethanol.* Attempts to prepare a crystalline oxalate of this base were unsuccessful, as were attempts to prepare crystalline succinates, tartrates and phthalates.

(±)-2′-Aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride. Using the method described for 2′-aminoethyl 1-methyl-1,2,5,6-tetrahydronicotinate, 1-methyl-1,2,5,6-tetrahydronicotinic acid hydrochloride (890 mg., 0·005 mole) and (±)-2-aminopropanol hydrochloride (1 g.) gave a crystalline product (825 mg. 61 per cent) which was recrystallised from ethanol (charcoal) to yield *(±)-2′-aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride*, m.p. 215° (decomp.). Found: C, 43·9; H, 7·4; Cl, 25·4; N, 10·0. $C_9H_{18}Cl_2O_2$ requires C, 44·3; H, 7·4; Cl, 26·2; N, 10·3 per cent.

(±)-2′-(1-Methyl-1,2,5,6-tetrahydronicotinamido)propanol. (±)-2-Aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride was treated with alkali as described for corresponding aminoethyl ester. The product was a hygroscopic, amorphous semi-solid which failed to form a crystallisable oxalate, succinate, picrate, tartrate or phthalate.

A solution of the dihydrochloride (27 mg., 0·1 millimole) in water (10 ml.) was then treated with aqueous alkali as described for the aminoethyl ester. A portion equivalent to 0·02 millimole had an initial titre of 0·809 ml. of potassium hydroxide solution and a titration curve typical of that for a diacidic base. After treatment with alkali and neutralisation the titre was 0·404 ml. and the curve characteristic of a monoacidic base,

indicating that the solution contained (±)-2′-(1-*methyl*-1,2,5,6-*tetrahydronicotinamido*)*propanol*.

Attempted preparation of (±)-2′-*aminopropyl*-1-*methylhexahydronicotinate hydrochloride*. (*a*) The acid chloride hydrochloride, prepared from 1-methylhexahydronicotinic acid hydrochloride (0·18 g., 0·001 mole), was heated with (±)-2-aminopropanol hydrochloride (0·14 g., 0·0012 mole) in the absence of solvent as described previously. Reaction occurred with evolution of hydrogen chloride, but the product was very hygroscopic and could not be crystallised.

(*b*) (±)-2′-Aminopropyl 1-methyl-1,2,5,6-tetrahydronicotinate dihydrochloride (0·27 g., 0·001 mole), was dissolved in water (5 ml.) and hydrogenated at atmospheric pressure in the presence of a platinum oxide catalyst. Reduction was complete in 4 hr. The product, on evaporation to dryness *in vacuo* was again amorphous, hygroscopic and could not be crystallised. The non-crystalline residue was treated with aqueous alkali, extracted with chloroform, the chloroform extract evaporated to dryness *in vacuo* and the residue treated with a slight excess of ethanolic solution of oxalic acid. The resultant oxalate could not be crystallised, nor could any other crystalline derivatives be prepared.

References

Adamson, D. W. (1949). *J. Chem. Soc., Supp. Issue No.* 1, S 144–155.
Akkerman, A. M. and Veldstra, H. (1954). *Rec. Trav. chim. Pays-bas*, **73**, 629–648.
Akkerman, A. M., de Jongh, D. K. and Veldstra, H. (1951). *Ibid.*, **70**, 899–916.
Baltzly, R., Dvorkovitz, V. and Phillips, A. P. (1949). *J. Amer. chem. Soc.*, **71**, 1162–1164.
Baltzly, R. and Phillips, A. P. (1949). *Ibid.*, **71**, 3421–3422.
Berlinguet, L. (1954). *Can. J. Chem.*, **32**, 31–39.
Bovet-Nitti, F. (1952). *R.C. Ist. Sup. Sanit.*, **15**, 989–1007.
Brinzinger, H. and Koddebusch, H. (1949). *Ber. dtsch. chem. Ges.*, **82**, 201–203.
Cain, C. K., Plampin, J. N. and Sam, J. (1955). *J. org. Chem.*, **20**, 466–474.
Chilton, J. and Stenlake, J. B. (1962). *J. Pharm. Pharmacol.*, **14**, 367–370.
Cookson, R. C. (1953). *Chem. and Ind.* (*Rev.*), 337–340.
Felkin, H. (1950), *C.R. Acad. Sci. Paris*, **230**, 304–306.
Garrett, W. J. and Embrey, M. P. (1958). *J. Pharm. Pharmacol.*, **10**, 325–327.
Gearien, J. E. and Liska, K. J. (1954). *J. Amer. chem. Soc.*, **76**, 3554–3555.
Hoffmann, K. and Schellenberg, H. (1944). *Helv. chim. Acta*, **27**, 1782–1790.
Ivanov, D. and Pshenichniĭ, G. (1937). *Ann. Univ. Sofia II. Faculté phys.-math. Livre* 2, **33**, 213–217; through *Chem. Abstr.* (1938), **32**, 3358.
de Jongh, D. K. (1956), *Arch. exp. Path. Pharmak.*, **228**, 106–123.
de Jongh, D. K. and van Proosdij-Hartzema, E. G. (1952). *J. Pharmacol.*, **105**, 130–138.
Knorr, L. and Rössler, P. (1903). *Ber. dtsch. chem. Ges.*, **36**, 1278–1283.
Koelle, G. B. and Kamijo, K. (1954). *J. Pharmacol.*, **112**, 444–461.
Mannich, C. and Ganz, E. (1922). *Ber. dtsch. chem. Ges.*, **55**, 3486–3504.
Marini-Bettolo, G. B. and Cavalla, J. F. (1954). *Gazz. chim. ital.*, **84**, 896–907.
Marini-Bettolo, G. B., Chiavarelli, S. and Bovet-Nitti, F. (1951). *Gazz. chim. ital.*, **81**, 587–595.
Marini-Bettolo, G. B., Chiavarelli, S. and Bovet-Nitti, F. (1952). *R.C. Ist. sup. Sanit.*, **15**, 804–807.
Norris, P. E. and Blicke, F. F. (1952). *J. Amer. pharm. Ass.*, **41**, 637–639.
Phillips, A. P. and Baltzly, R. (1947). *J. Amer. chem. Soc.*, **69**, 200–204.
Plieninger, H. (1953). *Ber. dtsch. chem. Ges.*, **86**, 25–31.
Preobrazhenskiĭ, N. A. and Fisher, L. B. (1941). *J. gen. Chem. Moscow*, **11**, 140–142.
Proosdij-Hartzema, E. G. van and de Jongh, D. K. (1954). *Arch. int. Pharmacodyn.*, **98**, 335–339.
Rosen, H., Blumenthal, A., Townsend, P. N., Tislow, R. and Seifter, J. (1956). *J. Pharmacol.*, **117**, 488–493.

Rothlin, E. and Cerletti, A. (1950). *Arch. int. Pharmacodyn.*, **82**, 118–125.
Schindler, O. and Voegtli, W. (1949). *Pharm. Acta Helvet.*, **24**, 207–216.
Stenlake, J. B. (1953). *Chem. and Ind. (Rev.)*, 1089–1090.
Stenlake, J. B. (1955). *J. chem. Soc.*, 1626–1628.
Stoll, A. and Hofmann, A. (1943). *Helv. chim. Acta*, **26**, 944–965.
Stoll, A., Petrzilka, T., Rutschmann, J., Hofmann, A. and Günthard, H. H. (1954). *Helv. chim. Acta*, **37**, 2039–2057.
Vogl, O. and Pöhm, M. (1952). *Mh. Chem.*, **83**, 541–543.
Votava, Z. and Podvalova, I. (1956). *Arch. exp. Path. Pharmak.*, **228**, 150.

DISSOCIATION CONSTANTS OF SOME COMPOUNDS RELATED TO LYSERGIC ACID

PART II. ERGOMETRINE, ERGOMETRININE AND ALKANOLAMIDES OF 3-DIMETHYLAMINOPROPIONIC ACID, 1-METHYLHEXAHYDRONICOTINIC ACID AND ARECAIDINE

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Dissociation constants have been recorded for ergometrine, ergometrinine and a number of alkanolamides of 3-dimethylaminopropionic acid, 1-methylhexahydronicotinic acid and arecaidine. The similarity of ΔpK (amide) values for the alkanolamides with that for ergometrine, offers evidence that such values may be taken as an indicator of amino-carboxyl distances.

IN a previous communication (Chilton and Stenlake, 1955) ΔpK (ester) values (the difference between the $pK'a_2$ value of an amino-acid and the pK'a value of its ester) for 3-dimethylaminopropionic acid, 1-methylhexahydronicotinic acid, ψ-ecgonine and arecaidine were shown to be similar, indicating that there is a similar relationship of amino- and carboxyl groups in all of these compounds (Neuberger, 1937) consistent with the adoption of a chair ring-conformation and an equatorial carboxyl substituent by the last three (structures I, II, and III respectively).

I II III

IV

V VI

A similar comparison of ΔpK (ester) values for lysergic and isolysergic acids (IV; R=OH) in aqueous solution was prevented by the low water-solubility of their esters. The higher water-solubility of ergometrine and ergometrinine (IV; R=NHCH(Me)CH_2OH), however, allowed determination of differences in pK'a between these alkanolamides and the corresponding acids (ΔpK (amide) values). Agreement between ΔpK (amide) values for ergometrine with those for the alkanolamides of 3-dimethylaminopropionic acid, 1-methylhexahydronicotinic acid and arecaidine (Table I) offers evidence that this value, like the ΔpK (ester) value, is a measure of amino-carboxyl distance in related molecules and that there is a similar steric relationship of amino- and carboxyl groups in all of these compounds. This would be in agreement with the previously postulated (Stenlake, 1953) adoption of a semi-chair conformation by ring D of lysergic acid and ergometrine in aqueous solution, combined with a equatorial substituent at C(8) (structure V corresponding to structures I and II for the other cyclic amino-acids).

TABLE I

DIFFERENCES IN PK'A VALUES BETWEEN AMINO-ACIDS AND THEIR ALKANOLAMIDES (Δ PK (AMIDE) VALUES)

Amino-acid	pK'a values of			ΔpK (amide)
	Acid	Ethanolamide	Propanolamide	
3-Dimethylaminopropionic acid	9·85	8·65		1·20
			8·82	1·03
1-Methylhexahydronicotinic acid	9·70	8·65		1·05
1-Methyl- 1,2,5,6- tetrahydronicotinic acid ..	9·07	8·02		1·05
			8·07	1·00
Lysergic acid (in 40 per cent cellosolve, 7·84; in 30 per cent cellosolve, 7·82; in 15 per cent cellosolve, 7·84)	7·83		6·79	1·04
Isolysergic acid (in 30 per cent cellosolve, 8·69; in 20 per cent cellosolve, 8·68)	8·68		7·37	1·31

The higher ΔpK (amide) value for ergometrinine (Table I) supports the validity of our interpretation of ΔpK (amide) values and the existence of an axial carboxyl substituent at C(8) in the isolysergic acid series (VI); it is analogous to the higher ΔpK (ester) value of ecgonine, which is known to have an axial carboxyl substituent as compared with ψ-ecgonine and other related molecules with equatorial carboxyl groups (Chilton and Stenlake, 1955). It would be expected that the closer proximity of ionised carboxyl and amino-groups produced by an axial configuration of the carboxyl group would have a base-strengthening effect in the free acid (Stenlake, 1953) whereas the effect of a proximate carboxypropanolamido-group on the ionisation of the basic nitrogen would only be the relatively weak one due to hydrogen bonding with the amido and hydroxyl hydrogen atoms. This hydrogen bonding would have the effect of increasing the pK'a value of the alkanolamide relative to that of the corresponding ester in which bonding could not occur, and would result in a ΔpK (amide) value lower than that of the ΔpK (ester) value for the same amino-acid. This is shown clearly in values quoted by Stoll and co-workers (1954) for the dihydroisolysergic acid series: for dihydroisolysergic

acid I and its monoethyl amide ΔpK (amide) = 1·97 while ΔpK (ester) = 2·85. The effect is much less marked in the corresponding dihydrolysergic acid derivatives, where an equatorial carboxyl substituent would result in wider separation of the interacting groups (dihydrolysergic acid I has ΔpK (amide) 1·17 and ΔpK (ester) 1·65). The difference in actual values between these figures and our own may be attributed to the use of 80 per cent cellosolve as solvent by Stoll. It follows that the ΔpK (amide) value would not be expected to vary so much with differences in distance between amino- and carboxyl groups as does the ΔpK (ester) value. The relatively small, though significant, differences between ΔpK (amide) values for lysergic and isolysergic acids would therefore probably have corresponded to a much greater difference in ΔpK (ester) values if these had been obtainable in an aqueous system and both methods should be equally valid as a means of comparing differences in distance between charged groups in related molecules.

Experimental

Dissociation constants were determined by titration of the hydrochlorides of the bases in aqueous solution (0·005 M) at 25° with carbonate-free potassium hydroxide solution as described by Chilton and Stenlake (1955). Lysergic and isolysergic acids, which are not soluble in water to this extent at 25°, were dissolved in a known slight excess of carbonate-free potassium hydroxide solution and immediately back-titrated with 0·05 N hydrochloric acid. The addition of a little ethyl cellosolve was found necessary to prevent precipitation during titration, but was considered to have little effect on ionisation, since pK'a values determined at a number of different low cellosolve concentrations showed no marked or consistent variation (Table I).

Preparation of Materials

2-(3'-*Dimethylaminopropionamido*)*ethanol*, (±)-2-(3'-*dimethylaminopropionamido*)*propanol and* 2-(1'-*methylhexahydronicotinamido*)*ethanol hydrochlorides*. The acid oxalates, prepared by the method of Chilton and Stenlake (1962), were converted to hydrochlorides as follows: 0·1 millimole of the oxalate dissolved in water (1 ml.) was treated with a very small excess of solution of calcium chloride (10 per cent). Precipitated calcium oxalate was removed by centrifugation, washed with water and the total aqueous solutions made up accurately to 5 ml. with water. Aliquot portions of 1 ml. (0·02 millimole) were used for titration.

2-(1'-*Methyl*-1',2',5',6'-*tetrahydronicotinamido*)*ethanol and* (±)-2-(1'-*methyl*-1',2',5',6'-*tetrahydronicotinamido*)*propanol hydrochlorides*. Prepared by treatment of aqueous solutions of the dihydrochlorides of 2-aminoethyl 1,2,5,6-tetrahydronicotinate and (±)-2-aminopropyl 1,2,5,6-tetrahydronicotinate respectively with a slight excess of sodium hydroxide followed by neutralisation with dilute hydrochloric acid as described by Chilton and Stenlake (1962). The neutralised solution was used directly for titration.

Ergometrine and ergometrinine hydrochlorides. Authentic samples of the bases, kindly given by Messrs. Burroughs Wellcome & Co. Ltd., were dissolved in a known slight excess of hydrochloric acid immediately before titration.

Lysergic acid was prepared from ergotoxine by the method of Stoll and Hofmann (1937), m.p. 239° (decomp.) from water. Stoll and Hofmann (1937) give 240–250° (decomp.).

Isolysergic acid was prepared from lysergic acid by the method of Smith and Timmis (1936), m.p. 238° (decomp.) from water, depressed on mixture with lysergic acid. Stoll and Hofmann (1937) give 240–245°. The equivalent weight and homogeneity of this acid and of the lysergic acid were confirmed from their neutralisation curves.

References

Chilton, J. and Stenlake, J. B. (1955). *J. Pharm. Pharmacol.*, **7**, 1004–1011.
Chilton, J. and Stenlake, J. B. (1962). *Ibid.*, **14**, 350-366.
Neuberger, A. (1937). *Proc. roy. Soc.*, A, **158**, 68–96.
Smith, S. and Timmis, G. M. (1936). *J. chem. Soc.*, 1440–1444.
Stenlake, J. B. (1953). *Chem. and Ind.* (Rev.), 1089–1090.
Stoll, A. and Hofmann, A. (1937). *Hoppe-Seyl Z.*, **250**, 7–10.
Stoll, A., Petrzilka, T., Rutschmann, J., Hofmann, A. and Günthard, H. H. (1954). *Helv. chim. Acta*, **37**, 2039-2057.

A NOTE ON THE ACTION OF GALLAMINE ON ISOLATED RABBIT AURICLES

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The action of gallamine triethiodide on the effects of methacholine and acetylcholine on isolated spontaneously beating rabbit auricles in oxygenated mammalian Ringer's solution at 30° has been investigated. Gallamine blocked the effects of both drugs. The effects of gallamine were similar to those of atropine, but gallamine was weaker and was more readily removed by washing.

When gallamine triethiodide is administered to anaesthetised human subjects, with the exception of those who have been given cyclopropane, it causes tachycardia (Doughty and Wylie, 1951). The mechanism by which it produces this effect is disputed. Riker and Wescoe (1951) and Wein (1951) suggested that the tachycardia was due to an atropine-like action, but Della Bella, Rognoni and Gopal (1961) state that gallamine has no atropine-like action on the heart. Since there was some doubt regarding the mechanism by which gallamine causes tachycardia, this subject has been reinvestigated.

Methods

Hearts were removed from two-month-old rabbits, which had been killed by stunning and by bleeding from the carotid arteries. After dissection and cleaning, unseparated right and left auricles were suspended in 50 ml. oxygenated mammalian Ringer's solution at a temperature of 30° in a constant-temperature organ bath. The auricular contractions were recorded using a Starling heart lever on smoked paper. In each experiment

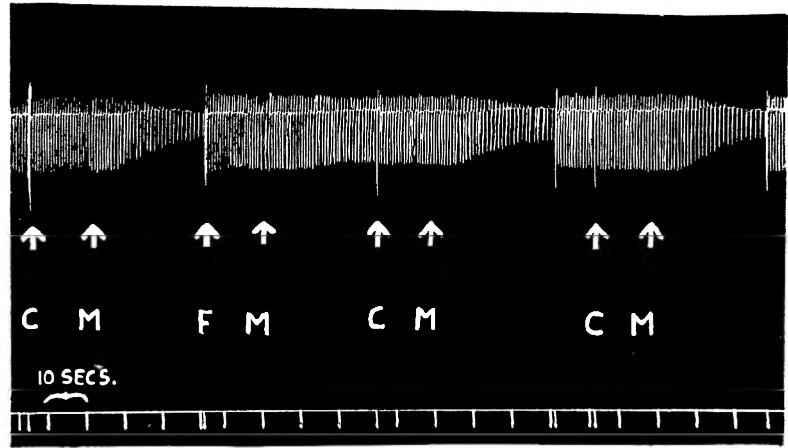

Fig. 1. Isolated spontaneously beating rabbit auricles in oxygenated mammalian Ringer solution at 30°. At M. methacholine chloride 10·0 μg. was added to the bath. At F, gallamine triethiodide 480·0 μg. in 0·6 ml. distilled water was added. At C, 0·6 ml. distilled water was added. Between additions of distilled water or gallamine, and methacholine, there was an interval of 2 min. Between additions of methacholine, there was a 5 min. interval.

the drug being investigated or a control, consisting of a volume of distilled water equal to that containing the drug, was added to bath 2 min. before the addition of acetylcholine or methacholine. Normal contractions of the auricles were recorded for 15 sec. Acetylcholine 20 μg. or methacholine 10 μg. was then added and the effect was recorded for 30 sec. Between each addition of acetylcholine or methacholine there was a 5 min. interval.

Results

Gallamine triethiodide, 480 μg., blocked the effect of 10 μg. methacholine (Fig. 1). A similar effect was seen with acetylcholine.

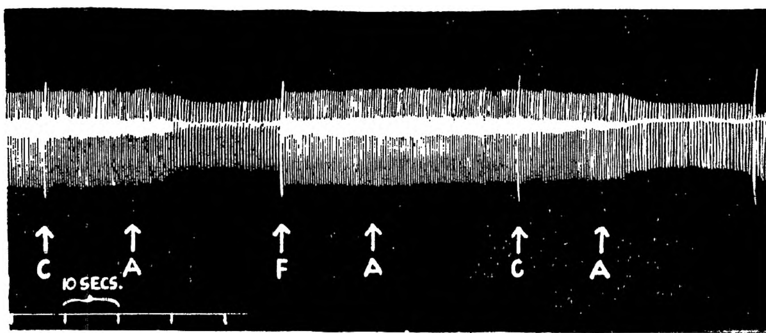

Fig. 2. Isolated spontaneously beating rabbit auricles in oxygenated mammalian Ringer solution at 30°. At A, acetylcholine chloride 20·0 μg. was add-d to the bath. At F, 480·0 μg. gallamine triethiodide in 0·6 ml. was added. At C, 0·6 ml. distilled water was added. Time intervals as in Fig. 1.

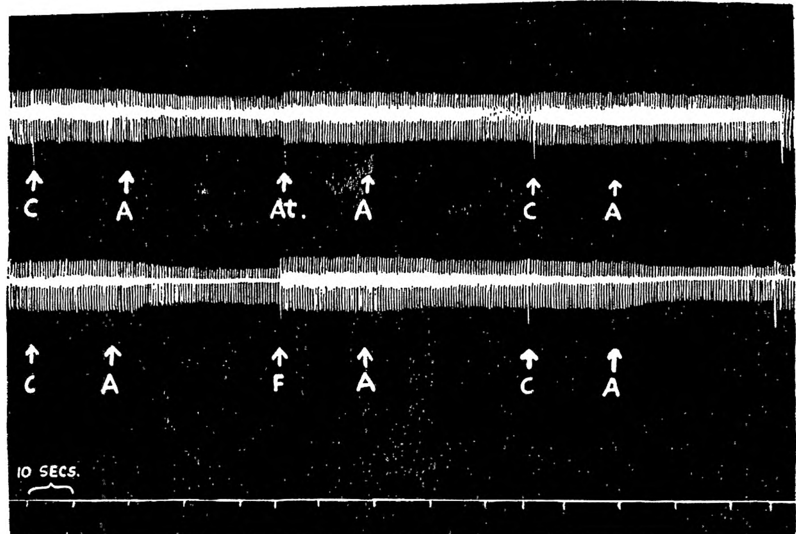

Fig. 3. Isolated spontaneously beating rabbit auricles in oxygenated mammalian Ringer solution at 30°. At A 20·0 μg. acetylcholine was added to the bath. At F, gallamine triethiodide 240·0 μg. in 0·3 ml. distilled water was added. At C, 0·3 ml. distilled water was added. At "At." 0·6 μg. atropine in 0·3 ml. distilled water was added. The time intervals as Fig. 1.

Gallamine triethiodide, 480 μg., completely blocked the effect of 20 μg. acetylcholine (Fig. 2). The effects of gallamine and atropine were compared using the same pair of auricles. Gallamine produced a qualitatively similar effect to that of atropine. However, two differences were noted. The effect of gallamine was very weak compared with that of atropine

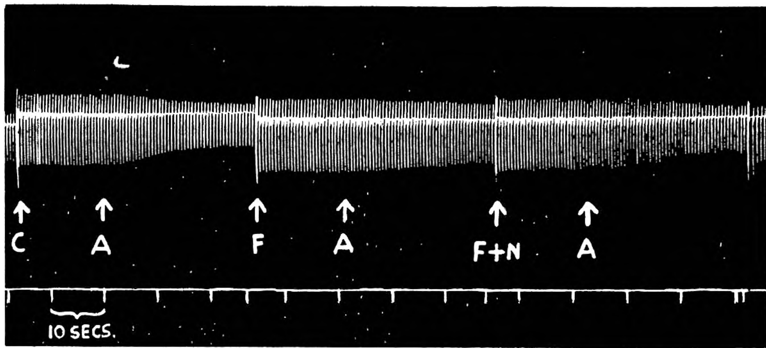

FIG. 4. Isolated spontaneously beating rabbit auricles in oxygenated mammalian Ringer solution at 30°. At A 20·0 μg. acetylcholine was added to the bath. At F, 320·0 μg. gallamine triethiodide in 1·0 ml. distilled water was added. At F + N, gallamine 320·0 μg. and neostigmine methylsulphate, 300·0 μg. in 1·0 ml. of distilled water was added to the bath. Time intervals as Fig. 1.

and also was readily reversed by washing whereas that of atropine persisted (Fig. 3). Neostigmine methylsulphate 300 μg. prevented 320 μg. gallamine from blocking the effect of 20 μg. acetylcholine (Fig. 4).

Discussion

Since methacholine has predominantly muscarine-like actions, it would appear that gallamine has a similar action on the heart to that of atropine, differing only from the latter by being weaker and being more readily reversible by washing. Mushin, Wein, Mason and Langston (1949) reported that gallamine does not have a significant effect on the blood pressure. It therefore appears that gallamine causes tachycardia by blocking the muscarine-like effects on the heart of acetylcholine, which is liberated from the post-ganglionic endings of the vagus nerve. This confirms the findings of Wein (1951) and of Riker and Wescoe (1951). Doughty and Wylie (1951) reported that the tachycardia could be reversed by administering neostigmine methylsulphate to their patients. This effect has been seen experimentally.

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References

Della Bella, D., Rognoni, F. and Gopal, K. (1961). *J. Pharm. Pharmacol.*, **13**, 93–97.
Doughty, A. C. and Wylie, W. D. (1951). *Proc. Roy. Soc. Med.*, **44**, 375–386.
Mushin, W. W., Wein, R., Mason, D. F. J. and Langston, G. T. (1949). *Lancet*, **1**, 726–728.
Riker, W. F., Jr. and Wescoe, W. C. (1951). *Ann. N.Y. Acad. Sci.*, **54**, 373–392.
Wein, R. (1951). *Proc. Roy. Soc. Med.*, **44**, 386–387.

COMMON DRUGS THAT MAY INVALIDATE SPECTROPHOTO-FLUOROMETRIC ASSAYS OF BLOOD GRISEOFULVIN

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Studies have been made of the potential effects of aspirin, salicylic acid, phenacetin, paracetamol, codeine, caffeine, theophylline and quinine on the spectrophotofluorometric assay of griseofulvin in blood. Aspirin, salicylic acid or quinine are likely to give falsely high values. Detection of this bias by inspecting the fluorescence spectra of the extracted griseofulvin is not always possible.

Blood griseofulvin levels are easily determined by the spectrophotofluorometric method of Bedford, Child and Tomich (1959). Because the antibiotic's only known metabolite, 6-desmethylgriseofulvin, does not fluoresce, and because the extraction procedure does not remove any fluorescent substances that may be normally present in the blood, the assay has been regarded as specific. Recently, however, when estimating the blood griseofulvin levels of human volunteers, we found that one subject had unexpectedly high values; examination of her blood extract showed that the fluorescence spectrum of the griseofulvin contained in the extract was grossly distorted. On being questioned, the subject stated that she had taken a large dose (2·4 g.) of aspirin to overcome toothache.

We decided to study the effects on the griseofulvin assay of smaller doses of aspirin, and also of other drugs that might be encountered.

Experimental and Results

Griseofulvin in 1 ml. whole blood is assayed by extracting the antibiotic into ether, evaporating the extract to dryness and dissolving the residue in 10 ml. of 1 per cent aqueous ethanol. The fluorescence of this alcoholic solution is then compared with that of a standard griseofulvin solution, both solutions being activated at 295 mμ and analysed at 450 mμ (Bedford, Child and Tomich, 1959).

In Fig. 1A are shown the activating spectra obtained on a Farrand spectrophotofluorometer for 1 per cent aqueous ethanol and for griseofulvin (0·3 μg./ml.), aspirin (10 μg./ml.) and salicylic acid (0·05 μg./ml.) in this solvent. The analysing wavelength was 450 mμ, which is maximal for griseofulvin but not for the two salicylates. The activating spectrum of griseofulvin contains two peaks, one at 295 mμ and the other at 335 mμ (uncorrected values); that of aspirin or salicylic acid has one only at 300 mμ.

The activating spectra shown in Fig. 1B were obtained on extracts of blood from a volunteer who had ingested 1 g. griseofulvin 4 hr. before venipuncture. The residues from the ether extracts were dissolved in 1 per cent ethanol, 1 per cent ethanol containing 0·05 μg./ml. salicylic acid or 1 per cent ethanol containing 0·10 μg./ml. salicylic acid. It can be

seen that the intensity of fluorescence at the activating wavelength of 295 mμ was increased from 25 arbitrary units (Fig. 1B——) to 43 (Fig. 1B---) and that the characteristic activating spectrum of griseofulvin was undistorted. A similar result was obtained when the residue from the ether extract was dissolved in 1 per cent ethanol containing 10 μg./ml. aspirin. However, the spectrum was entirely unlike that of griseofulvin when a sufficiently high concentration of aspirin or salicylic acid was present in the aqueous ethanol (Fig. 1B · · · · ·).

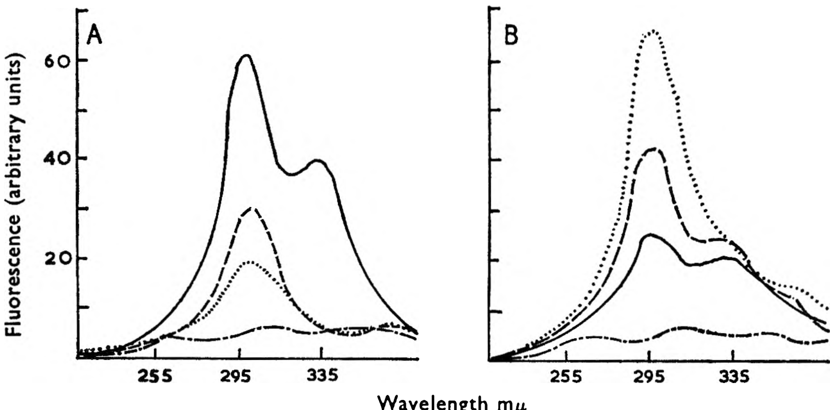

FIG. 1A. Scans of:

—— 1 per cent ethanol containing 0·3 μg. griseofulvin/ml.
...... 1 per cent ethanol containing 10·0 μg. aspirin/ml.
- - - - 1 per cent ethanol containing 0·05 μg. salicylic acid/ml.
—·—· 1 per cent ethanol.

All are scans of the activation spectrum with the analysing wavelength fixed at 450 mμ

FIG. 1B. Scans of:

—— griseofulvin extracted from human blood and dissolved in 1 per cent ethanol.
- - - - griseofulvin extracted from human blood and dissolved in 1 per cent ethanol containing 0·05 μg. salicylic acid/ml.
...... griseofulvin extracted from human blood and dissolved in 1 per cent ethanol containing 0·10 μg. salicylic acid/ml.
—·—· Extract of control human blood dissolved in 1 per cent ethanol.

All are scans of the activation spectrum with the analysing wavelength fixed at 450 mμ

In the experiments recorded above, salicylic acid was added to the 1 per cent ethanol used as the final solvent, but similar results were obtained when sodium salicylate was added at a concentration of 25 μg./ml. to whole blood containing griseofulvin.

Because aspirin and salicylic acid can invalidate blood griseofulvin assays, we studied the effects of other drugs that might be met in similar circumstances.

In Table I are given the fluorescence intensity values of 1 per cent aqueous ethanol solutions of griseofulvin, aspirin, salicylic acid, phenacetin, paracetamol, codeine, caffeine, theophylline and quinine; they were measured at both their own and at griseofulvin's fluorescence maxima.

Concentrations were such that readings were obtained on the most sensitive scale of the micro-ammeter. If it be assumed that a micro-ammeter reading of 10 or more units would bias the griseofulvin assay (micro-ammeter reading on normal blood extract = 5 or 6 units), then it is possible to calculate for each of the above-mentioned drugs the

TABLE I

FLUORESCENCE CHARACTERISTICS OF SOME PHARMACOLOGICAL AGENTS

Compound	Concentration µg./ml.*	Fluorescence maxima mµ†		Fluorescence intensity* at maxima	Fluorescence intensity‡ at griseofulvin's maxima
		Activating wavelength	Analysing wavelength		
Griseofulvin	0·3	295	450	62	62
1 per cent aqueous ethanol	—	—	—	—	5
Aspirin	10	300	405	32	20
Salicylic acid	0·05	300	405	65	35
Phenacetin	10	385	425	25	9
Paracetamol	50	330	400	32	18
Codeine (as phosphate)	2·5	260	400	28	6
Caffeine	10	325	395	26	11
Theophylline	10	315	435	20	19
Quinine (as sulphate) ..	0·004	340	380	36	11

* In 1 per cent ethanol.
† Uncorrected values.
‡ Arbitrary units on the most sensitive scale of the microammeter.

TABLE II

MAXIMUM CONCENTRATIONS OF SOME DRUGS LIKELY TO BE FOUND IN HUMAN BLOOD

Drug	Normal dose range (g.)	Maximum theoretical blood concentration—µg./ml.*	Maximum blood concentration not interfering with griseofulvin assay—µg./ml.†
Aspirin	0·3 -1·0	60–200 (=50–160 salicylic acid)	60 0·6
Phenacetin	0·3 -0·6	60–120	120
Paracetamol ..	0·3 -0·6	60–120	300
Codeine	0·01–0·06	2– 12	60
Caffeine	0·3 -0·6	60–120	120
Theophylline ..	0·06–0·2	12– 40	60
Quinine	0·3 -0·6	60–120	0·05
Tea or Coffee .. (per strong cup)	about 0·1 (as caffeine)	20 (as caffeine)	120

* Assuming rapid and complete absorption into a blood volume of 5 litres.
† Assuming 100 per cent recovery of drug from blood by the griseofulvin extraction procedure.

maximum blood concentration that would not falsify the results. These values, and the maximum blood concentrations likely to be encountered in practice, are given in Table II. The latter values have been calculated by assuming immediate and complete absorption into the blood stream of the maximum recommended dose.

DISCUSSION

Of the compounds tested, only aspirin, salicylic acid and quinine are likely to interfere in blood griseofulvin estimations. Lolli and Smith (1946) have shown that fasting subjects given 0·6 or 1·6 g. aspirin have maximum blood salicylate levels of 38 and 73 μg./ml. respectively. The ether extraction procedure in the griseofulvin assay of Bedford and others (1959) will remove 3 per cent of any salicylate present in the blood;

hence blood salicylate levels higher than 15 μg./ml. will falsify the griseofulvin estimations.

A given oral dose of quinine produces widely different blood levels in human subjects. One hour after a single dose of 0·5 g. a maximum concentration of about 10 μg./ml. is reached; this decreases to about 1·5 μg./ml. after 24 hr. (Sollman, 1957). Because normal doses of proprietary preparations containing quinine have about 30 mg. of the alkaloid, blood levels of 0·5 μg./ml. are theoretically possible. At this blood level 100 per cent of the quinine can be recovered in the griseofulvin assay of Bedford and others (1959), and it thus seems likely that quinine also could interfere in this assay.

The evidence indicating that salicylic acid can invalidate blood griseofulvin assays should be borne in mind when other spectrophotofluorometric assays are being conducted. When characterisation of the minute quantities of drug being assayed depends entirely on the appearance of its fluorescence spectra, then it is not enough just to ensure that metabolites and substances closely related to the drug do not fluoresce; compounds that, though not distorting the spectra, raise the peaks being measured must be taken into account.

References

Bedford, C., Child, K. J. and Tomich, E. G. (1959). *Nature, Lond.*, **184**, 364–365
Lolli, G. and Smith, R. (1946). *New Engl. J. Med.*, **235**, 80–84.
Sollman, T. (1957). A Manual of Pharmacology. Philadelphia: Saunders.

CHRONIC TOXICITY STUDIES ON FOOD COLOURS. V. OBSERVATIONS ON THE TOXICITY OF BRILLIANT BLUE FCF, GUINEA GREEN B AND BENZYL VIOLET 4B IN RATS

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The food colours Brilliant Blue FCF, Guinea Green B and Benzyl Violet 4B were fed to rats at concentrations of 0·03, 0·3 and 3 per cent of the diet for 75 weeks. Thirty rats were used at each feeding level. Brilliant Blue and Benzyl Violet had no adverse effects on growth at any of the levels used. With Guinea Green there was an initial depression of growth at the two higher levels accompanied by a decreased food efficiency and, in one group, a lower food consumption. There was an increase in mortality in female rats fed 3 per cent of all three colours. With Brilliant Blue, indications were that this increase was not related to treatment. In the groups fed 3 per cent Guinea Green and 3 per cent Benzyl Violet there was a total of eight malignant tumours. Five of these were derived from the epidermis. Further investigation of these two colours is required.

This report is one of a series covering the food colours permitted for use in Canada. Reports on eight other colours have been published (Allmark, Grice and Lu, 1955; Allmark, Grice and Mannell, 1956; Mannell, Grice, Lu and Allmark, 1958). Since the work began, four of the colours tested have been deleted from the Canadian list of permitted food colours. These are Oil Yellow OB, Oil Yellow AB, Oil Red XO and Orange SS.

In this experiment the effects of feeding Brilliant Blue, Guinea Green and Benzyl Violet on growth, food consumption, food efficiency, and mortality in rats were investigated. Histopathological and haematological studies were also done.

Methods

Details of the methods used have been given by Mannell and others (1958). The colours were fed at concentrations of 0·03, 0·3 and 3·0 per cent. Fifteen rats of each sex were included in each test group and in the control group, 300 rats in all. They were of Wistar origin and ranged from 30 to 37 days of age at the start of the experiment. Weekly records were kept of food consumption and food efficiency during the first year, while body weight was recorded weekly until the experiment was terminated at 75 weeks. Red blood cell counts, haemoglobin estimations and haematocrit readings were done at intervals throughout the test.

Rats that died on test were examined to determine, if possible, the cause of death. At the end of the experiment, the surviving rats were killed and a gross examination was made of all organs and tissues. A detailed examination was made of haematoxylin-eosin stained paraffin

sections from five representative male and female animals of each of the groups. The organs studied were, lung, heart, liver, spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testis, prostate, coagulating gland, ovary, uterus and thymus. In addition, all tissues in which any gross pathological change was observed, and all tumours, were examined histologically.

Results

Growth, Food Consumption, Food Efficiency

Brilliant Blue. This colour had no adverse effect on growth of either males or females at any of the levels fed. In fact, from the tenth to the twenty-sixth week, the males fed 0·3 per cent were significantly heavier than the control group (Table I). The females at this feeding level had a larger mean body weight than the controls after 5 and 10 weeks on test.

TABLE I

Body weight of rats fed brilliant blue, guinea green, or benzyl violet

Treatment	Dosage (per cent of diet)	Weight, g. (Mean ± S.E.) Initial	3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks	75 weeks
Males									
Control		72 ± 3	149 ± 4	184 ± 5	234 ± 4	268 ± 7	308 ± 10	332 ± 16	285 ± 23
Brilliant Blue	0·03	70 ± 3	150 ± 3	187 ± 4	242 ± 5	279 ± 7	321 ± 8	331 ± 16	325 ± 19
	0·3	70 ± 3	151 ± 4	192 ± 4	†259 ± 5	†300 ± 5	*341 ± 7	346 ± 9	320 ± 14
	3·0	69 ± 2	148 ± 4	185 ± 4	242 ± 6	280 ± 8	321 ± 8	336 ± 12	322 ± 9
Guinea Green	0·03	71 ± 3	146 ± 3	186 ± 3	247 ± 6	*289 ± 7	331 ± 7	360 ± 12	*359 ± 17
	0·3	71 ± 3	*134 ± 4	*171 ± 5	230 ± 6	270 ± 6	316 ± 8	367 ± 8	*348 ± 15
	3·0	72 ± 3	139 ± 6	175 ± 4	234 ± 6	271 ± 9	317 ± 11	342 ± 12	320 ± 17
Benzyl Violet	0·03	72 ± 3	148 ± 3	183 ± 2	244 ± 3	*287 ± 4	330 ± 5	357 ± 13	†397 ± 12
	0·3	72 ± 3	149 ± 4	191 ± 5	*252 ± 5	†293 ± 6	334 ± 8	352 ± 10	332 ± 15
	3·0	70 ± 3	142 ± 2	180 ± 3	238 ± 4	277 ± 5	322 ± 10	*376 ± 12	*367 ± 28
Females									
Control		62 ± 2	114 ± 2	127 ± 2	155 ± 3	177 ± 3	201 ± 3	223 ± 3	212 ± 7
Brilliant Blue	0·03	63 ± 3	114 ± 2	128 ± 3	154 ± 3	175 ± 4	202 ± 6	216 ± 7	212 ± 11
	0·3	65 ± 2	116 ± 2	†135 ± 2	*164 ± 3	183 ± 3	209 ± 4	221 ± 6	232 ± 5
	3·0	64 ± 2	114 ± 2	†135 ± 2	161 ± 3	179 ± 4	202 ± 4	217 ± 7	216 ± 10
Guinea Green	0·03	64 ± 2	114 ± 2	130 ± 2	156 ± 3	177 ± 3	203 ± 4	224 ± 5	225 ± 8
	0·3	64 ± 2	*108 ± 1	126 ± 2	155 ± 2	175 ± 3	198 ± 3	228 ± 5	*230 ± 5
	3·0	64 ± 2	*108 ± 2	125 ± 2	151 ± 3	*169 ± 3	194 ± 3	214 ± 4	210 ± 7
Benzyl Violet	0·03	64 ± 2	115 ± 2	*134 ± 2	159 ± 3	182 ± 3	201 ± 4	226 ± 4	216 ± 10
	0·3	64 ± 2	113 ± 2	132 ± 2	156 ± 3	175 ± 3	202 ± 4	228 ± 4	235 ± 8
	3·0	65 ± 2	113 ± 3	*134 ± 2	161 ± 3	178 ± 3	201 ± 3	223 ± 4	243 ± 26

* P less than 0·05. † P less than 0·01.

Food consumption was not reduced by the addition of Brilliant Blue to the diet up to the 3 per cent level (Table II). In the group of male rats fed 0·3 per cent of the colour a significant increase in food consumption occurred from the tenth to the twenty-sixth week. This coincided with the increase in weight for this group noted above.

Food efficiency was also increased in this same group for the same period (Table III). In no group fed Brilliant Blue was there a diminution of food efficiency at any time during the experiment.

Guinea Green. Male rats fed Guinea Green at 0·3 per cent in the diet had a smaller mean weight than their controls for the first 5 weeks of the test (Table I). By the tenth week the weight of this group was the same as the control value and at the end of the experiment had surpassed it. Food efficiency values followed a similar pattern (Table III). At the 3 per cent level, food efficiency was significantly reduced at 3 and 5 weeks (Table III), but the weight was not significantly affected (Table I). After the tenth week the only effect observed in the males on 3 per cent Guinea Green was an increase in food consumption at 16 and 26 weeks (Table II).

TABLE II

FOOD CONSUMPTION OF RATS FED BRILLIANT BLUE, GUINEA GREEN, OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Food consumption in g./rat/day (Mean ± S.E.)					
		3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks
Males							
Control		14·0 ± 0·36	14·3 ± 0·32	15·3 ± 0·26	15·7 ± 0·28	16·3 ± 0·28	17·3 ± 0·38
Brilliant Blue	0·03	14·2 ± 0·32	14·7 ± 0·26	15·8 ± 0·25	16·2 ± 0·32	16·9 ± 0·36	17·9 ± 0·37
	0·3	13·9 ± 0·27	14·6 ± 0·21	*16·0 ± 0·18	†17·0 ± 0·19	†17·3 ± 0·19	17·9 ± 0·16
	3·0	14·0 ± 0·32	†18·5 ± 0·36	15·8 ± 0·29	16·5 ± 0·31	16·8 ± 0·29	17·4 ± 0·26
Guinea Green	0·03	13·9 ± 0·21	14·5 ± 0·22	15·5 ± 0·20	16·1 ± 0·20	16·9 ± 0·16	17·8 ± 0·17
	0·3	13·3 ± 0·23	13·8 ± 0·24	15·2 ± 0·24	16·0 ± 0·25	16·6 ± 0·29	17·9 ± 0·22
	3·0	13·8 ± 0·34	14·7 ± 0·34	15·9 ± 0·31	*16·8 ± 0·31	*17·5 ± 0·33	18·2 ± 0·32
Benzyl Violet	0·03	14·6 ± 0·23	*15·2 ± 0·21	†16·3 ± 0·17	†17·0 ± 0·17	†17·6 ± 0·20	*18·3 ± 0·20
	0·3	14·5 ± 0·32	15·1 ± 0·36	*16·0 ± 0·29	*16·6 ± 0·29	17·1 ± 0·28	17·9 ± 0·24
	3·0	14·3 ± 0·22	*15·2 ± 0·24	†16·2 ± 0·16	†16·8 ± 0·17	*17·3 ± 0·25	*18·3 ± 0·17
Females							
Control		12·3 ± 0·21	12·0 ± 0·18	12·1 ± 0·14	12·2 ± 0·13	12·8 ± 0·14	13·7 ± 0·15
Brilliant Blue	0·03	12·4 ± 0·25	12·4 ± 0·28	12·4 ± 0·22	12·3 ± 0·20	13·1 ± 0·24	14·0 ± 0·25
	0·3	11·9 ± 0·15	12·1 ± 0·14	†12·6 ± 0·03	*12·7 ± 0·20	13·2 ± 0·20	13·8 ± 0·19
	3·0	11·9 ± 0·29	12·0 ± 0·24	12·3 ± 0·21	12·3 ± 0·23	12·6 ± 0·22	13·3 ± 0·21
Guinea Green	0·03	12·0 ± 0·21	12·0 ± 0·02	12·2 ± 0·18	12·2 ± 0·18	12·7 ± 0·19	13·5 ± 0·19
	0·3	11·7 ± 0·24	11·9 ± 0·04	12·4 ± 0·20	12·5 ± 0·20	13·0 ± 0·20	13·7 ± 0·18
	3·0	†10·9 ± 0·21	†11·3 ± 0·16	*11·6 ± 0·18	11·8 ± 0·17	12·3 ± 0·20	†13·0 ± 0·19
Benzyl Violet	0·03	12·1 ± 0·17	12·5 ± 0·17	*12·8 ± 0·21	*12·8 ± 0·24	13·4 ± 0·27	14·0 ± 0·21
	0·3	12·2 ± 0·23	12·2 ± 0·21	12·2 ± 0·19	12·1 ± 0·20	12·6 ± 0·23	13·4 ± 0·16
	3·0	12·7 ± 0·30	12·5 ± 0·20	*12·7 ± 0·18	12·6 ± 0·20	13·0 ± 0·24	13·3 ± 0·18

* P less than 0·05. † P less than 0·01.

Body weight of the females on Guinea Green was down at 3 weeks in the groups fed 0·3 per cent and 3 per cent of the colour, and at 16 weeks for the high level group only (Table I). Food consumption of the females on the 3 per cent level was reduced during most of the experiment (Table II). There were two instances of reduced food efficiency, at 3 weeks for the 0·3 per cent group and at 16 weeks for the 3 per cent group.

Benzyl Violet. There was no depression of growth in either males or females at any feeding level. As happened with Brilliant Blue there were instances, especially with male rats, where the test animals were heavier than the controls, at times markedly so (Table I).

As might be expected in view of the body weight results, increased food consumption was a consistent finding in males fed Benzyl Violet (Table II).

Food efficiency was lowered at the start of the test for both sexes at the 3 per cent level and for the males in the 0·03 per cent group. After 5 weeks all test groups were up to the control values (Table III).

TABLE III

CUMULATIVE FOOD EFFICIENCY IN RATS FED BRILLIANT BLUE, GUINEA GREEN OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Food efficiency (g. gained/100 g. food consumed. Mean ± S.E.)					
		3 weeks	5 weeks	10 weeks	16 weeks	26 weeks	52 weeks
Males							
Control		26·0 ± 0·42	22·5 ± 0·42	15·1 ± 0·32	11·1 ± 0·23	7·9 ± 0·17	4·1 ± 0·18
Brilliant Blue	0·03	26·8 ± 0·45	22·7 ± 0·51	15·2 ± 0·39	11·5 ± 0·25	8·1 ± 0·13	4·0 ± 0·21
	0·3	27·5 ± 0·64	*24·0 ± 0·52	†16·9 ± 0·31	†12·2 ± 0·18	†8·6 ± 0·16	4·2 ± 0·12
	3·0	27·1 ± 0·71	22·8 ± 0·62	15·7 ± 0·24	11·4 ± 0·23	8·2 ± 0·17	4·4 ± 0·14
Guinea Green	0·03	25·6 ± 0·64	22·8 ± 0·55	16·2 ± 0·47	*12·0 ± 0·33	†8·5 ± 0·16	4·4 ± 0·14
	0·3	†22·8 ± 0·30	*20·9 ± 0·52	14·9 ± 0·43	11·1 ± 0·23	8·1 ± 0·14	*4·5 ± 0·11
	3·0	†23·1 ± 0·71	†19·9 ± 0·64	14·5 ± 0·50	10·5 ± 0·42	7·7 ± 0·25	4·1 ± 0·15
Benzyl Violet	0·03	*24·7 ± 0·25	*20·8 ± 0·64	15·1 ± 0·31	11·3 ± 0·24	8·0 ± 0·14	4·2 ± 0·16
	0·3	25·2 ± 0·68	22·5 ± 0·77	16·0 ± 0·37	*11·9 ± 0·22	8·4 ± 0·17	4·3 ± 0·10
	3·0	*23·8 ± 0·81	*20·6 ± 0·62	14·8 ± 0·42	11·1 ± 0·29	8·0 ± 0·24	4·6 ± 0·19
Females							
Control		20·0 ± 0·38	15·3 ± 0·45	11·0 ± 0·20	8·4 ± 0·16	6·0 ± 0·08	3·2 ± 0·05
Brilliant Blue	0·03	19·9 ± 1·12	15·5 ± 0·93	10·5 ± 0·39	8·1 ± 0·29	5·8 ± 0·21	3·0 ± 0·14
	0·3	20·5 ± 0·60	16·4 ± 0·43	11·3 ± 0·25	8·3 ± 0·16	6·0 ± 0·12	3·1 ± 0·11
	3·0	20·1 ± 0·37	†17·0 ± 0·37	11·3 ± 0·23	8·4 ± 0·19	6·0 ± 0·12	3·2 ± 0·11
Guinea Green	0·03	19·7 ± 0·62	15·6 ± 0·26	10·7 ± 0·26	8·3 ± 0·19	5·9 ± 0·11	3·2 ± 0·08
	0·3	*17·9 ± 0·66	15·1 ± 0·43	10·5 ± 0·24	8·0 ± 0·22	5·7 ± 0·15	3·3 ± 0·10
	3·0	18·9 ± 0·91	15·4 ± 0·54	10·6 ± 0·24	*7·9 ± 0·15	5·8 ± 0·12	3·2 ± 0·10
Benzyl Violet	0·03	20·3 ± 0·66	16·1 ± 0·46	10·6 ± 0·28	8·2 ± 0·18	*5·6 ± 0·13	3·3 ± 0·09
	0·3	19·2 ± 0·66	15·9 ± 0·43	10·7 ± 0·30	8·2 ± 0·19	6·0 ± 0·14	*3·4 ± 0·06
	3·0	†18·2 ± 0·49	15·7 ± 0·26	10·8 ± 0·19	8·0 ± 0·14	5·8 ± 0·13	3·2 ± 0·08

* P less than 0·05. † P less than 0·01.

Mortality

As indicated in Table IV the mortality at the end of a year was low and the deaths were well distributed among the groups. At 75 weeks, statistical analysis (Duncan, 1953) indicated a significant increase in mortality of female rats on the 3 per cent level of each of the three colours. This significance was largely due to the very low death rate in the control group. When the mortality figures of males and females were combined there was no longer statistical significance in the results.

A summary of the necropsy findings in rats that died during the test is also given in Table IV. Sixty-six animals died of chronic respiratory disease. This disease is endemic in our rat colony and there appeared to be no connection between the colours fed and the amount of respiratory disease. Eight rats died from malignant tumours, two of renal failure, and one of starvation. Because of advanced autolysis, cause of death remained unknown in six animals.

Histopathology

The histopathological findings are summarised in Table V. Kidney changes are present to some degree in all rats of this age in our colony.

Only when the pathology of the kidney was marked was any note made of the change. In the Table "nephritis" includes glomerulonephritis and nephrosis. No attempt was made to assess observed change in the

TABLE IV

MORTALITY IN RATS FED BRILLIANT BLUE, GUINEA GREEN, OR BENZYL VIOLET

Treatment	Dosage (per cent of diet)	Total number of rats on test	Number of deaths at				Causes of death							
			52 weeks		75 weeks		Respiratory		Tumour		Other		Unknown	
			Males	Females	Males	Females	M	F	M	F	M	F	M	F
Control		30	2	0	6	1	6	1						
Brilliant Blue	0·03	30	1	1	7	4	6	4					1	
	0·3	30	0	0	5	5	3	5	1				1	
	3·0	30	3	2	5	6*	3	6	1		1			
Guinea Green	0·03	30	0	1	2	1	2	1						
	0·3	30	1	1	2	3	1	2				1	1	
	3·0	30	0	2	4	6*	4	1		3		1		1
Benzyl Violet	0·03	30	1	1	7	4	7	4						
	0·3	30	0	2	1	3	1	3						
	3·0	30	1	1	4	7*	3	3		3			1	1

* P less than 0·05.

TABLE V

SUMMARY OF HISTOPATHOLOGICAL FINDINGS IN RATS FED FOOD COLOURS FOR 75 WEEKS

Treatment	Control		Brilliant Blue						Guinea Green						Benzyl Violet					
Dosage (per cent of diet)			0·03		0·3		3·0		0·03		0·3		3·0		0·03		0·3		3·0	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of rats on test ..	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Number of survivors ..	9	14	8	11	10	10	10	9	13	14	13	12	11	9	8	11	14	12	11	8
Number examined ..	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Nephritis	3	4	1	3	—	—	1	2	1	2	1	2	4	2	2	2	1	3	3	4
Hydronephrosis ..	—	6	—	6	—	7	—	6	—	12	—	9	—	8	—	4	1	13	—	5
Splenitis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Myocarditis	—	—	1	1	—	—	1	—	—	—	—	—	2	—	—	—	—	—	—	—
Liver cell change ..	—	—	—	—	1	1	—	1	—	—	—	—	—	1	—	—	—	—	1	2
Testicular atrophy ..	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
Adrenal haemorrhage ..	—	3	—	3	—	—	1	1	—	2	—	1	—	—	1	1	—	1	—	2
Gastric hyperplasia ..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Tumours																				
Thyroid adenoma ..	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pituitary adenoma ..	—	1	—	—	2	—	2	1	—	1	—	1	1	—	—	2	—	1	—	—
Uterus polyp	—	4	—	4	—	3	—	1	—	4	—	5	—	—	—	—	—	1	—	—
Testis leydig tumour ..	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Mammary fibroadenoma	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Skin kerato-acanthoma	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
Squamous cell carcinoma	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1	—	—	1	2
Fibrosarcoma	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Kidney carcinoma ..	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Liposarcoma	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1
Lung carcinoma ..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Lymphosarcoma ..	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Malignant tumours per group	1	0	0	0	1	0	0	0	0	0	1	0	0	3	0	1	0	0	1	4

kidney and correlate this with levels of food colours fed, as was done by Mannell and others (1958). Adrenal haemorrhage, as previously described (Mannell and others, 1958), was a common finding in this group of rats. This change was not related to treatment.

With respect to neoplasms, pituitary adenomas and uterine polyps occur spontaneously in the colony. The majority of the malignant tumours was observed in rats fed 3 per cent Guinea Green or 3 per cent Benzyl Violet. Six tumours were derived from the epidermis, five of which were in female rats.

Haematology

There were slight differences noted in the results of the blood studies of the various groups but all values were within normal limits. This indicated that there were no adverse effects of these colours on the blood or blood-forming organs.

Discussion

We are aware of few published reports of toxicity studies on these three food colours. Willheim and Ivy (1953) fed Brilliant Blue and Guinea Green to rats at 4 per cent of the diet for as long as 20 months. They reported two tumours of the lymphatic tissue in nine rats fed Guinea Green and no tumours in ten rats with Brilliant Blue. Nelson and Hagan (1953) gave the same two colours to rats by subcutaneous injection for 94 to 99 weeks. With Guinea Green there was one tumour at the injection site and no distant tumours. With Brilliant Blue fibrosarcomas developed at the injection site in over 75 per cent of the animals.

In experiments in this laboratory (unpublished), no tumours were obtained with subcutaneous injections of Brilliant Blue over a 45-week period. The rats were observed for 71 weeks.

Hess and Fitzhugh (1955) studied the absorption and excretion of five triphenylmethane dyes including Brilliant Blue, Guinea Green and Benzyl Violet. When given orally to rats, the three colours were excreted almost completely in the faeces. None of the colours was found in the urine. Small amounts (less than 5 per cent) of Brilliant Blue and Guinea Green were found in the bile of dogs.

Our results indicate that Brilliant Blue and Benzyl Violet produced no deleterious effects on growth, food consumption and food efficiency in rats. With Guinea Green there was an initial depression of growth, due either to poorer food efficiency or, in one case, to reduced food consumption. In no instance did this effect last beyond the sixteenth week of feeding, and there was no effect at all at the lowest dietary level (0·03 per cent).

An increase in mortality was shown for female rats fed 3 per cent of all three colours. In the group fed Brilliant Blue the cause of death in all six animals that died was respiratory disease. This was not considered to be related to treatment. Nearly half of the deaths of females fed the other two colours at the 3 per cent level were due to tumours. This must be viewed with suspicion.

Malignant tumours were most frequent in the groups fed 3 per cent Guinea Green and 3 per cent Benzyl Violet. Six tumours were derived from the epidermis. Only one skin tumour has been observed in approximately 1,200 rats of a similar age in this colony. The incidence of skin

tumours in the Guinea Green group was 13 per cent and in the rats on Benzyl Violet was 20 per cent. In addition, two females fed 3 per cent Guinea Green had benign skin lesions, which meant that nearly 25 per cent of the rats in this group developed skin tumours. From contact with the food, the fur and skin of the rats eventually became stained with the food colours. It is possible that if these two colours act as carcinogens on the skin, the action is by direct contact rather than indirectly through oral administration. This possibility is presently being investigated. The poor absorption of these colours from the intestinal tract reported by Hess and Fitzhugh (1955), might be considered as evidence favouring a direct effect on the skin.

From the results of our experiments, there appears to be no evidence to contradict the safety of Brilliant Blue as a food colour. It is felt that the status of Guinea Green and Benzyl Violet needs further clarification.

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References

Allmark, M. G., Grice, H. C. and Lu, F. C. (1955). *J. Pharm. Pharmacol.*, **7**, 591–603.
Allmark, M. G., Grice, H. C. and Mannell, W. A. (1956). *Ibid.*, **8**, 417–424.
Mannell, W. A., Grice, H. C., Lu, F. C. and Allmark, M. G. (1958). *Ibid.*, **10**, 625–634.
Duncan, A. J. (1953). *Quality Control and Industrial Statistics*, 380–411, Homewood, Ill., U.S.A.: Richard D. Irwin Inc.
Willheim, R. and Ivy, A. C. (1953). *Gastroenterology*, **23**, 1–19.
Nelson, A. A. and Hagan, E. C. (1953). *Fed. Proc.*, **12**, 397.
Hess, S. M. and Fitzhugh, O. G. (1955). *J. Pharmacol.*, **114**, 38–42.

THE EFFECTS OF BRETYLIUM AND COCAINE ON NORADRENALINE DEPLETION

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Bretylium and cocaine possess the property of reducing the depletion of noradrenaline in rat hearts and spleens produced either by guanethidine or by reserpine. Their modes of action differ.

Boura and Green suggested in 1959 that bretylium exerts its hypotensive action by preventing the release of noradrenaline from the nerve terminals when the postganglionic sympathetic nerves are stimulated. It has also been suggested (Fleckenstein and Bass, 1953; Macmillan, 1959) that cocaine prevents the release of noradrenaline from its stores. Since drugs such as reserpine or guanethidine release noradrenaline from sympathetically innervated tissues, it is of interest to discover whether bretylium or cocaine could reduce the effect of reserpine or guanethidine in depleting the rat heart and spleen of noradrenaline.

Methods

Groups of 12 rats weighing 200–250 g. were used in most experiments. After treatment, they were killed by a blow on the head and their hearts and spleens removed as rapidly as possible. These tissues were weighed and stored at $-10°$. After extraction, their catecholamine content (adrenaline and noradrenaline) was estimated fluorimetrically as described by Cass and Spriggs (1961) and expressed in terms of noradrenaline.

Drugs were administered by subcutaneous injection. Reserpine, dissolved in the solvent of Pletscher, Shore and Brodie (1955), was given in doses of 0·1 mg./kg., except in the preliminary experiment where 3 daily doses of 1 mg./kg. were used. Guanethidine hemisulphate, dissolved in 0·01N HCl, was injected at a dose of 10 mg./kg. In the protection experiments, aqueous solutions of bretylium tosylate (30 mg./kg.) or cocaine hydrochloride (10 or 50 mg./kg.) were administered with the reserpine or guanethidine. All doses were calculated in terms of the base. Animals were killed either 6 or 18 hr. after the drugs.

In other experiments, a pressor effect indicative of stimulation of the sympathetic nervous system was induced in rats under urethane anaesthesia by the intravenous injection of 20 μg. of physostigmine (Varagić, 1955); cocaine (50 mg./kg.) was then injected subcutaneously and the response to physostigmine re-tested over the following 5 hr. at 30 min. intervals.

Results

Preliminary experiments showed that whereas bretylium in 3 daily doses reduced the effect of guanethidine (also in 3 daily doses) in depleting the rat heart of noradrenaline, it failed to alter that of reserpine (3 daily doses of 1 mg./kg.). The dose of reserpine was therefore reduced to a single dose of 0·1 mg./kg. in the later experiments.

Action of Bretylium

Eighteen hr. after a single dose of bretylium the noradrenaline contents of rat heart (0·59 ± 0·05 μg./g.) or spleen (0·39 ± 0·03 μg./spleen) were not significantly altered. When given with reserpine or guanethidine,

TABLE I

EFFECT OF COCAINE (10 MG./KG.) ON THE DEPLETION OF THE NORADRENALINE CONTENT OF RAT HEART AND SPLEEN PRODUCED BY RESERPINE (0·1 MG./KG.) OR GUANETHIDINE (10 MG./KG.). ANIMALS KILLED 6 OR 18 HR. AFTER THE INJECTIONS

Time (hr.)	Drug	Noradrenaline (per cent of control)			
		Heart		Spleen	
		No cocaine	Cocaine	No cocaine	Cocaine
6	Saline	100	89	100	110
	Reserpine	4	10	8	12
	Guanethidine	19	22	19	32
18	Saline	100	92	100	126
	Reserpine	<9	<9	9	14
	Guanethidine	<9	<9	11	16

bretylium significantly reduced (P <0·001) the release of noradrenaline produced by these agents. This is shown in Fig. 1. The reduction was greater in the heart than in the spleen, and in fact the noradrenaline content in the heart after bretylium and guanethidine did not differ significantly from the control value.

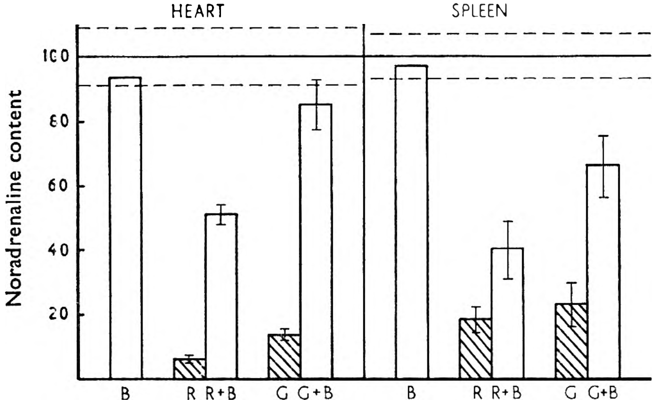

FIG. 1. Effect of bretylium (B, 30 mg./kg.) on the depletion of the noradrenaline content of rat heart and spleen produced by reserpine (R, 0·1 mg./kg.) or guanethidine (G, 10 mg./kg.). Animals killed 18 hr. after the injections; noradrenaline content expressed as percentages (± S.E.) of the control values.

Action of Cocaine

Cocaine (10 mg./kg.) also did not significantly alter the noradrenaline contents of the heart and spleen when estimated 6 or 18 hr. later. When given with reserpine or guanethidine, cocaine had no effect on the release

of noradrenaline produced by these agents (see Table I). When the larger dose of cocaine (50 mg./kg.) was given and the animals were killed 6 hr. later, significant reductions ($P < 0.05$) in the release of noradrenaline were found (Fig. 2). Again, cocaine did not significantly alter the noradrenaline contents of the heart or the spleen. The larger dose of cocaine also failed to reduce the pressor action of physostigmine in the anaesthetised rat: in fact, it slightly potentiated the response.

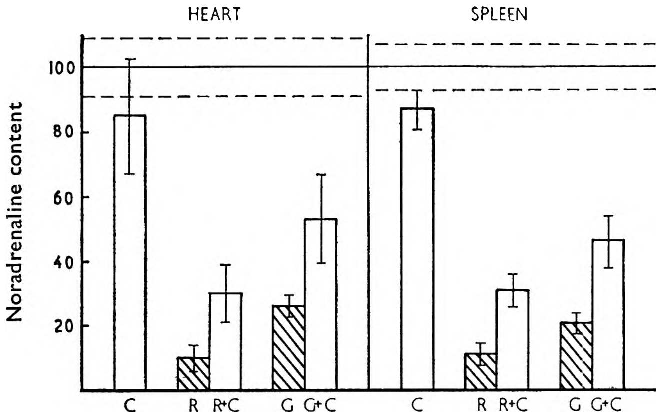

FIG. 2. Effect of cocaine (C, 50 mg./kg.) on the depletion of the noradrenaline content of rat heart and spleen produced by reserpine (R, 0·1 mg./kg.) or guanethidine (G, 10 mg./kg.). Animals killed 6 hr. after the injections; noradrenaline content expressed as percentages (± S.E.) of the control values.

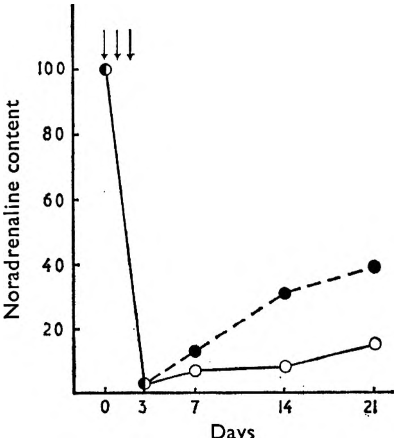

FIG. 3. Effect of daily doses of bretylium (30 mg./kg.) on the depletion and replacement of noradrenaline in rat heart produced by reserpine (3 daily doses of 1 mg./kg., at the arrows). Noradrenaline content expressed as percentages of the control values: reserpine and saline (○——○); reserpine and bretylium (●----●).

Replacement of Noradrenaline

Daily doses of bretylium for 21 days produced no alteration in the noradrenaline content of rat heart, yet given with 3 daily doses of reserpine

(1 mg./kg.) and then by itself for the next 18 days, it produced an increased rate of recovery of noradrenaline. This is shown in Fig. 3.

Discussion

Bretylium has been shown to reduce the loss of noradrenaline in the heart and spleen of rats produced by reserpine or guanethidine. This suggests that bretylium acts by preventing the release of the noradrenaline from its stores or the access of the releasing agent to these stores. However, Varagić, Lešić, Vuco and Stamenović (1961) reported that bretylium prevented the release from sympathetic nerve endings of noradrenaline produced by the intravenous injection of physostigmine into the intact rat. Moreover, Hertting, Axelrod and Patrick (1962) using radioactive techniques found that bretylium blocked both the release and uptake of ^{3}H-noradrenaline in rat heart. These same authors showed also that guanethidine 4 hr. after administration, partially inhibited the reserpine-induced release of noradrenaline. This observation adds weight to the suggestion of Cass and Spriggs (1961) that guanethidine has a dual mode of action; a primary bretylium-like action and a secondary reserpine-like action.

In the present experiments, large doses of cocaine also antagonised the action of reserpine or guanethidine in depleting tissue noradrenaline. Whereas Macmillan (1959) suggested that cocaine blocked the release of noradrenaline from the stores, Trendelenberg (1959) found that cocaine did not alter the release of noradrenaline during stimulation of the splenic nerves and that it potentiated the pressor effects which followed this stimulation. Later, Trendelenberg (1961) showed that cocaine antagonised the pressor action of tyramine and suggested that this was due to competition with tyramine, thus preventing its access to the noradrenaline store. Hertting, Axelrod and Whitby (1961) have recently shown that cocaine prevents the uptake of infused ^{3}H-noradrenaline. Further, the larger dose of cocaine used in the present experiments did not reduce the pressor response to injected physostigmine. Thus it seems likely that the action of cocaine is to block the access of noradrenaline, or of its releasing agents to the stores of noradrenaline; the release of noradrenaline after nerve stimulation is unaffected.

Although it has been shown that both bretylium and cocaine possess the ability to reduce the degree of depletion of noradrenaline after treatment either with reserpine or with guanethidine, they appear to do so by different mechanisms. Bretylium probably acts by preventing the release of noradrenaline and cocaine by preventing the access of the releasing agents to the noradrenaline store.

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References

Boura, A. L. A. and Green, A. F. (1959). *Brit. J. Pharmacol.*, **14**, 536–548.
Cass, R. and Spriggs, T. L. B. (1961). *Ibid.*, **17**, 442–450.

Fleckenstein, A. and Bass, H. (1953). *Arch. exp. Path. Pharmak.*, **220**, 143–156.
Hertting, G., Axelrod, J. and Patrick, R. W. (1962). *Brit. J. Pharmacol.*, **18**, 161–166.
Hertting, G., Axelrod, J. and Whitby, L. G. (1961). *J. Pharmacol.*, **134**, 146–153.
Macmillan, W. H. (1959). *Brit. J. Pharmacol.*, **14**, 385–391.
Pletscher, A., Shore, P. A. and Brodie, B. B. (1955). *Science*, **122**, 374–375.
Trendelenberg, U. (1959). *J. Pharmacol.*, **125**, 55–65.
Trendelenberg, U. (1961). *Ibid.*, **134**, 8–17.
Varagić, V. (1955). *Brit. J. Pharmacol.*, **10**, 349–353.
Varagić, V., Lešić, R., Vuco, J. and Stamenović, B. (1961). *Biochem. Pharmacol.*, **8**, 10.

A NOTE ON THE ACTIVITY OF SIX PENICILLINS AGAINST *ESCHERICHIA COLI*

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A quantitative comparison of the activity of six penicillins against *Escherichia coli* has been made. The order of their effectiveness in inhibiting growth was benzylpenicillin and phenbenicillin, phenoxymethylpenicillin, phenethicillin, propicillin, methicillin; and in inducing spheroplasts benzylpenicillin, phenoxymethylpenicillin, phenethicillin and phenbenicillin, propicillin, methicillin.

The isolation of the penicillin "nucleus" (6-aminopenicillanic acid) by Batchelor, Doyle, Naylor and Rolinson (1959) has rightly been acclaimed as a major advance in chemotherapy. Several new penicillins, which differ only in the nature of the side-chain R (I) have been prepared from this and are employed clinically.

One of the main antibacterial effects of benzylpenicillin is the induction of spherical bodies (spheroplasts) in Gram-negative bacteria treated with the antibiotic in a hypertonic medium containing a readily available source of Mg^{++} ions (Lederberg 1956, 1957). Other penicillins induce similar morphological changes in Gram-negative bacteria (Hugo and Russell 1960 a,b) but apart from the results obtained with methicillin (Hugo and Russell, 1960b) no quantitative comparison of this property appears to have been undertaken. The present report compares this function in six penicillins.

MATERIALS AND METHODS

Nutrient broth was prepared from Oxoid No. 2 granules; hypertonic medium for the investigation of spheroplast formation was nutrient broth containing 0·33M sucrose and 0·25 per cent w/v magnesium sulphate, $MgSO_4.7H_2O$; nutrient agar was prepared from Oxoid granules. The pH of all media after autoclaving was 7·4.

I

Me_2C—CH·COOH
S N
C CO
H CHNHR

The penicillins used, and their molecular weights, were: sodium benzylpenicillin (I, R=·CO·CH_2·Ph) (356·4), potassium phenoxymethylpenicillin (phenoxymethylpenicillin, Penicillin VK, I, R=·CO·CH_2·O·Ph) (388·5); potassium phenoxybenzylpenicillin (phenbenicillin, Penspek, I, R= ·CO·CH(Ph)·O·Ph) (464·8); potassium phenoxypropylpenicillin (propicillin, Ultrapen, I, R=·CO·CH(Et)·O·Ph) (416·5); potassium phenoxyethylpenicillin (phenethicillin, Broxil, I, R=·CO·CH(Me)·O·Ph) (402·5);

and the sodium salt of 6-(2,6-dimethoxybenzamido)penicillinic acid (methicillin, Celbenin, I, R = CO—C$_6$H$_3$(MeO)$_2$) (420·4). The concentrations of antibiotics are expressed as μmoles/ml.

The organism used was a laboratory strain of *Escherichia coli*. It was maintained in nutrient broth and at weekly intervals was plated on to nutrient agar. After overnight incubation of the plate at 37°, a new culture was initiated from a single colony.

When required, 10 ml. nutrient broth tubes were inoculated with one loopful of the stock culture and incubated for 18 hr. at 37°. 0·1 ml. was then added to (*a*) 10 ml. nutrient broth containing the desired concentration of antibiotic, these tubes being incubated at 37° for 24 hr. or (*b*) 10 ml. sucrose-Mg^{++}-broth containing the antibiotic; these tubes were incubated at 37° for 5 hr. when samples were examined by phase-contrast microscopy.

All experiments were repeated on two separate occasions.

TABLE I

MINIMUM CONCENTRATIONS OF PENICILLINS NEEDED TO INHIBIT GROWTH OF, AND INDUCE SPHEROPLASTS IN, *E. COLI*

Antibiotic	Minimum conc. (μmoles antibiotic/ml.) to inhibit growth within 24 hr. at 37°.	Minimum conc. (μmoles antibiotic/ml.) to induce spheroplasts within 5 hr. at 37°.
Benzylpenicillin	Between 0·25 and 0·5	Between 0·1 and 0·25
Phenethicillin	Between 1·0 and 2·0	Between 1·0 and 1·5
Methicillin	>4·0	>4·0
Phenoxymethylpenicillin	Between 0·5 and 0·75	Between 0·5 and 0·75
Phenbenicillin	Between 0·25 and 0·5	Between 1.0 and 1·5
Propicillin	Between 2·0 and 3·0	Between 2·0 and 3·0

RESULTS AND DISCUSSION

The minimum concentration of each penicillin required to inhibit the growth of, and to induce spheroplast formation in, *E. coli* is given in Table I. Benzylpenicillin and phenbenicillin are the most effective in inhibiting growth, although the result with the latter antibiotic must be accepted with some reservation, as the substance was stated by the manufacturers to be 92 per cent purity. Those impurities present could have contributed to the growth-inhibitory effect, while also being of such a nature that they do not affect spheroplast formation. This is supported by the antibiotic concentrations needed to induce spheroplasts (Table I), since phenbenicillin is less effective than either benzylpenicillin or phenoxymethylpenicillin in this respect. Benzylpenicillin is the most effective of the substances tested in inducing speroplasts, and of the phenoxypenicillins, phenoxymethylpenicillin is the most active, followed by phenethicillin, phenbenicillin and propicillin, in that order. Benzylpenicillin is at least 8–16 times as active as methicillin, not only in inhibiting growth but also in inducing spheroplasts. In a study of the antibacterial activity of four penicillins against sensitive and resistant staphylococci, streptococci, *Haemophilus influenzae* and neisseriae,

Williamson, Morrison and Stevens (1961) showed that against the Gram-negative organisms, benzylpenicillin was the most effective, followed by phenoxymethylpenicillin, phenethicillin and propicillin in that order. The present report shows complete agreement with this finding.

A comparison of the concentrations of benzylpenicillin needed to inhibit growth within 24 hr. and to induce spheroplasts within 5 hr. indicates that a lower dose is required in the latter case. This result was confirmed in two replicate experiments, and the apparent anomaly might be explained on the assumption of a penicillinase which would slowly destroy the antibiotic over the longer period of incubation.

Acknowledgements. The author is grateful to the following firms for generous samples. Beecham Research Laboratories, Ltd. (Broxil, Celbenin); Eli Lilly and Co., Ltd. (Penicillin V); Harvey Pharmaceuticals, Ltd. (Ultrapen); and Distillers Co. (Biochemicals) Ltd., (Penspek).

REFERENCES

Batchelor, F. R., Doyle, F. P., Naylor, J. H. C. and Rolinson, G. N. (1959). *Nature, Lond.*, **183**, 257–259.
Hugo, W. B. and Russell, A. D. (1960a). *Ibid*, **188**, 875.
Hugo, W. B. and Russell, A. D. (1960b). *Brit. med. J.*, **2**, 1085.
Lederberg, J. (1956). *Proc. Natl. Acad. Sci., U.S.*, **42**, 574–577.
Lederberg, J. (1957). *J. Bact.*, **73**, 144.
Williamson, G. M., Morrison, J. K. and Stevens, K. J. (1961). *Lancet*, **1**, 847–850.

LETTERS TO THE EDITOR

Heparin and Anaphylaxis

SIR,—It has been suggested that experimental anaphylactic shock may lead to vascular damage with thrombus formation notably in the rabbit (Sanyal and West, 1958; Waalkes and Coburn, 1959), and thus it seemed probable that some of the manifestations of this phenomenon may be ameliorated by pretreatment with anticoagulants. The methods for sensitisation and challenge were those previously described (Sanyal and West, 1958a, 1958b). In the rat, dog and the guinea-pig, administration of 5,000 I.U. of heparin per kg. weight, 30 min. before challenge did not modify the production of anaphylactic shock. In the rabbit, however, such a treatment afforded considerable protection against development of anaphylactic shock. In a series of 18 sensitised animals, 14 animals died within 15 min. after the injection of the challenging dose. In a series of 12 animals similarly sensitised, but injected with heparin as above, on challenge one animal died within this period; another died in 6 hr.; the rest survived.

The mechanism of the protective action of heparin has also been studied in this species. An action on antibody formation is excluded on theoretical grounds, inasmuch as heparin was given a short time before challenge. Heparin pretreatment did not prevent passive transfer of antibodies to guinea-pigs, nor did heparin prevent precipitin reaction *in vitro*. The uptake of antibodies by the virgin guinea-pig uterus *in vitro* was not modified in presence of heparin. Histamine and 5-HT have been suggested to be the two chief mediators of anaphylactic shock in this species; however, the antihistamine or anti-5-HT action of heparin was minimal. It was found that the histamine and 5-HT content of the lung tissue is raised after anaphylactic shock in this species (Sanyal and West, 1958b). In heparin pretreated animals, this rise was absent, either there was no change in the values or there was a slight reduction.

This action could be explained by the well-known anticoagulant action of heparin, as this rise is said to be due to formation of thrombus in the lung field.

It was also seen that during anaphylactic shock characteristic changes occurred in the ECG indicating disturbances of the coronary circulation (Mikulich, 1951)f Pretreatment with heparin did not prevent the almost immediate depression o- the S–T segment, and alterations in the shape of T-wave, but whereas in heparin. treated animals these changes were transient and passed off within 5–10 min., in the control group undergoing anaphylaxis the changes persisted for several hours in animals which survived. The earlier changes might be due to spasm of the coronary arteries (Mikulich, 1951) but if the later changes are supposed to be due to thrombus formation, as has been suggested for pulmonary circulation, the absence of delayed effects in heparin-treated animals is easily explained.

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REFERENCES

Mikulich, G. (1951). *J. Allergy*, **22**, 249–263.
Sanyal, R. K. and West, G. B. (1958a). *J. Physiol.*, **142**, 571–584.
Sanyal, R. K. and West, G. B. (1958b). *Ibid.*, **144**, 525–531.
Waalkes, T. P. and Coburn, H. (1959). *J. Allergy*, **30**, 394–407.

Mechanism of Increased Capillary Permeability Induced by *Echis carinatus* (Saw-scaled Viper) Venom: A possible new approach to the Treatment of Viperine Snake Poisoning

SIR,—Haemorrhages in various organs, in skin, and in mucous membranes, haematuria, and the accompanying shock, resulting from a widespread capillary endothelial damage, and the prolonged clotting time, determine to a large extent the ultimate outcome of the viperine snake poisoning (Ahuja and Singh, 1956). The exact mechanism by which the venom produces an increased capillary permeability is not known so far. It was, therefore, decided to determine whether or not the response is mediated through the release of 5-hydroxytryptamine (5-HT) or histamine, which have been reported to be responsible for the formation of oedema and increased capillary permeability induced by various substances (Spector, 1958; Jori, Bentivoglio and Garattini, 1961).

Capillary permeability was determined by injecting azovan blue dye, 20 mg./kg. intravenously (Parratt and West, 1957), and observing the extent and intensity of blue discoloration produced by 20 μg. each of the *E. carinatus* venom, histamine and 5-HT administered intradermally in the previously depilated abdominal skin of the rat. Male albino rats of Haffkine strain, weighing 150–200 g., were used. An interval of 15 min. was allowed for the dye to accumulate at the site of the drug administration, when the rats were killed and skin removed. The number of rats used as well as the results obtained in the control group, promethazine pretreated group, lysergic acid diethylamide pretreated group and the promethazine plus lysergic acid diethylamide pretreated group are summarised in Table I. It may be observed that the venom is almost as powerful as 5-HT, and approximately three times more potent than histamine, in increasing the capillary permeability in the rat. Promethazine pretreatment completely blocked the response to histamine, that to 5-HT remained unaltered, and that to the venom was significantly reduced. Lysergic acid diethylamide, on the other hand, completely blocked the response to 5-HT, slightly reduced that to histamine, and that to the venom was reduced to almost half. When both promethazine and lysergic acid diethylamide were given, the response to all the three drugs was completely blocked.

TABLE I

Treatment 30 min. before the test	Diameter (mm.) of area of blue discoloration 15 min. after the test drug administration in groups of 10 rats: 5-HT (20 μg.)	Histamine (20 μg.)	*E. carinatus* venom (20 μg.)
Control	20 (18–22)	6 (5–7)	18 (16–20)
Promethazine 10 mg./kg. i.p.	19 (18–20)	nil	6 (4–8)
Lysergic acid diethylamide 1 mg./kg. i.v.	nil	5 (4–6)	10 (8–11)
Promethazine 10 mg./kg. i.p. + Lysergic acid diethylamide 1 mg./kg. i.v.	nil	nil	1–2

The results suggest that the increased capillary permeability induced by the *E. carinatus* venom in the skin of the rat is mediated through a release of both histamine and 5-HT, and the effect is partially prevented by the use of an anti-histamine or an anti-5-HT drug alone, and completely blocked by the two drugs given together. Inasmuch as most of the symptoms in the viperine snake poisoning are due to an increased capillary permeability with the resultant

haemorrhages and shock, treatment with antihistamine and anti-5-HT drugs may prove to be an important advance in the clinical management of the snake poisoning.

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March 27, 1962

REFERENCES

Ahuja, M. L. and Singh, G. (1956). In *Venoms*, pp. 341–351. Washington: Amer. Assoc. for the Advancement of Sciences.
Jori, A., Bentivoglio, A. P. and Garattini, S. (1961). *J. Pharm. Pharmacol.*, **13**, 617–619.
Parratt, J. R. and West, G. B. (1957). *J. Physiol.*, **139**, 27–41.
Spector, W. G. (1958). *Pharmacol. Rev.*, **10**, 473–505.

The Activity of Ampicillin against *Escherichia coli*

SIR,—The isolation of the penicillin "nucleus," 6-aminopenicillanic acid, has resulted in the development of new penicillins with advantages over the older existing ones. It has become clear from recent work that the penicillins show much variation in their antibacterial activity (Garrod, 1960).

Until recently, the penicillins were regarded as having only a slight inhibitory action on the growth of Gram-negative bacteria in general. Infections caused by such organisms were normally treated with either chloramphenicol or the tetracyclines; a new penicillin, ampicillin, 6[D(−)α-aminophenylacetamido]penicillanic acid, known commercially as Penbritin, has now been formulated which is more effective than either of these antibiotics in infections caused by *Escherichia coli*, *Proteus sp.*, *Shigella sp.* and *Salmonella sp.* (Rolinson and Stevens, 1961; Brown and Acred, 1961).

One of the principal mechanisms involved in the antibacterial action of benzylpenicillin is its interference with, and inhibition of, bacterial cell wall synthesis, with the resultant formation of bacterial spheroplasts. These may be regarded as bacteria which are deficient in a portion of the cell wall responsible for rigidity. At least five other penicillins are known to have a similar antibacterial action (Russell, 1962), and experiments were made to investigate whether ampicillin also possessed this property. Accordingly, 0·1 ml. of a 17-hr. broth culture of *Escherichia coli* grown at 37° was added to 10 ml. tubes of broth containing 0·33 M sucrose, 0·25 per cent w/v $MgSO_4 \cdot 7H_2O$ and varying concentrations of ampicillin. After incubation of all tubes for 5 hr. at 37°, samples were examined by phase-contrast microscopy. It was found that the minimum dose of the drug needed to induce spheroplast formation was 10 μg./ml. The minimum inhibitory concentration of ampicillin against the same organism in nutrient broth was also 10 μg./ml., this reading being taken after 24 hr. incubation at 37°. Further, by means of the method described by Rolinson and Stevens (1961) it was possible, in the space of a few days, to "train" the organism to grow in the presence of 100 μg./ml. ampicillin. Whether or not bacterial resistance to this antibiotic will present a clinical problem remains to be seen. We wish to thank Dr. G. N. Rolinson for a gift of ampicillin.

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REFERENCES

Brown, D. M. and Acred, P. (1961). *Brit. med. J.*, 1, 197–200.
Garrod, L. P. (1960). *Ibid.*, 1, 527–529.
Rolinson, G. N. and Stevens, S. (1961). *Ibid.*, 1, 191–196.
Russell, A. D. (1962). *J. Pharm. Pharmacol.*, **14**, 390–392.

New Oral Hypoglycaemic Agents

SIR,—The clinical and experimental aspects of oral hypoglycaemic agents have recently been reviewed by Creutzfeldt and Söling (1961). It would appear that two classes of compounds, the sulphonyl-ureas (tolbutamide and related substances) and the guanidines (biguanide derivatives, such as Phenformin) have supplemented insulin therapy in diabetes mellitus to some extent. However, these compounds have their own limitations and generally insulin has to be administered with them. Consequently, efforts are continuing either to suitably modify these compounds or to arrive at newer compounds, which may replace insulin. Lightbody and Reid (1960) have reported the hypoglycaemic effect of ortho-cresotinic acid.

We have prepared six cogeners of salicylic acid and tested their hypoglycaemic effect on albino rabbits. The compounds were fed to normal healthy rabbits, not less than 1·5 kg., by a stomach tube, in a dose of 15 mg./kg., the solution being made in water with the aid of an equivalent amount of sodium bicarbonate. The effect of each drug on the blood-sugar level of the animals was observed for 5 hr., blood sugar being estimated by the method of Folin and Wu (King, 1951). Preliminary results obtained on these compounds are given in the Table.

TABLE

HYPOGLYCAEMIC EFFECT OF NEW SALICYLIC ACID DERIVATIVES IN NORMAL RABBITS

Compound	Maximum fall in blood sugar per cent. Mean of 3 animals	Mean time of maximum fall (hr.)
2-Hydroxy-4-methylbenzoic acid ..	24·4	2·6
2-Hydroxy-3-ethylbenzoic acid ..	27·8	1·66
2-Hydroxy-5-propylbenzoic acid ..	24·0	3·3
2-Hydroxy-3-propylbenzoic acid ..	33·24	1·33
β-Resorcylic acid	9·6	3·3
2,4-Diacetylresorcylic acid.. ..	18.9	4·5

The maximum hypoglycaemic percentage fall is more pronounced with 2-hydroxy-3-propylbenzoic acid, while diacetylresorcylic acid gives a more prolonged action. The series appears to be promising.

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REFERENCES

Creutzfeldt, W. and Söling, H. D. (1961). *Oral Treatment of Diabetes*, Berlin: Springer-Verlag.
King, E. J. (1951). *Microanalysis in Medical Biochemistry*, London : J. & A. Churchill Ltd.
Lightbody, T. D. and Reid, J. (1960). *Brit. med. J.*, 1, 1704.