REVIEW ARTICLE

THE RELEASE OF MEDICINAL SUBSTANCES FROM TOPICAL APPLICATIONS AND THEIR PASSAGE THROUGH THE SKIN

By D. H. O. Gemmell, B.L., F.P.S. and J. C. Morrison, B.Sc.

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

When medicinal substances in the form of topical applications like ointments and creams are applied to the skin the effect which is desired may be either local or systemic. Therefore a knowledge of the rate and extent of release of the medicament from the vehicle in which it has been incorporated is important. The large number of vehicles and bases now in use for the presentation of medicinal substances as skin preparations underlines the need for suitable methods which estimate the rate and extent of release of medicaments. There is a need, too, for precise methods for evaluating the amount and extent of penetration and systemic absorption of medicaments from their vehicles.

The Skin as a Barrier

The properties and structure of the skin influence the extent of penetration and absorption of a medicament applied to its surface. The skin consists of two layers, the outer epidermis and the inner dermis. The outer epidermis is composed of stratified epithelium rich in lipids and esters of cholesterol. The superficial layer is horny, tough, and keratinised, and also presents a greasy surface which prevents or delays the penetration of water and aqueous solutions. The skin is electrically polarised and behaves like a membrane with a negative charge on the outside. It is cation permeable and anion impermeable, and this is one explanation of the general impermeability of the skin to electrolytes, although substances in aqueous solution can be introduced by electrophoresis. But no systemic absorption results from penetration into the outer epidermis since it is avascular.

The inner dermis is composed of a meshwork of fibrous and elastic tissue which is well supplied with blood vessels and merges into, and is continuous with, the underlying fat. The control of the flow of blood by the variation of tone of these blood vessels regulates surface temperature; also they serve as the nutritional vessels of the dermis and should a substance penetrate the outer epithelium it may be rapidly absorbed in the capillary bed.

The greater part of percutaneous penetration takes place by way of the appendages of the skin—hair follicles and sebaceous and sweat glands—so that before penetration may take place, there is yet a further barrier to systemic absorption; this is the sebum, a secretion of glycerides and fatty acids.

The presentation of medicaments in ointments, creams or lotions may be considered from three aspects. Firstly, the liberation of the medicament from the base or vehicle; secondly, the penetration of the outer epidermis of the skin by the medicament; and thirdly, the absorption of the medicament

into the bloodstream. Among the factors influencing penetration and absorption through the intact skin are the mode of application, the vehicle, the various physico-chemical properties of the medicament, the base, dermal secretions such as sweat, sebum, tissue lipids, and the extracellular fluid.

Penetration readily occurs through broken skin because the protective barrier of outer layers has been removed. Perhaps unbroken skin is a rarer state than we imagine; the modern concept of hygiene demands, in varying degree, the removal of sebum with detergents, and the abrasion of the skin with various foreign tissues.

The Problem Defined

While the purpose for which a topical application is intended should be considered in the design of any test for the evaluation of its efficiency, the number of variables in the intended system is often such that to obtain any result it is more advantageous to investigate one aspect of the system—usually the rate of release. This has given rise to numerous *in vitro* techniques. These tests are of value where the medicament is required for local effect only, as with antiseptic ointments. When penetration and absorption of the medicament into the skin is important, histological and histochemical results are needed to measure the rate of release. The rate and degree of absorption of a medicament which gives rise to systemic effects can be assessed by clinical effects, or tests on animals in which the concentration in blood, urine, faeces or tissues is measured. Pathogenic skin conditions are numerous and preclude generalisations so most *in vivo* assessments are made on healthy intact skin to avoid increasing the number of variable factors in the system. This approach itself is open to criticism in that the true state of affairs is not extant but if the approach is to be rationalised, work must move to the specialised from the generalised rather than plunging directly into an assortment of individual skin conditions.

Recent reviews[1–13] have discussed the absorption of drugs, and it is proposed to limit this review to a survey and evaluation of the variety of methods which have been devised to estimate the rate of release, amount of penetration and absorption of the applied medicament from the vehicle. Chemical, physical, pharmacological, toxicological, histological, and microbiological methods have been adopted. In this range of tests and techniques, some are concerned with liberation, some with penetration and some with absorption. To rationalise the range of methods a classification has been attempted.

In Vitro Methods

Diffusion without a membrane

(i) Chemical and Physical
(ii) Microbiological

Diffusion with a membrane

(i) Chemical and Physical
(ii) Microbiological

In Vivo Methods

(i) Blood, urine, faeces analysis
(ii) Tissue analysis
(iii) Characteristic Reactions
(iv) Histological methods
(v) Clinical methods

Radio-active Tracer Methods

RELEASE OF MEDICAMENTS FROM TOPICAL APPLICATIONS

In Vitro METHODS

In vitro methods are of limited value but they are a means of assessing the ability of a vehicle or base to liberate medicament under the conditions of the test. Those reported are of a comparative nature, and most are empirical which prevents results from being compared with those from other techniques. Lockie and Sprowls[14] hold that the study of ointment bases by *in vitro* methods is essentially a study of diffusion rates, neglecting the importance of the base as an emollient or protective. The methods seem to fall into two categories of diffusion methods that use a membrane and those that do not. In both of these categories chemical, physical and microbiological estimations have been devised.

DIFFUSION METHODS WITHOUT A MEMBRANE

The assumption made in most of these tests is that the distribution of medicament between the vehicle or base and the area under treatment will be similar to the distribution between vehicle and medium of the test, although this is doubtful, particularly when one considers the complexity of skin fats with the simple media—water and saline—often used. The distribution will be a function of the partition coefficient, assuming the medicament is soluble in both phases; it will also be governed by the rate of diffusion of the medicament from within the base to the surface. A base in which the medicament is not soluble but dispersed, can make available only such material as can be leached from the surface layer by the extraction medium, unless this itself is soluble in the base. This factor seems to have been disregarded in some observations; it is, of course, the same factor which accounts for the very small quantities of medicament released particularly from mineral oil bases. With such base and medicament relations a badly made preparation presenting gross particles of medicament to the extraction medium will give anomalous results.

Chemical and Physical Estimations

Demonstrating the principle simply, Seelman[15] showed diffusion of salicylic acid from a lard base by covering the ointment with a solution of ferric chloride, when the colour diffused quickly throughout the supernatant; soft paraffin did not release the acid to give a colour.

Hawking[16] followed the course of the topical release of sulphonamides with a static method of measuring diffusion by estimating the amount of sulphonamide, from a dispersion, diffusing into a known surface area of agar or gelatin incorporating Erlich's reagent (an acid solution of *p*-dimethylaminobenzaldehyde). The sulphonamide reacts to give a yellow colour the depth of which was measured and an analysis of the results made. Waud and Ramsay[17] also used a static method, similar in technique, to measure the diffusion of sulphonamides from hydrophilic and non-hydrophilic bases with and without sodium lauryl sulphate. Lockie and Sprowls[14] sought a mathematical basis using the rate of diffusion of sulphonamides from bases into an agar gel incorporating Erlich's reagent. Hawking[16] also used a cellulose film cylinder into which gelatin was

poured and then 5 ml. of a suspension of sulphonamide added. The use of the cellulose container made possible the slicing, transversely, of the gelatin and, after standing the uncut cylinder and contents at room temperature, 3 mm. slices were cut and analysed for sulphonamide by a colorimetric method.

A colorimetric method was used by Bandelin and Kemp[18] to assay sulphonamide release. The sulphonamide ointments were applied evenly around the inside of standard test-tubes to which either saline or serum was added, the tubes were incubated at 37° for varying times after which the fluid was decanted and assayed colorimetrically. Surface-active agents markedly increased the rate of release of drug.

Howard[19] also used the colorimetric method to estimate the release of medicament from ointment spread on a watch glass into surrounding water at 37°. A criticism of these methods is that by allowing only static diffusion the rate of release would be less than would occur where the extraction fluid was in motion as with blood and serum. To overcome the disadvantages of Hawking's method, Fuller, Hawking and Partridge[20] estimated the rate of diffusion of sulphonamides from a glycerol-gelatin jelly into a stream of water moving at 20 ml./hour. With sulphanilamide, which is about 10 times more soluble in water than most other sulphonamides (except sulphacetamide), diffusion was rapid until the surface layer of the jelly was exhausted. After 25 hours, conditions approached equilibrium, probably as the result of diffusion from the inner layers of the jelly. A steady rate of diffusion was obtained with the less soluble sulphonamides. The method is simple and elegant, and although water and not saline or tissue fluid was used in the extraction, the authors applied the sulphonamide to a standard wound, exposing subcutaneous tissues in rabbits, and claim a satisfactory agreement between their *in vitro* and *in vivo* results. But this *in vivo* method is not, of course, a measure of absorption through intact skin.

Microbiological Assay Methods

Since topical applications are often intended to be antiseptic a number of methods and techniques have been devised which depend upon a form of microbiological assay. The familiar arguments for and against the methods of assessing disinfectants would equally apply here. The tests aim at assessing the antiseptic value and also give an indication of the rate and degree of release of the medicament from the vehicle, but, of course, different organisms and bases are used and comparisons should be made only when all the conditions of bioassay are met, which seldom occurs.

One of the earliest methods was devised in 1895[21] when glass coverslips were treated with a broth of *Serratia marcescens*, dried and then introduced into the ointment for a fixed period of time. The slips were then washed with ether, transferred to sterile broth and incubated. The effect of the ether on the organisms does not appear to have been investigated. Cold cream and lanolin were found to be the most effective bases for the release of the antiseptics used. Cheyne[22] spread antiseptic ointments on coverslips which were then laid under agar in a petri dish. After inoculating

the surface of the agar with *M. pyogenes var. aureus* and incubating, zones of inhibition were observed; lanolin was the most and the hydrocarbons the least efficient in releasing phenolic antiseptics. As these are far more efficient antiseptics in aqueous media, this result could be explained by the fact that cold cream and lanolin make phenol available to aqueous surroundings more easily than mineral oils by virtue of their own water content or their ability to incorporate water and thus give a greater zone of inhibition.

Reddish[23] devised the most widely used test for assessing the effectiveness of antiseptics included in ointment bases, and this with certain modifications was eventually adopted by the United States Food and Drug Administration[24] and remains the only official test in being. In the original, plates were inoculated with a culture of *M. pyogenes var. aureus* and spread with the test ointment melted at 37°, controls using base without medicament also being used. After incubation the width of the zone of inhibition was taken by Reddish to be an indication of antiseptic value. His suggestion that the nutrient agar of the petri dish simulated the conditions met with in wounds and skin since "it is permeable, semisolid, isotonic and constitutes a valuable laboratory means of approximating the conditions found in human and animal tissue" is open to question, as apart from physico-chemical differences there is no cell membrane to penetrate, no keratin, cell detritus, pus, skin flora, excretions or appendages, all of which are known to influence activity and absorption. The method is also limited to antiseptic preparations and is not applicable to preparations which have no antibacterial property.

Reddish and Wales[25] and other workers[14,26] using this and similar tests have shown the much greater efficiency of emulsified bases over fat, oil, and wax bases as antiseptic carriers. Reddish's test was modified by the use of weighed quantities of ointment spread over a definite area, and it was shown that antiseptic efficiency was diminished or entirely absent with fat, oil, or wax bases, while oil-in-water emulsion bases gave a greatly increased antibacterial value[27].

Pillsbury, Livingood and Nichols[28] proposed a technique in which the hands were scrubbed in a standard way and the number of organisms removed was estimated by colony count of incubated rinsings. Ointment was rubbed on each hand and forearm, allowed to remain for a stated time, removed and, after washing again, the number of organisms remaining estimated, comparison of diminution in count being the criterion of efficiency. The objections to this method are numerous; the main criticism is that it introduces too many variables to be of practical value as a reliable technique.

Diffusion Methods using Membranes

The use of a membrane is an attempt to simulate *in vitro* the barrier which is presented by the skin to a topical application. Both artificial and natural membranes have been used, and while the assumption is made that the process of penetration in the skin is similar to the quantitative diffusion through a membrane, this does not make any allowance for

differences in physico-chemical properties of "dead" membranes and living tissue, the latter presenting a much more complex system both physically and chemically.

Chemical and Physical Estimations

In an attempt to make a simple evaluation, Rae[29] incorporated sodium chloride into various ointment bases which were then introduced individually into glass tubing to one end of which was attached a cellulose film membrane. The ointment was gently forced to the membrane end of the tube and this was then immersed in distilled water for 24 hours. The chloride diffusing out was assayed with silver nitrate. Rae states that "the various results obtained probably represent what takes place when the ointment is applied to broken skin"—a quite uncritical statement in view of the method, "medicament" and bases employed. It is hardly surprising that the greatest release was from a 5 per cent pectin jelly. No temperature was stated, an artificial membrane and a completely ionised salt were used, and no allowances were made for the effect of electrolytes in blood or serum.

In 1891, Luff[30] immersed, in water at 37°, sheep's bladders in which were suspended ointments of paraffin, lard and lanolin base with potassium iodide, phenol and rescorcinol as medicaments. He found paraffin showed the quickest and lanolin the slowest rate of release, a result at variance with later workers.

Coran and Huyck[31] used a method suggested by Izgu and Lee[32] in which diffusion from ointment in a hollow cylinder, placed in the centre of a filter paper moistened with indicator solution, was estimated by measuring the distance to the outer edge of the indicated ring on the paper. Izgu and Lee used salicylates and ferric chloride impregnated paper; Coran and Huyck employed sulphonamides and Erlich's reagent, and assessed their results in similar manner to that adopted by Lockie and Sprowls and compared the results of these authors with their own.

Microbiological Assay

The rate of release of penicillin[33] and sulphonamides [34] was estimated from various bases placed in cellulose film bags immersed in saline at 37° for a fixed time. The saline was estimated for activity by the F.D.A. cup-plate method. Clarke and Davies[35], in an interesting modification of the cup-plate method, poured agar plates which were dried for 2 hours, after which 1 ml. of a 1 : 10 dilution of a 24 hour culture of *M. pyogenes var aureus* was added. Four sterile 1 inch squares of cellulose film were then placed on each plate, and after 45 minutes incubation the films were carefully spread with the preparations under test and the whole incubated overnight, the plates were then examined for zones of inhibition. With phenyl mercuric nitrate and proflavine sulphate an increasing rate of release was observable from fatty base, water in oil base, oil in water base to jelly. Velu and colleagues[36] examined the diffusibility of antibiotics from various ointments and creams by placing them in dialysing tubes and assaying samples at intervals.

In Vivo METHODS

Most absorption through the intact skin occurs by way of the appendages. Increases in rates of absorption are usually seen with the use of solvents or bases that remove the protective layer of sebum and skin fats and thus aid penetration; absorption is also increased when the medicament is itself absorbable. The steroids, salicylic and boric acid are good examples. Numerous methods with animals have been devised to estimate both penetration and absorption of medicament; generally the results of animal experiments may be extended to man.

Blood, Urine and Faeces Analysis

After its cutaneous application, the detection of a substance in the bloodstream, urine, or faeces offers the most conclusive proof of its absorption but care must be exercised in accepting these figures as a quantitative estimate. Blood levels have been accepted as a measure of the absorption of topically applied sulphonamides[37–39]. Woodward and others[40] determined sulphathiazole levels in blood samples and in catheterised urine samples from rabbits after the application of an ointment to a measured area of clipped skin. Bases containing propylene glycol were found to be superior to fat or oil bases when using the absorption of sulphathiazole as a criterion. The addition of certain surface-active agents improved absorption. A comparison of ointment bases using rats by Meyer and colleagues[41] employed phenolsulphophthalein and potassium iodide as chemical tracers. The time taken for the first colouration of the urine in sodium hydroxide with phenolsulphophthalein was noted, while for potassium iodide a chemical analysis was made. These results and those obtained with the same bases from the agar plate method were correlated. Lund[42] studied the absorption of calcium and sodium penicillins from ointment bases by assaying the drug in urine samples.

That mercury is absorbed percutaneously there is no doubt: the mechanism is presumed to be by combination of the mercury with the sebaceous fatty acids to produce oil-soluble salts. The rate of absorption is modified by the choice of base. Mercury is conveniently estimated in the body fluids, tissues and excreta by routine chemical analysis[43].

Tissue Analysis

If selective absorption of medicament occurs in particular organs or tissues misleading information will be obtained by estimations of blood, urine or faeces levels. Laug and his colleagues[44] found one hundred times as much mercury in the kidney as in the rest of the body and urine after the application of mercury ointments. Differences in the efficiencies of the ointments were observed. Lang and Kunze also estimated the absorption of lead by the kidney, liver, muscle and lung from different vehicles applied to rats[45].

Care must also be exercised in accepting systemic blood levels as an estimate of the amount of absorption. Since the blood level represents drug in transfer it accurately reflects several unknown transfer processes. The first is the rate of absorption from the skin site; the second is the

storage in depots like fat (hexobarbitone) or heart and kidney (mercury); the third is the rate of excretion in bile and urine. Frequently, as in the classical instance of sulphonamides, the kidney is functioning both as an organ of excretion by glomerular filtration and of conservation by tubular resorption. This latter mechanism is common to many drugs[46].

An ingenious method of measuring release and penetration of drugs devised by Hunter and Smith[47] uses chick embryos. The authors injected into the natural air sac of fertilised eggs ointment bases containing antibiotics. The chorio-allantoic membrane served as the medium for penetration, toxicity was determined by the number of embryos remaining alive after varying times. An assessment of release of the antibiotics was made by assaying aliquot portions of the allantoic fluid. The authors justify their method by a number of arguments. The method employs a living membrane devoid of glandular ducts or hair follicules which eliminates absorption through such appendages. The living tissue is covered by a relatively dry non-living membrane which is thin and therefore allows rapid analysis, and this give a biological system from which small samples can be assayed *in vivo* without excretion problems. The technique is simple and the method cheap. However, the lack of keratin in the non-living shell membrane and chorio-allantoic membrane and the use of embryonic tissue with remote phylogenetic relation to mammals constitute salient criticisms of this method giving statistics of doubtful application to man.

Characteristic Reactions

Pharmacological or physiological end points have been adopted as measure of the time taken for the passage of a drug through the skin to produce a systemic effect. Macht[48] used strychnine to measure the action of various ointment bases on the course of penetration of alkaloids. Convulsions were taken as the end point in tests on rats and mice. Macht concluded that the use of ointments did little to improve and may even have hindered penetration. Walzer[49] demonstrated the percutaneous absorption of an antigen by first sensitizing passively a skin site to the antigen and then proving absorption by the production of an urticarial reaction at another site 24 to 48 hours later.

The effect of the passage of hormones through the skin has been the subject of wide research; a few of the many references will be quoted. In 1929 Zondek[50] demonstrated the physiological effects resulting from the passage of sex hormones through the skin. Moore and others[51] discussing the effects of androgens and oestrogens on the skin of guinea pigs stated that "there is yet a lack of appreciation of the readiness with which substances are taken up by the skin and are effective in the body". Nelson, Greene and Wills[52] were critical of this work as the number of animals in the experimental groups used by these workers was small and the data not statistically analysed. This latter criticism can be applied to much of the work on percutaneous absorption, and was one of the criticisms of the Hadgraft, Somers and Williams[53] paper presented at the 1956 British Pharmaceutical Conference, although in an earlier paper Hadgraft and Somers[54] attempted a statistical examination but made the point that

different bases were used which influenced rate and magnitude of release, and with test and standard not having the same contribution the principles of bioassay were confounded. They concluded in this work that a very large number of animals would be needed to obtain a high degree of precision. Smith, commenting on the paper, considered the assay valid because the *same* medicament was being compared in different bases.

The method presented by Hadgraft and Somers to the British Pharmaceutical Conference in 1954 was an interesting application of characteristic effects. Eserine potentiates the effect of acetylcholine which induces the secretion of opaque reddish-brown tears in the rat. The acetylcholine 50 μg. was injected subcutaneously, then the rats were inuncted on a shaved area on the back with bases containing eserine. The response was given an arbitrary classification of numbers based on the amount and colour of secretion. The drawback of this method was the difficulty of carrying out this procedure on a number of animals large enough to give reliable results, and the authors contended that calculation of a statistic was not in accordance with bioassay principles, paraffin used as a standard being compared with other bases. From their results it was concluded that the absorption of eserine was better from lard, arachis and castor oil than from white soft paraffin. Only oily or fatty bases were used. It would have been interesting to see the results of release of eserine from oil-in-water emulsion bases, ethanol or ether in which the solubility is different.

Zondek[55], making use of ethanol (96 per cent), ether and benzene as vehicles for the application of oestrogen was able to detect only little difference between the cutaneous and subcutaneous administration of the drug necessary to produce oestrus in castrated mice. Nelson and his colleagues[52] have shown that percutaneous administration of testosterone was more effective in ethanol than in oil as measured by the increase in weight of the prostate and seminal vesicles of rats. Here two factors were probably extant, the defatting action of ethanol and a partition coefficient more favourable to release of the steroid from this solvent than from oil.

Heparin creams have been tested by German workers who noted a significant increase in the coagulation time of the blood of the rabbits used[56].

Another interesting application of a characteristic reaction has been used to measure the effectiveness of ointments containing local anaesthetics. Using a modified Harding-Wolff-Goodell pain threshold apparatus, Lucas and Guth[57] measured the response to the stimulus of radiant heat on the blackened tails of rats to which were applied Simple Ointment, Hydrophilic Ointment and Bentonite Ointment U.S.P. XIV with the anaesthetics incorporated. The base alone was used as control. With this pain threshold method, Brockmeyer and Guth[58] showed that bentonite was an efficient base for the release of cyclomethycaine. These results are subject to the obvious criticism that heat has been used and absorption no doubt facilitated by the increased peripheral blood supply which will result. Heat will also reduce the viscosity of the bases which will assist release. The comparison is legitimate but may prove unreal.

The protective effect of topically applied chemotherapeutic agents has been used to demonstrate absorption and could be used to evaluate vehicles. Zondek[59] studied the application of external disinfectants, in particular *p*-chloroxylenol, in protecting rats infected with streptococci and pneumococci, and this protective effect has been utilised in clinical trials. Green[60] investigated the release of sulphonamides from creams by two methods using mice. Neither involves skin penetration, and therefore only measures rate of release of medicament from depots within the body. In the first method, groups of 5 mice were injected with 0·1 ml. of the cream intraperitoneally and blood levels estimated for 6 hours after injection. The other method was to measure the survival times of mice, infected with *Str. pyogenes* in the thigh muscle after subcutaneous injections of the creams. Infected untreated animals were used as controls.

Histological and Histochemical Methods

A variety of procedures under this heading has been adopted to evaluate drug release from vehicles. Biopsies were made at intervals by Strakosch[61] to assess the time to produce keratolysis by sulphur, salicylic acid, rescorcinol, and other medicament-containing bases.

Duemling[62] adopted a method similar to that of Eller and Wolff[63] in which a study was made of permeability and absorbability of preparations into the skin. Shaved rabbits were treated with test materials on one side of the back, the other side being used as control. Skin biopsy showed much more rapid penetration and a greater depth was obtained when fatty substances were applied with wetting agents.

The penetration of sulphathiazole through the intact skin of rats, rabbits, and men, was measured by means of tissue analysis, and the results obtained from the application of wet dressings, iontophoresis and ointments have been compared[64]. Penetration was examined from water-in-oil and oil-in-water emulsions of sulphanilamide; increase in concentration of sulphanilamide failed to increase the degree of penetration. The use of solubilisers and wetting agents did not give improved results, and injured skin took up greater amounts of sulphanilamide than intact skin. Of the sulphonamides tested, the very soluble sodium sulphacetamide had the greatest tissue concentration[65,66].

Dyes[67–71] and fluorescent materials[67,68,72] have been used to demonstrate skin penetration. Harry[67] examined histologically human post-mortem material, and rabbits, rats and guinea pigs after application of fats, oils, emulsions and aqueous solutions containing dyes and active chemical agents. He concluded that oil, fats and aqueous preparations in general do not penetrate the intact epidermis to any appreciable extent, but that lipids such as cholesterol and lecithin could increase the penetrating property of liquid paraffin, and the presence of polar groups conferred penetrant properties upon mineral oils and greases.

Clinical Methods

Clinical methods have amply demonstrated the systemic absorption of medicaments, but many of the methods do not effect an evaluation of

drug release. In 1911, Wild[73] used an "analysis by difference" technique which he continued with Roberts[74]. The method is primitive, and the results should be viewed with caution. A known weight of ointment was inuncted on the arm for a given time, the excess ointment was then removed by a tared razor blade, and the weight difference was the quantity absorbed.

Moncorps[75,76] carried out the first comparative clinical trials when he measured the amount of salicylic acid eliminated after its cutaneous application in various bases. The absorption of salicylates and iodides has been examined extensively. Brown and Scott[77] studied the absorption of salicylates from various vehicles using the hands as the area of application. The excretion of salicylate in the urine was assayed. They observed a trend to increasing absorption coinciding with a downward trend of partition coefficients. In a general discussion on cutaneous absorption they point out that it has generally been considered that a substance must possess both oil and aqueous solubility to penetrate the human skin, and that an extremely high or low partition coefficient is less conducive to absorption than intermediate values. Zondek[59] showed that the blood of patients who had received percutaneous treatment with *p*-chloroxylenol was able to inhibit the growth of staphylococci and that an application of this medicament brought about disinfection of the urinary tract because of its presence in the urine.

Using phenolsulphophthalein excretion, after application to the skin, and its colorimetric estimation in the urine, it was demonstrated by Nadkarni and others[78] that a vehicle containing propylene glycols promoted greater absorption than an oil or paraffin base. Propylene glycol and surface-active agents have been shown to aid the passage through the skin of acetylcholine chloride, pilocarpine nitrate, atropine sulphate, hyoscine, ephedrine and histamine phosphate, the substances themselves being used as pharmacological indicators[79]. Greatest absorption was shown to have occurred in the most hairy areas of the body, while no evidence of absorption was noted when the drugs incorporated in penetrating types of vehicles were applied to the palms of the hands and the soles of the feet, areas where hair follicles are normally absent. Here the keratinous layer is thicker than elsewhere on the body, but the results support the contention that absorption occurs only via the appendages.

Jacobi and Lantzsch[80] proposed a photometric method of estimating the penetration of ointments and creams after 10 drops of a 1 per cent solution of Sudan Red 5B in mineral oil had been admixed with them. 0·1 g. of ointment was used on a circle of the skin of 4 cm. in diameter on the inside of the underarm. Filter paper squares measuring 25 cm.^{2} were laid upon the treated skin for 30 seconds by means of the cuff of a blood pressure apparatus. This procedure was repeated with fresh paper until no more colour could be removed from the skin. Untreated skin, treated skin before and after removal of the surplus ointment, and an arbitrary standard (barium sulphate) were given a photometric value, and from these a value for the absorption or penetration of the ointments tested was calculated.

Radioactive Tracer Methods

The application of tracers to the study of permeability in biological problems arose from the pioneer work of Hevesy. Isotopic techniques have now been introduced to evaluate drug release from topical applications and probably offer the most accurate *in vivo* means of assessment, and they are of particular value where the biochemical processes following absorption are being investigated.

Where the information which is obtained is of the same nature as that from chemical analysis, tracer techniques should not necessarily displace accepted analytical or quantitative procedures and in general they should only be used where results will be more accurate than those obtained by other methods. Up to the present time only relatively simple compounds have been used for the investigation of absorption through the skin: perhaps with a reduction in cost and greater availability of labelled compounds, their penetrant and medicinal properties will be increasingly examined in animals. It is doubtful, with the evidence of cumulative effects, that further work on man will be permitted.

Barker, Christian and De Kay[81] modified the agar cup plate method to estimate the release of ^{131}I incorporated as salts in hydrophilic bases. The agar was not seeded with a test organism but weighed amounts of ointment were placed in each plate, and after incubation strips of agar were removed and the counts per minute determined. In all cases the iodine diffused throughout the medium. Agreement with the cup plate method was found in all but U.S.P. XIV hydrophilic ointment, where the zone of inhibition was the smallest with the official method while by the radioactive tracer method the release of iodine was the greatest.

Johnston and Lee[82] using sodium chloride activated by deutron bombardment in a cyclotron tested absorption from fatty bases. The salt was dissolved in water, incorporated into the bases and a weighed portion tested for activity. One gram was inuncted into the upper arm of young males and the counts per minute from the left hand measured. Urine samples were also taken. Anhydrous lanolin gave the best results.

Czetsch-Lindenwald[83] in his experiments was unable to demonstrate the absorption of ointment bases containing deuterium oxide as a tracer, and it was suggested that only penetration had taken place although Szczesniak and others[84] were able to demonstrate the penetration of deuterium oxide through the skin of rats by measuring the content in the blood.

Cyr and others[85] studied the absorption of sodium iodide, labelled with ^{131}I, from lard, woolfat and soft paraffin when applied to albino rats. The ^{131}I was determined in the thyroid, after killing the animals; the amounts passing through the skin were small, a result also observed by Hadgraft and colleagues[53]. Skauen and others[86] used a similar technique and found hydrous wool fat to be more efficient in releasing ^{131}I than lard.

The permeability of frog skin has been examined extensively by means of tracer techniques. The ability of frog skin to allow the passage of both liquids and gases is well known, but as this ability decreases as the phylogenic series is ascended the results obtained by this method are hardly

applicable to man. Nevertheless, an evaluation of the effectiveness of antiperspirant preparations using isolated frog membrane and radioactive iodine as a tracer showed that astringents increased the rate of iodine ion penetration[87,88].

Other isotopes have been used in tracer work on absorption. For example, Loeffer and Thomas[89] used radioactive strontium in the form of the chloride for the determination of absorption through rat skin, both intact and broken, and the use of radioactive zinc in the form of the sulphate has shown that absorption was least from fat bases but was increased by the addition of soap[90].

Edwards[91] has studied the absorption of topically applied amino acids in guinea pigs using methionine labelled with ^{35}S. The presence of ^{35}S in cystine in newly grown hair was demonstrated.

Hadgraft, Somers and Williams[53] using diiodofluorescein-^{131}I have examined percutaneous absorption in rats from five different bases containing the tracer. The radioactivity of the blood was assessed, and only very small quantities of radioactivity were detectable. The results suggest that absorption was better from hydrous ointment and cetomacrogol than from lard, white soft paraffin and hydrous emulsifying ointment. The authors suggest that this would be expected by the theory that the skin surface is repellant to aqueous solutions, and when the external phase is oily, as in hydrous ointment, or the base has both lipo- and hydro-philic properties as cetomacrogol, miscibility with the sebum is facilitated, which allows the medicament to come into contact with the absorbing cells at the base of the follicles.

Clinically Lange and Evans[92] applied an ointment of lanolin containing radon to patients, and the amount of radon in the expired air was taken as a measure of the amount passing into the bloodstream. The application of thorium X to human skin has demonstrated that penetration is by way of the appendages[93]. This confirmed work by Witten and others[94,95] who examined biopsies of tissue which had been treated with thorium X in various vehicles.

Present Status of the Problem

This survey reveals that while a certain elegance of technique has been evolved, a fundamental basis is still lacking upon which experimental work, to be of real value, can be placed.

In vitro methods begin at a disadvantage by being *in vitro* while the variable reception encountered in different animal species can also make for misleading results *in vivo*. Few authors have set out to make any but an arbitrary assessment based upon their own terms. The results are usually not comparable with other methods.

It is possible that an explanation in terms of Brodie's[46] extrapolation of early theories might offer a basis for experiment. Many of the reports reveal that lipid-soluble drugs, provided they are made available by a vehicle allowing diffusion, can pass rapidly into the body after application to the intact skin. Harry[67] in 1941 commented "It is a remarkable fact that nearly every substance at present known to be absorbed is oil-soluble

(salicylic acid, phenol, rescorcinol, β-naphthol, iodine, vitamins A and D, while mercury can combine with fatty acids to produce oil-soluble salts)". These examples and those of steroids and recently the organo-phosphorus insecticides suggest that in alignment with these ideas some physico-chemical factor common to all should be considered. Brown and Scott observed that in the absorption of boric acid by the skin "a highly specific property of boric acid appears to be involved". We have already noted that the skin behaves like a membrane with a negative change on the outside. This means that electrolytes are not likely to be permeable and that anions will be repelled in addition. It would seem that the intact skin, having the characteristics of a lipoid membrane, allows the passage of lipid-soluble drugs in their undissociated form while restricting the entry of the dissociated form.

The medicament must first pass through the fatty protective layer in the healthy epidermis before either reaching the keratin or penetrating the appendages. Defatting the skin does much to increase absorption assuming the medicament is absorbable, and this would account for the better absorption usually encountered from ointments incorporating surface-active materials or vehicles which defat. The vehicle must not, of course, prevent release of the medicament by any physico-chemical means.

The non-penetrability of saturated mineral oils may be explained as resulting from their general inertness. The introduction of polar groups has been shown by Harry to confer penetrant properties. This observation suggests that the passage through the membrane is not wholly mechanical; some form of active transport may also be involved. At least, physico-chemical activity is essential for the reactions which are associated with cells and interfaces.

The early workers did not have sufficient evidence to suggest that any but the simplest principles were involved. It was soon observed that defatting solvents like chloroform or ethanol enhanced penetration. But many results are anomalous because in the attempts to study release of the medicament little if any attention seems to have been given to whether the medicament could diffuse from anywhere but the surface of the base. Wolfhügel and von Knorre[96] in 1881 found that the amount of phenol liberated from a 5 per cent solution in oil was negligible, and this was clearly shown by Koch[47], who demonstrated that this preparation did not kill spores of *Bacillus anthracis* after 110 days immersion. The principle of diffusion and partition coefficient was recognised, but that diffusion of the medicament from within the base is necessary seems often to have been overlooked. Seelman[15] demonstrated this very simply by placing moistened blue litmus paper on a slide covered with official boric acid ointment. The litmus was still blue after several hours, showing that no penetration or release of acid had occurred. The work on sulphonamide release suggests that the activity of the preparations is often that of the surface layer. As Fuller and colleagues[20] showed, even in a base of glycerol-gelatin a flow rate of 20 ml./hour removed sulphonilamide faster than it could diffuse from within the base, and it was 25 hours before anything approaching a stable system resulted.

This relation between vehicle and base should be recognised, and a knowledge of the physico-chemical properties of both base and medicament should form the basis for a more fundamental approach to the problem of drug release.

The ideal would be to so tailor the medicament molecule and so choose a vehicle that all but one of the basic requirements of percutaneous absorption can be met by physico-chemical factors. Unfortunately the requirement—that of absence of other than the desired effect on the body—is the stumbling block of many a good intention, and, although a compromise would almost invariably result, preparations based on the study of recognised principles should give more reliable effects than those based on empiricism.

It would seem that a standard *in vitro* method of estimating the availability of the medicament is still required. This should be a diffusion technique of the simplest system—a standard weight and volume of medicated base available over a standard area above which is a simple fluid from which samples for assay can be withdrawn at known distances from the base.

In vivo methods must of necessity vary with the purpose of the medicament. Where activity in the skin only is required, histological methods offer the best results. Systemic effects should be assessed quantitatively by biochemical estimation or when more specific knowledge is required by a radio-active tracer technique.

References

1. Eller and Wolff, *J. Amer. med. Ass.*, 1940, **114**, 2002.
2. Rothman, *Handbuch der normalen und pathologischen Physiologie*, 1929, **4**, 107.
3. Neuroth and Lee, *J. Amer. pharm. Ass.* (*Pract.*), 1945, **6**, 285.
4. Calvery, Draize and Laug, *Physiol. Rev.*, 1946, **26**, 495.
5. Busse, *J. Amer. pharm. Ass.* (*Pract.*), 1943, **4**, 314.
6. Guillot, *J. Physiol., Paris*, 1954, **46**, 31.
7. Lane and Blank, *Arch. Derm. Syph.*, 1946, **54**, 497, 650.
8. Mehlhose, *Pharmazie*, 1947, **2**, 202.
9. Vallette and Cavier, *J. Physiol. Path. gen.*, 1947, **39**, 137.
10. Sollman, *Manual of Pharmacology*, 7th Ed., p. 30.
11. Hadgraft and Somers, *J. Pharm. Pharmacol.*, 1956, **8**, 625.
12. Ashley, *Austral. J. Pharm.*, 1955, **36**, 989.
13. de Roeck, *Pharm. Tijdschr. Belg.*, 1955, **32**, 233.
14. Lockie and Sprowls, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 222.
15. Seelman, *J. Amer. med. Ass.*, 1938, **110**, 1127.
16. Hawking, *Quart. J. Pharm. Pharmacol.*, 1941, **14**, 226.
17. Waud and Ramsay, *Canada Med. Ass. J.*, 1943, **48**, 121.
18. Bandelin and Kemp, *J. Amer. pharm. Ass., Sci. Ed.*, 1946, **35**, 65.
19. Howard, *New Engl. J. Med.*, 1945, **232**, 698.
20. Fuller, Hawking and Partridge, *Quart. J. Pharm. Pharmacol.*, 1942, **15**, 127.
21. Breslauer, *Z. Hyg.*, 1895, **20**, 165.
22. Cheyne, *Lancet*, 1915, **1**, 419.
23. Reddish, *Proc. Amer. Drug. Mfg. Ass.*, 1929, **16**, 116.
24. Ruehle and Brewer, *U.S. Dept. Agric., Circ.* No. 198, 1931.
25. Reddish and Wales, *J. Amer. pharm. Ass.*, 1929, **18**, 576.
26. Huyck, Hirose and Reyes, *ibid.*, 1946, **35**, 129.
27. Husa and Radin, *ibid.*, 1932, **21**, 861.
28. Pillsbury, Livingood and Nichol, *Arch. Derm. Syph.*, 1942, **45**, 61.
29. Rae, *Brit. J. Derm. Syph.*, 1944, **56**, 92.
30. Luff., *Pharm. J.*, 1891, **50**, 206.
31. Coran and Huyck, *J. Soc. cosmet. Chem.*, 1956, 7, 20.
32. Izgu and Lee, *J. Amer. pharm. Ass.* (*Pract.*), 1954, **15**, 396.

33. Aymer and Ferlauto, *J. Amer. pharm. Ass., Sci. Ed.*, 1947, **36**, 211.
34. Zheutlin and Fox, *J. Invest. Derm.*, 1948, **11**, 161.
35. Clark and Davies, *J. Pharm. Pharmacol.*, 1949, **1**, 521.
36. Velu, Claude, Peyre and Viennet, *Ann. pharm. franç.*, 1953, **11**, 675.
37. Magner and O'Sullivan, *Canad. med. Ass. J.*, 1944, **50**, 118.
38. Legroux, *Mem. Acad. Chim.*, 1940, **13**, 415.
39. Zondek, Bromberg and Shapiro, *Proc. Soc. exp. Biol., N.Y.*, 1942, **50**, 116.
40. Woodward, Wright, Evenson, Ofner, Kramer, Jenner and Johnson, *Fed. Proc.*, 1944, **3**, 87.
41. Meyers, Nardkarni and Zopf, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 231.
42. Lund, *Arch. Pharm. Chem.*, 1951, **58**, 595.
43. Cole et al., *Arch. Derm. Syph.*, 1928, **17**, 625.
44. Laug, Vos and Kunze, *J. Amer. pharm. Ass., Sci. Ed.*, 1947, **36**, 14.
45. Laug and Kunze, *J. Ind. Hyg. Toxicol.*, 1948, **30**, 256.
46. Brodie and Hogben, *J. Pharm. Pharmacol.*, 1957, **9**, 345.
47. Hunter and Smith, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 125.
48. Macht, *J. Amer. med. Ass.*, 1938, **110**, 409.
49. Walzer, *Arch. Derm. Syph.*, 1940, 41, 692.
50. Zondek, *Klin. Wschr.*, 1929, **8**, 2229.
51. Moore, Lamar and Beck, *J. Amer. med. Ass.*, 1938, **111**, 11.
52. Nelson, Greene and Wells, *Endocrinology*, 1940, **26**, 651.
53. Hadgraft, Somers and Williams, *J. Pharm. Pharmacol.*, 1956, **8**, 1027.
54. Hadgraft and Somers, *ibid.*, 1954, **6**, 944.
55. Zondek, *Lancet*, 1938, **1**, 1107.
56. Pichotka and Mayer, *Arzneimitt.-Forsch.*, 1954, **4**, 277.
57. Lucas and Guth, *J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 344.
58. Brockemeyer and Guth, *ibid.*, 1955, **44**, 706.
59. Zondek, *Nature, Lond.*, 1942, **149**, 334.
60. Green, *Quart. J. Pharm. Pharmacol.*, 1946, **19**, 107.
61. Strakosch, *Arch. Derm. Syph.*, 1944, **49**, 1.
62. Duemling, *ibid.*, 1941, **43**, 264.
63. Eller and Wolff, *ibid.*, 1939, **40**, 900.
64. Clark, Strakosch and Nordlum, *Proc. Soc. exp. Biol., N.Y.*, 1942, **50**, 43.
65. Strakosch and Clark, *Amer. J. med. Sci.*, 1943, **20P**, 518.
66. Strakosch and Clark, *ibid.*, 1943, **206**, 610.
67. Harry, *Brit. J. Derm. Syph.*, 1941, **53**, 65.
68. Butcher, *J. Invest. Dermatol.*, 1953, **21**, 243.
69. Seki, *Japan. J. Pharm. Chem.*, 1951, **23**, 138.
70. MacKee, Hermann, Baer and Sulzberger, *J. Lab. clin. Med.*, 1943, **28**, 1642.
71. Cullumbine, *Brit. J. Derm Syph.*, 1946, **58**, 291.
72. Helander, *Nature, Lond.*, 1945, **155**, 109.
73. Wild, *Brit. med. J.*, 1911, **1**, 161.
74. Wild and Roberts, *ibid.*, 1926, **1**, 1076.
75. Moncorps, *Arch. exp. Path. Pharmak.*, 1929, **141**, 25.
76. Moncorps, *ibid.*, 1931, **163**, 26.
77. Brown and Scott, *J. Pharmacol.*, 1934, **50**, 32, 373.
78. Nadkarni, Meyers, Carney and Zopf, *Arch. Derm. Syph.*, 1951, **64**, 294.
79. Shelley and Melton, *Fed. Proc.*, 1947, **6**, 199.
80. Jacobi and Lantzsch, *Pharm. Zentralh.*, 1952, **91**, 6.
81. Barker, Christian and De Kay, *J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 601.
82. Johnson and Lee, *ibid.*, 1943, **32**, 278.
83. Czetsch-Lindenwald, *Pharm. Ind.*, 1943, **10**, 29.
84. Szczesniak, Sherman and Harris, *Science*, 1951, **113**, 293.
85. Cyr, Skauen, Christian and Lee, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 615.
86. Skauen, Cyr, Christian and Lee, *ibid.*, 1949, **38**, 618.
87. Lux and Christian, *Amer. J. Physiol.*, 1950, **162**, 193.
88. Urakami and Christian, *J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 179.
89. Loeffler and Thomas, U.S. Naval Radiol. Defense Lab., AD-225(*b*), 1950, p. 25.
90. Severan and Tack, *Pharm. Tijdschr. Belg.*, 1952, **29**, 41.
91. Edwards, *Nature, Lond.*, 1954, **173**, 1042.
92. Lange and Evans, *Radiology*, 1947, **48**, 514.
93. Graul, *Strahlentherapie*, 1953, **92**, 197.
94. Witten, Ross, Oshry and Hyman, *J. Invest. Dermatol.*, 1951, **17**, 311.
95. Witten, Bauer, Holmstrom and Loevinger, *ibid.*, 1953, **20**, 93.
96. Wolfhugel and von Knorre, *Mitt. Kaiser Gesundheit*, 1881, **1**, 635.

RESEARCH PAPERS

THE HYPOTHERMIC AND SEDATIVE ACTION OF RESERPINE IN THE MOUSE

BY A. W. LESSIN AND M. W. PARKES

From the Biological Laboratories, Research Department, Roche Products Limited, Welwyn Garden City

Received May 13, 1957

The sedative action of reserpine has been studied by its effects upon the body temperature of the mouse, in comparison with chlorpromazine and 5-hydroxytryptamine (5-HT). Reserpine and chlorpromazine produce hypothermia and sedation in the mouse by interference with the mechanism of temperature regulation, but 5-HT acts in a different manner. The central stimulants, lysergic acid diethylamide, amphetamine and tetrahydro-β-naphthylamine, antagonised the hypothermic effects of reserpine, chlorpromazine and 5-HT. Iproniazid antagonised the hypothermic effects of reserpine, while potentiating those of chlorpromazine and 5-HT. This evidence does not seem to support the hypothesis that the sedative action of reserpine is mediated by 5-HT.

IT has been shown that reserpine causes the disappearance of 5-hydroxytryptamine (5-HT) from various tissues[1–8]. Brodie and his colleagues have consequently suggested that the sedative actions of reserpine are mediated by released 5-HT[9]. In support of this hypothesis they have put forward the evidence that both reserpine and 5-HT have sedative actions in the mouse and can be antagonised by lysergic acid diethylamide (LSD)[10,11].

We have already shown[12] that reserpine, chlorpromazine and 5-HT all lower body temperature in the mouse. Moreover, it was shown for the first two agents that this hypothermic effect may be used as a measure of sedative action. We have further examined the properties of all three agents to determine the extent to which resemblances between the actions of reserpine and 5-HT support Brodie's hypothesis.

METHODS

Rectal temperatures were measured in mice as previously described[12]. Groups of 5 mice had mean temperatures recorded. Except where otherwise stated the room temperature was 22°. All drugs were injected intraperitoneally, in a volume of 0·2 ml./20 g. mouse; they were dissolved in water or saline with the exception of reserpine, which was dissolved in 2N acetic acid, neutralised as far as possible and diluted with water.

RESULTS

Sedative Effects

The first visible sign after injection of moderate doses of reserpine in the mouse is ptosis of the eyelids and this occurs after 10 to 20 minutes. After 30 to 120 minutes sedation commences and body temperature falls,

the mice presenting a characteristic hunched attitude with piloerection, and crowding together in a corner. When handled, they respond normally but become inactive after a short interval. As the body temperature continues to fall the response to handling becomes weaker, although it never completely disappears. Six hours after doses of 3 mg./kg. or more the mice have temperatures approaching that of the room and may be placed on their sides, although attempts to rise are eventually successful.

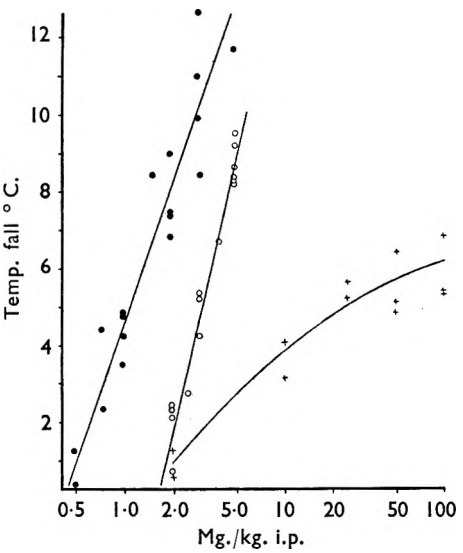


FIG. 1. The effect of chlorpromazine, reserpine and 5-hydroxytryptamine upon the rectal temperature of mice. Each value for temperature fall is the lowest recorded for a group of 5 mice after injection of the drug, and is expressed as the difference from the mean value for an untreated group.

● Reserpine; $b = 12{\cdot}0 \pm 1{\cdot}9$ ($P = 0{\cdot}95$)

○ Chlorpromazine; $b = 17{\cdot}8 \pm 2{\cdot}2$ ($P = 0{\cdot}95$)

+ 5-HT.

By contrast, the fall in temperature with chlorpromazine is immediate, and the predominant feature is muscular weakness, the mice lying outstretched and making no attempts to crowd together. Responses to handling are typically reduced and with doses above 5 mg./kg. the mice barely respond and are unable to resume an upright position when placed on their sides, although they make feeble efforts to do so.

The fall in temperature with 5-HT is also immediate and the mice appear characteristically limp. The appearance of mice sedated with any of these three agents allows them to be readily distinguished.

The maximum fall in rectal temperature was determined for several doses of reserpine, chlorpromazine and 5-HT. As we have shown[12], this occurs at about 6 hours after injection with reserpine and between 1 and 3 hours with chlorpromazine, depending upon dose. With 5-HT, maximum effect occurs after 20 minutes to 1 hour, again depending on dose. Figure 1 shows that a regression of maximum temperature fall with dose occurs for each substance, although with 5-HT the regression on log dose did not appear to be linear over the whole range.

The delay in onset of hypothermia after intravenous injection of up to 2 mg./kg. reserpine in mice was found to be similar to that observed after intraperitoneal injections. This is in agreement with the delay in onset of other effects of reserpine administered by various routes[13]. A very large dose (20 mg./kg. i.p.), however, produced an immediate effect, the temperature falling 4°, within 30 minutes. It was also found that a more rapid fall was produced by small doses when the mice were

kept at room temperatures below 22° and the extent of the fall was greater than at higher room temperatures.

Effect of External Temperature

As we have already shown, no hypothermia occurs in mice with reserpine or chlorpromazine at an ambient temperature of 32°[12]. On the other hand, 50 mg./kg. 5-HT lowered body temperature under these conditions, the mice being limp and sedated. At 38–40°, reserpine and

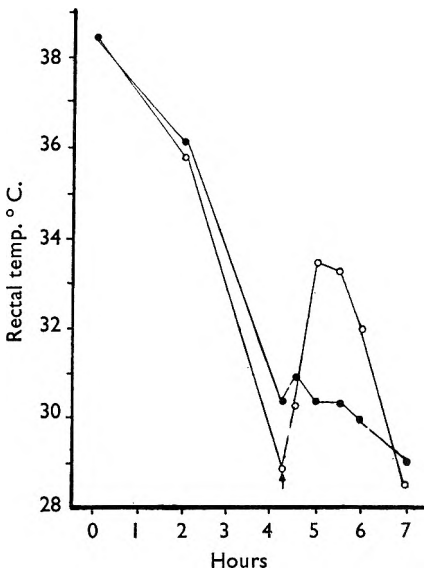


FIG. 2. The effect of LSD on the hypothermic action of reserpine.
●——● Reserpine, 2 mg./kg. i.p. (At arrow—water 0·15 ml./20 g. i.p. given 4¼ hr. later.) ○——○ Reserpine, 2 mg./kg. i.p. (At arrow —0·75 mg./kg. LSD in 0·15 ml./20 g. i.p. given 4¼ hr. later.)

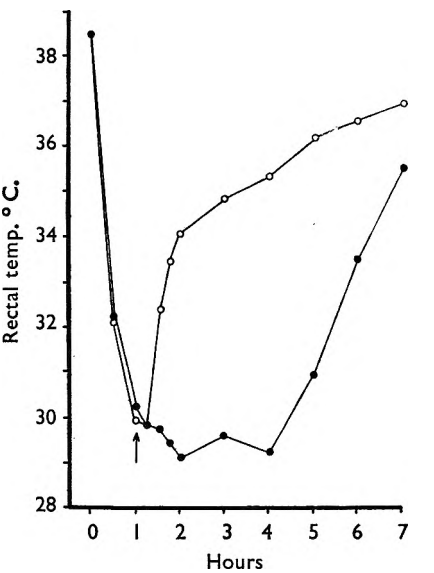


FIG. 3. The effect of LSD on the hypothermic action of chlorpromazine.
●——● Chlorpromazine 5 mg./kg. i.p. (At arrow—water 0·15 ml./20 g. i.p. given 1 hr. later.)
○——○ Chlorpromazine 5 mg./kg. i.p. (At arrow—0·75 mg./kg. LSD in 0·15 ml./20 g. i.p. given 1 hr. later.)

chlorpromazine caused a rise in the rectal temperature of mice, an effect similar to that previously reported for reserpine in other species[14]. At this temperature reserpine still caused ptosis of the eyelids but no appreciable sedation, while chlorpromazine produced excitement, as reported by Berti and Cima[15]. Mice given reserpine and kept at an ambient temperature of 32° for periods up to 4 hours showed no sedation, but their temperature fell and they became sedated immediately on removal to a room temperature of 22°.

Effects of LSD, Amphetamine and Tetrahydro-β-naphthylamine

The hyperthermic action of amphetamine was readily confirmed in mice, but LSD, which raises body temperature in rabbits[16] produced only a slight fall in mice in doses from 0·25 to 10 mg./kg., after a short

period of motor stimulation. However, a rise in temperature was produced by 5 mg./kg. LSD in mice kept at 32°. Similar results were obtained with tetrahydro-β-naphthylamine (50 mg./kg.) and it is concluded that stimulants do not readily raise the body temperature of mice at 22°, but are able to do so at thermal neutrality. LSD given 4 hours after reserpine, 1 hour after chlorpromazine or 30 minutes before 5-HT, clearly antagonised the hypothermic effects of all three agents (Figs. 2, 3 and 4). In each case, signs of sedation were also visibly reduced; it was noticeable that ptosis of the eyelids caused by reserpine was also abolished. The antagonism of LSD towards reserpine lasted about an hour, body temperatures subsequently falling to the level of the control group, whereas with chlorpromazine and 5-HT this subsequent fall did not occur. Similar antagonism of the effects of reserpine and chlorpromazine was observed with 50 mg./kg. tetrahydro-β-naphthylamine and 10 mg./kg. amphetamine.

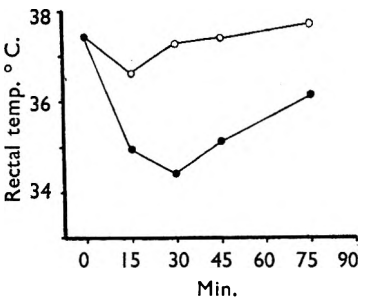


FIG. 4. The effect of LSD on the hypothermic action of 5-HT.
●——● 5-HT 20 mg./kg. i.p.
○——○ 5-HT 20 mg./kg. i.p. 30 min. after LSD 0·75 mg./kg.

Effect of Enzyme Inhibitors

Iproniazid has been reported to antagonise sedation due to reserpine in the rabbit[17] and in the mouse[18,19]. We have observed a corresponding antagonism towards effects on the body temperature of the mouse, hypothermia and sedation being completely prevented by 100 mg./kg. iproniazid, given to mice 24 hours before reserpine[20]. Iproniazid, given before chlorpromazine or 5-HT potentiated their hypothermic effects (Figs. 5, 6).

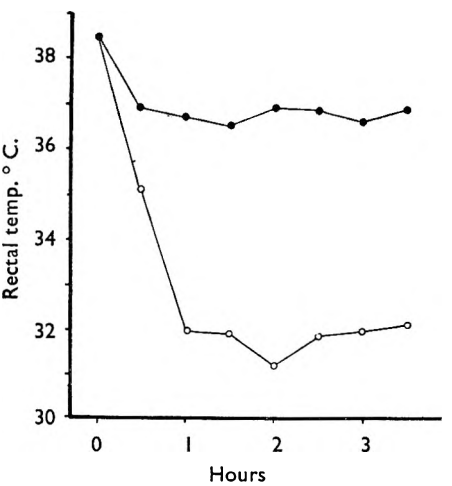


FIG. 5. The effect of iproniazid on the hypothermic action of chlorpromazine.
●——● Chlorpromazine 3 mg./kg. i.p.
○——○ Chlorpromazine 3 mg./kg. i.p. 1½ hr. after iproniazid 100 mg./kg. i.p.

Iproniazid is not only a powerful inhibitor of monoamine oxidase, but has been shown to inhibit the enzyme present in liver microsomes which plays a part in barbiturate detoxication[21]. In this respect it resembles the inhibitor SKF 525A[22] and it was therefore of interest to determine the effects of this inhibitor on the hypothermic agents. Table I summarises the results obtained with both inhibitors, and Figure 7

illustrates the strong potentiation of the effect of chlorpromazine by SKF 525A. The effect of SKF 525A upon reserpine was less marked, while 5-HT was unaffected. Neither iproniazid nor SKF 525A affected body temperature in the doses used.

TABLE I

EFFECTS OF ENZYME INHIBITORS UPON HYPOTHERMIA CAUSED BY RESERPINE, CHLORPROMAZINE AND 5-HYDROXYTRYPTAMINE

Enzyme Inhibitor	Effect upon hypothermia by		
	Reserpine	Chlorpromazine	5-HT
Iproniazid	Antagonism	Potentiation	Potentiation
SKF 525A	Potentiation	Potentiation	No effect

DISCUSSION

Although there is a superficial resemblance between the effects of injections of reserpine and 5-HT in the mouse, in that both lower body temperature and produce sedation, we have shown that a number of differences exist. The effects of reserpine appear to be exerted upon thermoregulation, since at thermoneutrality, the temperature at which heat loss is at a minimum, neither hypothermia nor sedation are produced[12], while at ambient temperatures above 37° hyperthermia occurs. With 5-HT, on the other hand, both temperature fall and sedation occur at thermoneutrality, implying either a fall in heat production or an increase in heat loss. It is possible that this action is largely a peripheral one.

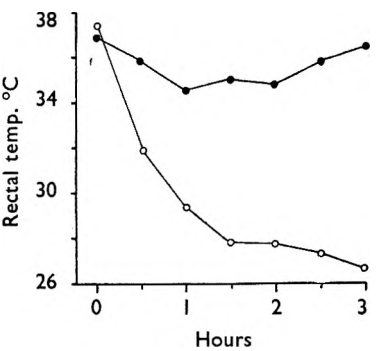


FIG. 6. The effect of iproniazid on the hypothermic action of 5-HT.
●——● 5-HT 25 mg./kg. i.p.
○——○ 5-HT 25 mg./kg. i.p. 1½ hr. after iproniazid 100 mg./kg. i.p.

Although we have confirmed that the 5-HT antagonist, LSD, reduces the sedative action of reserpine and 5-HT, as measured by its antagonism towards the hypothermia caused by these agents, it is significant that the hypothermia of chlorpromazine was also antagonised in the same way. Moreover, amphetamine and tetrahydro-β-naphthylamine were also effective in antagonising the hypothermic effect of reserpine, chlorpromazine and 5-HT.

We conclude, therefore, that the antagonism of LSD towards reserpine and 5-HT is not specific, but may be due to its stimulant action, which it exerts in common with amphetamine and tetrahydro-β-naphthylamine. There is thus no reason to regard this antagonism as related to the anti-serotonin properties of LSD. A similar conclusion concerning the central actions of LSD was reached by Gaddum and Vogt[23] from their studies of intraventricular injections.

The similarities between the sedative properties of reserpine and 5-HT, which were considered by Brodie and colleagues[3] to support the hypothesis that reserpine action is mediated by 5-HT, have been shown to extend to chlorpromazine also. Moreover, reserpine and 5-HT differ in certain important respects; their mode of action appears to be different, and iproniazid, which antagonises the action of reserpine, potentiates that of 5-HT. With reserpine there is a fall in the 5-HT content of the brain[24], the maximum sedative effect of the drug occurring when brain 5-HT is at its lowest level. The prevention of reserpine sedation by iproniazid is associated with retention of brain 5-HT[24] and this has been related to the amount of inhibition of monoamine oxidase in the brain[20]. Thus, if brain 5-HT is concerned in reserpine sedation it is more likely that the effect is due to a deficiency in 5-HT than to mediation by the amine.

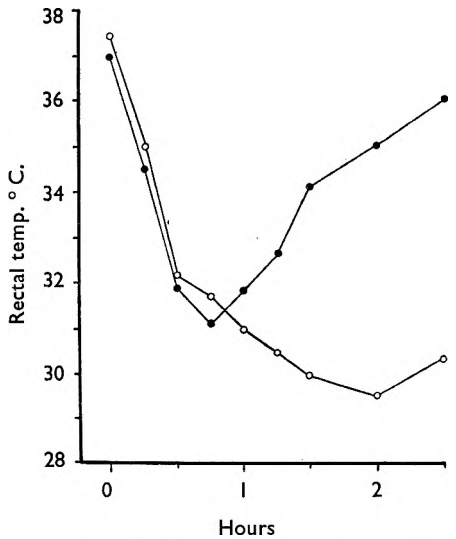


Fig. 7. The effect of SKF 525A on the hypothermic action of chlorpromazine.

●——● Chlorpromazine, 2 mg./kg. i.p.

○——○ Chlorpromazine, 2 mg./kg. i.p. 40 min. after SKF 525A, 10 mg./kg. i.p.

References

1. Pletscher, Shore and Brodie, *Science*, 1955, **122**, 374.
2. Pletscher, Shore and Brodie, *J. Pharmacol.*, 1956, **116**, 84.
3. Brodie, Pletscher and Shore, *Science*, 1955, **122**, 968.
4. Erspamer, *Lancet*, 1956, **2**, 511.
5. Erspamer, *Experentia*, 1956, **12**, 63.
6. Naess and Schanche, *Nature, Lond.*, 1956, **177**, 1130.
7. Paasonen and Vogt, *J. Physiol.*, 1956, **131**, 617.
8. Hardisty, Ingram and Stacey, *Experientia*, 1956, **12**, 424.
9. Hess, Shore and Brodie, *Fed. Proc.*, 1956, **15**, 437.
10. Shore, Silver and Brodie, *Experientia*, 1955, **11**, 272.
11. Shore, Silver and Brodie, *Science*, 1955, **122**, 284.
12. Lessin and Parkes, *Brit. J. Pharmacol.*, 1957, **12**, 245.
13. Plummer, Earl, Schneider, Trapold and Barrett, *N.Y. Acad. Sci.*, 1954, **59**, 8.
14. Bein, Gross, Tripod and Meier, *Schweiz. med. Wschr.*, 1953, **83**, 1007.
15. Berti and Cima, *Arzneimitt-Forsch.*, 1955, **5**, 73.
16. Horita and Dille, *J. Pharmacol.*, 1955, **113**, 29.
17. Brodie, Pletscher and Shore, *ibid.*, 1956, **116**, 9.
18. Chessin, Dubnick, Kramer and Scott, *Fed. Proc.*, 1956, **15**, 409.
19. Besendorf and Pletscher, *Helv. Physiol. Pharmacol. acta*, 1956, **14**, 383.
20. Davison, Lessin and Parkes, *Experientia*, 1957, In the press.
21. Fouts and Brodie, *J. Pharmacol.*, 1955, **115**, 68.
22. Cooper, Axelrod and Brodie, *ibid.*, 1954, **112**, 55.
23. Fastier, *Experientia.*, 1956, **12**, 351.
24. Gaddum and Vogt, *Brit. J. Pharmacol.*, 1956, **11**, 175.
25. Pletscher, *Experientia.*, 1956, **12**, 479.

CRYSTALS IN THE LEAF OF *LOBELIA INFLATA* LINN.

By T. E. Wallis

From the Museum of the Pharmaceutical Society of Great Britain

Received June 19, 1957

The leaves of *Lobelia inflata* Linn. have been said either to contain crystals of calcium oxalate or to have no crystals of any kind. Other descriptions note the presence of droplets of oil in the mesophyll. This anomalous situation needs clarifying. It is shown that the crystals are fat.

The presence or absence of crystals in the leaf of *Lobelia inflata* Linn. has been variously recorded by the writers of textbooks of pharmacognosy. Some authors give no description of the histology of the leaf, others state that calcium oxalate or crystals are absent, e.g., Zörnig[1] 1925; Gilg, Brandt and Schürhoff[2] (although crystals appear to be shown in their figure) 1927; Karsten and Bencke[3] 1928; Brandt and Wasicky (in Thoms)[4] 1931; Gathercoal and Wirth[5] 1956; Trease[6] 1952. Others again state that calcium oxalate in microcrystals is present, e.g., Koch[7] 1914; Flück, Schlumpf and Siegfried[8] 1935. Wasicky[9] 1936 makes the rather curious statement that "in cold chloral hydrate there crystallise out in the mesophyll after some time large needles, rods and sphaerites."

For some years past I have noticed in some sections of leaves of *Lobelia inflata* the presence of small crystals often almost filling certain cells of the palisade and spongy parenchyma of the mesophyll; in other similar sections, crystals appeared to be absent. When checking the details of microscopical structure for inclusion in the British Pharmaceutical Codex, I decided that this anomaly ought to be investigated.

The occurrence of crystals was reported in 1914 and 1935 (see above); the presence of droplets of oil was first recorded in the British Pharmacopoeia 1932[10]. When crystals were described they were said to be calcium oxalate either by direct statement or by inference. Wasicky in 1936 (see above) appears to refer to some decomposition product formed on standing with chloral hydrate.

The crystals present are small slender prisms about 10 to 15μ long and 2μ thick, often arranged in fan-shaped groups of about 2 to 6; they occur in many cells of the palisade tissue and in cells scattered throughout the spongy parenchyma; the cells containing them are often almost completely filled with the crystals (see Fig. 1). They are well seen in sections mounted in glycerol or in cold chloral hydrate solution and they polarise brightly. When these preparations are warmed to the boiling point, the crystals are no longer seen, but abundant droplets of oil are present and are stained deeply with Soudan red III. These facts and observations suggested to me that the globules of oil and the crystals might have some relationship to each other and that the crystals might possibly be regarded as crystals of fat which become globules of oil when the preparations are warmed.

To test the proposition that the crystals and globules of oil are the same substance, a rough sketch of a section was made superimposed by an eyepiece-scale and the positions of the crystals were marked; after warming droplets of oil were found in the same positions and no polarisable particles could be found, thus confirming the suggestion that the crystals are fat.

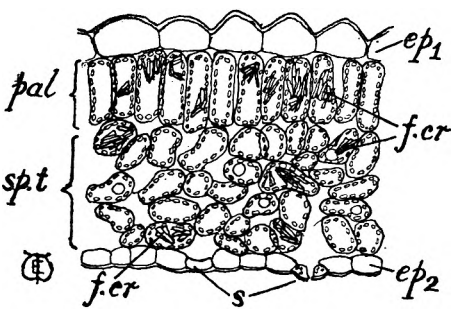


FIG. 1. Transverse section of the lamina of a leaf of *Lobelia inflata* Linn. × 200; ep_1, upper epidermis; ep_2, lower epidermis; *f.cr*, fat crystal; *pal*, palisade tissue; *s*, stoma; *sp.t*, spongy tissue.

Since the presence of crystals of calcium oxalate has been recorded by Koch and others, experiments were made to show the absence of calcium oxalate. All the preparations made in the course of this work were examined in polarised light as well as by ordinary illumination; this was done because the crystals are not always clearly visible in ordinary light.

EXPERIMENTS TO SHOW THE ABSENCE OF CALCIUM OXALATE

1. A transverse section was mounted in strong hydrochloric acid (s.g. 1.16) and after standing for ten days, the crystals showed no change, whereas calcium oxalate is rapidly dissolved under these conditions.

2. A transverse section was mounted in sulphuric acid 20 per cent v/v and no apparent change was observed during four days and no formation of the characteristic crystals of calcium sulphate was seen, showing an absence of calcium salts.

It is therefore evident that calcium oxalate is absent from the leaf.

FURTHER EXPERIMENTS TO CONFIRM THE FATTY NATURE OF THE CRYSTALS

1. A piece of leaf was soaked in chloroform for a few hours, a transverse section was then cut and mounted in chloroform with a little glycerol and crystals were absent.

2. A piece of leaf was soaked in alcohol, 95 per cent, for 24 hours after which a transverse section was cut and mounted in glycerol, when only a very few small bright specks were visible in polarised light, showing that the crystals dissolve in alcohol.

3. A transverse section was mounted in caustic potash, 5 per cent, and numerous crystals were seen similar to those seen in mounts in glycerol

or cold chloral hydrate. On warming to the boiling point, the crystals were replaced by globules of oil which stained deeply with Souden red III.

Hence the crystals present must be fat.

Conclusion

It is therefore evident that: (i) crystals are abundantly present in the mesophyll of the leaf of *Lobelia inflata*; (ii) calcium oxalate is absent from the leaves; (iii) the crystals present are composed of fat.

References

1. Zörnig, *Tabellen für das pharmakognostische Praktikum*, Berlin, 1925, p. 77.
2. Gilg, Brandt and Schürhoff, *Lehrbuch der Pharmakognosie*, 4th edn. Berlin, 1927, p. 434.
3. Karsten and Bencke, *Lehrbuch der Pharmakognosie*, 4th edn. Jena, 1928, p. 222.
4. Brandt and Wasicky, in Thoms, *Handbuch der Pharmazie*, vol. V, Berlin, 1929, p. 1635.
5. Gathercoal and Wirth, *Pharmacognosy*, 3rd edn. London, 1956, p. 433.
6. Trease, *Textbook of Pharmacognosy*, 6th edn. London, 1952, p. 761.
7. Koch, *Pharmakognostischer Atlas*, Leipzig, 1914, p. 153.
8. Flück, Schlumpf and Siegfried, *Pharmakognostischer Atlas*, Basle, 1935, p. 260.
9. Wasicky, *Leitfaden für die pharmakognostischen Untersuchungen*, vol. II, Leipzig, 1936, p. 341.
10. *British Pharmacopoeia*, 1932, p. 275.

MORPHINE ANTAGONISM

By F. H. Shaw, S. Gershon and G. A. Bentley

From the Department of Pharmacology, University of Melbourne, and Nicholas Institute for Medical and Veterinary Research

Received May 14, 1957

A series of compounds which antagonise the depressant activity of morphine in dogs is described. The most active compounds are the previously described 2 : 4-diamino-5-phenylthiazole and tetrahydro-5-aminoacridine. A mixture of both substances is more active than either alone. Certain antihistamine drugs display antimorphine activity.

The action of a series of compounds which antagonised the narcotic property of morphine was described by Shaw and Bentley[1]. One, 2 : 4-diamino-5-phenylthiazole (amiphenazole, Daptazole, DHA.245) has now found clinical application as a partial morphine antagonist in the treatment of intractable pain of terminal carcinoma[2], post-operative pain[3], and pain in childbirth[4].

Amiphenazole is a partial morphine antagonist which counteracts the narcosis of morphine and to a lesser extent the respiratory depression, but has little effect on the nausea and vomiting. It does not affect the analgesia produced by morphine. In animals the drug is a respiratory stimulant[5] but less regularly in man. It has a low toxicity, the LD50 being 309 (oral) and 250 (i.p.) mg./kg. in rats and guinea pigs. The animals die after mild convulsions.

The disadvantage of amiphenazole, clinically, is that whilst the elimination of morphine-induced narcosis is usually complete it is not as efficacious in combating vomiting and respiratory depression. However, up to the present time no death has been reported when a combination of amiphenazole and large doses of morphine has been used. Also amiphenazole in solution in water is not stable for more than a few days. A search has been made now for additional compounds without these disadvantages.

Methods

Healthy dogs of both sexes were used. A mixture of morphine 100 mg. and hyoscine 6 mg. in varying doses produced uniform narcosis in individual animals. The narcosis aimed at was such that the dog would make only a slight movement of the head when stimulated by movement or a sudden sharp noise. Dogs which could not be brought to this state, even with large dosages were rejected. The required dose of morphine varied around 15 mg./kg. The mixture was usually administered subcutaneously, occasionally intramuscularly. The test compound was administered $\frac{3}{4}$–1 hour later. If soluble the compound was usually administered intravenously; if insoluble, a suspension was made in gum tragacanth and given intramuscularly. The antimorphine activity was assessed as follows: good arousal, the animal stood up and walked

spontaneously; mild arousal, the animal sat up spontaneously but only walked when stimulated or called; slight arousal, the animal raised only its head when stimulated.

Some animals were used repeatedly twice a week for several months. No tolerance to the action of morphine appeared when the drugs were given at this interval.

Most of the dogs under morphine-hyoscine had an elevated respiratory rate. Any further respiratory stimulation was noted. If a compound failed to arouse a dog a standard dose of amiphenazole (20 mg./kg.) was usually given. A test compound was not reported to be without activity if this dose of amiphenazole itself failed to rouse the animal.

Results

The compounds of greatest interest are described below; others are mentioned briefly in Table I.

Cyclizine Chloride (*Marzine*). In view of its antiemetic effect against morphine-induced vomiting in man cyclizine was tested and found, surprisingly, to be almost equal to amiphenazole and tetrahydroaminacrin (THA) in producing arousals. Eleven morphinised dogs were given doses (intravenously) ranging from 10–30 mg./kg. The response was an improvement in the level of consciousness in all cases. The effects on respiration were minimal. Muscle tone was strong enough in one dog only to permit it to stand. The dogs seemed to remain analgesic to crude stimuli such as pricking or crushing of the paw or tail. The delayed effect varied from relapse after half an hour to maintenance of the alert state.

Chlorcyclizine (*Perazil*). Chlorcyclizine, a more potent antihistamine drug than cyclizine, was investigated. Given intravenously to nine morphinised dogs in a dose range of 10–30 mg./kg. the response was an immediate return to consciousness and improvement in respiration. Muscle tone was scarcely affected and hypotonia predominated. The animal was able to lift its head and right its body, but was unable to support its weight on its legs and walk about as is usual with amiphenazole and THA. The dog responded to orders and sounds by moving its head and appeared to possess a considerable degree of comprehension.

Avil (*p*-amino salicylate of 1-phenyl-1-pyridyl-2′: 3-dimethylamino propane) was given to 5 dogs with dosage range 20–30 mg./kg. intravenously. Full consciousness was regained immediately in all dogs; respiration and general muscle tone was slightly improved.

Less satisfactory effects were seen with diphenhydramine (Benadryl) and pyribenzamine.

n-Methyl piperidyl-(3)-methyl phenothiazine deepened the narcosis. Methyl phenidate (Ritalin) in a dosage of 2 mg./kg. to two dogs produced an immediate return to consciousness and some improvement in muscle tone. In one animal there was an improvement in respiratory function.

Amiphenazole and Tetrahydroaminacrin. Shaw and Bentley[1] have described both substances, and preferred the less toxic amiphenazole for clinical use.

TABLE I

SERIES OF COMPOUNDS INVESTIGATED

Compound	Dose in mg./kg.	Arousal	Excitation	Respiratory Effect
Methylacridine	12 i.v.	None	Marked	Resp. very deep, fast. Effect wore off after about 20 min.
	6 i.v.	None	Excitation followed immediately by deepening of coma	Much deeper, faster
9-Amino-5:6:7:8-tetrahydrophenanthridine	5 i.v. in HCl	Transient	Convulsed	Extreme, transient stimulation rate and volume
5-Amino-1-methyl-8:9-benzacridine	4·5 i.v.	None	None	Deepened, became slower; irregular late
5-Amino-6:7-benzacridine	1 in HCl	Mild	Severe convulsion	Deeper, then rate decreased markedly
	0·5 in HCl	Slight, with regression about 1 hour	Twitches developed	Very slow, became very shallow also
	1 in HCl	Good, with total regression after about 20 min.	Excitement twitches	Slowed; then became irregular
5-Amino-2:3:6:7 dibenzacridine	4-5 i.m. in 7 doses at 20 min intervals	Slight	None	Gradually became more forced and irregular
Aminodiazine	10 i.v.	None	Twitches developed	Immediate deepening (irregular)—persisted; very slow and regular later
	17 i.v.	None		Deeper, irregular
Aminomerazine	20 i.v.	None	—	No action
	20 i.v.	None	—	No action
	20 i.v.	None	—	No action
2-Aminopyridine	8·5 i.v.	Slight	Twitches developed	Slower and deeper immediately, then irregular
	2·5 i.v.	None	Great excess of tone in whole body. Convulsion	Deepened, became irregular
	5 i.v.	None	Convulsions	Very deep, then became irregular
	2·5 i.v.	Mild arousal	—	
	7 i.v.	Good arousal with total regression	Extreme excitement with severe twitches	Very deep
2-Aminothiazole	30 i.v.	Slight	—	Deepened, slow and regular
	38 i.v.	None	—	Temporarily deeper
	40 i.v.	None	—	Very deep, then slow and irregular
2-Amino-4:6-dimethylpyrimidine	17 iv.	None	—	Very deep for short while, then shallow and irregular
	20 i.v.	None	Severe tremors and excess tone in hind leg	Deep and regular
3:4-Dimethyl-5-aminoisoxozole	12 iv.	None	—	—
	20 i.v.	None	—	—
	20 i.v.	None	—	—
2-Amino-5-phenylthiazole	20 i.m.	None	None	Irregular
	40 i.m.	None	Developed tremors in hind legs	No effect
2-Amino-4-phenylthiazole	15 i.m.	Slight	—	—
	25 i.m.	Mild, gradual	—	—
	40 i.m.	Mild, gradual	—	Slower, very deep
	30 i.m.	Mild	Slight twitching	Rapid
*iso*Cytosine	8·5 i.v.	—	—	None
	20 i.v.	—	—	—
2-Amino-4-phenylpyrimidine	10 i.v.	—	—	Good volume regular, a few deep resps
	20 i.v.	Good with slight regression	—	—
	25 i.v.	Mild	Mild fit with excitation, then short deep coma followed, by arousal	Good volume
	20 i.v.	None	Injection followed by dry gagging then deep coma	Shallow, fairly fast
5-Amino-1:2:8:9-dibenzacridine	3 i.m.	Slight after ½ hour	—	—
	6 i.m.	Slight	—	Faster, deeper
	8 i.m.	Slight	—	Irregular
	6 i.m.	Mild, gradual	—	Irregular
	10 i.m.	None	—	Shallower
Aminoacridine pKa 4·4	20 i.v.	None	—	None
	30 i.v.	Slight	—	None
Aminoacridine pKa 5·9	15 i.v.	None	—	None

TABLE I—*continued*

Compound	Dose in mg./kg.	Arousal	Excitation	Respiratory Effect
ıtylaminoacridine pKa 9·4	5	Good	Convulsed, then cardiac failure and death	—
acetylaminoacridine pKa 5·0	17	Mild, delayed	—	None
Methyl-5-diacetyl-aminoacridine	8	None	—	—
	10	None	—	None
2:3:4-Tetrahydro-5-diacetylaminoacridine	12	Slight	—	None
4-Diamino-5-(*p*-chlorophenyl) thiazole pKa 7·5	50	None	—	None
4-Diamino-5-(*o*-chlorophenyl) thiazole	30	Mild, regression later	Convulsed during injection, and again 20 mins later	None
4-Diamino-5-(*o*-methoxyphenyl) thiazole	30	Mild	—	None

See also Tables II and III.

ote:—Excitation means fasciculation or other movements if the animal remains unconscious or convulsions, etc., if the animal is first aroused.

Thirty dogs provided a control series with amiphenazole, twenty-two gave a good or mild arousal with 8 to 40 mg./kg., eight, did not show any arousal, even with 60 mg./kg.

In a group of 4 dogs, it was observed that admixtures of amiphenazole and THA enabled the amount of each to be reduced, sometimes to less than half, yet to produce the same degree of arousal. (Table II). The optimum ratio would appear to be amiphenazole:THA, 2:1 by weight.

TABLE II

AROUSAL OF DOGS FROM ADMIXTURES OF AMIPHENAZOLE AND THA

Dog	Amiphenazole mg./kg.	THA mg./kg.	THA + Amiphenazole mg./kg.
I	40 B	10 B 14 A 15 B 20 A	12 + 20 B
II	40 A 60 A	10 A 14 A 14 B 20 A	5 + 10 B 2·5 + 5 B 5 + 10 B 15 + 30 A
III	40 A 60 A	10 B 15 A 17 A 20 A	3 + 15 B 5 + 15 A 5 + 10 B 10 + 20 A 15 + 30 A
IV	40 B	10 A 13 A 20 B 25 A	10 + 7 B 15 + 30 A 6 + 12 B 10 + 20 A

A = Good arousal B = Mild arousal

It was felt that the animals treated with the mixture were more normal in their gait and alertness than those treated with either drug alone. The respiration also appeared to be more normal in depth and rate.

From earlier experiments it was known that THA, was the more convulsive drug. An attempt was now made to see if amiphenazole would lower the motor excitement induced by THA. The dose of THA,

producing mild convulsions was estimated in four dogs. This dose found to be approximately 5 mg./kg., was then given simultaneously with 30 mg./kg. of amiphenazole. The degree of convulsions produced was the same as that given by THA alone. The non-protective action of amiphenazole was confirmed by a similar procedure with 30 rats.

La Barre told us that the barbiturate antagonist bemegride, β-methyl-β-ethyl glutarimide (Megimide) antagonised the respiratory depression in animals anaesthetised with Chlovalox and morphine. Therefore this compound was tested for its ability to arouse morphinised dogs together with three other related glutarimides with barbiturate-antagonistic ability. These were: *N*-methyl-β-methyl-β-ethyl glutarimide and methyl-β-methyl-β-ethyl glutarimide, and β-methyl-β-propyl glutarimide. The results are shown in Table III.

TABLE III

EFFECT OF BEMEGRIDE AND RELATED GLUTARIMIDES ON THE AROUSAL OF DOGS ANAESTHETISED WITH MORPHINE AND CHLOVALOX

Compound	Dose mg./kg.	Arousal	Excitation	Respiration
Bemegride	10	Good	Extremely violent convulsions, ending in death	Marked stimulation
	1·5	Moderate, regression later	Convulsions	Marked stimulation
	0·5	Moderate, regression later	Twitching	Moderate stimulation
N-Methyl-β-methyl-β-ethyl glutarimide	1·6	Mild, regression later	Convulsions	Moderate stimulation
β-Methyl-β-propyl glutarimide	5	None	—	Brief moderate stimulation
α-Methyl-β-methyl-β-ethyl glutarimide	5	None	—	Brief moderate stimulation

DISCUSSION

It is not difficult to obtain substances which antagonise the narcotic action of morphine in animals[1]. This is in distinction to the difficulty of obtaining an antagonist to the barbiturates, where only a few out of over 100 compounds tested possessed this property[7,8]. Furthermore, in man, the barbiturate antagonist bemegride counteracts the depression of vital reflexes rather than eliciting a return to consciousness[9]. Amiphenazole is particularly useful in restoring consciousness to morphinised dogs and man[2]. There seems to be only slight structural specificity required for a morphine antagonist. In the grossest sense any compound with one or more *N*-containing rings (5 or 6 membered) and one or more amino side chains would appear to be a potential morphine antagonist. What is surprising is the discovery that certain antihistamine drugs also possess this property. Cyclizine and chlorcyclizine are as effective as amiphenazole and THA in dogs (and cyclizine in man). Three other antihistamine substances investigated were also very effective. Amiphenazole and THA possess only slight antihistaminic activity.

In a previous paper[1] it had been pointed out that all the highly active morphine antagonists in this series were basic compounds with acid dissociation constants of 8 or higher. However, until recently, very few compounds of low basicity had been available. This deficiency

has now been partly removed, through unfortunately a number of the compounds listed in Table I were in such short supply that it was not possible to measure their dissociation constants. Table I gives a list of some of the compounds with their dissociation constants. By adding these figures to those already presented[1], it can be seen that morphine antagonism is conspiciously absent from the weakly basic compounds in this series. However, it must be emphasised again that all compounds with a high basicity are not antagonistic towards morphine.

Proflavine (pK 10) and acriflavine (pK 11) are notable examples of this phenomenon. It is also interesting to note that the three derivatives of 2:4-diamino-5-phenylthiazole (pK 8) have almost negligible activity. Their dissociation constants are only slightly less than that of the parent compound, and it would be surprising if this small reduction in basicity was the sole cause of the reduced biological activity. Other factors, such as lipid solubility would also play their part.

The moderate activity of the poorly ionised compound 5-diacetylaminoacridine was unexpected. However, since its activity appeared some 5–10 minutes after injection, it seems possible that it is hydrolysed in the body to the active parent compound 5-aminoacridine.

The four glutarimide derivatives must be considered separately. They are all very weakly acidic compounds, with dissociation constants above 10, i.e., at biological pH they are completely unionised.

A surprising aspect of this work is the marked antimorphine effect of certain compounds with antihistaminic activity. This prompted us to test the antihistaminic activity of amiphenazole and THA. The action is very weak indeed. On the guinea pig intestine amiphenazole at a concentration of 10^{-5} abolishes the action of histamine for 5 minutes, the corresponding figures for THA, are $10^{-5\cdot5}$ and 6. These drugs do not influence the action of histamine on the cat's blood pressure. *p*-Chlor-2:4-diamino-5-phenylthiazole is also a weak antihistamine drug. Whilst the antihistamine drugs appear to antagonise the narcotic action of morphine in dogs they do not usually bring about a complete return of tone and voluntary movement in the animal.

Acknowledgements. This work was carried out with the aid of a grant from the National Health and Medical Research Council of Australia. We wish to thank the following for gifts of substances used: Dr. Cymerman-Craig, Professor W. Davies and Nicholas Pty. Ltd.

References

1. Shaw and Bentley, *Austral. J. exp. Biol.*, 1955, **33**, 143.
2. Mckeogh and Shaw, *Brit. med. J.*, 1956, **1**, 142.
3. Hugin, *Schweiz med. Wsch.*, 1956, **86**, 1100.
4. Holmes, *Lancet*, 1956, **1**, 765.
5. Shulman and Trethewie, in the press.
6. Shaw, *Nature, Lond.*, 1954, **174**, 402.
7. Somers, *ibid.*, 1956, **178**, 996.
8. Shaw, *Med. J. Aust.*, 1955, *ii*, 889.

WATER-SOLUBLE CELLULOSE DERIVATIVES

Factors Affecting the Viscosity of Aqueous Dispersions. Part I

By R. E. M. Davies and J. M. Rowson

From the Museum of the Pharmaceutical Society of Great Britain

Received May 3, 1957

The effects of temperature and of the presence of acid and alkali on the viscosity of aqueous dispersions of methyl-, methylethyl- and sodium carboxymethylcellulose have been studied. Irreversible viscosity decreases may occur on heating dispersions of all three. The magnitude of the decrease varied with the temperature and time of heating, and with the viscosity grade and type of derivative. The efflux time reductions caused by heat in the presence of acid were greater than in neutral or alkaline dispersions. Viscosity changes may also occur in unheated dispersions in the presence of acid and alkali. The magnitude of the changes was related to the pH value of the dispersion, the time of storage and the nature of the derivative. There were no significant pH changes in the dispersions either on heating or after storage.

By substituting alkoxyl for a certain proportion of the hydroxyl groups in the anhydroglucose unit of the cellulose molecule, derivatives can be prepared which disperse in water to give viscous colloidal solutions. This property of water-solubility in cellulose ethers has been attributed to the ability of the substituent groups to prise apart adjacent cellulose chains, thereby rendering the remaining unsubstituted hydroxyl groups accessible to hydration[1,2]. Water solubility depends partly on the chain length of the molecule, but to a greater extent on the nature of the substituent radicals, their evenness of distribution throughout the chain and their numerical ratio to the unsubstituted hydroxyl groups[2,3]. Some derivatives, such as methyl- and methylethylcellulose, are soluble in cold water only; others, for example, sodium carboxymethylcellulose, are soluble both in cold and hot water, and this forms a convenient basis of classification. An important property of these ethers is the viscous nature of their aqueous dispersions. Although this viscosity is to some extent dependent on the degree of substitution, in the absence of certain complicating factors, it is essentially a function of the chain length of the molecule. Consequently, processes involving controlled degradation can be applied during manufacture to produce several viscosity grades of the same derivative[4].

Monographs on methyl- and sodium carboxymethylcellulose were included for the first time in the United States Pharmacopeia in the fifteenth revision and their possible uses as gelling, suspending and emulsifying agents are now being recognised in Britain. Their suitability as such largely depends on the stability of the viscosity of their dispersions. From the literature it is apparent not only that gross variations in viscosity can occur, but that the various ethers behave differently. However, no published comparison which provides a basis for a selective and informed approach to formulation has been found. Further, although

viscosity variations might be expected to occur in the absence of visible reaction products, much of the work on the incompatibilities of these materials has been based on observations of precipitate-formation. These considerations led us to study the conditions under which viscosity changes occur in aqueous dispersions of the three most commonly used cellulose derivatives. The effects of concentration, temperature and the presence of acid and alkali are discussed in the present paper.

Materials and Apparatus

Three viscosity grades of methylcellulose* and of sodium carboxymethylcellulose† (hereinafter referred to as low, medium and high) and one grade of methylethylcellulose‡ were studied in detail. All tests were made on single production batches of these materials. The batches were well mixed and their uniformity confirmed by viscosity determinations on several random samples.

The dispersions were made with Purified Water B.P. The chemicals, with stated exceptions, were of A.R. quality. Viscosities of dispersions containing added acid and alkali were measured with a U-tube viscometer (Ostwald No. 3). Others were measured with a Redwood No. 1 viscometer. All measurements were made at 25° except those on the effects of temperature variation.

pH measurements were made with a glass electrode on a Cambridge meter.

Preparation of Dispersions

Dispersions of all three ethers were prepared as follows: Half the required amount of distilled water containing 0·002 per cent phenylmercuric nitrate B.P. was added at 80° to 90° to weighed quantities of the cellulose derivative in a beaker. The mixture was stirred at intervals for 30 minutes and then made up to volume with distilled water at room temperature. The dispersion was then left to stand with occasional stirring for 24 hours.

With the low viscosity grade of methylcellulose and the sample of methylethylcellulose anomalous viscosity readings were observed; these were traced to undispersed fibre. The difficulty was finally overcome by passing the dispersions through a hand-operated homogeniser. The viscosities of the mucilages were not altered by this treatment.

One other variation was observed. This was a progressive decrease in viscosity for the first few viscosity readings. The following are typical: 457, 454, 452, 447, 446, 446 seconds. There was no evidence that the effect was due to the shearing of gelatinous aggregates, and Heymann's[5] work on methylcellulose sols does not suggest that thixotropic phenomena are encountered at temperatures much below gel point. Other investigators[6], however, considering that the colloidally dispersed particles exist

* Low: Celacol M20 (British Celanese Ltd.).
Medium: Celacol M2500 (British Celanese Ltd.).
High: Methocel 4000 (Dow Chemical Co.).

† Cellofas B, low, medium and high (Imperial Chemical Industries Ltd.).

‡ Edifas A (Imperial Chemical Industries Ltd.).

as flexible coils, have advanced the hypothesis that such coils would be deformed in passing through a streaming field and require a definite relaxation time to return to their original shapes. The pattern of results is similar to that observed by Middleton[7] in the course of viscosity determinations on tragacanth mucilage using a falling sphere viscometer, although his explanation of "stream orientation" would not seem to be applicable in the present instance.

Percentage solutions of the derivatives were made on the basis of the dry weight, calculated by drying at 105° for 3 hours. Methyl- and methylethylcellulose samples contained from 5 to 8 per cent moisture and sodium carboxymethylcellulose from 12 to 15 per cent.

The mean efflux time is from two samples. The average efflux time is from three estimations. Variation between samples falls within $\pm$ 3 per cent.

Experimental and Results

Effect of Concentration

Within the range of concentrations examined, a sensibly linear relation was shown to exist between the concentrations of dispersions and the logarithms of the differences between their efflux times and the efflux time of water. Samples prepared by dilution of more concentrated dispersions gave efflux times similar to those of extemporaneously-prepared dispersions of the same concentration.

Effect of Temperature

On heating dispersions of methylcellulose the viscosity gradually decreases until a temperature is reached (about 50°) at which the ether separates. At this point, depending on the concentration of the dispersion, either discrete gel particles or an opaque continuous gel is formed, and, in the latter case, further small increases in temperature produce large increases in viscosity. On cooling, the gel reverts to a sol. This inverse sol-gel transformation of methylcellulose has been extensively studied by Heymann[5]. We obtained typical heating-cooling curves. The gelation temperature varies inversely with the concentration of the dispersion and the viscosity grade of the derivative. Similar results were obtained with methylethylcellulose, except that the gel differed physically from that produced by methylcellulose, being softer and more easily disrupted. With sodium carboxymethylcellulose, efflux times decreased progressively with rise of temperature, no gel being formed since the derivative is soluble in hot water. With the exception of gelled methylcellulose samples, in which pronounced hysteresis phenomena were observed, heating and cooling curves with dispersions of all three derivatives were nearly coincident.

Effect of Time and Temperature of Heating

Dispersions of methyl- (medium), methylethyl-, and sodium carboxymethylcellulose (medium) of approximately equal efflux times were stored at 50°, 98° and 115° for periods of 15, 30, 45, 60, 120 and 240 minutes. The

dispersions stored at 50° and 80° were rapidly pre-heated to temperature in a water bath before being transferred to an electric hot-air oven. The dispersions at 98° were heated in a free-steaming autoclave, and those at 115° in an autoclave under pressure. Heating at 115° was subsequently extended to a range of concentrations of methylethylcellulose and of the high and low viscosity grades of the two other derivatives. Also studied were the effects of autoclaving dispersions prepared with powdered and fibrous batches of methylcellulose, and dispersions of all three derivatives which had been made acid or alkaline. The influence of heat on the dry materials was also investigated. Efflux times and pH values were measured before and 24 hours after heating the dispersions. The results may be summarised as follows:

Heating dispersions of the three derivatives at 50° (a temperature at which methyl- and methylethylcellulose samples begin to gel) for periods of up to 4 hours had no effect on their efflux times.

Heat at 80° for periods of up to 4 hours had little effect on the efflux times of methyl- and methylethylcellulose dispersions. The efflux times of sodium carboxymethylcellulose dispersions, however, began to decrease after as little as 15 minutes' heating (by 14 per cent), and, after 4 hours' heating, the efflux time had decreased by 33 per cent (Fig. 1).

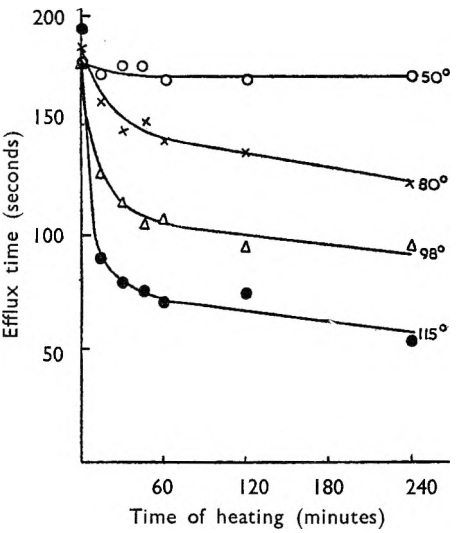


FIG. 1. Variation of efflux time of a sodium carboxymethylcellulose sol (medium) with temperature and time of heating.

Heating at 98° for periods of up to 4 hours had little effect on the efflux times of methyl- and methylethylcellulose dispersions. Samples of the former exhibited considerable syneresis at the end of the heating period. With sodium carboxymethylcellulose, 15 minutes heating reduced the efflux time by 27 per cent and 4 hours heating reduced it by 46 per cent (Fig. 1).

The efflux times of dispersions of all three derivatives were lowered by heating at 115°. For methylcellulose the decreases after 15 minutes and 4 hours were 6 and 43 per cent respectively. The corresponding decreases with methylethylcellulose were 11 and 38 per cent and for sodium carboxymethylcellulose 53 and 72 per cent (Fig. 2).

When a range of concentrations of the various viscosity grades of the three derivatives was heated at 115° for 30 minutes the greatest decrease in efflux time among the methylcellulose samples was exhibited by the high viscosity grade, the reduction being 16 to 33 per cent, compared with 10 and 11 per cent for the low and medium grades. Under the same

conditions, dispersions of sodium carboxymethylcellulose (low) decreased in efflux time by up to about 50 per cent and darkened in colour. With the medium grade the decreases were greater (up to 65 per cent) and in the high grade were lower (maximum 39 per cent).

There was little difference between the efflux time decreases brought about by heating dispersions made with powdered and fibrous samples of four viscosity grades of methylcellulose for 2 hours at 115°.

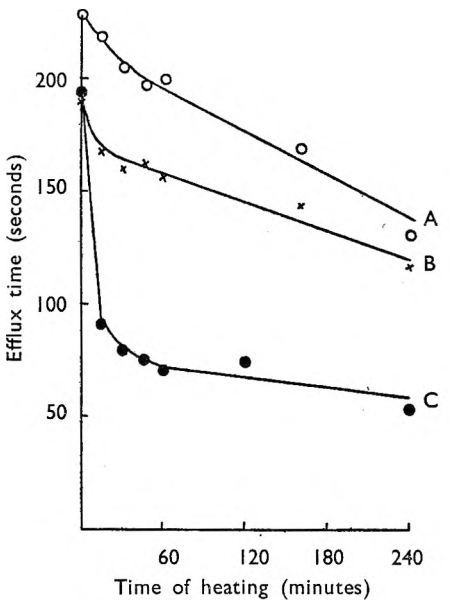


FIG. 2. Variation of efflux time with time of heating at 115°. A, Methylcellulose (medium); B, Methylethylcellulose; C, Sodium carboxymethylcellulose (medium).

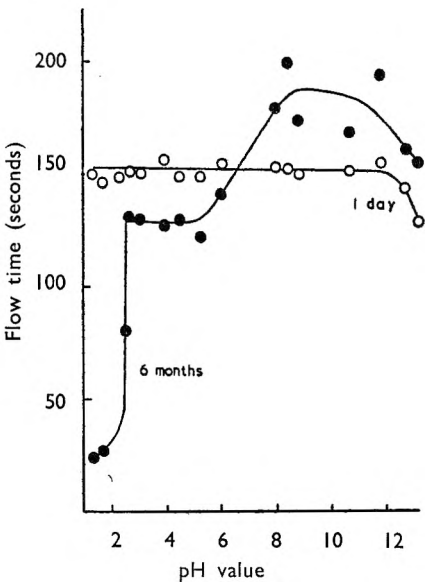


FIG. 3. Variation in flow time of a methylcellulose sol (medium) with pH.

When dispersions which had been made acid with hydrochloric acid (methyl- and methylethylcellulose, pH 3, sodium carboxymethylcellulose, pH 5) or alkaline with sodium hydroxide (pH 10) were heated for ½ hour at 115° together with control samples (methylcellulose, pH 6–7·6; methylethylcellulose, pH 4·7; sodium carboxymethylcellulose, pH 6·5–7·2), the results were as follows:

Methylcellulose (low): Acid, alkaline and control samples remained unaffected; (medium)—acid dispersions showed a flow time decrease of 56 per cent, compared with 4 and 5 per cent, respectively, in the control and alkaline samples; (high)—acid dispersions decreased in flow time by 56 per cent, compared with 19 per cent (control) and 17 per cent (alkaline). *Methylethylcellulose*: The flow time decrease in the acid dispersion (16 per cent) was twice as great as in the control and alkaline samples. *Sodium carboxymethylcellulose*: The flow time decreases with all three grades were greatest in the acid samples (about 60 per cent). In the control and alkaline samples the decreases were about 50 per cent.

When dispersions were prepared with samples of the derivatives which had been heated at 105° for 4 hours, their efflux times were, in all cases, lower than those of dispersions of the same concentration prepared with non-heated material. For methylcellulose the percentage decreases were 20, 25, and 18, respectively, for the low, medium and high grades; for methylethylcellulose, 20; for sodium carboxymethylcellulose, 30, 34, and 18, for the low, medium and high grades, respectively.

There was no significant variation in the pH values of any of the above dispersions on heating.

Effect of Acid and Alkali

One hundred ml. quantities of dispersions of the medium grades of methyl- and sodium carboxymethylcellulose and of methylethylcellulose were prepared in such concentration that, when diluted with an equal volume of water, the dispersions would have a flow time of between 100 and 200 seconds. Each of these "double-strength" dispersions was then diluted with an equal volume of distilled water to which had been added varying quantities of 0·1N hydrochloric acid or 0·1N sodium hydroxide solution. The pH values of the dispersions were measured initially and at intervals during storage. Flow times were determined initially and after 1 week, 2 months, 4 months and 6 months. All the dispersions were stored in clear glass jars, with screw caps, in daylight, on a shelf. Airtight seals were obtained by dipping the necks of the jars in molten paraffin wax. The results were as follows:

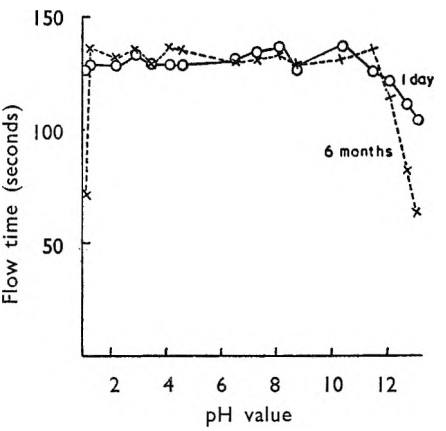


FIG. 4. Variation of flow time of a methylethylcellulose sol with pH.

Methylcellulose: On the first day there were no marked differences in the flow times of dispersions with pH values between 1 and 12. In more alkaline samples the flow time was slightly below the average level. At the end of 7 days there was a marked drop in the flow times of dispersions with pH values below about 2·5. In the remaining samples there was a small diminution in flow times. At the end of 2 months the dispersions below pH 2·5 showed further large decreases in viscosity. There was little difference in the remainder of the samples. At the end of 4 months the viscosities of the most acid dispersions were found to have continued to decrease. The remainder of the acid-containing dispersions remained the same but alkaline samples became more viscous. After 6 months' storage, the two most acid samples had diminished in flow time by over 80 per cent. Between pH 2·5 and pH 6 the losses averaged about 20 per

cent. The increase in viscosity of the alkaline dispersions was marked, all having exceeded their original flow times—two by one third (Fig. 3).

Methylethylcellulose. Dispersions of pH 1·25 to 11·5 retained their original viscosities for 6 months, and flow times of acid, neutral or alkaline samples were the same. The viscosity of a dispersion of pH 1·08 decreased slowly on storage, and, after 6 months, the flow time was reduced by one-half. Dispersions with a pH above 11·5 had, originally, a flow time that was lower than the average for the series. This difference became more marked on storage and the flow time of the most alkaline sample after 6 months was about half that of the dispersions between pH 1·25 and 11·5 (Fig. 4).

Sodium carboxymethylcellulose. On preparation, the most viscous dispersions were those with pH values between 5 and 7. There was a sharp fall in the viscosity of dispersions below pH 5 and above pH 10. In the two most acid samples (pH 1·05 and pH 1·42), carboxymethylcellulose was immediately precipitated and the viscosity was reduced to a minimum. At the end of a week there was a general reduction in the viscosity of the samples. Dispersions with a pH below 7 continued to decrease in viscosity on further storage, and, at 6 months, those samples that had not precipitated had decreased in flow time by about two-thirds. The flow time, of the alkaline dispersions, however, did not alter appreciably after the first week of storage, and, at the end of six months, those with pH values between 7 and 12 had the highest flow times of the series (about 60 per cent of the original) (Fig. 5).

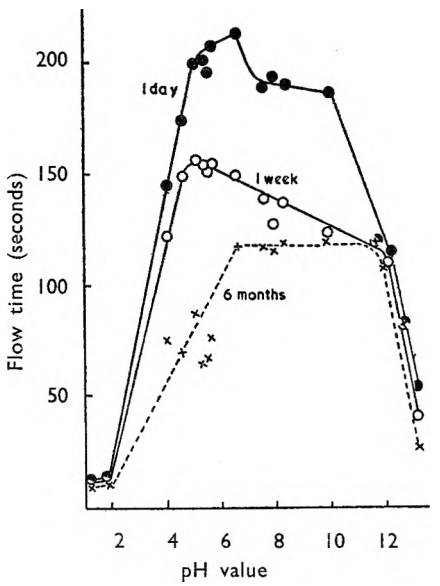


FIG. 5. Variation in flow time of a sodium carboxymethylcellulose sol (medium) with pH.

There was no significant variation in the pH values of any of the above dispersions on storage.

DISCUSSION AND CONCLUSIONS

Effect of temperature. The gradual decrease in the viscosity of methylcellulose sols on raising the temperature has been attributed by Heymann[5] to a decrease in the effective volume of the particle on dehydration. The hydrated particle cannot be quantitatively defined, but is built up of water molecules adsorbed with decreasing bonding energy as the thickness of the hydration layer increases. At low temperatures there is no marked limit to the hydration layer but a more or less smooth transition to free

water. On heating, water molecules are shed from the outer layer, decreasing the size of the hydrated particles while increasing their freedom of motion, until a point is reached where the particles come within each other's sphere of influence and coagulate[1]. Provided the temperature and duration of heating are kept within certain limits, the sol-gel transformation is strictly reversible and the dispersion on cooling regains its original viscosity. The diminution of viscosity which occurs on prolonged heating, however, is a separate and quite distinct process which, in the case of methylcellulose, was considered by Heymann to be due to depolymerisation. Brown and Houghton[8] who investigated similar viscosity losses in heated sodium carboxymethylcellulose sols, compared the reaction with the denaturation of proteins. They suggested that the chemical changes involved are probably somewhat similar and might be due to the breaking of cross-linkages or hydroxyl bonds, or to the evolution of carbon dioxide. This latter, which assumes a change in the structure of the ether, has been contested by Caldwell and Watters[9] who found that when sodium carboxymethylcellulose which had undergone this lowering of viscosity was precipitated, dried and redispersed, it regained its original viscosity.

The present work shows that whereas dispersions of methyl- and methylethylcellulose are comparatively stable towards heat, the viscosity of sodium carboxymethylcellulose dispersions are irreversibly lowered by short periods of heating at relatively low temperatures. With the methyl- and methylethyl derivatives marked losses in viscosity occurred only in the samples heated at 115°, but, even at this temperature, the decrease generally is not large for heating periods appropriate to a sterilisation process. It is probable that the relatively large fall in efflux time which occurred in dispersions of the high viscosity grade of methylcellulose is simply a manifestation of the greater susceptibility of the larger molecule to degradative changes. The results of the experiments with sodium carboxymethylcellulose confirm those of Brown and Houghton. Not only is the temperature at which deterioration sets in lower with sodium carboxymethylcellulose than with the two other derivatives, but the viscosity decreases are much greater. The apparent stability of the highest viscosity grade of sodium carboxymethylcellulose, relative to the two lower grades of this derivative, is surprising.

With all three derivatives deterioration varies with both temperature and time. The decrease in efflux time was most rapid during the first 15 to 30 minutes of heating. Dispersions of methyl- and methylethylcellulose continued to decrease in efflux time beyond this point, whereas sodium carboxymethylcellulose dispersions became stabilised. This latter observation lends support to the suggestion[8] that prolonged heating may reduce the viscosity of sodium carboxymethylcellulose dispersions to a limiting value.

Although acid or alkali in the quantities added had no immediate effect on the efflux times of dispersions of the three derivatives, the presence of acid in dispersions of the two higher grades of methylcellulose brought about a marked reduction in viscosity when the temperature was held at 115° for 30 minutes. These circumstances favour the degradation

of the polymer and treatment with acid at high temperatures is one method of bringing about depolymerisation[4]. Its effectiveness with the lowest viscosity grade of methylcellulose provides another illustration of the stability of a molecule of relatively short chain length. That the inclusion of acid did not bring about such a marked effect with sodium carboxymethylcellulose may be explained partly by the fact that the system acts as a buffer[10]; although the effect of heat alone on this derivative is so great that the limiting viscosity is nearly approached in the absence of acid.

It appears that sodium carboxymethylcellulose in the dry state is less readily degraded by heat than are its aqueous dispersions. The effect of heating the dry material for 4 hours at 105° is comparable with that of heating an aqueous dispersion for ½ hour at 98°. With methyl- and methylethylcellulose, the result of heating the dry derivatives at 105° for 4 hours is roughly comparable with that of heating a dispersion at 115° for 2 hours.

Effect of acid and alkali. Provided the pH is not below 3, such viscosity losses as do occur in dispersions of methylcellulose in the presence of acid or alkali can, for most practical purposes, be ignored. The most interesting feature of this part of the study was the rise in viscosity which occurred in the presence of alkali after 4 months' storage. Campbell and others[11] have observed the viscosity of methylcellulose dispersions to increase as the pH increases, but do not state whether the rise was immediate, or delayed. Methylethylcellulose dispersions are seen to be even more stable, with the viscosities remaining unaffected over a wide pH range. The behaviour of sodium carboxymethylcellulose sols, in which the viscosity varied widely with pH and time of storage, provides a striking contrast. The results obtained with this derivative are in general agreement with those of Brown and Houghton[8] who attributed the loss of viscosity on the alkaline side to the replacement of unsubstituted hydroxyl groups by –ONa. On the acid side, the lowering of viscosity is due to the precipitation of carboxymethylcellulose. However, even neutral dispersions containing no added material undergo a loss of viscosity on storage, probably as a result of some degree of depolymerisation. This "ageing" process does not appear to be accelerated by the presence of alkali, but on the acid side the more progressive decrease suggests that here the two effects potentiate each other.

References

1. Ott, *Cellulose and Cellulose Derivatives*, Vol. 5, Interscience Publishers Inc., New York, 1943, p. 796.
2. Bock, *Industr. Engng Chem.*, 1937, **29**, 985.
3. Ott, *Chem. and Ind.*, 1949, **68**, 915.
4. Trail, *J. Soc. chem. Ind., Lond.*, 1934, **53**, 338T.
5. Heymann, *Trans. Faraday Soc.*, 1935, **31**, 846.
6. Hermans, *Kolloid-Z.*, 1944, **106**, 22, through *Chem. Abstr.*, 1944, 38, 3533 (8).
7. Middleton, *Quart. J. Pharm. Pharmacol.*, 1936, **9**, 493.
8. Brown and Houghton, *J. Soc. chem. Ind., Lond.*, 1941, **60**, 254.
9. Caldwell and Watters, *Research, Lond.*, 1948, **1**, 248.
10. Shaw, *Proc. S. Dak. Acad. Sci.*, 1945, **25**, 57.
11. Campbell, Burlage and Lloyd, *J. Amer. pharm. Ass.* (*Pract.*), 1955, **16**, 38.

THE EXAMINATION OF A BACTERIOLOGICAL PEPTONE

By A. F. S. A. Habeeb*

From the Department of Pharmacy, Faculty of Pharmacy, Kasr El Ainy, Cairo, Egypt

Received May 6, 1957

Paper chromatography has been used for the qualitative examination of the peptides in three batches of "Oxoid" bacteriological peptone. Batches A, B and C have shown in addition to the free amino acids 54, 49 and 50 peptides respectively. Variation occurs in the peptide content of the three batches of peptone examined.

In a previous paper[1] the separation of the free amino acids and those in a peptide form in "Oxoid" bacteriological peptone were reported. In the present paper the qualitative identification of the constituent peptides by paper chromatography is described.

Experimental

Three batches were subjected to separation into basic, acidic, neutral and aromatic groups as previously described using the method of Fromageot, Justiz and Lederer[2]. Each was examined by placing 0·003 ml. at 1·5 cm. distance on a line drawn 2 cm. from the bottom of a Whatman No. 1 paper $11\frac{1}{4}$ in. × $18\frac{1}{2}$ in. The basic, neutral and aromatic groups were developed three times with a butanol—acetic acid—water system[3], This multiple development has been used for sugars[4] and amino acids[5], and was used here satisfactorily with peptides, the spots being more compact and the separation more complete. For the acidic group a butanol—acetic acid—water system gave trailing spots but it was found that a benzyl alcohol—acetic acid—water system, 4:1:5 by volume, gave better results.

After development the paper was dried for 30 minutes at 60°. Two strips were cut from either end of the paper sheet and the presence of the different fractions revealed by the ninhydrin colour reaction. Each group gave rise to several fractions, each representing either a single amino acid or a mixture of amino acids and peptides. Using the two strips as guides, horizontal strips were cut from the remnants of the paper and each fraction eluted with water[6]. Each eluate was evaporated to dryness at room temperature in a vacuum desiccator over silica gel.

The residue was dissolved in 0·08 ml. 10 per cent *iso*propanol and 0·003 ml. samples were subjected to chromatography with firstly butanol —acetic acid—water, secondly phenol saturated with buffer pH 6·2, the paper being buffered at pH 6·2 and thirdly a *m*-cresol—ammonia[7] 0·03 per cent system to examine its homogeneity. Buffered paper gave good separation but the resulting eluate contained salts which resulted in unsatisfactory chromatograms, and extraction of the dried eluate with acetone containing 1 per cent v/v concentrated hydrochloric acid[8] failed to extract

*Present address, Research Fellow, Sterling Chemistry Laboratory, Yale University, New Haven, Conn., U.S.A.

the peptides completely. The *m*-cresol—ammonia 0·03 per cent system was used for separative chromatography in place of phenol buffered paper. Finally each fraction was developed using a solvent system that produced the best separation into subfractions. Each subfraction was completely hydrolysed with concentrated hydrochloric acid and subjected to two dimensional paper chromatography.

Table I shows the number of fractions, subfractions and peptides present in each group from the three batches of bacteriological peptone.

TABLE I

ANALYSIS OF A BACTERIOLOGICAL PEPTONE

	Number of Fractions			Number of Subfractions			Number of Peptides		
Batch	A	B	C	A	B	C	A	B	C
Basic Group	10	8	7	19	14	17	15	12	11
Acidic Group	6	4	4	10	13	13	8	12	13
Neutral Group ..	9	7	9	29	30	24	22	17	15
Aromatic Group ..	7	7	8	10	8	17	9	8	11
TOTAL	32	26	28	68	65	71	54	49	50

RESULTS

Tables II-V show the amino acid content of the peptides separated from the acidic, basic, neutral and aromatic groups respectively of batch A. Arbitrary figures ranging from 1–10 indicate the relative amounts of amino acid on the chromatogram judged from the size of the spot and the intensity of the colour; "trace" represents a very weak spot. Some subfractions show richness in a particular amino acid which is most probably due to its presence in the free state but contaminated with a peptide. Some peptides occur in different subfractions, this may be the result of their forming trails due to their length or to their having very close R_F values.

The three batches of peptone showed the same free amino acids, but very few similar peptides like those in the fractions F1–1 of the basic group (see subscript of Table II), F1 and F3–1 of the acidic group, F1 of the neutral group and F1–1 and F1–2 of the aromatic group. The majority of the peptides in batch B and C differ from those of batch A; in some cases the variation is slight. Batches A and B are poor in ornithine while rich in arginine; the reverse is true for batch C.

DISCUSSION

The method of Fromageot and his colleagues did not effect complete separation of the peptides into groups. Silica gel satisfactorily separated the basic peptides in the three batches of peptone, shown by the preponderance of the basic amino acid in their hydrolysates, while acid alumina adsorbed only acidic peptides yet separated them incompletely. Charcoal separated the majority of peptides containing aromatic amino acids. The non-specific adsorption of peptides on the different adsorbents may be due to the complexity of the mixture and to the length of

TABLE II

THE HYRDOLYSATES OF FRACTIONS* OBTAINED FROM ACIDIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1	0·16–0·24(b)	2	2	—	2	1	—	2	1–2	1	—	1	2	1	—	—	—	—	—
F2–1	0·16–0·26(b)	2	2	—	2	1	—	2	2	1	—	1	2	1	—	—	—	—	—
F3–1	0·09–0·18(b)	1	2	—	1	1	—	2	2	1	—	1	2	1	—	—	—	—	—
F4–1	0·11–0·17(b)	1	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F4–2	0·17–0·29(b)	2	4	—	—	—	—	2	2	1	1	1	2	1	—	—	—	1	1
F5–1	0·11(b)	trace	1	—	trace	—	—	trace	trace	—	—	—	trace	—	—	—	—	—	—
F5–2	0·17(b)	1	(6)†	—	—	—	—	1	1	—	—	—	1	1	—	—	—	—	—
F5–3	0·19–0·36(b)	1	5	—	1	1	—	2	1	1	1	1	2	1	—	—	—	—	—
F6–1	0·15–0·26(b)	2	3	—	—	1	—	2	2	1	—	1	2	—	—	1	1	1	1
F6–2	0·26–0·59(b)	1	2	—	1	—	—	2	2	2	1	2	2	1	—	trace	—	—	—

* In the fractions shown, the first figure indicates the number of the fraction and the second the number of the subfraction.
† The number in parentheses indicates that the amino acid is most likely to be present in the free state.
(b) R_F in butanol—acetic acid—water.
(c) R_F in *m*-cresol—ammonia.

TABLE III

THE HYDROLYSATES OF FRACTIONS* OBTAINED FROM BASIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1–1	0·0·14(b)	1	2	—	4	2	—	2	2	1	—	1	1	1	—	trace	—	1	1
F1–2	0·2(b)	—	2	—	2	1	—	1	1	1	—	1	1	—	—	—	—	—	—
F2–1	0·29–0·4(c)	—	—	(2)	(4)	1	—	—	—	—	—	—	—	—	—	—	—	—	—
F2–2	0·4–0·51(c)	—	1	1	(5)	1	1	1	1	trace	—	trace	1	trace	—	—	—	—	—
F2–3	0·51–0·91(c)	—	1	trace	3	1	1	1	1–2	trace	—	trace	1	trace	—	—	—	—	—
F3–1	0·55–0·71(c)	—	1	1	1	(4)	1	1	1–2	trace	—	trace	1	trace	—	—	—	1	1
F3–2	0·71–0·91(c)	—	1	1	3	4	2	1	4	1	—	1	1	1	—	—	—	1	1
F4–1	0·53–0·71(c)	—	1	—	1	2	—	1	1	—	—	—	1	—	—	—	—	—	—
F4–2	0·71–0·97(c)	—	1	1	2	3	1	1	3	1	—	1	1	trace	—	—	—	—	—
F5	0·13–0·2(b)	1	1	—	3–4	2	1	2	2	2	—	1	1	1	—	—	—	—	—
F6	0·13–0·2(b)	1	1	—	3–4	2	1	3	2	2	—	1	2	1	—	—	—	—	—
F7–1	0·2(b)	—	—	—	1	—	—	1	2	1	—	1	1	—	—	—	—	—	—
F7–2	0·23–0·31(b)	1	—	—	2	1	1	1	2	1	—	1	1	—	—	—	—	—	—
F8–1	0·14–0·23(b)	—	—	—	3	1	—	1	1	—	—	—	1	—	—	—	—	—	—
F8–2	0·3(b)	—	1	—	4	2	1	2	2	1	—	2	2	1	—	—	—	1	1
F9–1	0·19(b)	—	—	—	3	1	—	1	1	—	—	—	1	—	—	—	—	—	—
F9–2	0·3–0·46(b)	—	1	1	1	1	—	1	1	1	—	2	1	—	—	—	—	—	—
F10–1	0·15(b)	—	—	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F10–2	0·34–0·52(b)	—	1	—	1	1	—	1	1	1	1	1	1	trace	—	—	1	1	1

* See footnote to Table II.

TABLE IV

THE HYDROLYSATE OF FRACTIONS* OBTAINED FROM NEUTRAL GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1	0–0·11(b)	1	2	1	2	1	—	3	2	1	—	1	2	1	—	—	—	—	—
F2	0–0·12(b)	1	2	1	2	1	—	1	1	—	—	—	1	—	—	—	1	—	—
F3–1	0·13(c)	2	3	trace	1	1	—	1	1–2	—	—	—	1–2	1	—	—	—	—	—
F3–2	0·13–0·25(c)	1	2	—	2	1	—	1	2	1	—	1	1	1	—	—	—	—	—
F3–3	0·25–0·69(c)	1	2	trace	2	1	—	2	2	1	—	1	1	1	—	—	—	—	—
F4–1	origin(c)	1	3	—	1	—	—	1	1	—	—	—	2	1	—	—	—	—	—
F4–2	0·02–0·07(c)	trace	2	—	1	—	—	(4)	2	1	—	1	(4)	1	—	—	—	—	—
F4–3	0·07–0·13(c)	trace	trace	—	—	—	—	(4)	—	—	—	—	(4)	—	—	—	—	—	—
F4–4	0·13–0·34(c)	—	2	1	1	—	—	1	2	1	—	—	1	—	—	—	—	—	—
F4–5	0·4–0·57(c)	trace	2	—	1	trace	—	1	1	1	—	1	1	—	—	—	—	—	—
F5–1	origin(c)	1	4	1	1	1	—	1	2	trace	—	trace	2	1	—	—	—	—	—
F5–2	0·03–0·11(c)	trace	1	—	trace	—	—	1	2	trace	—	trace	1	(3)	—	—	—	—	—
F6–1	origin(c)	1	4	—	1	trace	—	1	4	1	—	1	2	1	—	—	—	—	—
F6–2	0·03–0·14(c)	trace	2	—	—	1	—	2	(5)	1	—	1	2	1	—	—	—	—	—
F6–3	0·14–0·28(c)	trace	2	—	1	1	—	1	1–2	1	—	1	1	1	—	—	—	1	1
F6–4	0·28–0·77(c)	trace	2	—	1	1	—	1	2	1	—	1	1	1	—	—	—	1	1
F7	0·15–0·29(c)	—	2	trace	1	1	—	2	2	1	1	1	2	1	—	1	—	—	—
F8–1	0·18(b)	—	1	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F8–2	0·28(b)	—	1	—	1	—	—	1	3	1	—	1	1	1	1	—	—	—	—
F8–3	0·34(b)	1	3	—	1	1	—	1	2	1	—	1	1	trace	1	—	—	—	—
F9–1	0·37(b)	1	1	—	1	1	—	1	1	1	—	1	1	—	(4)	—	—	—	—
F10–1	0·16(b)	—	1	—	1	—	—	1	1	—	—	trace	1	—	—	—	—	—	—
F10–2	0·33–0·37(b)	—	—	—	—	—	—	1	—	1	—	—	1	—	1	—	—	—	—
F10–3	0·37–0·47(b)	1	2	—	1	trace	—	2	2	(7)	(3)	2	1	1	1	—	—	—	—
F11–1	0·15(b)	—	1	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F11–2	0·38(b)	—	2	—	trace	trace	—	1	1–2	1	1	1	1	1	—	—	—	—	—
F11–3	0·43–0·6(b)	trace	2	—	1	1	—	1–2	3	2	—	(8)	1	1	—	trace	1	1	1
F11–4	0·6–0·71(b)	trace	2	—	1	1	—	1–2	3	2	—	3	1	1	—	trace	trace	1	1

* See footnote to Table II.

TABLE V

THE HYDROLYSATES OF FRACTIONS* OBTAINED FROM AROMATIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1–1	0–0·12(b)	1	2	—	2	1	—	2	2	1	—	1	2	1	—	1	1	1	1
F1–2	0·12–0·15(b)	1	2	—	1–2	1	—	1–2	2	1	—	1	1	1	—	1	1	1	1
F1–3	0·15–0·3(b)	1	2	trace	2	1	—	2	2	2	1	2	1–2	1	—	1	1	1	1
F2	0·17(b)	1	2	trace	2	1	—	2	2	2	1	2	1–2	1	—	1	1	1	1
F3	0·21–0·31(b)	1	2	trace	1–2	1	—	1	2	2	—	2	1	1	—	1	1	1	1
F4	0·27–0·41(b)	1	2–3	1	2	1	—	1–2	2	2	1	2–3	1	1	—	(3)	1	1	1
F5–1	0·16(b)	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F5–2	0·33–0·53(b)	1	2	trace	2	1	—	1	2	2	1	3	1	1	—	2	1–2	1	1
F6	0·5–0·63(b)	1	2	—	1	1	—	1–2	2	2	1	3	1–2	1	—	trace	(3)	1	1
F7	0·52–0·8(b)	1	2	1	1	1	—	1–2	2	3	1	4	1–2	1	—	1–2	2	1	1

the peptide chain involved. But this method of separation is suitable for a preliminary group separation of such a complex mixture of peptides.

Chromatography using a multiple development technique resulted in the separation of each group into several fractions that were subsequently separated into their constituent peptides.

Batches A, B and C showed respectively 54, 49 and 50 peptides; few were identical while the remainder showed some variation either in their amino acid content or in the relative strength of some amino acids. This variation may be due to slight differences in the conditions under which the peptone was prepared or to the synthesis of some new peptide bonds during the enzymatic hydrolysis of the blend of proteins used in the manufacture of the peptone.

The manufacturers consider that ornithine present is most likely produced from arginine as one part of the hydrolysis takes place under alkaline conditions. Similarly α-amino butyric acid may be produced from threonine as there is no satisfactory evidence that it occurs in any native protein.

The unknown ninhydrin positive spots previously reported[1] were not found.

Acknowledgements. I wish to express my thanks to Prof. E. Shotton for his encouragement and for the facilities to carry out this work in the Department of Pharmaceutics, School of Pharmacy, University of London.

References

1. Habeeb and Shotton, *J. Pharm. Pharmacol.*, 1956, **8**, 197.
2. Fromageot, Justiz and Lederer, *Biochim. biophys. Acta*, 1948, **2**, 487.
3. Partridge, *Biochem. J.*, 1948, **42**, 238.
4. Jeanes, Wise and Dimler. *Analyt. Chem.*, 1951, **23**, 415.
5. Csoban, *Magyar Kém Folyoirat*, 1950, **56**, 449, through *Chem. Abstr.*, 1952, **46**, 1384.
6. Dent, *Biochem. J.*, 1947, **41**, 240.
7. Sanger and Tuppy, *Biochem. J.*, 1951, **49**, 463.
8. Boulanger and Biserte, *Bull. Soc. Chim. Biol.*, 1949, **31**, 696.

ANALYSIS OF PROMAZINE AND CHLORPROMAZINE IN PHARMACEUTICAL PREPARATIONS

By J. B. Milne and L. G. Chatten

From the Pharmaceutical Chemistry Section, Food and Drug Directorate, Ottawa, Canada

Received April 25, 1957

The titration of promazine and chlorpromazine in acetone as the hydrochlorides or free bases has been applied to the assay of pharmaceutical products. The gravimetric procedure of Blazek and Stejskal has been used to verify the results.

Promazine and chlorpromazine are members of the phenothiazine nucleus type of antihistamines and tranquillising drugs for which several methods of analysis have been proposed. Durost and Pascal[1] estimated chlorpromazine in biological fluids by a colorimetric method using the carmine red colour produced by concentrated sulphuric acid. Blazek and Stejskal[2] used the precipitate formed in acid solution with silicotungstic acid as a gravimetric method. Sandri[3] used the oxidation of the phenothiazine nucleus with bromate and estimation of the excess bromate. This method has been used for determining chlorpromazine, promethazine and diethazine 10-(2-diethylamino-1-ethyl) phenothiazine). A non-aqueous titration of promethazine hydrochloride has been offered by Kleckner and Osol[4] and an aqueous back titration method is in commercial use. These methods were investigated and compared with non-aqueous methods developed in this laboratory, for the assay of pharmaceutical preparations.

Experimental

Reagents. Acetone, A.C.S. grade, hexane, B.D.H. certified reagent, glacial acetic acid, A.C.S. grade, chloroform, A.C.S. grade, 6 per cent mercuric acetate in glacial acetic acid, 0·05N perchloric acid in dioxane standardised against potassium acid phthalate, A.C.S. grade, 0·2N potassium hydroxide in water, 0·1 per cent methyl red in glacial acetic acid.

Apparatus. Fisher titrimeter model No. 9–311A with glass indicator electrode and fibre type calomel or silver-silver chloride electrodes; a semimicro-burette to measure 0·01 ml.

Non-aqueous procedures with Crystalline Promazine and Chlorpromazine Hydrochlorides

Method I. Accurately weigh and dissolve about 50 mg. of crystals in 40 ml. of acetone. Add 0·5 ml. of mercuric acetate solution, 3 drops of methyl-red indicator and titrate with perchloric acid in dioxane. The indicator colour change at the end point is from orange to salmon pink and it is preceded by a brilliant pink cone. The blank on 40 ml. of acetone is also estimated.

Method II. Accurately weigh about 50 mg. of crystals and transfer to a separating funnel containing 20 ml. of water. Add 4 ml. of potassium

hydroxide solution and extract four times with 20 ml. portions of hexane with vigorous shaking. Combine the hexane extracts, add 40 ml. of acetone, 3 drops methyl red indicator and titrate with perchloric acid in dioxane. The indicator colour change at the end point is the same as that in method I but all the colours are of a lighter shade. Estimate the blank on 80 ml. of hexane-40 ml. of acetone using the same end point.

Non-aqueous procedures with Pharmaceutical Preparations

Tablets. Weigh and powder 20 tablets. Extract an accurately weighed amount of the tablet mass containing 20 mg. of the active ingredient with 10 ml. acetone by stirring electromagnetically for 10 minutes. Filter out insoluble material using a fine sintered glass filter and suction. Wash the residue and container with 30 ml. acetone. Proceed by method I, beginning at "Add 0·5 ml. of mercuric acetate solution . . ." *Ampoules.* Take the contents of 5 ampoules and transfer an aliquot containing 25 mg. of the active ingredient to a separatory funnel. Add 2 ml. of 0:2 N potassium hydroxide solution and extract with 4 to 20 ml. portions of hexane with vigorous shaking. Proceed by method II, beginning at "Combine the hexane extracts . . ." *Suppositories.* Dissolve suppositories containing 25 mg. of chlorpromazine in 40 ml. of hot acetone. Titrate with perchloric acid in dioxane while hot using 3 drops of methyl red indicator. The end point is the same as that for ampoules. Determine the blank in 40 ml. of acetone as in procedure I.

Gravimetric procedures with Pharmaceutical Preparations

The gravimetric method of Blazek and Stejskal[2] was applied to a sample containing approximately 20 mg. of chlorpromazine or promazine with the following procedures to obtain the solution for precipitation and to wash the final precipitate.

Tablets. Extract the tablet mass with acetone and filter, washing the residue and container with sufficient acetone. Evaporate this solvent and replace with 20 ml. of water containing 1 ml. concentrated hydrochloric acid. Wash the final precipitate with 20 ml. of chloroform and 40 ml. of water. *Ampoules.* The ampoule solution previously basified with 2 ml. 0·2N potassium hydroxide solution is extracted four times with 20 ml. portions of hexane. Extract the combined hexane extracts twice with 10 ml. portions of water containing 0·5 ml. of concentrated hydrochloric acid per 10 ml. Wash the precipitate with 40 ml. of water. *Suppositories.* Dissolve suppositories in 15 ml. of hexane and extract twice with 10 ml. portions of water containing 0·5 ml. concentrated hydrochloric acid per 10 ml. of water. Combine the acid extracts and proceed with the precipitation. Wash precipitate with 20 ml. of hexane and 50 ml. of water.

Discussion

Promazine and chlorpromazine both contain two tertiary amino groups; one attached to an alkyl chain, the second incorporated in the central ring of the phenothiazine nucleus. Only the former can be titrated. These

drugs are present in pharmaceutical products as the hydrochloride or the free base and both forms can be titrated directly by non-aqueous methods. Titration of hydrochlorides by the use of mercuric acetate, was devised by Pifer and Wollish[5]. The titrant used in all instances was perchloric acid in dioxane.

Since the drugs are present as the hydrochlorides and estimation of the drug as free base took longer than as hydrochloride, the hydrochloride assay is preferred for tablets. Several solvents for the extraction of the hydrochloride from the tablet mass were investigated. Magnesium stearate, a common lubricant, created a major difficulty since it titrated as a

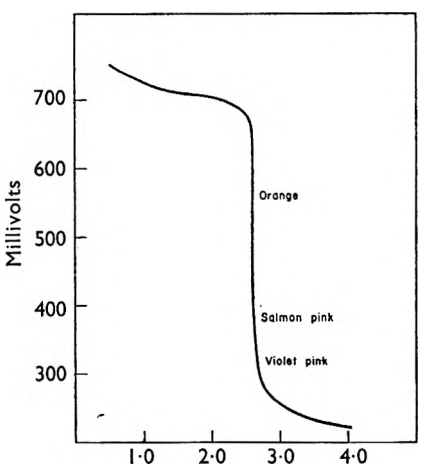
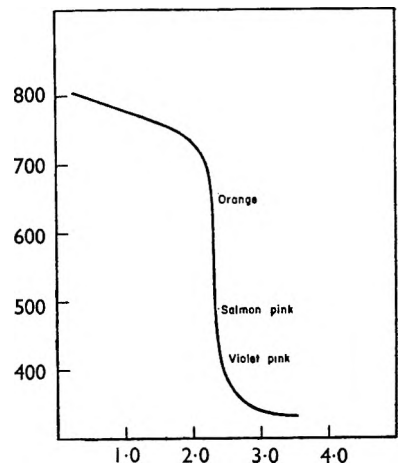


FIG. 1. Titration of chlorpromazine hydrochloride, extracted from tablet, in acetone using a glass: calomel electrode combination.



ml. of 0·05N perchloric acid in dioxane

FIG. 2. Titration of promazine base, extracted from tablet, in hexane-acetone (2:1) using a glass: calomel electrode combination.

base with perchloric acid. Glacial acetic acid, the solvent used by Kleckner and Osol for titration of promethazine hydrochloride unfortunately dissolves this lubricant. But we found that magnesium stearate was practically insoluble in acetone giving a titration of only 0·005 ml. of 0·05N perchloric acid for 10 ml. of acetone saturated with the salt. Mercuric acetate was slightly basic in this solvent but the error caused by its presence was insignificant. The preferred solvent for mercuric acetate and the indicator was glacial acetic acid, since methanol gave a diffuse end point. Methyl red, bromcresol green, bromcresol purple and bromphenol blue can be used as indicators, but methyl red is superior. The colour change of methyl red at the end point was selected as being from orange to salmon pink. The colour at the true end point lies between orange and pink and may be described as the first change of the initial orange towards the pink. Although this colour change is distinct it is difficult to describe. For this reason the salmon pink end point was selected with full realisation that it was 0·005 ml. of titrant beyond the true end point.

The active ingredient in ampoules was measured as the base. Extraction was with hexane in preference to ethyl ether to reduce the carry over of water and basic substances. The active ingredient could not be titrated successfully as the free base in glacial acetic acid. Hexane-acetone (2 : 1) was found to be a good solvent and not only permitted potentiometric measurements but also eliminated the necessity to replace the hexane. Methyl red dissolved in glacial acetic acid proved to be a good indicator for this titration also. Procedure II for the pure drug and the recoveries by this procedure have been included to indicate that the hexane extraction was complete and the assay procedure for ampoules is reliable.

All indicator end points were checked potentiometrically with a glass electrode in combination with a fibre type calomel or a silver-silver chloride electrode. Figures 1 and 2 show the titration curve of the hydrochloride in acetone and the free base in hexane : acetone (2 : 1) respectively using the glass-calomel electrode combination and a burette measuring 0·05 ml. Figure 3 illustrates the titration curve of the hydrochloride in acetone using the glass silver-silver chloride electrode combination and a burette calibrated to 0·01 ml. With the calomel electrode if the flow of potassium chloride became excessive the end point could not be observed and for this reason the silver-silver chloride electrode was preferred.

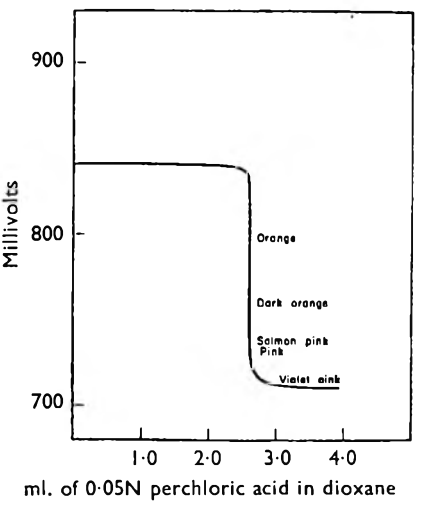


FIG. 3. Titration of crystalline promazine hydrochloride in acetone using a glass : silver-silver chloride electrode combination.

The recoveries of the pure active ingredient by either method I or II were 1 per cent high. The partial titration of the second amino group may provide a possible explanation but other than this no answer can be given.

In Durost and Pascal's colorimetric method[1], some dependence of optical density on concentration of chlorpromazine was found. However, the optical density was so sensitive to time of colour development and age of initial aqueous chlorpromazine solutions that this method was considered unsatisfactory. A second reagent used for colour development, ferric chloride in dilute hydrochloric acid was also found unsuitable. Several difficulties were inherent in the back titration procedure. A large quantity of product was needed for accurate analysis, and the extraction of the tablet mass with water was found to be unsatisfactory. Ethyl ether was used to extract the drug from basic solution but this step was also objectionable since undesirable basic substances could be carried over into the ether layer by the water which is slightly soluble in ethyl ether.

TABLE I

COMPARATIVE RECOVERIES OF NON-AQUEOUS AND GRAVIMETRIC METHODS

Form	Non-aqueous methods			Gravimetric method
	No. of estimations	Mean recovery per cent	Standard deviation	Mean recovery per cent
Pure chlorpromazine hydrochloride by method I	6	101·4	0·130	—
Pure promazine hydrochloride by method I	6	101·05	0·150	—
Pure chlorpromazine hydrochloride by method II	6	101·3	0·225	—
Chlorpromazine tablets	10	106·02	0·812	105·4
Promazine tablets	5	96·23	0·472	96·15
Chlorpromazine ampoules	5	110·06	0·689	110·8
Promazine ampoules	5	97·92	0·502	100·1
Chlorpromazine suppositories	5	102·2	*	102·2

* Single suppositories analysed and therefore standard deviation not representative of method itself.

Although the recoveries obtained by the oxidation method were in closer agreement with those of the gravimetric and non-aqueous methods (Table I), we found that they were not reproducible. Blazek and Stejskal's gravimetric assay[2] was found to be reliable and accurate but like all gravimetric methods it was time-consuming.

REFERENCES

1. Durost and Pascal, *Ann. pharm. franc.*, 1953, **11**, 615.
2. Blazek and Stejskal, *Analyt., Abstr.*, 1956, Feb., No. 530.
3. Sandri, *II Farmaco, Ed. Sci.*, 1955, **10**, 444.
4. Kleckner and Osol, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 573.
5. Pifer and Wollish, *Analyt. Chem.*, 1952, **24**, 300.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

11-Desmethoxyreserpine, Fluorometric Determination of. J. A. Gordon and D. J. Campbell. (*Analyt. Chem.*, 1957, **29**, 488). The method described uses the fluorescence resulting from the reaction of ceric salts with 11-desmethoxyreserpine. To a solution of the latter containing 0·5 to 4·5 μg. in 5N acetic acid is added 1·0 ml. of a solution of 0·001N ceric sulphate in 0·1N sulphuric acid and the solution made up to 10 ml., the tube is immersed in a boiling water bath for 1 hour, cooled, the volume readjusted to 10 ml. and the fluorescence measured against a quinine standard (0·25 μg./ml. of quinine sulphate in 0·1N sulphuric acid). To an aqueous extract prepared from tablets or to parenterals and elixirs, sodium bicarbonate is added to retain 3:4:5-trimethoxybenzoic acid or 11-desmethoxyreserpic acid before extraction of the 11-desmethoxyreserpine with chloroform. An aliquot part of the chloroform solution is evaporated to dryness under nitrogen and the residue taken up in 5N acetic acid. The process is being applied to mixtures of 11-desmethoxyreserpine and reserpine. The activation and fluorescent spectra of 11-desmethoxyreserpine and the fluorescent spectrum of cerate-treated 11-desmethoxyreserpine are given. D. B. C.

Morphine-Marmé Complex. L. Levi. (*Analyt. Chem.*, 1957, **29**, 470). A complex of formula $(C_{17}H_{19}NO_3)$ (CdI_2) is formed when morphine hydrochloride reacts with aqueous cadmium iodide or cadmium iodide-potassium iodide solution. Since the sensitivity of the reaction varies with the amount of potassium iodide in the reagent and the reagent-reactant ratio of the final system, conditions have been worked out to ensure maximum sensitivity which enables 0·1 mg. of morphine to be readily detected. The reagent solution contains 0·2M CdI_2 and 0·8M KI, and the morphine hydrochloride solution under test was 0·0053M, the best result being obtained when three volumes of the morphine solution interacted with one volume of reagent. Any deviation from these conditions reduced sensitivity. On a micro scale, about 0·03μl. of reagent is added to 0·1 μl. of a 0·1 per cent morphine hydrochloride solution. A picture of the crystalline complex is given together with the ultra-violet and infra-red absorption spectra, the X-ray defraction pattern, optical rotation and the solubility behaviour. D. B. C.

Nerve Gases, Detection and Estimation of, by a Fluorescence Reaction. B. Gehauf and J. Goldsen. (*Analyt. Chem.*, 1957, **29**, 276.) The reaction depends upon the oxidation of indole to the highly fluorescent indoxyl and indigo white by alkaline peroxide in the presence of nerve gases such as sarin, soman and tabun. These have the structure either of the type R(R'O)(PO)F or $R_2N(R'O)(PO)CN$. The final stage of the reaction is indigo which is non-fluorescent, but a reaction based upon indigo formation is far less sensitive than one based upon the intermediate transient fluorescent products. The sensitivity of the fluorescent reaction is claimed to be at least 50 times greater than that of the best available colour reaction. Under the conditions used it

was possible to determine 0·05 μg. of sarin, $CH_3(C_3H_7O)(PO)F$, in 10 ml. of solution, and refinements in the technique might decrease this figure to 0·001 μg. The difficulty of the method arises from the short duration, 30 to 60 seconds, of the fluorescence, but methods for stabilising this fluorescent stage are suggested. A complete study of interference has not yet been made. D. B. C.

Poppy Capsules, Isolation of Alkaloids in, with Ion Exchangers. J. Böswart and A. Jindra. (*Československ. Farm.*, 1957, **6**, 82.) The extraction of morphine from poppy capsules is studied, a number of different solvents being used under various conditions. Hot aqueous or methanolic alkaline (containing sodium carbonate or ammonia) solutions give the best results. Pure methanol extracts only 50 per cent of the morphine. Morphine is absorbed from methanolic extracts by the strong anion exchangers Lewatit MN and Amberlite IRA-400, from which it is eluted by N hydrochloric or 0·5N acetic acid. The cation exchanger Wofatit F absorbs morphine from aqueous extracts and it is eluted by a 5 per cent aqueous or methanolic solution of ammonia. For the analytical separation of morphine, the total opium alkaloids are first separated on Wofatit F. The solution of alkaloids is then passed through a column of Lewatit MN and the column is eluted with a 5 per cent solution of ammonia; the morphine, which is retained while the other alkaloids pass through, is finally eluted with N hydrochloric acid and determined polarographically by the method of Holubek (*Pharm. Zentralh.*, 1955, **94**, 347). E. H.

Solanaceous Alkaloids, Purity of. J. Büchi and H. Schumacher. (*Pharm. Acta Helvet.*, 1957, **32**, 75.) The aim of this investigation was the separation and quantitative determination of small amounts of subsidiary alkaloids in commercial alkaloidal products. To this end three paper chromatographic procedures were designed, (A) employing an organic solvent as mobile phase, (B) employing water as mobile phase and (C) employing an optically active acid (di-*p*-toluyl-l-tartaric acid) and an organic solvent as the mobile phase. These enabled a complete separation of the solanaceous alkaloids to be made including hyoscyamine and atropine. By means of procedures B and C, the optical isomers (+)- and (−)-hyoscyamine were separated for the first time. The best reagent for the determination of the alkaloids proved to be Dragendorff's reagent which revealed the presence of 5 μg. of atropine, hyoscyamine, scopolamine and apoatropine and 3 μg. of tropine. It was possible to chromatograph comparatively large amounts of alkaloids e.g., 500 μg., and to separate therefrom the amounts indicated above. It is suggested that these assays for accompanying alkaloids are more sensitive than those officially prescribed and could be used to supplement them. D. B. C.

Sulphadiazine, Sulphathiazole and Sulphadimidine in Tablets, Identification of. F. Abaffy and S. Kveder. (*Acta pharm. Jug.*, 1956, **6**, 200.) Samples of sulphadiazine, sulphathiazole, sulphadimidine, sulphaguanidine, sulphamerazine, sulphanilamide and phthalylsulphathiazole were submitted to partition chromatography on Whatman No. 1 paper using the descending technique, with a 2:1 mixture of butanol and water as the developing solvent. R_F values are given for the three buffer solutions used as the stationary phase. Tablets containing sulphadiazine, sulphathiazole and sulphadimidine were extracted with pyridine and the extract examined by paper chromatography, using the circular, ascending and descending techniques. The sulphonamides were

separated by using paper impregnated with McIlvaine's buffer solution pH 8. The position of the sulphonamide spots was detected by applying a 1 per cent ethanolic solution of *p*-dimethylaminobenzaldehyde, drying and exposing the paper to the vapour of hydrochloric acid. G. B.

Vitamin D and Related Compounds, Determination of. W. H. C. Shaw and J. P. Jefferies. (*Analyst*, 1957, **82**, 8.) A method is described for the assay of the individual components of the mixture of compounds formed during irradiation of the provitamins D (ergosterol or 7-dehydrocholesterol) involving preliminary precipitation of the unchanged provitamins with digitonin, and then the chromatographic separation of the other constituents into two bands. The first band contained the precalciferols 2 and 3 and the lumisterols 2 and 3, the second contained calciferol, the tachysterols 2 and 3 and vitamin D_3. Formulae are given for calculating the percentage of the constituents of each band from ultra-violet absorbtion data. As a check a further colorimetric assay was done on each band using antimony trichloride in chloroform which gave the percentage of precalciferol in the first band and the sum of the vitamin D and tachysterol in the second. The solvent used was 6 per cent v/v of acetone in light petroleum, boiling range 40 to 60°. The size of the sample used was about 30 mg., and experiments were done on known irradiation compounds to ensure that recovery was good. In most cases it exceeded 95 per cent, and was never below 80 per cent. The method was also applicable to the determination of the precalciferols and vitamins D in preparations of the pure vitamins D, but in its present form is unsuitable for materials of low potency or those containing vitamin A. Products obtained under different conditions of irradition were examined, and the results discussed in relation to the structural changes taking place during, and subsequent to irradiation, and in relation to the results of biological assays. D. B. C.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Phenylalanine Inhibition of Tyrosine Metabolism in Liver. I. J. Bickis, J. P. Kennedy and J. H. Quastel. (*Nature Lond.*, 1957, **179**, 1124.) Studying the factors which influence tyrosine breakdown to acetoacetate in isolated liver tissue, results have been obtained which indicate that (–)-phenylalanine exercises a considerable inhibitory action on tyrosine metabolism in liver slices. Experiments were carried out with freshly cut rat liver slices incubated in the presence of solutions of (–)-tyrosine, potassium α-ketoglutarate and sodium ascorbate at 37° in the conventional Warburg manometric apparatus. The acetoacetate formed was measured by addition of aniline at the end of the incubation period, the citrate salt being used. The carbon dioxide thus liberated was a measure of the acetoacetate produced. It was found that both (–)-tyrosine and (–)-glycyl-tyrosine yield substantial increases of the rates of acetoacetate formation. The addition of (–)-phenylalanine brings about a negligible rise in acetoacetate formation. When (–)-phenylalanine was mixed with (–)-tyrosine or (–)-glycyl-tyrosine the rate of acetoacetate formation from the latter amino acids was decreased almost to the level found with (–)-phenylalanine alone. This inhibitory effect of phenylalanine is unlikely to be due to competition for the α-ketoglutarate which brings about the preliminary transamination before oxidation takes place.

It may be due to competition with tyrosine for transport into the liver cell but it may also be due to the formation of phenylpyruvate produced either by oxidation of (–)-phenylalanine in the liver or by transamination. Results from experiments with radioactive (–)-tyrosine and (–)-phenylalanine lead to the conclusion that phenylalanine exercises an inhibition of tyrosine metabolism in the liver, the inhibition being effected by phenylpyruvate produced in the tissue from the phenylalanine either by oxidation or by transamination. The inhibition by phenylpyruvate is not due to isotopic dilution by hydroxyphenylpyruvate as there is no evidence that this substance is formed in the rat liver; nor does phenylpyruvate give rise to significant quantities of acetoacetate. The results do not rule out the possibility that phenylalanine may itself exert an inhibitory effect on tyrosine breakdown; but if so, the inhibition takes place only with intact cells, for no such effect is observed in the rat liver extract. The uptake of free tyrosine by rat liver slices amounts approximately to that calculated for passive diffusion of the amino acid into the tissue, and the uptake is not adversely affected by the presence of phenylalanine or phenylpyruvate. Incorporation of tyrosine into proteins of the liver slices is, however, definitely affected by phenylalanine and phenylpyruvate. Whether the inhibitory effect of (–)-phenylalanine on tyrosine incorporation is due to competition for incorporation or competition for activation preliminary to incorporation, is a matter for further investigation. These results have a bearing on the interpretation of the metabolic findings in phenylketonuria. In this disorder, which is characterised by mental defect, the conversion of phenylalanine to tyrosine is reduced or arrested. Thus the blood phenylalanine concentration is increased many times over the normal value. Phenylpyruvate is excreted in large amounts. An interference by phenylalanine or by phenylpyruvate with hormone production may throw some light on the problem of the connection between phenylketonuria and mental defect.

M. M.

BIOCHEMICAL ANALYSIS

Ammonia, Determination of, in Blood. D. G. Nathan and F. L. Rodkey. (*J. Lab. clin. Med.*, 1957, **49**, 779.) A colorimetric microdiffusion technique is described for the analysis of ammonia in blood. Pipette 3 ml. of 20 per cent trichloroacetic acid into a centrifuge tube. Cool in ice and add 3 ml. of freshly drawn blood. Shake and store in ice. To assay, shake, centrifuge and record the total volume in the tube and the volume of the precipitate. Decant the clear supernatant fluid. Transfer 1 ml. aliquots of the supernatant to separate 25 ml. penicillin bottles. Moisten a glass rod, previously placed in the rubber stopper, with one drop of 1M citric acid. Distribute the acid in a thin film covering the rod to within 5 mm. of the stopper (acidified rod). Add 1 ml. of saturated potassium carbonate solution. Avoid mixing the two solutions. Insert the acidified rod and stopper tightly. Take care to avoid contact of the acidified rod with either the bottle or its contents. Prepare blank and standard samples with 1 ml. of ammonia free water, or 1 ml. of ammonium sulphate solution containing 1 μg. of NH_3-N, in place of the filtrate. Rotate all the samples for 30 minutes on a rotator. Remove the stoppers and acidified rods from the bottles and place in Pyrex Klett tubes. Wash each rod with 2 ml. of a mixture of equal parts of ninhydrin solution and pH 5 citrate buffer. Cap the tubes and place in a boiling water bath for 30 minutes. Remove from the bath and cool. Add 50 per cent ethanol to make a total volume of 10 ml. in each tube, cover with parafilm and mix by inversion. Clean the outside of the tubes and read in a Klett colorimeter with a No. 56 filter. Subtract the reading of the

blank from the readings of the unknown and standard solutions. The concentration of blood ammonia nitrogen is calculated from the NH_3-N in 1 ml. of the filtrate by the following formula.

$$(NH_3\text{-}N)_{B1} = \frac{(NH_3-N)_F\,(V_t-0{\cdot}10V_p)}{(V_t-V_{tca})}$$

V_t = total volume in centrifuge tube (supernatant plus precipitate).

V_p = total volume of precipitate.

V_{tca} = volume of trichloroacetic acid added to centrifuge tube.

$(NH_3\text{-}N)_F$ = concentration of ammonia in the supernatant fluid expressed as μg. of NH_3-N per ml. of fluid.

$(NH_3\text{-}N)_{B1}$ = concentration of ammonia in blood as μg. of NH_3-N per ml. of blood.

The venous blood ammonia concentration of normal fasting human subjects was found to be $0{\cdot}77 \pm 0{\cdot}28$ μg. NH_3-N per ml. G. F. S.

Glucose and Fructose, Simultaneous Measurement of. W. L. Brown, M. K. Young and L. G. Seraile. (*J. Lab. clin. Med.*, 1957, **49**, 630.) A method is described for the simultaneous determination of glucose and fructose in biological materials, based on the development of colour with an anthrone reagent at 75° and 100°. A good precision is obtained. For estimation in blood, pipette 0·25 ml. of plasma into 5 ml. of water in a test-tube, add 1 ml. of 10 per cent zinc sulphate and allow to stand for five minutes. Now add 1·0 ml. of 0·5 N sodium hydroxide, cap the tube with parafilm, shake vigorously and centrifuge. Place two 1 ml. aliquots in cuvettes, immerse in a beaker of cold water and shake during the addition of 10 ml. of 0·2 per cent anthrone reagent (prepared by dissolving 2 g. of anthrone in 250 ml. of concentrated sulphuric acid and adding 500 ml. of concentrated sulphuric acid previously diluted by adding to 250 ml. of water and cooling). One of the cuvettes is then placed in a water bath at 75° and the other in a water bath at 100°. Heat for seven minutes and cool for ten minutes. Read the optical densities of both solutions in a spectrophotometer at 630 mμ and compare with reagent blanks and duplicate standards of glucose and fructose treated in the same way. The standards contain 60 μg./ml. and are prepared daily from stock solutions containing 100 mg. of glucose or fructose in 0·25 per cent benzoic acid solution. The concentrations of glucose and fructose are calculated by substitution in the following equations.

$$\text{Glucose conc./ml.} = \frac{(\text{O.D.U.}_{100} \times KF_{75})-(\text{O.D.U.}_{75} \times KF_{100})}{(KF_{75} \times KG_{100})-(KF_{100} \times KG_{75})}$$

$$\text{Fructose conc./ml.} = \frac{(\text{O.D.U.}_{75} \times KG_{100})-(\text{O.D.U.}_{100} \times KG_{75})}{(KF_{75} \times KG_{100})-(KF_{100} \times KG_{75})}$$

when O.D.G. = optical density of glucose standard

O.D.F. = optical density of fructose standard

O.D.U. = optical density of unknown mixture.

$$KG_{75} = \frac{\text{O.D.G. at } 75^\circ}{\text{conc. glucose/ml.}} \qquad KG_{100} = \frac{\text{O.D.G. at } 100^\circ}{\text{conc. glucose/ml.}}$$

$$KF_{75} = \frac{\text{O.D.F. at } 75^\circ}{\text{conc. fructose/ml.}} \qquad KF_{100} = \frac{\text{O.D.F. at } 100^\circ}{\text{conc. fructose/ml.}}$$

G. F. S.

Salicylic Acid, Determination of, in Plasma. S. P. Chiang and S. Freeman. (*J. Lab. clin. Med.*, 1957, **49**, 481.) A micromethod is described for the separation of salicylates from plasma proteins and for their estimation. Twenty μl. of plasma is applied to a strip of 3 MM Whatman filter paper, previously extracted with ethanol and dried. Dry at room temperature and add 20 μl. of 6N hydrochloric acid on the same spot as the plasma. Allow to dry. Carry out ascending paper chromatography in test tubes containing 1 ml. of ethanol until the solvent front has travelled to a marked 50 mm. line. Dry in air and repeat this procedure three more times. Cut from the paper strip an area 3 mm. above and 7 mm. below the solvent line. Place in a test tube, add 500 μl. of water and 20 μl. of a 1 per cent solution of ferric nitrate in 0·07N nitric acid. Mix frequently over 30 minutes. Place in a microcell and read the optical density at 540 mμ in a spectrophotometer. Compare with standards prepared by adding 20 μl. of standard solutions of salicylic acid containing 100, 200 and 400 μg./l. added to strips of filter paper on which 20 μl. of salicylate free plasma has been dried. The standards are treated the same way as the test. The results are calculated from the difference in optical density obtained with the patients plasma before and after treatment with salicylates by reference to the standard curve. G. F. S.

Salicylsulphonic Acid Test for Protein in Urine. D. N. Baron. (*Brit. med. J.*, 1957, **1**, 628.) The use of a proprietary tablet, containing salicylsulphonic acid, effervescing agents and a trace of bromcresol green (Altest), is described for the detection of protein in urine. Place about 4 ml. of the filtered urine in a test-tube and add one test tablet. When the tablet is dissolved shake the tube and examine the fluid, which should be yellow. If green the fluid is still alkaline and another one or two tablets are added until yellow. Protein is present when the mixture is cloudy or shows a white precipitate. The test can detect 0·005 g. of protein per 100 ml. of urine, which is comparable with the salicylsulphonic acid solution test, but slightly less sensitive than the boiling test. G. F. S.

CHEMOTHERAPY

Phenazines with high Antituberculosis Activity. V. C. Barry, J. G. Belton, M. L. Conalty, J. M. Denneny, D. W. Edward, J. F. O'Sullivan, D. Twomey and F. Winder. (*Nature, Lond.*, 1957, **179**, 1013.) These riminocompounds are obtained by condensing *o*-phenylenediamine derivatives with diarylamino-*o*-quinones. This paper is concerned with the biological properties of one of these derivatives, B.663. The methods used in screening for antituberculosis activity in mice were as follows: groups of 8 to 10 mice were infected intravenously with the isoniazid-sensitive Ravenel Rv bovine strain of *M. tuberculosis* or with an isoniazid-resistant variant of that strain. Drugs were administered in the diet (*a*) for 14 days commencing on the day of infection, that is, "protective screening", (*b*) for 14 days commencing on the sixth day after infection, that is, "established disease screening" and (*c*) as in (*a*) but using the isoniazid-resistant variant. Median survival times of the control groups (both isoniazid-sensitive and -resistant were about sixteen days. It was found that B.663 is more active than isoniazid on a weight for weight basis and considerably more active on a molar basis. The activity is greater than that observed with other antituberculosis substances such as *p*-aminosalicylate, streptomycin or the thiosemicarbazones. B.663 showed a striking effect in established tuberculosis of guinea pigs. It was also found to accumulate in the tissues of mice and

guinea pigs. Prophylactic experiments showed that 10 mg./kg./day for 14 days, terminated as long as 4 weeks before infection, confers protection. Part of the action of these rimino-compounds may be due to their withdrawal of hydrogen from the respiratory chain and part due to their peroxide formation. M. M.

Threo-β-Phenylserine, the Antiviral Action of. L. Dickinson and M. J. Thompson, with an appendix by J. S. Nicholson, (*Brit. J. Pharmacol.*, 1957, **12**, 66.) L-*Threo*-phenylserine and esters of *threo*-phenylserine were the most active of a series of compounds tested against influenza A virus in tissue culture. Substitution of the β-OH or α-NH_2 group abolished activity. The activity of phenylserine was reversed competitively by phenylalanine. Phenylserine had no action on free virus or on the adsorption of virus to host cells. It prevented virus growth if added during the first half of the latent period. Phenylalanine appears to be necessary for virus synthesis and could be supplied by glycylphenylalanine, phenylalanylglycine or phenylalanine ethyl ester, but not by *N*-acetylphenylalanine. Phenylserine had no significant action against ectromelia infections in mice, even when the amino acid content had been depleted by starvation. *Threo*-β-phenylserine was relatively non-toxic to the host cells investigated, compared with *p*-fluorophenylalanine which has been reported to be active against poliomyelitis virus at 0·04 to 0·1 mg./ml., but cytostatic at 0·1 mg./ml. G. P.

PHARMACY

Albomycin and Grisein, Similarity of. E. O. Stapley and R. E. Ormond. *Science*, 1957, **125**, 587.) Albomycin, isolated from *Actinomyces subtropicus* by Soviet workers and grisein, isolated from *Streptomyces griseus* by Waksman and colleagues are red-coloured substances containing amino acids and iron, having similar activity against a number of species of bacteria. Samples of the two substances were compared to establish whether or not they are identical. Paper and column chromatography and counter-current distribution analysis showed that both antibiotics consist of four active substances, of which a highly active one (A) and a weakly active one (D) are stable, while a less stable component (C) was found to break down during purification, with the formation of more (A). Cross-resistance between the antibiotics was demonstrated in 12 antibiotic-resistant strains of *Escherichia coli*. Cross-resistance also developed to viomycin, but not to 12 other antibiotics produced by actinomycetes. It is concluded that the two antibiotics are closely similar chemically, and identical in their antimicrobial activity. G. B.

PHARMACOLOGY AND THERAPEUTICS

Chlorhexidine for Local Treatment of Burns and Scalds. J. C. Grant and J. C. Findlay. (*Lancet*, 1957, **1**, 862.) A method is described for the treatment of burns using a 0·5 per cent solution of chlorhexidine. The patient after admission to hospital is sedated, and, if necessary, given intravenous fluid. The burn is cleaned with 1 per cent cetrimide, rinsed with normal saline and covered with gauze soaked in 0·5 per cent chlorhexidine solution, using aseptic precautions. The gauze dressings are resoaked with the chlorhexidine solution twice daily, and twice a week are removed to allow inspection of the lesion and removal of separating sloughs. The areas are not left exposed until epithelial cover is complete. In 108 treated patients results have been excellent and

infection rare. Superficial burns heal rapidly by regeneration of the surviving epithelium, and in the deep burns, after separation or removal of the sloughs, a clean granulating surface presents, which is eminently suitable for grafting. The practical advantages of the method over aseptic and antibiotic methods are discussed. G. F. S.

Diamorphine and Pholcodine, Comparison of, as Cough Suppressants. E. S. Snell and P. Armitage. (*Lancet*, 1957, **1**, 860.) A controlled clinical trial is reported of a comparison of the relative effectiveness as cough suppressants of heroin linctus B.P.C., a pholcodine preparation and a placebo in 45 patients with chronic coughs. The mixtures were numbered 1, 2 and 3 and the patients instructed to take 120 minims at night using each mixture for two nights. They were asked to state their preference, which was recorded. It became clear that patients were rarely able to state their preferences since they usually gave a tie for first or last place, so for statistical analysis a new type of "restricted sequential procedure" was adopted, which is described. At the 5 per cent level of significance there was no evidence of a difference between the heroin and pholcodine preparations, but both were more effective than the placebo. G. F. S.

Dipipanone Hydrochloride, Analgesic Activity of. D. A. Cahal. (*Brit. J. Pharmacol.*, 1957, **12**, 97.) The analgesic activity of dipipanone hydrochloride was assessed against ischaemic muscular pain in healthy human volunteers. The drug was administered by subcutaneous injection, 0·5N-saline being used as a control since pain caused by the drug indicated its use rather than normal saline. Observations of pain threshold were made at ten minute intervals during the first hour of the action of the drug, and thereafter at hourly intervals. Peak effects were registered about two hours after administration at all dose levels of the drug. The lowest dose causing a significant rise in pain threshold was 10·0 mg. The effects of increase in dose showed a linear function between log dose and response. The most common side effects observed were drowsiness, euphoria, nausea and vertigo. Two of the twenty-six subjects were hypersensitive to the drug. The drug appears to be a histamine liberator, indicated by the generalised pruritus in some subjects and weal and flare production on intradermal injection, together with incidence of epigastric discomfort and headache. Injection of the drug in all subjects was painful and often the pain was very persistent. Many subjects had tenderness and induration at the sites of subcutaneous injection for many weeks. After intradermal injection, slight ulceration often occurred, the ulcers healing only slowly and leaving pigmented scars. G. P.

Ethylcrotonyl Carbamide, a New Sedative. T. Canbäck, N. Diding and C. G. Lindblad. (*Svensk farm. Tidskr.*, 1957, **9**, 221.) This compound (Ektylcarbamide) may be obtained by dehydrobromination of carbromal. It shows *cis-trans-* isomerisation, and two stable forms may be obtained, melting at 198° and 158° respectively. It has been reported that, with rats, a sedative effect is obtained with oral administration of 1 per cent of the LD50, while 48 per cent had no permanent effect: 90 per cent is required for full hypnotic action. The wide margin between sedative and hypnotic action indicates that the compound should be more reliable than other sedatives. The present authors have partially confirmed this, in that they have found the minimum lethal dose (LD50) for mice to be 1 g./kg. G. M.

Histamine Release Inhibition *In Vitro* and Antianaphylactic Effects *In Vivo* of some Chemical Compounds. F. C. McIntire, R. K. Richards and L. W. Roth. (*Brit. J. Pharmacol.*, 1957, **12**, 39.) Some 2,500 compounds were tested for inhibitory activity on the anaphylactic reaction *in vitro* on the "platelet fraction" of blood from a rabbit sensitised to egg white, and *in vivo* in guinea pigs similarly sensitized. Antihistamine activity was also measured *in vivo* on guinea pigs against histamine aerosols. Several compounds were effective inhibitors of the reaction *in vitro*, but proved very toxic *in vivo* and in maximum tolerated doses none of these gave significant protection in sensitised animals when the antigen was given intravenously. When the antigen was administered as an aerosol three of the substances afforded some protection, but none of these showed any promise of being useful clinically. A number of other drugs, among them aspirin and sodium salicylate, reported to alleviate allergic symptoms or prevent anaphylactic shock, failed to inhibit histamine release *in vitro*, although in rabbits the antianaphylactic action of aspirin was confirmed *in vivo*. The histamine release by antigen from the blood of rabbits which had been treated with sufficient aspirin to protect them against anaphylaxis was not inhibited when the blood was tested *in vitro*. These results are inconsistent with the view that aspirin protects against anaphylactic shock by interfering with the antigen-antibody reaction. Substitution of a carbomethoxy group for a carbamide group in the β-position of certain quaternary pyridinium compounds changed an antianaphylactic action to one of histamine release, suggesting the possibility of a common basic mechanism of histamine release and of prevention of release, probably at the cell membrane. None of the substances active against antigen aerosols had any effect on the actions of histamine aerosols.

G. P.

5-Hydroxytryptamine, Role of, in the Inflammatory Process. B. Gőzsy and L. Kátó. (*Science*, 1957, **125**, 934.) Experiments in rats have shown that while histamine and 5-hydroxytryptamine are liberated from mast cells on injury, the two substances show a different behaviour in the defence processes. 5-HT. was found to be more potent than histamine in inducing an accumulation of Indian ink or trypan blue at the site where they were administered percutaneously. The phagocytic activity of the monocytes *in vitro* was influenced by histamine, but not by 5-HT. These two substances therefore play a different role on the defence mechanisms. G. F. S.

Iron Preparations, Intramuscular, Local Effects and Mechanism of Absorption of. C. R. Beresford, L. Golberg, and J. P. Smith, (*Brit. J. Pharmacol.*, 1957, **12**, 107.) The retention in muscle of four iron-polysaccharide complexes after intramuscular injection deep into the gluteals of the rabbit was not closely related to diffusibility of the complexes in agar. There was also considerable variation among the compounds in the extent of the retention. The major part of the absorption of the iron occurred during the first 72 hours after injection. An acute inflammatory reaction was induced by the drug at the site of injection, and this appeared to govern, by increased lymphatic transport of the iron complex, the absorption after injection. The local inflammatory response in rats was accompanied by degenerative changes in the muscles, but regeneration was rapid, tissue repair being complete in less than one month, leaving no residual damage to the muscles, nerves or neighbouring tissues. Rapid fixation by tissue macrophages impeded absorption and, with some complexes, this factor may make much of the injection inaccessible. G. P.

Poliomyelitis Vaccine, British, Assessment of. Report to the Medical Research Council by the Poliomyelitis Vaccines Committee. (*Brit. med. J.*, 1957, **1**, 1271.) The report describes an investigation of the protective effect against clinical poliomyelitis in children of the vaccinations carried out in Great Britain under the auspices of the Ministry of Health and the Department of Health for Scotland in May and June, 1956. The vaccine used was a formolized vaccine containing MEF-1 type II and Saukett type III strains as used in American trials but with Brunenders type I strain instead of the more virulent Mahoney type I strain used in America. 178,161 children received two injections, each of 1 ml., at intervals of at least 3 weeks. In a sample 1 per cent, visited the day after receiving the first injection, local reactions were found to the extent of 2 to 3 per cent but were always mild. Only 6 children developed illness possibly related to the inoculation and in none of them was there a definite clinical or laboratory relation. Controls were children registered with a local authority for vaccination. During 6 months following the vaccinations poliomyelitis was reported in 512 registered children, vaccinated and unvaccinated. Among 74,660 vaccinated children aged $5\frac{1}{2}$ to $9\frac{1}{2}$ years, there was 1 case of paralytic poliomyelitis, corresponding to an attack rate of 1·3 per 100,000. The corresponding figure for unvaccinated children was 8·2 per 100,000. Among 74,024 children aged $1\frac{1}{2}$ to $5\frac{1}{2}$ years the corresponding figures were 4·1 and 20·1 per 100,000 respectively. The incidence of non-paralytic poliomyelitis was not influenced by the vaccination. 32,379 children received a single injection only and there was insufficient evidence on which to judge the effect. Although the protection conferred by the vaccine was not complete, the incidence of paralytic disease in the vaccinated children was only about one-fifth of the incidence in the unvaccinated. The degree of protection was probably quite substantial over the ages $1\frac{1}{2}$ to $9\frac{1}{2}$ years and although the number of observations was small it appeared that the Brunenders strain conferred protection against the prevailing type I infection. H. T. B.

Reserpine, Antileukaemic Action of. A. Goldin, R. M. Burton, S. R. Humphreys and J. M. Venditti. (*Science*, 1957, **125**, 156.) The antileukaemic action of reserpine was studied in mice inoculated with a suspension of leukaemic cells and left until the local tumour had reached an approximate diameter of 12 mm. and the systemic disease had developed. These mice were then randomised and the designated groups were treated with a single injection of reserpine. A single treatment produced an almost threefold increase in the remaining lifetime of mice with advanced leukaemia. Inhibition of the growth of the local tumour was observed consistently in the reserpine-treated mice. Reserpine also appeared to retard the usual terminal leucocytosis in the peripheral blood. A regime of daily treatment with small doses of reserpine may be superior to a large single dose. Several derivatives of reserpine also possessed some antileukaemic activity, e.g., rescinnamine, deserpedine and *iso*reserpine. The mechanism by which reserpine exerts its antileukaemic action is not known and whether the action is direct or mediated through the host is not clear. 5-Hydroxytryptamine administration alone did not influence the course of the leukaemia. Both leukaemic and non-leukaemic animals suffered severe depression at the higher dose levels of reserpine employed. This was overcome with (+)-amphetamine which did not affect the course of the experiment in any way. G. P.

Tranquillising Drugs; Allergic Reactions to. C. Bernstein and S. D. Klotz. (*J. Amer. med. Ass.*, 1957, **163**, 930.) Meprobamate gave rise to allergic reactions in 8 patients observed by the authors and in 7 other patients reported to them. The reactions included urticaria, fever, arthralgia, purpura and, in a woman being treated for lupus erythematosus, a flare-up of new skin lesions. Several of the patients with these reactions had previously used mephenesin, and there is a possibility that mephenesin may pre-sensitise patients to meprobamate. Reserpine and chlorpromazine have also caused side-effects essentially different and more variable in type. Bearing in mind the enormous quantities of these tranquillising drugs which are now being used, the incidence of allergic reactions has been extremely low, but the hazard must be kept in mind as some of the symptoms, especially the fever, may confuse the issue in the clinical course of a patient under treatment. S. L. W.

***Veratrum album*, Pharmacological Study of a New Acetone Fraction of Alkaloids of.** V. Trčka and M. Vaněček. (*Československ. Farm.*, 1957, **6**, 68.) Results of pharmacological tests on an acetone fraction prepared from the roots of *Veratrum album* are reported. Hypotensive studies were carried out on rabbits, cats and dogs. In comparison with Puroverin (proveratrines A and B), the preparation, termed "VER V", had one-tenth to one-fifth of the hypotensive activity in dogs, but its toxicity in mice was only one-thirtieth of that of Puroverin. Its emetic effect on dogs was also less marked. E. H.

APPLIED BACTERIOLOGY

Bacteria, Viable Count of, a New Technique. A. Guha. (*Nature, Lond.*, 1957, **179**, 1360.) The author describes a method by which viable cells may be identified in a culture of viable and non-viable bacteria when counted microscopically. Cultures of *E. coli* were prepared in a medium containing 1 per cent 2:3:5-triphenyltetrazolium hydrochloride. Counts of the culture were made by the haemocytometer method, using 4 per cent formalin as the dilution liquid. Bacteria which were living before the formalin treatment were distinguished by a reddish tinge, the colour being due to red formazan bodies which are produced by reduction of the tetrazolium salt by the mitochondria. Dead bacteria in the culture appeared completely transparent, so that both a total and viable cell count could be obtained. It was shown that the stained cells, not treated with formalin, were capable of growth. Unstained cells did not grow. The tetrazolium salt was apparently devoid of toxic effects to the bacteria used in these experiments. B. A. W.

***Mycobacterium tuberculosis*, Cultivation of, on Semi-solid Agar Media for Rapid Drug Sensitivity Tests.** R. Knox and R. Woodroffe. (*J. gen. Microbiol.*, 1957, **16**, 647.) This paper reports results supporting previous descriptions by the same authors of the use of semi-solid media for rapid cultivation of *M. tuberculosis* in the performance of drug sensitivity tests. Media used were those of Kirchner (without penicillin), Dubos and Fisher, all being used as semi-solid media (0·125 per cent agar) and as liquid media. Sensitivity of *M. tuberculosis* to isoniazid, streptomycin and aminosalicylic acid were determined. The organisms used were *M. tuberculosis* H37Rv strain, variants of this strain made resistant to isoniazid and streptomycin, and aminosalicylic acid resistant strains of *M. tuberculosis*. Using semi-solid media and heavy inocula, results could be read in 2 days with a hand lens and in 3 to 5 days with unaided vision. With liquid media results were less easy to read and interpret, the

(ABSTRACTS *continued on page* 704.)

PHARMACOPOEIAS AND FORMULARIES

THE BRITISH PHARMACEUTICAL CODEX 1954, SUPPLEMENT 1957*

REVIEWED BY H. TREVES BROWN

Few of those whose responsibility it is to keep up to date with modern drugs can rely on memory for the necessary information on the latest additions to therapeutic resources. The number of drugs being introduced and the rapidity with which they can become established is reflected in the Addendum 1955 to the British Pharmacopoeia 1953, in which no less than 10 of the drugs were not described in the B.P.C. 1954. It is not only a matter of knowing the purpose and manner of use of the new drugs that secure recognition in the Pharmacopoeia but also of assessing the value of the numerous compounds offered by an enterprising pharmaceutical industry. An authoritative reference book surveying the most important introductions is needed and the Supplements to the British Pharmaceutical Codex, in the compilation of which a judicious process of selection is exercised, provides exactly what is required between issues of the main volume.

The Supplement 1957 to the B.P.C. 1954 contains monographs on 44 new substances and they illustrate the variety of fields in which research is enabling progress in therapeutics to be achieved. Among the new substances described are antibiotics, anti-inflammatory agents related to cortisone, antihistaminic compounds, ganglion-blocking agents, anti-convulsants and the insulin zinc suspensions. It is perhaps a surprise to find, not quite buried among these pharmacologically active compounds, monographs on spearmint oil and sulphan blue. The oil is required for the preparation of spearmint water, no doubt a useful, if not a novel flavouring agent in 1957. The dye is described as being "used in the colouring of medicines", and its inclusion in the Supplement is perhaps a non-scientific concession to pharmaceutical elegance which has the merit of serving a dual purpose since the blue colour it normally imparts can be changed to green by use with tartrazine.

In the Formulary section of the book a few new preparations have been introduced. It is noteworthy that reserpine is the only instance of a substance which is the subject of a new general monograph being represented by a tablet in the Formulary section. This is probably a reflection of what seems to be the growing practice of manufacturers not to make a new substance available except in the form of compounded preparations. The compilers have appreciated that in such instances the inclusion of a B.P.C. tablet serves no useful purpose. The alleged dangers of borated dusting powders have been recognised to the extent of reducing the boric acid content of these preparations to 5 per cent and including a formula

* Published by direction of the Council of the Pharmaceutical Society of Great Britain, pp. xiii + 124 (including Index). The Pharmaceutical Press, London, 1957. 27s. 6d.

for a dusting powder containing only zinc oxide, starch and talc. For the same reason provision is made in four of the Standard Dressings for replacing boric acid by a less suspect antiseptic. Additions to the Surgical Dressings Section include monographs on lint and gauze made from rayon and on penicillin gauze dressing.

In addition to the inclusion of monographs on new substances and preparations, the Supplement brings the B.P.C. 1954 up to date by giving details of all the alterations and amendments made since the 1954 book was issued. Most have been published previously but it is convenient to have them bound in one volume with the new material.

The Supplement will be welcomed by those whose daily work could not be done without the Codex, and the Editor is to be congratulated on having welded a compilation, to which many different hands and minds have contributed, into a unified and consistent whole.

BOOK REVIEWS

MATHEMATICS AND STATISTICS FOR USE IN PHARMACY, BIOLOGY AND CHEMISTRY. By L. Saunders and R. Fleming. Pp. x + 257 (including Index). The Pharmaceutical Press, 17, Bloomsbury Square, London, W.C.1. 1957. 27s. 6d.

This is a very useful book for anyone working in a biological field who wants to improve his mathematical knowledge. Its fifteen chapters proceed from arithmetic, algebra, and graphs through differential calculus, trigonometry and probability to statistical analysis and applications. Additional mathematical theorems and techniques and statistical tables are provided in a number of appendices. To cover all this ground in so few pages is a remarkable achievement, and the authors deserve much credit for their concise and readable English. Since the material is so highly compressed, there are few explanatory passages to ease the reader's difficulties when the mathematical logic is not self-evident. On the other hand, worked examples are clearly set out and give guidance in practical applications, particularly of the statistical material. For the honours students for whom it is intended, this book is a valuable supplement to other teaching; it is also likely to be very useful to research workers for reference, particularly because it contains so much material in such a small volume. The production and the appearance are excellent.

MILES WEATHERALL.

GLOSSARY OF INDIAN MEDICINAL PLANTS. By R. N. Chopra, S. L. Nayar and I. C. Chopra. Pp. xx + 330. Council of Scientific and Industrial Research, India, 1956.

An all-India survey of medicinal and poisonous plants was begun more than thirty years ago to investigate the extensive native materia medica. Attempts have been made to establish the true botanical identity of each of these drugs and to set in order the lists of vernacular names. A herbarium, the first of its kind in India, of some 1,600 species of medicinal plants has been collected throughout the sub-continent and a museum of vegetable drugs is being organised.

The Glossary under review is the work of this survey. It is a list of some 2,000 native Indian medicinal plants and the information concerning them, ". . . based on a critical study of the literature . . ."; it also includes some of

the imported drugs sold in the Indian bazaars. The plants are arranged in alphabetical order of genera (the plant family being given in parenthesis). Many of the common alternative botanical names are quoted, with cross references to the accepted names: important vernacular names commonly used in different regions of India are also recorded and indexed. The various parts of the plant which comprise the drugs are then named together with a condensed list of medicinal uses. The active principles of the drugs are briefly named, together with references to published work thereon up to 1953, and an index of chemical constituents is provided. Finally, the distribution of the plants in different regions of India is stated but no descriptions of the drugs nor of the plants are given.

The volume is well produced, it is a valuable addition to our references on vegetable drugs and its compilation is the result of a very large amount of useful work. By its very nature, however, it must leave more questions unsolved than answered; thus the reader has no means of identifying an unknown drug or of confirming the characters of a named specimen by means of this volume, nor of tracing such a description in the literature; for the references quoted are almost entirely on chemical composition. These are by no means exhaustive and are (one imagines, purposely) uncritical: thus three separate sets of references are quoted to show that the principal alkaloid of *Datura metel* is atropine, is hyoscyamine, is scopolamine, respectively. Space could have been saved by a more critical appraisal of such contradictory information and might well have been used in giving references to publications on drug morphology and anatomy where known (and much does exist in widely scattered publications). An indication of toxicity or dosage might also have been given, for some relatively harmless materials are recorded along with other highly toxic drugs, e.g., *Illicium verum* and *I. religiosum* are both described as "stomach, carmin." These criticisms should not belittle the valuable nature of this book, and it is to be hoped that the detailed investigation of the rich field of Indian medicinal plants will be continued actively and critically.

J. M. ROWSON.

(ABSTRACTS *continued from page* 701.)

end point defining the initial minimal inhibitory concentration being much sharper in semi-solid media. Moreover, only in semi-solid media could be distinguished the later growth of colonies which gave a measure of the variability of resistance within a culture. These presumptive resistant colonies appeared most often with Kirchner semi-solid medium containing isoniazid and less frequently with the other 2 drugs, but the retesting of cultures from the colonies of the isoniazid medium showed that drug resistance did not always occur. If, however, the drug was not added to the inoculated medium until the 2nd or 3rd day (by this time minute colonies had appeared) a few large colonies containing truly resistant organisms later developed. Differing rates of decay of isoniazid in the different media were observed. The end points obtained with all 3 drugs in the semi-solid media were sharp and consistent. With aminosalicylic acid, the inhibitory concentration varied greatly with the inoculum size and with different strains and was attributed in part to the antagonism of *p*-aminobenzoic acid. The agar concentration used appeared to be optimal: discrete colonies developed and yet the culture was sufficiently fluid to be sucked up in a dropping pipette when picking out single colonies for subculture. The authors conclude that for all 3 drugs the Fisher semi-solid agar gave the most satisfactory results. B. A. W.

FRANK HARTLEY

Chairman, 1957