REVIEW ARTICLE

THE MODE OF ACTION OF STREPTOMYCIN

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The Effect of Environment on the Action of Streptomycin

To place in their right perspective the various effects of streptomycin on bacterial metabolism it is necessary to be acquainted with the environmental conditions in which they are displayed. As with many other chemotherapeutic substances the action of streptomycin may be influenced by its surroundings. These outside influences are many, and before any attempt is made to give an account of how streptomycin may interfere with the chemical activities of the cell the more important of them will be considered. For simplicity these several factors will be dealt with separately.

The Size and Age of the Inoculum

For most organisms the activity of streptomycin is inversely proportional to the size of the inoculum[1,2]; this relation however is merely a reflection of the greater number of resistant organisms present in the larger inocula and not an adsorption phenomenon[1]. This variation in the activity of streptomycin is most pronounced at low pH[3] and under such circumstances may be due to an antagonist produced by and carried over from the parent culture[1].

Streptomycin exerts its maximum effect upon young actively growing cultures[4,5]. It is, however, effective against older organisms and resting cells in concentrations higher than those inhibiting young cultures[6,7]. This difference in activity does not appear to be so great as that for penicillin[5], and is dependent upon the test organisms, being most noticeable with *Escherichia coli.*

The Effect of Natural Substances and of their Breakdown Products

Serum

Reports on the effect of serum on streptomycin activity vary. In general the sensitivities of Gram-negative organisms and of *Micrococcus pyogenes* var. *aureus* are unaffected by the addition of serum to nutrient broth, even in concentrations as high as 50 per cent. However, all streptococci show a marked increase in resistance with as little as 1 per cent serum. Growth curve studies show that this effect on sensitivity is greater than any growth-stimulating effect of the serum. It may be that this resistance is due to an alteration in the metabolic pathway so that the more susceptible system or systems, which alone are available to the organism when growing in nutrient broth, are by-passed[7].

Products of Bacterial Metabolism

Of the existence of streptomycin antagonists produced as the result of the metabolism of some of the normal inhabitants of the intestinal tract and of *M. pyogenes* var. *aureus* there is no doubt[1,8–11], but there is no justification in referring to them as "streptomycinases"[8,12]. Lightbown[9] succeeded in isolating in crystalline form the antagonist present in culture filtrates of *Pseudomonas aeruginosa* and showed that it antagonises 500–1000 times its weight of dihydrostreptomycin when *Bacillus subtilis* or *Bacillus pumilus* is used as the test organism. Chemical analysis strongly suggests that the antagonist is a mixture of 4-hydroxyquinoline-*N*-oxides[13]; synthetic 2-heptyl-4-hydroxyquinoline-*N*-oxide antagonises approximately 200 times its weight of dihydrostreptomycin[14]. Bergman and colleagues[15] found that the filtrate of *Ps. aeruginosa* antagonises neomycin suggesting involvement of the common streptidine group. If this antagonist affects neomycin and streptomycin in the same way then the inactivated portion of the streptomycin molecule plays no part in the inhibition of those reactions which are circumvented by the streptomycin-resistant organisms. The antagonist is still active towards neomycin when this antibiotic is tested against streptomycin-resistant organisms.

The highly active antagonist isolated from filtrates of *Ps. aeruginosa* by Lightbown[9] might provide an explanation for the findings of Elias and Durso[16] and Reimann, Elias and Price[17] on the ineffectiveness of streptomycin in the treatment of typhoid fever. With antibiotic therapy the intestinal flora changes, and the incidence of *Alcaligenes faecalis*, *Streptococcus faecalis* and *Ps. aeruginosa* increases. It is reasonable to suppose that *Ps. aeruginosa*, its growth unimpeded by the streptomycin-sensitive commensals of the intestinal tract, produces greater quantities of the antagonistic substance, rendering an otherwise adequate concentration of streptomycin ineffective. This explanation would be in keeping with the absence of antagonists in normal and pathological fluids[12]. This point might be proved by looking for an antagonist in the bowel contents of persons treated with streptomycin who show a preponderance of *Ps. aeruginosa* in the faeces.

Peptones and Amino Acids

With the exception noted by Davis and Sevag[18] the inclusion of peptone in the culture medium is said to reduce the efficiency of streptomycin[19–21]. Davis and Sevag[18] brought evidence to suggest that streptomycin was more effective in the presence of complex nutrients than in their absence. However it is doubtful whether the two media used for comparison are indeed comparable in the effect of these nutrients, since the salt concentrations in the two media were so vastly different; that of the salt-glucose medium being 70-fold that of the nutrient broth. Although they draw attention to the influence of electrolytes on the action of streptomycin, this influence is mentioned only to be dismissed. The addition of 3000 μg./l. of Difco proteose peptone to a salt-glucose medium, similar to that of Davis and Sevag[18], increases the bactericidal concentration of dihydrostreptomycin 4-fold when the inoculum used is the smallest

capable of growth in the test medium. Indeed the addition of 500 μg./l. of this peptone to the salt-glucose medium enables an inoculum 10^3 times smaller to withstand a standard concentration of dihydrostreptomycin. This same concentration of peptone has a trifling effect on growth[21].

The amino acids methionine, cysteine, tyrosine, proline and aspartic acid are antagonistic towards streptomycin[19,22]. Except for cysteine, the mechanism of antagonism is not known although they may be used by the organism in alternative metabolic paths.

Nucleoproteins and Nucleic Acids

Precipitable complexes are formed between streptomycin and cell constituents[23,24], proteins and nucleoproteins[25], dexoxyribosenucleic acid[26,27] and ribosenucleic acid[28]. There is general agreement about the formation of a streptomycin-desoxyribosenucleic acid conjugate but reports vary on the formation of a streptomycin-ribosenucleic acid complex. Whether the conjugation of streptomycin with nucleic acids plays any part in the mode of action of streptomycin is debatable[25,29] although there is no real evidence that will strictly eliminate it[30].

Other Substances

It has been reported that a number of purines[31] and phospholipids, in particular lipositol, antagonise streptomycin[32]. Neither finding has been substantiated[8,9,33].

Salts

The addition of many salts commonly used in culture media reduces the activity of streptomycin and the degree of reduction is dependent upon the salt concentration[1,21]. Although Berkman, and colleagues[1,25] suggest a desorption of the streptomycin from the bacterial cell surface to be the most likely explanation, Wasserman, Lessner and West[34] believe the action to be more subtle when they state "the salts reversing the antibiotic injury are physiologically active rather than acting solely in a physical manner".

The effect of certain salts, for example, phosphates, might well be due to their influence on the pH of the medium. However the effect of ions like the phosphate ion can be readily distinguished from that of the hydrogen ion. At any fixed pH the activity of streptomycin in fluid media is inversely proportional to the phosphate concentration[21,29].

The inhibitory effect of a salt is an expression of the effect of the constituent ions, and where both ions of a salt show interference their effect may be additive[29].

Glucose

The antagonistic action of glucose and certain other sugars towards streptomycin[4,35,36] has been attributed to a lowering of the pH of the medium, as a direct result of the production of acidic products of carbohydrate metabolism; to the reducing character of glucose; and to its growth promoting properties. However Sykes and Lumb[37] state that

"the effect of glucose on the apparent potency of streptomycin depends on the organism, and the culture medium in the assay. It seems doubtful, therefore, that acid production by the organism or reducing action is solely responsible for the phenomenon encountered".

Reducing Substances other than Glucose

Bondi, Dietz and Spaulding[38] were the first to draw attention to the inhibitory effect of reducing agents on the activity of streptomycin, although Waksman and Schatz[35] mention the inhibitory action of thioglycollate. Their investigations included observations with cysteine, sodium thioglycollate, stannous chloride, sodium bisulphite, sodium formate and sodium thiosulphate. Of these, cysteine was the most active. Seeing a parallel between the effect of reducing substances and anaerobiosis, they postulated an interference by the former with the action of streptomycin on an oxidative enzyme system rather than a direct effect on the drug itself.

Much work has been carried out on the effect of sulphydryl compounds on the action of antibiotics. Cavalitto[39] and Bailey and Cavalitto[40] have shown that streptomycin, although reacting slowly with sulphydryl groups, does so much more readily with those sulphydryl compounds containing basic amino groups in the vicinity of the –SH group. Occurrence of a reaction is dependent upon there being an unsubstituted sulphydryl group and the affinity is approximately proportional to the proximity of a basic amino group. This scale of relative affinities explains why the inactivation of streptomycin by cysteine is exhibited only when the antibiotic and inhibitor are brought into contact before assay. When cysteine is used as an antagonist of streptomycin already exposed to the bacterial cell the greater "attraction" of the polypeptide –SH groups and neighbouring $-NH_2$ groups of the cell or its enzymes overwhelms the "attraction" of the cysteine –SH group.

Cysteine does not, however, inactivate the reduced form of streptomycin, dihydrostreptomycin[41]. Gray and Birkeland[41] interpreted this to indicate that the aldehyde group was not necessary to the action of streptomycin for it is just this group that is reduced to give dihydrostreptomycin. Bailey and Cavalitto[42], examining the effect of cysteine on the two forms of the antibiotic, found that when susceptible bacteria were grown in media containing either form in the presence of cysteine antibiotic activity was neutralised. They postulated that the organisms oxidise dihydrostreptomycin to streptomycin which is then inactivated by the cysteine in the medium. In view of the findings reported in the previous paragraph this inactivation must take place before the streptomycin becomes "attached" to the enzyme or enzymes of the cell.

The role of free sulphydryl groups in enzyme and cell activity is well established. Nevertheless, Henry and colleagues[43] working with purified preparations of enzymes requiring free sulphydryl groups obtained no inhibition of their activity with streptomycin. Mamelek and Quastel[44] drew attention to the role of –SH groups in the metabolism of anaerobes. The failure of streptomycin to inhibit the activity of such organisms[2,45] lends support to the work of Henry and colleagues[43].

In conclusion, therefore, it is evident that with our present state of knowledge a complete assessment of the role of cysteine in streptomycin antagonism is impossible.

Oxidising Substances

Certain oxidising agents, in particular potassium permanganate and potassium periodate destroy streptomycin[46].

Ketone Reagents

Streptomycin[4] but not dihydrostreptomycin, in which the aldehyde group has been reduced[3], is completely inactivated by a variety of ketone reagents especially hydroxylamine and semicarbazide.

Organic Acids

Green, Iverson and Waksman[47] investigated the effect of organic compounds, in particular acids, on the activity of streptomycin in the presence of different sources of nitrogen. Those substances showing antagonism did not effect the potency of the antibiotic. Of the organic acids tested pyruvate and fumarate exerted the greatest effect; a second group, succinate, formate, maleate and malonate were less antagonistic and a third group consisting of lactose and glucose, sodium glycerophosphate, lactate, propionate and acetate had no effect. It was only in media with complex nitrogenous sources that these substances showed any real effect, suggesting to the authors "that the complex nitrogenous materials contained some substance which was necessary for the utilisation of the pyruvate or fumarate in the presence of streptomycin". The work of English and McCoy[48] with *M. pyogenes* var. *aureus* suggests that this substance may well have been biotin. An alternative explanation for their findings is that streptomycin primarily affects the synthetic processes of the cell. In a simple medium the inhibitory action of pyruvate and fumarate, participants in the streptomycin-sensitive oxidative mechanisms, is thereby masked.

Hydrogen Ion Concentration

To the alkaline side of neutrality decrease in the hydrogen ion concentration has little effect on the activity of streptomycin[49] but with increasing acidity its efficiency is reduced[3,21,35]. From a theoretical consideration of the effect of the hydrogen ion concentration on the activity of streptomycin Abraham and Duthie[3] and Eagle, Levy and Fleischman[49] concluded that it was the cationic form of the drug which was active. They differed about the site of action; Abraham and Duthie[3] favouring a surface, Eagle and colleagues[49] an intracellular site.

Gaseous Environment

Streptomycin is less effective under anaerobic conditions[2,4,21,39,43,50,51] and unless the medium is heavily buffered[21] the gas replacing the air may have a profound effect[4]. This is particularly noticeable with CO_2 which also lowers the pH of the medium. The reduced activity of streptomycin

in the absence of air may well be linked with a slower rate of growth of the test organism. Hurowitz and colleagues[52] found aerated cultures of *E. coli* B, having a growth rate approximately three times that of unaerated cultures, to be three times as sensitive to dihydrostreptomycin as unaerated cultures.

Finally streptomycin is unique among the antibiotics in general clinical use in being more effective against facultative anaerobes growing aerobically than against the same organisms growing anaerobically[2,21]. In this context it is of interest that streptomycin, unlike the other antibiotics, is inactive against the strict anaerobes[2,21,45].

The Present State of Knowledge on the Mode of Action of Streptomycin

Bacteriostatic and Bactericidal Action of Streptomycin

Under standard cultural conditions the antibacterial effects of streptomycin are dependent upon three factors, namely, its concentration, the size of the inoculum and the length of time of exposure of the cells to the antibiotic. The effect of the size of the inoculum has already been discussed.

With fixed inoculum size and time of exposure the effects of streptomycin are dependent upon the concentration of the antibiotic to which the organisms are exposed. These effects can be conveniently divided into three groups, each increasing with increasing streptomycin concentration. In sub-inhibitory concentrations streptomycin may alter the morphology of susceptible bacteria[45,53,54]. Minimum inhibitory concentrations produce bacteriostasis, and maximum concentrations are bactericidal[35].

With a given inoculum the bacteriostatic concentration of streptomycin can become bactericidal when the length of incubation is sufficiently prolonged (Smith and Waksman[55] for *Mycobacterium tuberculosis*; Hall and Spink[51] for *Brucella abortus*). However, Costil and Canlorbe[56] reported that the minimum bactericidal dose for *Myco. tuberculosis* varied widely and bore no relation to the amount required for bacteriostasis.

Comparison of the Effect of Streptomycin upon Resting Bacteria with that upon Growing Organisms

The concentration of streptomycin necessary to reduce the viable count of a resting-cell suspension is far greater than that necessary to sterilise a growing culture[6,53,57].

Although bacteria growing in the presence of streptomycin develop resistance, no such resistance is demonstrable in cells exposed, in the resting state, to the action of the antibiotic[6,53,58]. Does this suggest streptomycin interference with nucleic acid or protein (enzyme) synthesis?

The Action of Streptomycin on Cellular Metabolism

In any attempt to disentangle the mass of published data on the mode of action of streptomycin upon bacterial metabolism it is essential to pay attention to the organism used for each investigation, as this has a very

great bearing upon the effect produced. Most of the work has been carried out using *E. coli* and when the results obtained with this and other Gram-negative bacilli are carefully assessed a rational picture is forthcoming.

It is fundamental to any coherent argument on the mode of action of streptomycin to remember that synthesis is dependent upon either oxidation or fermentation for its energy. The action of the drug could conceivably take place in two ways; it might be catastrophic and inhibit oxidative or fermentative energy-producing mechanisms first and foremost, or it might be less drastic and interfere with synthesis at concentrations less than those inhibiting the energy-producing mechanisms. The synthesising activities of the bacterial cell are greatest during growth, they are least or non-existent in "resting-cell" suspensions metabolising a fixed substrate, for example, oxidising pyruvate. Streptomycin is effective against growing organisms which are actively engaged in synthesis, in concentrations less than those inhibiting the oxidation of fixed substrates. For example, Oginsky, Smith and Umbreit[59] found that the growth of the "Gratia" strain of *E. coli* was inhibited by 9 μg. streptomycin/ml. whereas 20 μg. streptomycin/ml. gave only a 60 per cent inhibition of the oxidation of a mixture of oxalacetate and pyruvate by a "resting cell" suspension of the same organism.

Clifton[60] postulated that substances which both increased oxygen uptake and interfered with cellular metabolism exerted their effects by inhibiting the synthesis of cell components from the available substrate. Such cell components must include proteins, nucleic acids and the constituents of both. Interference with the synthesis of these complex compounds can take two forms, the prevention of assimilation of their components, for example, amino acids, when supplied in the medium, or the inhibition of their synthesis from simpler substances. The problem then is to enquire what evidence exists for (*a*) increased oxygen consumption and (*b*) either impaired assimilation of nitrogen, amino acids and purines or interference with the synthesis of amino acids, purines and those substances of which these are the units; or a combination of both.

Increased oxygen uptake by "resting cell" suspensions of *Eberthella typhosa* and *E. coli* when metabolising glucose, intermediates of the di- and tricarboxylic acid cycles, glycerol and glutamate in the presence of streptomycin has been observed[61–63]. It is only fair to mention that the results reported for glucose are at variance with one another. This stimulation of the oxygen consumption of *E. coli* when metabolising glycerol[62] and glutamate[63] has led to the suggestion that streptomycin can uncouple the oxidative assimilatory processes from oxidative dissimilation—a dinitrophenol-like effect.

Having the prerequisite streptomycin interference with the coupled oxidative assimilatory-dissimilation mechanism, what further evidence is there for disturbed nitrogen metabolism? Bernheim and DeTurk[64,65] reported that streptomycin interfered with nitrogen (NH_3) assimilation by *Ps. aeruginosa* during the oxidation of succinate and other components of the tricarboxylic acid cycle. Working at different pH values they were

able to observe inhibition of ammonia assimilation without concomitant inhibition of the oxidation of the components of the tricarboxylic acid cycle. Again, Rosenblum and Bryson[66], examining the action of streptomycin in relation to the rates of metabolism in *E. coli*, were led to suggest that "the inhibitory mechanism may be more closely related to nitrogen assimilation than to respiration". That streptomycin may interfere with amino acid synthesis is shown by the reports that, with *E. coli*, methionine, cysteine, tyrosine, proline and aspartic acid are inhibitory to the action of the drug[19,22]. Indeed the emergence of resistance in a salt-glucose medium was linked with the ability of the cells to dispense with the necessity for cysteine, proline and aspartic acid although increased growth was obtained in the presence of these three amino acids together with serine, glutamic acid and arginine[22]. Finally, Williamson[21] examined the bactericidal action of dihydrostreptomycin on *Aerobacter aerogenes* growing in a salt-glucose medium and in a peptone-glucose medium, both of which were adequately buffered with phosphate, and compared the effect obtained under aerobic and anaerobic conditions. The results showed that dihydrostreptomycin was more active and was unaffected by the atmosphere when tested against this organism growing in the simpler medium. The activity of dihydrostreptomycin has been related to the rate of growth[52] which itself is related to the degree of oxygen tension[67]. If this is so then the action of dihydrostreptomycin in a synthetic medium would appear to be unrelated to these two factors and possibly be more closely associated with nitrogen assimilation or synthesis. It is to be noted that the addition of 500 μg. peptone/l. to the salt-glucose medium enabled an inoculum 10^3 times smaller to withstand a standard concentration of the antibiotic. This same concentration of peptone had a trifling effect on growth.

Interference with amino acid synthesis may be at enzyme level or, possibly at nucleic acid level, for the latter have been considered as templates for enzyme synthesis[68]. The inhibition of streptomycin by sulphydryl compounds[39,40] might suggest its site of action to be at enzyme level. However, Henry and colleagues[43] working with purified enzyme preparations, one of which succinic dehydrogenase, depends upon the integrity of its –SH groups, obtained no inhibition of their activity with streptomycin.

Precipitable complexes are formed between nucleic acids and streptomycin and although the picture is far from clear "we cannot critically eliminate this combining power of streptomycin as a factor in its action"[30]. Indeed, Pratt and Dufrenoy[69] go so far as to state "evidence in the literature suggests that the bacteriostatic effect of streptomycin follows the formation of a ribonucleic acid-streptomycin complex or salt which is not amenable to enzymatic degradation". Further support for an interference at nucleic acid level, accepting the template theory, is provided by the work of Fitzgerald and Bernheim[70] who submitted evidence to suggest that streptomycin prevented adaptive enzyme formation in non-pathogenic sensitive mycobacteria. Being unable to demonstrate adaptive enzyme formation in sensitive virulent mycobacteria they were guarded in their final opinion when they stated "it is probable therefore that the inhibition of adaptive enzyme formation is only one aspect of the

mechanism of streptomycin action"[71]. Vignais and Vignais[72] brought evidence to suggest that streptomycin interfered with the synthesis of enzyme systems concerned in the oxidation of acetate by *E. coli*. Finally, an increase in the ribosenucleic acid content of *Haemophilus pertussis* and *M. pyogenes* var. *aureus* resistant to streptomycin was noted by Smolens and Vogt[73]. This observation is of added interest when it is remembered that ribosenucleic acid controls growth rate and that the activity of streptomycin is proportional to the rate of growth[52]. That desoxyribosenucleic acid interference may be the basis of resistant mutation is indicated by the production of a resistant strain of *Haemophilus influenzae* from a sensitive strain by brief exposure to a desoxyribosenucleic acid-containing extract from another streptomycin-resistant strain[74].

This tracing of the mode of action of streptomycin back to beyond the energy producing mechanisms to the nucleic acid templates from which the enzymes necessary to synthetic processes have their beginnings is based upon some very fragmentary evidence. Nevertheless it may offer some explanation for the discrepancy between growth inhibitory experiments and metabolic investigations. But it must be remembered that it is from the latter that we have obtained the clearest picture of at least one action of streptomycin—its interference with carbohydrate metabolism.

Henry and colleagues[43,75] were the first to report that bacteriostatic concentrations of streptomycin non-competitively inhibit the oxidation of certain carbohydrate intermediates in susceptible strains of *M. pyogenes* var. *aureus*, *Bacillus cereus* and *Shigella sonnei*. Except with *B. cereus* metabolising acetate, this inhibition is never complete. This would indicate "either that some intermediate reaction is only partially blocked or that the step blocked is in parallel with others not affected". With inhibition there was a concomitant accumulation of acetate which could result from either mechanism. Limited interference with the anaerobic metabolism of *B. cereus* and *Sh. sonnei* was also noted. Geiger[76], working with *E. coli*, noted that streptomycin interfered with amino acid oxidation. This, in turn, was dependent upon a product of the oxidation of a number of carbon compounds all of which are associated with the metabolism of glucose.

Umbreit[77] put a different interpretation on the findings of Geiger[76], suggesting that streptomycin might interfere with the formation of the intermediate upon which depended the oxidation of the amino acids. However his own experimental work as a direct result of this concept, led him to the conclusion that streptomycin prevented the condensation of the intermediate (oxalacetate) with the keto acid produced as the result of the deamination of the amino acid.

In a series of extensive investigations Oginsky, Smith and Umbreit[59,78,79], have clarified the position about the role of streptomycin in the inhibition of the oxidation of pyruvate and oxalacetate by *E. coli*, strains "Gratia" and "Murray". In those cells capable of oxidising acetate *per se* (i.e., aerated cells), no streptomycin inhibition of the oxidation of pyruvate and oxalacetate, either singly or in combination, was demonstrable. However in cells not oxidising acetate (i.e., cells grown in deep

culture) inhibition of the oxidation of these carboxylic acids, either singly or in combination, did occur. Nevertheless, neither citrate nor *cis*-aconitate formation was affected. Hence streptomycin inhibition is best seen in those cells where the major portion of pyruvate cannot be directed through acetate oxidation, and must of necessity be metabolised by the streptomycin-sensitive mechanism. Under these circumstances accumulation of acetate occurs[43]. Umbreit and colleagues[79] studied citrate formation by the streptomycin-sensitive "Gratia" strain of *E. coli* and by one of its resistant variants. In the presence of streptomycin neither the sensitive parent nor its resistant variant exhibited any reduction in citrate formation, although pyruvate and oxalacetate oxidation was inhibited in the sensitive parent strain but unaffected in the resistant variant. From these observations Umbreit and colleagues[79] were led to postulate a pathway other than acetate-oxalacetate condensation as a means of entry for pyruvate into the terminal respiratory system. They concluded by stating "a pyruvate-oxalacetate reaction not forming citrate must be postulated to account for the results obtained, and it is this reaction that is inhibited by streptomycin".

In a further communication Umbreit[80] reported the isolation and identification of a seven—C condensation compound of pyruvate and oxalacetate, 2-phospho-4-hydroxy-4-carboxy-adipic acid whose formation is inhibited markedly by streptomycin. He was, however, guarded in his conclusions about its role in cellular metabolism.

Smith and colleagues[78] extended these investigations to include resistant and dependent variants of *E. coli*, strains "Gratia" and "Murray". Neither variant possesses the ability to effect the pyruvate-oxalacetate condensation. They have been able to eliminate the streptomycin-sensitive reaction and, possibly concomitantly, to develop a by-pass mechanism for end-products metabolised by the "pyruvate-oxalacetate reaction".

In *Myco. tuberculosis*, avian type, Kirchberg strain, the oxidation of the higher fatty acids is partially inhibited by streptomycin. The site of inhibition appears to be the oxidation of the breakdown-products of the fatty acids rather than the acids themselves. A resistant variant also oxidised the fatty acids but without comparable streptomycin inhibition. The antibiotic also inhibited stearate oxidation by *E. coli*. In this organism the oxidation proceeds to completion presumably by a pathway similar to the tricarboxylic acid cycle. Again, a resistant variant showed loss of the major portion of the oxidative mechanism for higher fatty acids[81].

Finally Umbreit and Tonhazy[82] examined the effect of streptomycin on animal tissues. When the permeability barriers at the cell wall and mitochondria were overcome they found that the antibiotic inhibited the pyruvate-oxalacetate condensation. In the intact animal these permeability barriers prevent streptomycin from attacking this condensation.

Besides the effect of streptomycin on the oxidation of carbohydrates, in particular pyruvate, Barkulis[83], working with *E. coli* strain N, demonstrated strong inhibition of the phosphoclastic split of pyruvate into

formate and acetate, the main pathway by which this strain fermented pyruvate. This inhibition of fermentation occurred only when the biological system contained bicarbonate and an active oxalacetate decarboxylase[84]. The antibiotic also affected other fermentation pathways. Both a resistant and dependent mutant fermented pyruvate slowly and, as with the oxidative mechanism, streptomycin failed to exhibit any inhibition. Zebovitz and Moulder[85] confirmed the conditions necessary for streptomycin inhibition of fermentation and suggested that two competitive enzyme systems are present in *E. coli* for the anaerobic breakdown of pyruvate; one streptomycin resistant, deteriorating with age (? oxalacetate decarboxylase—reviewer) and inhibited by CO_2; the other streptomycin sensitive (? phosphoclastic mechanism—reviewer), stable and unaffected by CO_2. Hence in freshly harvested cells streptomycin inhibition necessitates CO_2 or bicarbonate in the system thus driving fermentation via the streptomycin-sensitive route; aged cells having lost this system exhibit streptomycin inhibition of fermentation under all conditions.

Many are the sites at which the cell is vulnerable to the action of streptomycin and those of importance in the Gram-negative bacilli can now be summarised. They are listed in order of decreasing vulnerability and in doing so the writer is well aware of the flimsy evidence for some of the steps.

1. Streptomycin-nucleic acid complex formation thereby destroying the template for protein synthesis suggested by the impaired adaptive enzyme formation. Interference with nucleic acid control of cellular metabolism is seen also in the relation between cellular response to streptomycin inhibition and growth rate.
2. Inhibition of protein and amino acid synthesis. Inhibition of nitrogen assimilation. Streptomycin -SH group interference with enzymatic activity.
3. Uncoupling of oxidative assimilatory-dissimilation processes, that is, the transfer of energy made available by the energy-producing systems to the energy-requiring mechanisms.
4. Inhibition of energy-producing oxidative and fermentative mechanisms.

It will be observed that certain reported observations, for example, the interference with pantothenate synthesis[86,87], and the inhibition of diamine oxidase[88], have been omitted; that little or no mention has been made of the action of streptomycin on Gram-positive organisms. Both omissions are intentional. A reasoned appraisal of the literature, permitting a concise picture to be drawn, is possible only with *E. coli* and other Gram-negative bacilli. If believing that the mode of action of a drug should be specific and different from any other drug[89] then the broad basis upon which this picture has been drawn may not be acceptable. In answer to this criticism the reviewer suggests that "mode of action" must take into account all aspects of that action however common they may be.

This account of the mode of action of streptomycin may give a sense of completeness to our knowledge. In that, it is false for there are many links in the chain of argument which require strengthening. This can

come only from further work. Little, beyond the proved efficacy of streptomycin as an antituberculous agent, is known about the mode of action of this antibiotic upon *Myco. tuberculosis.* Until the metabolism of the Mycobacteria is more fully explored it would seem that this state of affairs is not likely to be remedied. The activity of streptomycin is closely linked with the rate of growth and the rate of metabolism. The obvious correlation is ribosenucleic acid yet the reported studies on streptomycin and nucleic acids are contradictory. Perhaps along with the resolution of nucleic acid metabolism will go the solution of this problem. That different rates of growth may explain the different levels of sensitivity of facultative anaerobes growing in various concentrations of air or oxygen; growth rate will not explain the resistance of the strict anaerobes. These organisms must lack the essential streptomycin-sensitive system or systems. What those systems are we do not know. Is the rapid development of resistance associated with streptomycin interference with desoxyribose-nucleic acid as suggested by the work of Alexander and Leidy[74]? Our greatest knowledge of the action of streptomycin comes from the study of its effects on carbohydrate metabolism; nevertheless, it has led us into unknown fields of metabolism. Until the effect of CO_2 on pyruvate fermentation and the role of 2-phospho-4-hydroxy-4-carboxy-adipic acid in pyruvate oxidation are more fully understood progress in this field will be hampered. Can the streptomycin antagonists explain the failure of the drug to combat organisms sensitive to streptomycin *in vitro?* Such are some of the problems to which, as yet, no answer is forthcoming.

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RESEARCH PAPERS

ANTITUSSIVE AND OTHER PHARMACOLOGICAL PROPERTIES OF THE DIETHYLAMINOETHOXYETHYL ESTER OF αα-DIETHYLPHENYLACETIC ACID, (OXELADIN)

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The diethylaminoethoxyethyl ester of αα-diethylphenylacetic acid (oxeladin) appears to possess similar antitussive activity to carbetapentane and to be slightly less active than codeine phosphate. Sensory nerve endings in the trachea and bronchial tree are not the site of action. The acute toxicity is not significantly different from that of carbetapentane. The ester is two to four times as toxic as codeine phosphate, according to species and route of administration. It has local anaesthetic properties, being approximately twice as active as procaine hydrochloride by the guinea pig intracutaneous wheal test. Only excessive amounts appear to have a constipating effect in normal mice. A spasmolytic effect has been demonstrated with the isolated guinea pig ileum and isolated rabbit duodenum. It has, however, a spasmogenic effect on the jejunum of the anaesthetised cat. Intravenous injection in the anaesthetised cat produced a transient fall in blood pressure of the same magnitude as that produced by codeine phosphate. It has been administered to rats over a prolonged period with no untoward effect on the growth rate and the haemopoietic system.

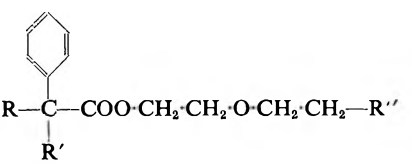
THE cough reflex serves a useful purpose in removing irritants from the larger bronchi and upper air passages. Frequently, however, coughing is due to hyper-excitability of the throat and bronchi and this results in a dry, unproductive cough causing considerable distress. A useless cough of this nature is usually treated with one of the opium alkaloids or their derivatives which have a depressant action on the cough centre in the medulla. They have the disadvantages that in addition to a liability to cause addiction, they have a respiratory depressant action, produce drowsiness and cause constipation.

The search for a non-opiate type of antitussive is hindered by the lack of a completely satisfactory method for testing new compounds, as evidenced by the multiplicity of methods used in recent years. These have differed in the means used to elicit coughing, in the species employed and according to whether the animal was conscious or anaesthetised. Winter and Flataker[1] induced coughing in conscious dogs by means of an aerosol of sulphuric acid, and in a later paper[2] they described the exposure of conscious dogs and guinea pigs to an aerosol of ammonia solution. The direct introduction of sulphur dioxide into the trachea of the anaesthetised cat by May and Widdicombe[3] and Green and Ward[4] and the guinea pig[4] has also been employed. Mechanical stimulation of the trachea has been carried out in the anaesthetised cat[3,4] and also in the

anaesthetised dog and guinea pig[4]. Finally, various forms of electrical stimulation have also been used; Stefko and Benson[5] used electrodes embedded in the trachea of conscious dogs and Chakravarty, Matallana, Jensen and Borison[6] produced coughing by electrical stimulation of the medulla of the decerebrate cat. The most widely used method, has been that of Domenjoz[7] (see refs. 4, 8, 9), involving electrical stimulation of the superior laryngeal nerve of the anaesthetised cat.

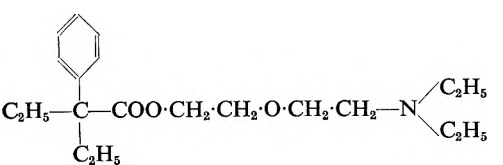
We used this latter method in our investigations for a number of reasons. It is easy and two compounds can be compared at several dose levels in the same animal, the cough being reproducible over a prolonged period. In addition, Green and Ward[4] found that the experimental cough produced by stimulation of the superior laryngeal nerve responded to various drugs in the same way as cough caused by mechanical and chemical irritation of the bronchial system. They concluded that the same afferent fibres are probably involved. They also compared the results of others with their own and found that, in general, the activities of recognised antitussives did not vary to any great extent either with the stimulus used for initiating the reflex or with the species and that results obtained in lightly anaesthetised animals are similar to those in conscious animals. The method is also of value since stimulation of the central cut end of the superior laryngeal nerve excludes any compound having a purely local action on the sensory nerve endings in the respiratory tract.

Twelve compounds, prepared by Dr. V. Petrow and his colleagues of the B.D.H. Chemical Research Department, have been examined for antitussive activity. These may be considered to be derived from the ethoxyethyl ester of phenylacetic acid and have the general formula I.



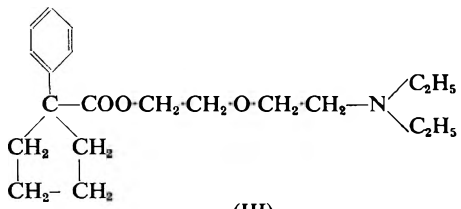
(I)

The diethylaminoethoxyethyl ester of αα-diethylphenylacetic acid (II, oxeladin, appeared the most promising in preliminary studies and was selected for further investigation. A related compound, the diethylaminoethoxyethyl ester of 1-phenyl-1-*cyclo*pentane carboxylic acid (III, carbetapentane), also possesses antitussive properties[9] and was used as a reference compound.



(II)

Oxeladin



(III)

Carbetapentane

Comparisons with codeine phosphate were also made.

Oxeladin has a molecular weight of 335. The free base is a yellow, slightly viscous fluid with an acrid odour and a bitter, aromatic flavour. It is soluble in dilute hydrochloric acid, ethanol and acetone but is insoluble in water. It is stable in acids but is unstable in alkalis. It is non-hygroscopic and is stable up to 150°.

METHODS

Antitussive Activity

Male and female cats weighing 2·8 to 4·4 kg. were anaesthetised with 5 per cent sodium pentobarbitone, 25 to 90 mg./kg., injected intraperitoneally. The anaesthetic was administered in divided doses until an adequate yet light anaesthesia was obtained. The right or left superior laryngeal nerve was identified by means of its sinuosity ventral to the carotid artery at the level of the larynx. The nerve was dissected and, in the earlier experiments, placed intact over platinum electrodes of the open-jaw type. In later experiments, the nerve was cut close to the larynx and the central end laid across the electrodes. This avoided muscle twitches around the larynx due to stimulation of efferent nerve fibres. More recently, platinum electrodes described by Bülbring[10] for the rat phrenic nerve-diaphragm preparation were used to stimulate the cut end of the nerve which was kept moist with normal saline. These electrodes gave better contact. The trachea was cannulated in the majority of experiments and although the larynx, vocal cords and glottis could not participate in the cough response, auditory recognition of a cough was possible. Diaphragmatic movements were recorded on smoked paper by an isometric lever attached by a thread to the abdominal wall just caudal to the xiphisternum. The animal was then left undisturbed for at least forty-five minutes to lighten the degree of anaesthesia. Thereupon, the nerve was stimulated for periods of five seconds with an electronic stimulator (Newton Victor Ltd.), the pulses being of 10 m. sec. duration and at a frequency of five per second. The stimulus intensity required to elicit a reproducible cough was determined and was found to be within the range 0·5 to 4 volts. Stimuli of this intensity were applied for five seconds at five minute intervals throughout the remainder of the experiment. The compounds were injected via the cannulated femoral vein at times halfway between two successive stimuli. At least three successive satisfactory cough responses were obtained before any dose

was injected. The effects of two compounds were usually compared at several dose levels in each animal, the doses being injected in a random order. Oxeladin and carbetapentane were injected in 5 per cent acacia and codeine phosphate in aqueous solution.

Acute Toxicity

Intravenous toxicity was estimated in male albino mice weighing approximately 20 g. Oxeladin and carbetapentane were emulsified in 5 per cent acacia and codeine phosphate was dissolved in distilled water. The volume administered was adjusted to 0·2 ml./20 g. weight. Each compound was examined at three or four dose levels which increased in geometrical progression by a factor of 4/3. Oral toxicity was estimated in male albino mice weighing approximately 20 g. and in female albino rats weighing approximately 120 g. The animals were starved overnight before use. The volume administered was adjusted to 0·5 ml./20 g. weight for both mice and rats. Each compound was examined at four or five dose levels increasing by a factor of 7/5 for mice and 4/3 for rats. Ten animals were used at each dose level in all experiments. LD50s were calculated by the method of Litchfield and Wilcoxon[11] from the mortalities in seven days.

Subacute Oral Toxicity

This was investigated in immature male albino rats. The compounds were administered by stomach tube five days a week for eleven weeks. Each compound was given at three dose levels (7, 20 and 60 mg./kg. weight/day) such that the daily dose corresponded to approximately 5, 15 and 45 per cent of the acute oral LD50 in mice. The animals were weighed at weekly intervals and the doses were related to the individual body weight at the beginning of each week. All volumes were adjusted to 25 ml./kg. weight. Ten animals were used at each dose level and a further group of ten animals served as controls; these were given only the vehicle, 5 per cent acacia. The animals were kept under identical conditions and were allowed Diet 41 and water *ad lib.* At the end of the experimental period haemoglobin estimations, total red and white cell counts and differential white cell counts were made on the control animals and the survivors on the high dose of each compound. Histological examination of liver, spleen, kidney, lung and stomach from three animals in these groups was also made.

Local Anaesthetic Activity

This was measured in shaved guinea pigs using the intracutaneous wheal method of Bülbring and Wajda[12]. The animals were divided into three groups of eight given either high, medium or low concentrations of the compounds. Four animals from each group were injected with the citrate salt of oxeladin in the anterior part and with procaine hydrochloride in the posterior part of the shaved area. The positions of the compounds were reversed in the remaining animals of the group. The order of injections was unknown to the observer. Each dose was injected

intracutaneously in the mid line in 0·2 ml. of normal saline. The resultant wheal was outlined in ink and the response to six pin pricks within this area was determined five minutes after injection and then at five minute intervals for thirty minutes. The number of negative responses to the total of 36 stimuli was recorded. The mean of the eight results for each concentration was estimated and plotted against the log of the concentration.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Segments of rabbit duodenum were suspended in Ringer-Locke solution in a 70 ml. bath maintained at a temperature of 37°. A mixture of oxygen and 5 per cent carbon dioxide was bubbled through the solution. Normal rhythmic contractions were obtained and varying volumes of 0·1 and 1·0 per cent solutions of the water-soluble citrate salt of oxeladin were added at intervals. The compound was allowed to act for two minutes before washing out.

Isolated guinea pig ileum. Spasmolytic activity was estimated using the isolated guinea pig ileum. A 4 cm. segment was suspended in Ringer-Locke solution in a 25 ml. bath maintained at 37°. A mixture of oxygen and 5 per cent carbon dioxide was bubbled through the solution. Sub-maximal doses of acetylcholine (0·3 μg.) were added at three minute intervals and allowed to act for thirty seconds before washing out. Varying amounts of methantheline bromide and the citrate salt of oxeladin were added thirty seconds before the addition of acetylcholine. At least three normal responses to acetylcholine were obtained between doses of the spasmolytics. The heights of the contractions immediately before and after the addition of the spasmolytic were measured. The inhibition per cent was plotted against log dose and the amount causing 50 per cent inhibition calculated.

Spasmolytic activity was also estimated against a barium chloride spasm. The guinea pig ileum was suspended in oxygenated Tyrode solution and sub-maximal doses of barium chloride (2·0 mg.) were allowed to act for 45 seconds at three minute intervals. Varying amounts of papaverine hydrochloride and the citrate salt of oxeladin were added one minute before the addition of barium chloride.

Defaecation. The method was based on that described by Lou[13] for the assay of vegetable purgatives. Male albino mice weighing approximately 20 g. were divided into four groups of nine animals. Three groups were given varying amounts of oxeladin corresponding to approximately 5, 15 and 45 per cent of the acute oral LD50 in mice. The compound was administered orally as its citrate salt in aqueous solution, the volume being adjusted to 0·5 ml./20 g. weight. The fourth group served as controls, being given a similar volume of water. Fifteen minutes after administration, the mice were placed in individual compartments over a wire grid and the faeces collected on blotting paper. The fifteen minutes interval was considered advisable as defaecation frequently occurred after the initial handling. The mice were given a paste of diet 41 and water throughout the experiment. The total number

of faecal pellets from each animal was counted at 8 and 24 hours after administration.

Cat Jejunum in situ. The cat was anaesthetised with ether followed by chloralose, 60 mg./kg. intravenously. A water-filled balloon was inserted into the jejunum through an abdominal incision and contractions were recorded by means of a water and air transmission system connected to a small piston recorder. The pressure in the balloon was approximately 10 cm. of water. When spontaneous rhythmic contractions were established, the citrate salt of oxeladin was injected into the cannulated femoral vein.

Effect on Blood Pressure and Respiration of the Anaesthetised Cat

Male and female cats weighing approximately 3 kg. were anaesthetised with ether followed by chloralose, 60–80 mg./kg., intravenously. The carotid arterial blood pressure was recorded by means of a mercury manometer, and 500 units of heparin were injected intravenously to prevent clotting. Respiration was recorded by means of a float recorder connected via uni-directional valves[14] to a tracheal cannula. The compounds were injected into the cannulated femoral vein.

Results

Antitussive Activity

The usual cough response after electrical stimulation of the superior laryngeal nerve consisted of a series of inspiratory gasps culminating in one or more explosive expirations. The sound produced by the violent expiratory component could be readily recognised and was similar to a normal cough, even in those animals with a cannulated trachea. The cough was recorded as a considerable excursion of the isometric lever following movements of smaller magnitude during the inspiratory gasps. The effect of an antitussive compound was, in completely effective doses, to abolish both the inspiratory gasps and the violent expiratory components. The cough sound was consequently absent and there were no movements of the isometric lever. After smaller doses, or sometimes during recovery from a large dose, the violent expiratory component was again abolished but the series of inspiratory gasps was unaltered or

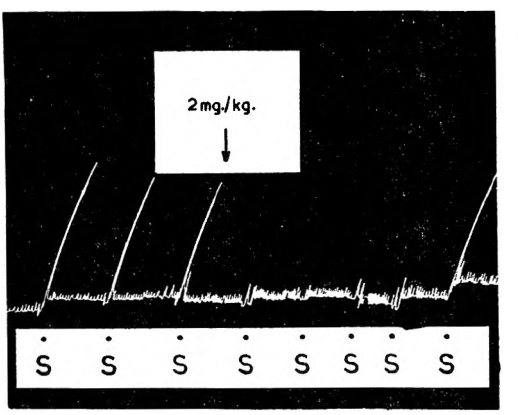


FIG. 1. Effect of intravenous injection of oxeladin at arrow in a 2·9 kg. cat anaesthetised with sodium pentobarbitone, 65 mg./kg. intraperitoneally. Movements of the xiphisternum after electrical stimulation (at S) of the superior laryngeal nerve for 5 seconds at 5 minute intervals.

slightly moderated. The excursions of the isometric lever were therefore markedly reduced but not abolished, whilst the cough sound was absent. Figure 1 shows a typical result, there being no response to the first two stimuli after injection of oxeladin, whilst the third and fourth stimuli resulted in a few inspiratory gasps.

Although oxeladin was compared directly with either codeine phosphate or carbetapentane in each animal, the results are difficult to evaluate because of the variability of the effects within the individual animal. This may be attributed to a changing level of anaesthesia throughout the experiment and possibly to residual effects from previous doses. The kymograph records of each experiment were carefully examined and the effects of each dose evaluated according to two criteria.

TABLE I

APPROXIMATELY EQUI-EFFECTIVE DOSES OF OXELADIN, CODEINE PHOSPHATE AND CARBETAPENTANE AGAINST THE COUGH PRODUCED BY ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN ANAESTHETISED CATS

Experiment	Compound		
	Oxeladin mg./kg.	Codeine phosphate mg./kg.	Carbetapentane mg./kg.
1	2	2	
2	2	1	
3	4	1·5	
4	1	2	
5	2	2	
6	4		4
7	4		2
8	6		8
9		1·5	3
		3	6
10		4	4
11		2	4

These were, intensity of effect, according to whether there was complete suppression of the cough reflex or whether only the expiratory component was prevented; and the duration of effect. The amount of each compound producing approximately the same intensity of effect for approximately the same duration was then noted. The results of this analysis are shown in Table I. It appears that the relative activity of two compounds varies widely in different animals, and this confirms the observation of Toner and Macko[8] who used a similar experimental procedure. They found that in some animals one compound was active whilst another was inactive but that the converse was true in other animals. It is not possible, therefore, to arrive at a strictly quantitative assessment of the relative activities of the three compounds examined. Inspection of the results in Table I indicates that oxeladin and carbetapentane are probably equally active, and that codeine phosphate may be slightly more active.

All three compounds were effective against stimulation of the central cut end of the nerve as well as against stimulation of the intact nerve. The site of action, therefore, would not appear to be on sensory nerve endings in the upper respiratory tract.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Oxeladin had little or no effect on the normal rhythmic contractions of three preparations in amounts below 0·1 mg. Amounts from 0·1 to 0·8 mg. produced varying degrees of reduction in tone associated with some reduction in amplitude of contractions. Larger amounts produced considerable loss of tone and

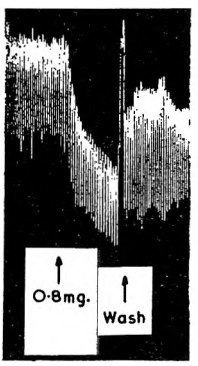


FIG. 3. Effect of oxeladin at arrow (0·8 mg. in 70 ml. bath for 2 minutes) on pendular movements of isolated rabbit duodenum.

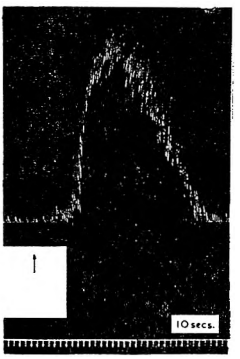


FIG. 4. Effect of intravenous injection of oxeladin at arrow on intact jejunum of 2·7 kg. cat anaesthetised with chloralose, 60 mg./kg. intravenously.

almost total inhibition of contractions. Spontaneous rhythmic contractions were readily resumed on washing-out after amounts up to 0·8 mg., but after larger amounts two or three wash-outs were required. Figure 3 records a typical response.

TABLE V

THE EFFECT OF OXELADIN AND METHANTHELINE BROMIDE ON ACETYLCHOLINE-INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

Compound	Dose µg.	Reduction per cent	Dose causing 50 per cent reduction, µg.
Oxeladin	10 20 40	22 57 91	17·5
Methantheline bromide	0·02 0·04 0·08	27 65 97	0·03

Isolated guinea pig ileum. The spasmolytic activity of oxeladin is recorded in Tables V and VI. It appears that oxeladin has approximately 1/600 of the activity of methantheline bromide against acetylcholine induced contractions and approximately 2·5 times the activity of papaverine hydrochloride against barium chloride.

Defaecation. There was a 50 per cent reduction in the number of faecal pellets passed in eight hours after 200 mg./kg. of oxeladin compared

with the controls. During the following sixteen hours, however, there was no significant reduction in the number of faecal pellets. Oxeladin had no effect at lower doses. The results are recorded in Table VII.

TABLE VI

THE EFFECT OF OXELADIN AND PAPAVERINE HYDROCHLORIDE ON BARIUM CHLORIDE INDUCED CONTRACTIONS OF ISOLATED GUINEA PIG ILEUM

Compound	Dose µg.	Reduction per cent	Dose causing 50 per cent reduction, µg.
Oxeladin	10	22	28
	20	31	
	40	65	
	80	99	
Papaverine hydrochloride	50	34	74
	100	64	
	200	93	

Cat jejunum in situ. In a cat weighing 2·7 kg. anaesthetised with chloralose, 0·5 and 1·0 mg./kg. of oxeladin produced a considerable increase in the tone of the jejunum. The increase was gradual and commenced after a latent period of about one minute. Maximum tone was maintained for a brief period and was followed by a gradual return to the initial level. Figure 4 illustrates a typical result. 0·25 mg./kg. had no effect.

TABLE VII

THE EFFECT OF OXELADIN ON THE NUMBER OF FAECAL PELLETS PASSED BY MALE ALBINO MICE DURING TWO PERIODS AFTER ORAL ADMINISTRATION

Dose mg./kg.	Mean number of faecal pellets per animal	
	0–8 hours	8–24 hours
0	23	76
20	20	85
70	18	76
200	12	71

Effect on Blood Pressure and Respiration of the Anaesthetised Cat

The effect of oxeladin was examined in three cats weighing 2·7 to 3·0 kg. Amounts corresponding to 1·0 and 2·0 mg./kg. caused a rapid fall in blood pressure with recovery within a few minutes, whilst smaller amounts had little or no effect. Equal amounts of codeine phosphate produced effects of similar magnitude. The fall in blood pressure was not abolished by section of both vagi. Oxeladin, 2 mg./kg., caused a slight increase in respiratory minute volume. Figure 5 compares the effects of oxeladin and codeine phosphate in the same animal.

DISCUSSION

It appears that oxeladin is a potent antitussive in the anaesthetised cat. In general, the pharmacological properties which we have examined are similar, both qualitatively and quantitatively, to those of carbetapentane which it resembles structurally. Both compounds are two to four times as toxic as codeine phosphate, according to species and route of administration, and both appear to be only slightly less active than codeine phosphate in suppressing experimental cough in the anaesthetised

Acute Toxicity

The results are recorded in Table II. Similar estimates of LD50 were obtained for oxeladin and carbetapentane in all experiments. They are two to three times as toxic as codeine phosphate after oral administration in mice and rats and are about four times as toxic as codeine phosphate intravenously in mice. Lethal doses of oxeladin and carbetapentane caused the mice to convulse within a few minutes. The convulsions were interrupted by short periods of quiescence during which the

TABLE II

THE ACUTE TOXICITY OF OXELADIN, CODEINE PHOSPHATE AND CARBETAPENTANE. THE MEDIAN LETHAL DOSE ESTIMATED BY THE GRAPHICAL METHOD OF LITCHFIELD AND WILCOXON[11]

Compound	Species	Route of administration	LD50 mg./kg.	Limits of error (P = 0·95), mg./kg.
Oxeladin	Mouse	Intravenous	13	12–15
Codeine phosphate ..			53	43–65
Carbetapentane ..			13	11–15
Oxeladin	Mouse	Oral	130	108–156
Codeine phosphate ..			300	252–357
Carbetapentane ..			130	108–156
Oxeladin	Rat	Oral	183	136–247
Codeine phosphate ..			420	341–517
Carbetapentane ..			150	117–192

TABLE III

THE EFFECT OF CONTINUED ORAL ADMINISTRATION OF OXELADIN AND CARBETAPENTANE ON THE MEAN WEIGHTS OF GROUPS OF TEN MALE ALBINO RATS

Compound	Dose/day mg./kg.	Time in weeks											
		0	1	2	3	4	5	6	7	8	9	10	11
—	—	97	116	132	155	174	195	200	214	226	234	236	243
Oxeladin	7	97	113	132	152	174	187	191	201	213	220	226	234
	20	96	112	134	*156	172	196	208	219	230	235	240	248
	60	96	*113	140	151	171	198	207	219	229	238	241	244
Carbetapentane	7	95	113	133	151	164	183	191	198	213	218	228	234
	20	97	111	132	*150	155	189	198	207	*212	205	212	221
	60	96	111	*119	138	162	184	196	206	210	222	226	232

* One animal died during the preceding seven days.

righting reflex was absent. Gasping respiration, which persisted for several minutes, preceded death. A few minutes after lethal doses of codeine phosphate, the mice showed increased activity with the tail generally held erect. This was followed by convulsions which terminated suddenly in death.

Sub-acute Oral Toxicity

Table III shows the effect of varying doses of oxeladin and carbetapentane on the mean weights of rats during a period of eleven weeks. After four weeks the group given 7 mg./kg./day of oxeladin gained weight somewhat more slowly than did the control group. This does not appear to be due to cumulative toxicity, however, as the animals given 20 and 60 mg./kg./day gained weight at the same rate as the

control group. All three groups given carbetapentane gained weight at somewhat slower rates than the controls. A limited number of deaths occurred during the treatment period as indicated in the Table, but these may have been due to an intercurrent infection. Histological examination of liver, spleen, kidney, lung and stomach from animals given the high

TABLE IV

THE EFFECT OF CONTINUED ORAL ADMINISTRATION OF OXELADIN AND CARBETAPENTANE (60 MG./KG. FIVE DAYS A WEEK FOR ELEVEN WEEKS), ON THE MEAN HAEMATOLOGICAL VALUES OF MALE ALBINO RATS

Compounds	No. of rats	Haemoglobin per cent	Erythrocyte count cells/cu. mm.	Leucocyte count cells/cu. mm.	Lymphocytes per cent	Polymorphs per cent	Monocytes per cent	Eosinophils per cent
—	10	109	7,270,000	17,000	83	14	<2	1
Oxeladin	9	110	7,440,000	17,600	82	17	<1	<2
Carbetapentane	8	110	7,270,000	17,000	84	14	<1	<1

doses of oxeladin and carbetapentane revealed no abnormalities compared with the control group. There were also no significant differences in the haematological values of the animals in these three groups at the end of the treatment period. The mean values are recorded in Table IV.

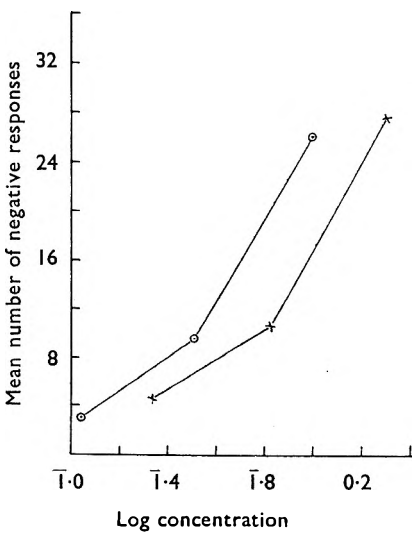


FIG. 2. Local anaesthetic activity of oxeladin (⊙——⊙) and procaine hydrochloride (×——×). Guinea pig intracutaneous wheal test.

Local Anaesthetic Activity

Figure 2 shows the mean number of stimuli giving negative responses plotted against log concentration of oxeladin and procaine hydrochloride. The dose-response line does not appear to be linear over the complete range of concentrations examined, but the two curves are parallel. Thus the relative activities of the compounds can be determined from the concentrations producing 50 per cent anaesthesia. The value for oxeladin was 0·58 per cent and for procaine hydrochloride 1·10 per cent; oxeladin appears to be about twice as active an infiltration local anaesthetic as procaine hydrochloride. It also appears to be absorbed from the conjunctiva as a 1 per cent solution produced local anaesthesia in the rabbit's eye. This was not fully investigated. There was no evidence that the varying concentrations of oxeladin produced local irritation or tissue damage.

cat. Levis, Preat and Moyersoons[9] claimed that carbetapentane is 1·5 times as active as codeine phosphate as an antitussive, but our results have not confirmed this, possibly due to differences in the criteria used for interpreting the results. Oxeladin and carbetapentane also have similar local anaesthetic activity, being two and three times as active, respectively, as procaine by the intracutaneous wheal test in guinea pigs. Finally, the two compounds have similar spasmolytic properties, being weakly active against acetylcholine-induced contractions of the guinea pig ileum and about three times as active as papaverine against barium chloride-induced spasm.

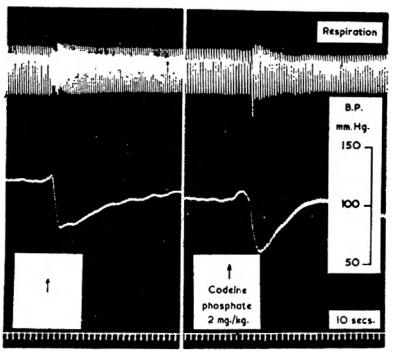


FIG. 5. Effects of intravenous injection of oxeladin at arrow and codeine phosphate on blood pressure and respiration of 3·0 kg. cat anaesthetised with chloralose, 80 mg./kg. intravenously.

As a result of their investigations on a series of compounds, Levis, Preat and Moyersoons[9] concluded that carbetapentane possessed greater antitussive activity than the *cyclo*propane, *cyclo*butane and *cyclo*hexane analogues. It appears from our experiments, however, that the *cyclo*pentane ring is not essential for maximal antitussive activity. Oxeladin differs from carbetapentane in that it is derived from αα-diethylphenylacetic acid instead of from 1-phenyl-1-*cyclo*propane carboxylic acid. Our results indicate that there is no marked alteration in activity consequent upon opening the *cyclo*propane ring.

The daily oral administration of oxeladin for eleven weeks did not appear to produce any untoward effects in immature rats, and no irritant effects have been observed after administration by oral and intracutaneous routes. A weak spasmolytic action was observed with isolated guinea pig ileum and rabbit duodenum preparations but, in contrast, oxeladin had a spasmogenic action on the jejunum of the anaesthetised cat. Only large amounts appeared to cause constipation in normal mice. In view of its efficacy in the suppression of experimentally-induced cough and the absence of undesirable pharmacological effects at therapeutic dose levels, oxeladin has been submitted for clinical trial.

Acknowledgements. The authors thank Professor T. Crawford for carrying out the histological investigation.

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THE ANTICOAGULANT ACTIVITY AND TOXICITY OF LAMINARIN SULPHATE K

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Four samples of sulphated laminarin, with differing degrees of sulphation, and two samples of fucoidin were examined for anticoagulant activity. Laminarin sulphate K, with 1·83 sulphate groups per glucose unit, showed anticoagulant activity about a third as potent as heparin. It did not cause agglutination of rabbit platelets either *in vivo* or *in vitro*, but it proved fatal to rabbits when given intravenously twice daily for periods of seven to twelve days, at a dose approximately three times the clinical dose. It was extremely toxic to guinea pigs in single small intravenous doses and produced the "anaphylactoid" phenomenon associated with some other toxic synthetic anticoagulants. Laminarin sulphate K was too toxic to justify clinical trials.

LAMINARIN is a polysaccharide obtained from *Laminaria cloustoni* frond[1,2]. It is insoluble in cold water, but can be sulphated to produce water-soluble compounds with varying numbers of sulphate groups per glucose unit[3]. Fucoidin is a polysaccharide sulphate from the brown seaweeds and is believed to occur in the intercellular mucilage[4,5].

Four laminarin sulphates, with varying numbers of sulphate groups per glucose unit, and two samples of fucoidin were obtained from the Institute of Seaweed Research, Inveresk, Midlothian, Scotland.

The *in vitro* anticoagulant activity of these compounds was estimated by the sulphated whole-blood method of Adams and Smith[6], and the results are recorded in Table I in terms of the International Heparin Unit. Only laminarin sulphate K, which had the highest sulphur content, was sufficiently active to warrant further examination.

METHODS

Anticoagulant activity in rabbits. Clotting times were measured by the capillary tube technique on samples taken from the ear vein at 0, 15, 30 and 60 minutes, after the intravenous injection of heparin (100 units per mg.) at 1 mg./kg., and laminarin sulphate K at 3 mg./kg. Crossover tests were made with seven rabbits.

Acute intravenous toxicity. The acute intravenous toxicity was estimated in mice and guinea pigs and compared with heparin. Animals were kept for seven days after the injections.

Chronic intravenous toxicity. Rabbits were injected twice daily, on 5 days of the week, with laminarin sulphate K at 12·5 and 15 mg./kg. and heparin at 5 mg./kg. This was approximately three times the estimated clinical dose of the anticoagulants. Animals which died were examined after death.

Platelet counts. The effect of laminarin sulphate K on the platelet count of rabbit blood was measured both *in vivo* and *in vitro*. The

counting fluid devised by Lempert[7] was used, but no brilliant cresyl-blue was included as quite small amounts of heparin and laminarin sulphate K caused precipitation of the dye. In the *in vitro* experiments, which were similar to those of Astrup[8], rabbit blood was collected by heart puncture into one tenth of its volume of a 3·5 per cent sodium citrate solution.

TABLE I

SEAWEED ANTICOAGULANTS

Compound	Per cent sulphur	Number of sulphate groups per glucose unit	*In vitro* activity units per mg. (International Heparin Units)
Laminarin sulphate K	16·8	1·83	35
Laminarin sulphate L	14·5	1·37	9·4
Laminarin sulphate M	8·84	0·62	1·4
Laminarin sulphate N	5·97	0·37	<1·3
Fucoidin F13	9·33	*—	8·9
Fucoidin A	*—	*—	9·0

* Information not available.

Saline, heparin solutions or laminarin sulphate K solutions were added to the citrated blood, and platelet counts were made on each of the samples after 15 minutes. For the *in vivo* experiments, platelet counts were made before the intravenous injection of the anticoagulants and 15 minutes later.

RESULTS

Anticoagulant activity in rabbits. The results (Table II) showed that laminarin sulphate K was about a third as active as heparin (100 units per mg.). The clotting times suggested that the action of laminarin

TABLE II

MEAN CLOTTING TIMES OF SEVEN RABBITS AFTER INTRAVENOUS INJECTION OF HEPARIN AND LAMINARIN SULPHATE K

	Mean clotting time in minutes			
Time after injection, in minutes	0	15	30	60
Heparin 1 mg./kg.	1½	14	6	3½
Laminarin sulphate K 3 mg./kg.	2	11	9	5

sulphate K was slightly more prolonged than heparin, a finding which was confirmed at higher dose levels. The anticoagulant activity of laminarin sulphate K in rabbits could be neutralised by the intravenous injection of protamine sulphate.

Effect of laminarin sulphate K on platelets in vitro *and* in vivo. Laminarin sulphate K at 1 mg. and 4 mg./ml. of rabbit blood had no effect on the platelet count *in vitro*. There was also no evidence that laminarin sulphate K, in doses up to 500 mg./kg. intravenously in rabbits, caused any pronounced drop in the platelet count 15 minutes after

injection. In the course of all the platelet counts there was no indication that laminarin sulphate K caused precipitation or aggultination.

Acute Toxicity

From Table III it will be seen that the intravenous average lethal dose in mice of laminarin sulphate K was about 1000 mg./kg., and that the substance was about twice as toxic as heparin. The way in which the animals reacted to the two compounds was quite different. The toxic effect of heparin appeared immediately after injection, whereas the animals dosed with laminarin sulphate K showed no effects for one to two hours.

TABLE III

ACUTE INTRAVENOUS TOXICITY OF LAMINARIN SULPHATE K AND HEPARIN IN MICE AND GUINEA PIGS

Animals	Substance	I.V. dose mg./kg.	Deaths
Mice	Laminarin sulphate K	1000 1500	6/10 9/10
	Heparin (100 units/mg.)	1500 2500	2/10 5/5
Guinea pigs	Laminarin sulphate K	20 40	8/11 3/5
	Heparin (100 units/mg.)	250	0/5

The intravenous toxicity of laminarin sulphate K was about fifty times greater in guinea pigs than in mice (Table III). While laminarin sulphate K was only twice as toxic as heparin to mice, it was more than twelve times as toxic in guinea pigs. Thus 20 mg./kg. of laminarin sulphate K produced toxic effects in all guinea pigs, while 250 mg./kg. of heparin produced no reactions of any kind.

All the guinea pigs which received laminarin sulphate K showed the same signs to a greater or lesser degree. There were signs of pulmonary oedema ("bubbly breathing") shortly after injection, a bout of jactitating movements and finally limpness, with the animal lying on its belly or side. Death occurred in some animals within an hour of injection, but in others a day or two later. These symptoms were similar to those reported by Walton and Ricketts in guinea pigs given certain synthetic anticoagulants[10], and termed by them "anaphylactoid" phenomena.

Chronic Toxicity in Rabbits

Three animals were given 5 mg./kg. of heparin intravenously twice daily for 5 days weekly for 3 weeks, and were killed at the end of this time by intravenous sodium pentobarbitone. Throughout this period the animals were in good health.

Nine rabbits were given 12·5 or 15 mg./kg. of laminarin sulphate K intravenously twice daily on five days of the week. Seven died after seven to twelve days of dosing. The other two were killed by intravenous sodium pentobarbitone when moribund, in order to obtain fresh autopsy material. Three died overnight, and autolytic changes had occurred by

the time they were examined. The other six were examined very soon after death and the outstanding macroscopical and pathological findings were recorded. The most consistent feature was a pronounced loss of tone of the ileum and sometimes colon, and the presence of liquid contents in these parts of the gut. In three animals a small ulcerated area was found in the stomach. Diarrhoea was a prominent symptom in six of the nine test rabbits, but was absent in the three heparin controls.

Microscopic changes in the mucous membrane of the small intestine were present in all animals, but were slight in the controls. In three animals there was an associated change in the submucosa suggesting that the necrosis was inflammatory. Degenerative changes in the convoluted tubules of the kidney were present in four animals, and in one rabbit this was associated with distension of the glomerular capillaries by fibrin clots. Fibrin clots were also seen in the pulmonary arteries of two animals, and in the portal vein of one.

Discussion

Our results suggest that laminarin sulphate K is an anticoagulant with about one third the activity of heparin.

Hawkins and O'Neill[9] tested four sulphated derivatives of laminarin, one of which contained 1·7 sulphate groups per glucose unit and was one third as active as heparin. It was probably similar in composition to laminarin sulphate K, with 1·83 sulphate groups. They found that a single intravenous injection of this substance in rats and dogs did not affect the platelet counts.

Many of the synthetic anticoagulant polysaccharide polysulphuric acid esters are known to be toxic because they cause agglutination of the blood platelets and precipitate fibrinogen[10–17].

Astrup[8] showed that rabbit blood platelets could be agglutinated *in vitro* by several synthetic anticoagulants, including "Paritol", a compound which has been in clinical use. Piper[11] recorded very great reductions in the platelet counts of rabbits after the intravenous injection of several synthetic sulphated polysaccharides in doses as low as 1 mg. per kg. Using similar *in vitro* and *in vivo* techniques, we found that laminarin sulphate K had no effect in doses higher than those which these two workers had used for their compounds. With *in vivo* tests we used very high doses without causing abnormal effects. We concluded that laminarin sulphate K did not cause platelet agglutination.

The chronic toxicity tests in rabbits, however, indicated that laminarin sulphate K was too toxic for clinical use. The occurrence of diarrhoea in six of these animals was very interesting, since this was one of the reasons why "Treburon" (a polyhexuronic acid ester) was abandoned clinically[19].

Walton[18] found that the higher molecular weight members of a series of dextran sulphates precipitated human fibrinogen and caused platelet agglutination in human blood. Injected intravenously into guinea pigs these same compounds caused an "anaphylactoid" response[10]. This is Walton and Ricketts' term for the typical symptoms of jactitating movements and respiratory distress that follow an intravenous injection of a

toxic anticoagulant, and is not to be confused with anaphylactic shock. They found that two other toxic synthetic anticoagulants, a sulphuric ester of xylan, and sodium polyanethol sulphonate ("Liquoid"), also produced the "anaphylactoid" phenomena in guinea pigs. We found that laminarin sulphate K at the very low doses of 20 mg./kg. and 40 mg./kg. produced the same toxic reaction, which supported our conclusion that this substance possesses some of the toxic properties shown by other synthetic heparin-like anticoagulants, and is too toxic for clinical use.

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THE ASSAY OF HISTAMINE, 5-HYDROXYTRYPTAMINE, ADRENALINE, AND NORADRENALINE, ON THE BLOOD PRESSURE OF THE FOWL

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Histamine and 5-hydroxytryptamine, 1 μg./kg. depress, and (−)-adrenaline and (±)-noradrenaline 0·5 μg./kg. raise, the blood pressure of fowls under pentobarbitone anaesthesia. With each compound the response in mm. Hg is linearly related to log dose. The responses have been used satisfactorily for the assay of these compounds.

ACTIONS and side-actions of new drugs destined for clinical trial are usually examined in cats, dogs, and sometimes monkeys, because the physiology of these species most closely resembles that of man. However, the use of these species for biological assay and for the preliminary screening of new drugs appeared justifiable, at the present time, only if no satisfactory alternative method is known.

Highly sensitive methods exist for the assay on isolated tissues of many naturally occurring pharmacologically active substances. Nevertheless, it is often desirable to examine such compounds either in tissue extracts, or preferably after separation from other constituents of the extracts, on a variety of preparations. That the fowl might yield a suitable whole animal preparation for this purpose has therefore been examined.

METHODS

Fowls of various breeds, weighing from 1·0 to 1·6 kg., were anaesthetised by the intramuscular injection of 1·0 ml./kg. of 6 per cent (w/v) pentobarbitone sodium in a solvent composed of 10 parts (v/v) ethanol, 20 parts propylene glycol, and 70 parts water. Full surgical anaesthesia developed in 30 to 40 minutes, and could be maintained by the intramuscular injection of approximately 0·3 ml./kg. of this solution per hour. The tongue was drawn forward, and a tracheal cannula was passed through the larynx under direct vision. Gentle positive ventilation was begun at once, and was continued throughout the experiment. The fowl was placed on its side with its legs extended at right angles to its back. Feathers were plucked from over the presenting femur. An incision was made through skin parallel to, and 1 cm. behind, this bone. The skin was retracted and the muscles were separated to expose the ischiadic artery and vein. Both vessels were cannulated. Drugs were injected into the venous cannula. Mean arterial pressure was recorded from the arterial cannula by means of a Condon rat manometer[1]. Heparin was used as anticoagulant.

Dose-effect curves relating graded responses of the fowl's blood pressure to drugs were determined as follows: Three or more doses of drug were selected, each of which produced a measurable but submaximal effect.

These doses were given repeatedly in a random order; the responses obtained were measured to the nearest millimetre. The results were analysed by standard statistical methods.

Assays. Two suitable doses, which produced submaximal effects, were chosen for both standard and test solutions. The ratio of the high dose to the low dose was the same for both standard and test solutions. Letters were assigned to these doses, which were given in the order of Latin squares. Drugs were injected at regular intervals which varied from two minutes in experiments with histamine to four minutes in experiments with adrenaline. Responses were measured to the nearest millimetre.

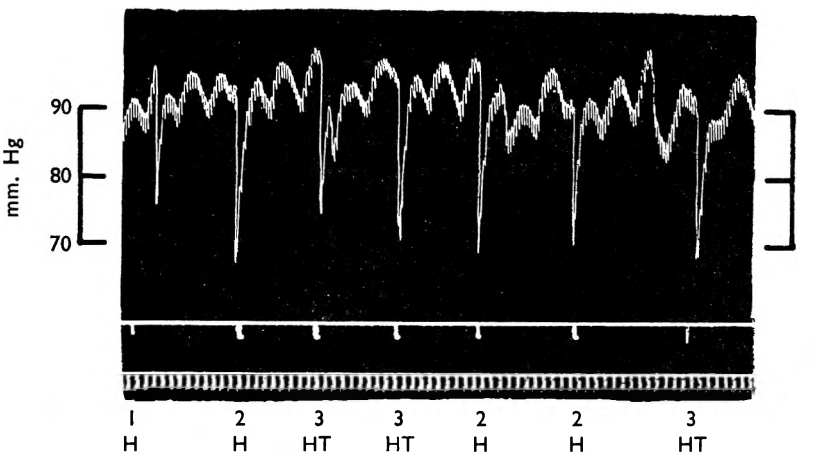

FIG. 1. The relative potency of histamine and 5-hydroxytryptamine on the fowl's blood pressure. Record of blood pressure from a fowl, wt. 0·9 kg. Histamine, H, 5-hydroxytryptamine, HT, injected i.v. Doses in μg. Time trace, 10 seconds.

Assay results were calculated by the method described by Schild[2], except that the following formula was used to determine the standard error of the mean: $V_m = \frac{S^2}{b^2}\left(\frac{1}{n_s}+\frac{1}{n_u}\right)+\frac{(\bar{Y}_s+\bar{Y}_u)}{b^2}\cdot V_b$, where S = root of the mean square for error, n_s and n_u are the numbers of observations made on the standard and test solutions respectively, $\bar{Y}_s$ and $\bar{Y}_u$ are the means of all responses to standard and to test solutions respectively, b is the slope of the log dose effect curve, and V_b is the variance of this slope.

RESULTS

Dose Effect Curves for the Action of Drugs on the Blood Pressure of the Fowl

Well ventilated fowls maintained a steady resting blood pressure, at a level between 80 and 120 mm. Hg, for many hours. The repeated injection of depressor drugs caused a small wave-like variation in the resting blood pressure which was maintained throughout the estimation of dose-effect curves, and throughout assays, of depressor drugs. These waves (Fig. 1) occurred at rates of approximately 2 to 3 a minute, and were central in origin. They did not seriously affect estimations of depressor

action. Pressor drugs did not induce variation in the resting blood pressure, and abolished that due to depressor drugs.

Histamine. The intravenous injection of histamine caused an abrupt fall of blood pressure. Submaximal depressor responses were proportional to the log dose of histamine used, and lasted for less than 1 minute. This fall was followed by a rise in blood pressure above the normal resting value before the latter was restored. The whole biphasic

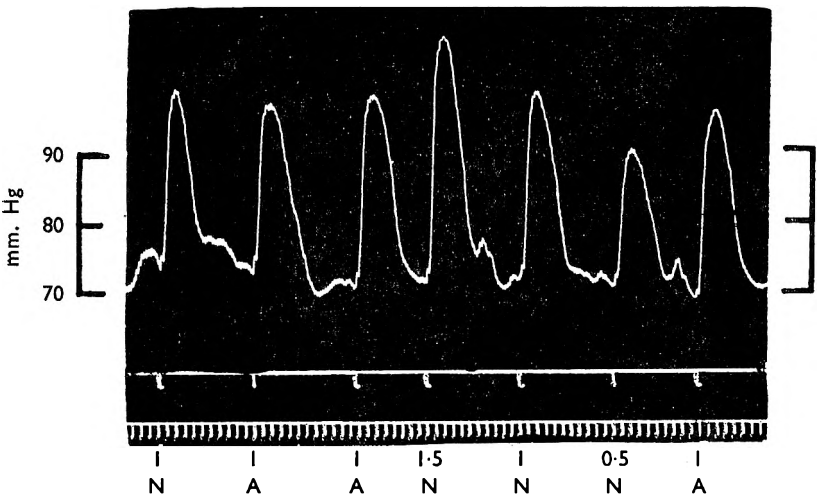

FIG. 2. The relative potency of adrenaline and noradrenaline on the fowl's blood pressure. Record of blood pressure from a fowl, wt. 1·3 kg. (−)-Adrenaline, A, and (±) noradrenaline, N, injected i.v. Doses in μg. Time trace, 10 seconds.

response lasted less than 2 minutes. In thirty-two determinations of the log dose effect curve for the action of histamine on the blood pressure of the fowl, the mean weight of histamine acid phosphate required to depress the blood pressure by 20 mm. Hg. was 3·23 μg./kg., standard error 0·31. The mean slope of the curves was 38·48, standard error 2·39.

5-Hydroxytryptamine. Depressor responses to the intravenous injection of 5-HT had duration similar to, but were followed by a secondary rise in blood pressure less than that of, responses to histamine in the same bird. Tachyphylaxis occurred during the first 5 to 10 responses to 5-HT, but thereafter responses to this drug remained constant when it was injected at 3 minute intervals. When the response to 5-HT had become constant, the effect of 1 μg. histamine base equated approximately with that of 1·5 μg. 5-HT. The slopes of the dose effect curves were however a little steeper for 5-HT than for histamine in the three birds in which this comparison was made (Fig. 1).

Adrenaline and noradrenaline injected intravenously caused very similar pressor responses of the blood pressure. The effect of 1 μg. (−)-adrenaline roughly equated with that of 1 μg. (±)-noradrenaline in each of three birds, and the dose effect curves given by these two drugs did not differ significantly from parallel (Fig. 2).

The Assay of Drugs on the Blood Pressure of the Fowl

Histamine. Twenty-eight assays were made in which the concentration of histamine acid phosphate in both the standard and the test solutions was known. The tracing obtained in a single 2 + 2 assay, in which each dose was given four times, is shown in Figure 3. The standard solution contained 15 μg./ml., and the test solution 18 μg./ml. The concentration of the test solution calculated from the data provided by this assay was 18·5 μg./ml. The calculated slope, b, of the dose effect curve was 44·4. The value s/b was 0·14 where $s = \sqrt{\text{mean square for error}}$.

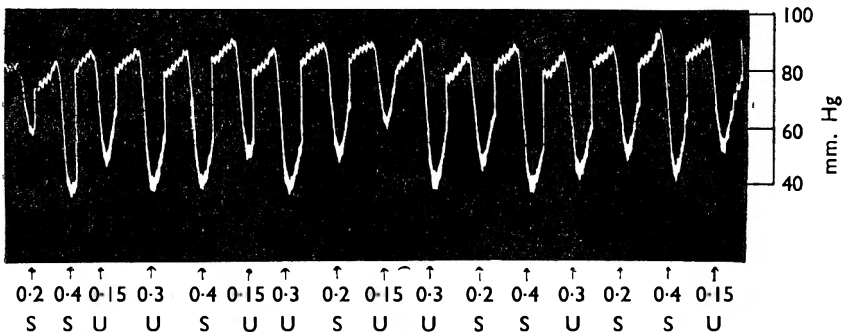

FIG. 3. Record of a 2 + 2 assay of histamine on the blood pressure of the fowl, wt. 1·6 kg. Injections of either standard, S, or test, U, solutions were made i.v. at 2 minute intervals. Doses in ml.

The Fiducial limits, P = 0·95, were however 13·0 to 26·5 μg./ml. The precision of this assay could have been much increased by repetition of the randomised groups of doses, to a total of 12 or 16 trials at each dose level. A summary has been made of twenty-eight such 2 + 2 assays of histamine acid phosphate in which only four randomised groups of doses

TABLE I

SUMMARY OF THE RESULTS OF 2 + 2 ASSAYS OF HISTAMINE ON THE FOWL'S BLOOD PRESSURE

	Nos. of randomised dose groups used	
	Four	Twelve
Mean found as per cent true	103·93 ± 2·34 (28)*	99·77 ± 0·48 (6)*
Limits of error per cent (P = 0·95):		
Determined directly	−27·7 to 31·1	−14·4 to 16·9
Determined indirectly	−23·8 to 31·1	−14·1 to 15·8
Value s/b	0·01 ± 0·08 (28)*	0·08 ± 0·03 (6)*

* Mean ± standard error (number of observations).

were given. This summary is found on the left side of Table I. The right side of the Table shows a similar summary of the results of six assays which differed only in that twelve randomised groups of doses were employed. It is evident that whereas assays consisting of only four groups of randomised doses can be relied upon to detect a 30 per cent difference between standard and test solutions, not less than twelve such groups are needed to ensure the detection of a 15 per cent difference between

samples. The preparation proved hardy, and gave good discrimination between doses for 5 to 7 hours: responses were evoked every two minutes.

5-Hydroxytryptamine. Three assays of 5-HT were made on the fowl's blood pressure. In each experiment 5-HT was injected at 3 minute intervals. Tachyphylaxis developed for the first 15 to 25 minutes, but thereafter constant submaximal responses to fixed weights of 5-HT were demonstrated, and the preparations proved satisfactory for assay purposes.

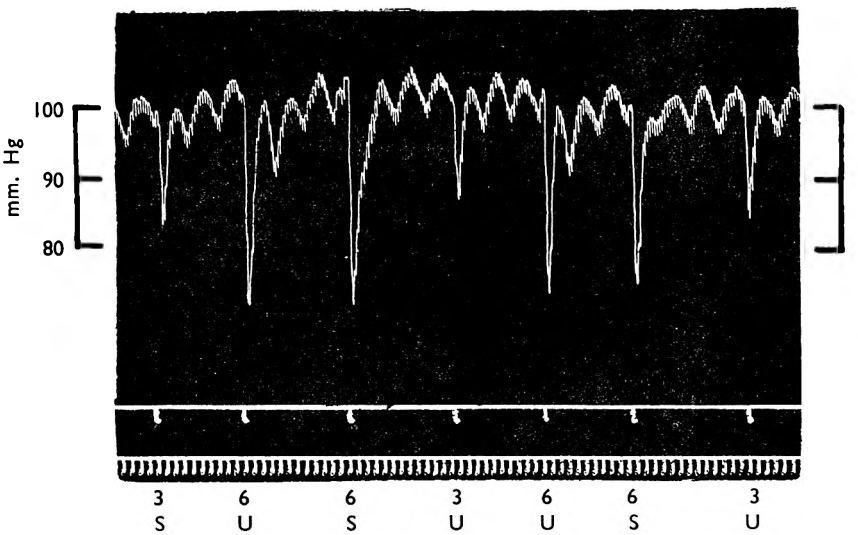

FIG. 4. Part of a record of a 2 + 2 assay of 5-hydroxytryptamine on the blood pressure of a fowl, wt. 1·3 kg. Injections of either standard, S, or test, U, solutions were made i.v. at 3 minute intervals. Doses in ml. × 10.

Figure 4 shows part of a typical tracing obtained during one of these 2 + 2 assays of 5-HT. In this assay both standard and test solutions contained 10 μg. 5-HT/ml. Twelve groups, each of four randomised doses, were employed. The concentration of 5-HT found in the test solution by experiment was 10·2 μg./ml. The Fiducial limits of this mean (P = 0·95) were 9·75 to 10·8 μg./ml. The value obtained for b was 45·8, and for s/b, 0·02. Evidently, on the blood pressure of the fowl, assays of 5-HT are more precise than assays of histamine.

Adrenaline and noradrenaline. The pressor actions of adrenaline and noradrenaline on the fowl's blood pressure were compared in three assays. In these both the concentrations of adrenaline in the test solutions and of noradrenaline in the standard solutions were known. The dose effect curves for the two drugs did not differ significantly from parallel. 1 μg. (±)-noradrenaline was found equally effective with 1·17 ± 0·08 (3) μg. (−)-adrenaline. The precision of these assays were greater than those of histamine, and less than those of 5-HT on the fowl's blood pressure.

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TOXICOLOGICAL HAZARDS OF MERCURIAL PAINTS

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The toxicological hazards of ingestion of mercury by absorption through the skin from mercurial ointments and through the lungs from mercury laden air are reviewed. Mice living for 6 months in cages painted with antifungal mercurial paints show no significant Hg levels in the kidneys, liver, lung or spleen. From this it is inferred that no toxic hazard to humans working in rooms treated with these mercurial paints should result. Using the literature figure of 100 μg. Hg/c.m. of air as the upper safe limit for continuous exposure of man for 8 hours per day and assuming that 25 per cent of the inhaled mercury is absorbed it has been deduced that the maximum safe continuous peroral intake of mercury is 1·3 μg./kg./day. This figure should assist in deciding the maximum permissible amount of mercury salts in food and drinking water. Guinea pigs have been given 150 times this dose daily for 6 months and found to remain in perfect health. The hazard arising from the possibility of contamination of foodstuffs by flaking of an antifungal mercurial paint is thought to be negligible.

UNTIL recent years hydrargyrism was a hazard confined to industrial workers continually handling mercury and its salts, and to people undergoing inunction therapy with mercurial ointments. During the last two decades, however, mercurial preparations have gained wide use as bactericides and fungicides both in the medical and industrial fields; accordingly the proportion of the population now exposed to toxic hazard has considerably increased. These uses have been previously reviewed[1,2]. More recently there has been a trend to incorporate organo-mercurial compounds in paints and varnishes particularly for use in industrial premises, for example, breweries and bakeries, where walls are prone to troublesome fungal growths because of humid conditions. Apart from the possible hazard to personnel manufacturing and applying these paints—hazards which can be largely controlled by suitable precautions—it is natural to enquire into the general health risk entailed by the widespread use of mercurial additives in paint. Hazard can arise from the continual breathing of the air inside the painted room if the organo-mercurial compound should have any appreciable volatility, and from flaking of the paint with consequent possible contamination of foodstuffs. The work now reported was undertaken to add further information to our knowledge of these hazards.

It is pertinent to the object in mind briefly to review previous work on the toxicological hazards met with in the use of mercury and its derivatives in order to arrive at threshold figures below which risk of toxaemia may be considered negligible. It is remarkable that up to the present time no Official Committee has laid down upper permissible limits for mercury in foodstuffs or drinking water.

Various general reviews[3–10] on metallic poisons have appeared which give reference to mercurialism. Two methods have been used for assessing mercury accumulation in the body after a known exposure: (i) the determination of the amount of mercury in the urine and faeces over a long period until the value falls to zero and (ii) the analysis of the organs for mercury in those cases where autopsy can be performed. Stock[11], from extensive analyses of 67 human cadavers, showed that the kidney and hypophysis are the organs in which circulating mercury preferentially accumulates. Sollemann and Schneiber[12], in post mortem analyses of suicides who had used corrosive sublimate, found the highest concentration of mercury in the kidney (38 μg./g.).

Considerable work has been carried out on absorption of mercury through the skin, as determined by the urinary excretion of mercury, during the inunction therapy of syphilis and psoriasis; the rate of absorption depends to a considerable extent[13] upon the ointment base employed. In this connection it is necessary to know the mercury content of normal urine; a reliable estimate would appear to be 5 to 20 μg./l. Thus, Borinski[14] reported that 38 of 75 normal humans excreted as much as 10 μg. of mercury daily in their urine and faeces despite absence of any known exposure to mercury; while Lane[10] found the average urinary level to be 20 μg./l. in psoriasis patients who had not been previously treated with mercurials. Cole and others[15] examined six men who had been given 18–30 rubs with mercurial ointments and found that after four weeks the median figures for elimination of mercury were about 100 μg./day in the urine and 300 μg./day in the faeces. Inman, Gordon and Trinder[16] record that, in a series of psoriasis patients inuncted twice daily for 6 weeks with 2 per cent ammoniated mercury in soft paraffin over an area averaging 65 per cent of the total body area, the urinary level of mercury was about 130 μg./l. during the first week, rising to 500 μg./l. by the sixth week and then falling slowly to the normal figure in 7 to 9 months. No clinical toxicity was observed; nevertheless Inman and colleagues call attention to the risk of toxicity which the prolonged use of mercury ointments entails and consider a urinary level in excess of 300 μg./l. to be potentially dangerous. Examples[17,18] of the nephrotic syndrome attributable to mercurial ointments have been described and the opinion[19] has been held that syphilitic nephrosis is more likely to be caused by the mercury than by the syphilis. There is much evidence, however, that it is necessary to apply mercurial ointments for longer than 6 months before symptoms appear.

The distribution of mercury in mice, after the subcutaneous injection of mercury and calomel ointments has been determined by Maren, Epstein and Hand[20]. It would appear from their results that mercury levels in the kidney are a fairly reliable index of the extent of chronic absorption in the case of exposure to metallic mercury but with exposure to calomel, liver analyses are also necessary. Maren and colleagues comment upon the fact that there was no significant loss of weight in the experimental mice or, indeed, any other gross sign of toxaemia observable despite the high concentrations of mercury in the liver and kidney. Laug,

Vos, Umberger and Kunze[21] determined the absorption of various mercurial ointments in rabbits after inunction into a clipped area of the back comprising about 8 per cent of the total body area; after 24 hours the animals were killed and the organs analysed. With calomel the concentration of mercury in μg. Hg/g. of wet tissue was much the highest in the kidney (kidney, 21–26; liver, 0·78–1·02). Covering the inunction site was found to increase absorption fourfold.

Much work has been done on the hazard involved in breathing air containing mercury vapour. Stock and Zimmerman[22] kept animals in cages through which was passed air which had been loaded with mercury vapour at 50°; substantial quantities of mercury were found in the bodies at autopsy. Frazer, Melville and Stahl[23] exposed dogs to air containing 1890 μg. Hg/c.m. for 8 hours a day for 40 days: there was no ostensible sign of mercurialism but the daily excretion of mercury was about 500 μg. for dogs weighing 12 kg., showing that on an average 24 per cent of the mercury in the inhaled air had been absorbed. Attention[24] has previously been called to the risk arising from the spilling of metallic mercury in chemical and physical laboratories, particularly in the neighbourhood of steam pipes. The results of exposure of 38 men to various atmospheric concentrations of mercury has been reported by Shepherd, Schumann and Flinn[25] who found no evidence of mercurialism in men constantly exposed to an atmosphere containing 70 μg. of Hg/c.m.

Bidstrop, Bonnell, Harvey and Locket[26] observed symptoms of chronic mercury toxaemia in 27 of 161 men repairing D.C. meters; 58 men working on the repair of A.C. meters (which do not contain mercury) were unaffected. It was observed[26] that excretion of more than 300 μg. of Hg/day is accompanied by manifest symptoms of hydrargyrism and the excretion level was related to the concentration of mercury in the atmosphere. A concentration of 100 μg. Hg/c.m. of air was considered the upper safe limit; this is identical with the figure which had been previously laid down by the American Bureau of Standards[27] for the upper safe limit for continuous exposure for 8 hours per day. By assessing the rate of normal quiet breathing for an adult man at about 8 litres/minute and assuming from the work of Stock and Zimmerman[22] that 25 per cent of the inhaled mercury is absorbed, it is deducible that a man breathing air containing 100 μg. of Hg/c.m. for 8 hours a day will absorb about 100 μg. of Hg/day. For a 70 kg. adult this is a mercury intake of 1·3 μg./kg./day. In the light of present knowledge this must be taken as the upper safe limit for continuous daily absorption and it gives a basis for assessing the possible hazard of working in a room which had been painted with, or of continually consuming food which has been contaminated with a mercurial paint.

Experimental

Groups of 20 mice were kept in cages 18 in. × 15 in. × 10 in. (46 × 38 × 25 cm.), the four sides and ceiling of which had been heavily coated with the mercurial antifungal paint. The front of the cages (18 in. × 15 in.) had a small wire grid 8 in. × 5 in. for admission of light and air. The

painted surfaces were covered with heavy gauge wire mesh ½ in. away from the paint, in order to prevent the ingestion of paint by contact or gnawing. The room temperature was 21°. The animals were kept in the cages for 6 months, weighed as a group each week and fed and watered *ad libitum*. At the end of this period 4 animals were taken at random from each cage, killed, the organs examined histologically and analysed for mercury content by the dithizone procedure.

The fungicidal organomercurials used were selected from substances now in commercial use and were as follows:

Paint No. 1. Emulsion paint containing 0·1 per cent w/w phenylmercuric dinaphthylmethane disulphonate. *Paint No.* 2. Emulsion paint containing 0·65 per cent w/w phenylmercuric 8-hydroxyquinolinate.

TABLE I

MERCURY CONTENT OF ORGANS OF MICE WHICH HAD BEEN KEPT IN CAGES PAINTED WITH MERCURIAL PAINT FOR 6 MONTHS

	Liver		Kidney		Lungs	
Paint No.	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)
1	1·87	0	0·38	0	0·28	0
	1·56	0	0·42	1	0·35	0
	1·32	0	0·38	0	0·21	0
	1·63	0	0·46	0	0·27	0
2	1·47	0	0·23	0	0·17	0
	1·87	0	0·41	0	0·27	0
	1·54	2	0·53	1	0·23	0
	1·16	0	0·29	0	0·18	0
3	1·85	0	0·45	0	0·31	0
	1·58	2	0·45	0	0·22	0
	1·74	0	0·46	0	0·39	0
	1·45	0	0·37	0	0·20	0
4	2·02	0	0·41	0	0·37	0
	1·67	0	0·29	0	0·24	0
	1·75	0	0·47	1	0·32	0
	1·90	3	0·40	3	0·26	0

* μg. Hg in total organ.

Paint No. 3. Oil paint containing 0·24 per cent phenylmercuric *p-tert.*-octyl phenate. *Paint No.* 4. Oil paint containing 1·2 per cent phenylmercuric *p-tert.*-octyl phenate. The first three of these paints have the mercury content which is recommended for use; the last has four times this content. At the end of the 6 months' period the average weight in each group had increased from 20–23 g. to 30–34 g. The mice appeared normal, there being no visible sign of toxaemia. The only deaths which occurred were from fighting and cannibalism; these were 1 in Group 1; 3 in Group 2; 4 in Group 3 and 1 in Group 4. In the four groups there were 5 healthy litters during the period. In the group of 20 controls kept in ordinary cages the average weight increase was from 20–22 g. to 30–33 g.; there were 3 deaths in this group and 2 litters.

Histological examination of the kidneys, lungs, liver and spleens showed no abnormality; the mercury content of the organs is shown in Table I.

In order to obtain information on the risk involved if old paint should flake and accidentally find its way into foodstuff, small daily doses of

phenylmercuric dinaphthylmethane disulphonate were fed to guinea pigs. This was the only one of the three additives which is water soluble and hence easy to administer over a long period. A group of four guinea pigs was given 50 g. of bran containing the pure organomercurial each morning; after this was eaten the animals were given greenstuff and oats *ad libitum*. The dose of the organomercurial amounted to 0·5 mg./kg./day (Group A). A further group of four guinea pigs was given 0·05 mg./kg./day in the same manner (Group B). The animals were weighed each week and after six months two from each group were killed and their

TABLE II

MERCURY CONTENT OF GUINEA PIG ORGANS AFTER FEEDING WITH MERCURIAL EACH DAY FOR 6 MONTHS

Daily dose	Guinea pig No.	Liver		Kidney		Lung		Spleen	
		Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)
Group A .. .. 0·5 mg./kg./day ..	1	22·4	3	4·5	58	4·0	0	0·6	0
	2	35·8	4	5·4	77	5·0	1	0·5	0
Group B .. .. 0·05 mg./kg./day ..	5	23·6	0	3·9	7	4·7	1	0·6	0
	6	29·2	1	4·8	7	5·0	0	0·8	0

* Per g. wet tissue.

TABLE III

MERCURY CONTENT OF GUINEA PIG ORGANS AFTER FEEDING WITH MERCURY FREE DIET FOR A FURTHER 6 WEEKS

Group	Guinea pig No.	Liver		Kidney		Lung		Spleen	
		Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)	Weight (g.)	Hg* (μg.)
Group A	3	26·5	0	4·4	11	5·0	0	0·7	0
	4	32·8	2	5·1	9	5·2	1	0·6	4
Group B .. .	7	27·1	0	4·1	2	4·8	1	0·8	0
	8	28·4	0	4·6	4	5·6	0	0·7	1

* Per g. wet tissue.

organs examined and analysed. The remainder were kept on normal diet for a further 6 weeks, killed and the organs examined and analysed to gain information on the rate of elimination of mercury.

In both groups the average weight increased regularly from 325–350 g. to 650–700 g., a rate of increase which is normal for guinea pigs of this age over a 6 month period. At the end of this period all the animals were alert, active and behaved normally; their fur had an excellent bloom. In the first group each animal had received an average of 100 mg./kg./6 months; in the second group 10 mg./kg./6 months. The single dose toxicity figures of this compound have previously been reported by Goldberg, Shapero and Wilder[28,29] to be: LDO 50 mg./kg.; LD50 70 mg./kg. and LD100 80 mg./kg. Table II shows the mercury content of the organs at the end of the initial 6 months and Table III the analyses after a further 6 weeks on the mercury free diet during which time elimination of the accumulated mercury was taking place.

It can be seen from Table II that, after oral ingestion of phenylmercuric dinaphthylmethane disulphonate, mercury accumulates principally in the kidney and liver; at the top dose (0·5 mg./kg./day) the amounts being about 360 μg. in the total kidney and 100 μg. in the total liver; none appears to be deposited in the lung and spleen. After 6 weeks on mercury-free diet these amounts had been reduced to about 50 μg. in the total kidney; the liver, lung and spleen being free from significant mercury.

Conclusions

The results in Table I indicate that there is no measurable or significant volatility of mercurial from any of the paints used and accordingly that there should be no health hazard to personnel working in a room the walls of which have been painted with these preparations. Even with the paint containing four times the recommended amount of mercurial additive (Group 4) the amount of mercury found in the kidneys and liver was negligible excepting in one animal where 3 μg. was found in each organ. It is probable that this animal had gained access to and gnawed the paint since in no case was mercury found in the lungs.

The possibility of food contamination with flakes of the mercurial paint is a more difficult hazard to assess although a number of relevant comments can be made. The dose of mercurial in Group A guinea pigs corresponds to an oral intake of mercury of 200 μg./kg./day for 6 months and Group B, 20 μg./kg./day of mercury for 6 months. These intakes are respectively 150 and 15 times the amount, namely, 1·3 μg./kg./day (100 μg./man/day), which is accepted as the safe upper limit for continuous intake of mercury in inspired air. An intake of 100 μg./man/day by mouth must therefore be considered to be within the safe limit. It is pertinent to consider the probability of this dosage being exceeded by contamination of foodstuffs. The approximate area covered by 1 kg. of paint is 200 sq. ft. (200,000 sq. cm.); with the highest amount of mercurial additive recommended, 0·1 per cent combined mercury in the wet paint, the amount of mercury in the surface of the painted area is about 5 μg./sq. cm. Accordingly, all of the mercurial in the paint which might flake off 20 sq. cm. of surface could be consumed per day by the same person indefinitely without exceeding the accepted upper safe limit. It must be admitted that the probability of this amount being exceeded or even reached in the most careless establishment is very remote.

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THE EFFECT OF ULTRA-VIOLET IRRADIATION ON CARDENOLIDES

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Solutions of digoxin, digoxigenin and digitoxigenin in ethanol were subjected to ultra-violet irradiation in the absence of oxygen. Chromatographic analysis showed that the starting material was transformed into several other compounds some of which may be intermediate in the reaction. Cardiotonic activity was absent from the irradiated material. There was no longer the characteristic unsaturated lactone ring of the cardenolides after irradiation. Upon analysis a crystalline compound isolated from irradiated digoxigenin corresponded to the original molecule with an added molecule of water.

IT is a pharmacopoeial recommendation[1] that cardiac glycosides should be stored in the absence of light and it has also been noted in these laboratories that digitalis extracts left exposed to light show decreased cardiac activity. These observations suggested an examination of the effect of ultra-violet irradiation upon these drugs and the present paper describes some preliminary observations.

Recently developed paper chromatographic methods have enabled the progress of such changes in the molecule to be followed by observing the decrease in concentration of the starting material with time of irradiation and the appearance of new spots upon the chromatogram corresponding to the irradiation products as they are formed.

The method consisted of the irradiation of dilute solutions of various cardenolides in 90 per cent ethanol by immersing a quartz lamp into the solution, the whole being maintained at or close to 0°. Oxygen is excluded from the system by bubbling nitrogen slowly through the alcoholic solution, and samples are removed at intervals for chromatographic study.

The first compound studied was a pure (chromatographically homogenous) sample of digoxin.

In preliminary experiments with a long time of irradiation it was found that a marked decrease of digoxin occurred after 20 to 30 minutes of irradiation, and with a solution of 50 mg. in 100 ml. of solvent the loss was clearly detectable after 15 minutes.

Parallel with the fall in digoxin three or possibly four new compounds were seen on the chromatograms. These were designated Digoxin Irradiation products A, B, C and D in order of increasing R_F values on the chromatograms. Two of these, DIA and DIB which were much more polar and stayed nearer the starting line formed the main products of the reaction. The quantities of these materials increased as irradiation continued whereas a third compound DIC, travelled more closely with digoxin and could be detected only after approximately half the digoxin had been changed. The amount of this material also increased progressively

and made it difficult to determine the point at which all the digoxin had disappeared.

From several such experiments it appeared that, under the conditions employed, all the digoxin was transformed in 5 to 6 hours and half was converted after 1 to 2 hours. When irradiation was further extended to 20 to 24 hours the material DIA decreased as also did DIC, whereas material DIB remained unaffected or even increased slightly in amount.

In addition to these three products it was often possible to detect a further one or two spots placed between DIA and DIB on the chromatogram but the concentration of these materials was low and they further decreased with prolonged irradiation.

A sample of digoxin which was irradiated in this way for 24 hours was tested for cardiotonic activity using the isolated papillary muscle of the cat as described by Cattell and Gold[2]. With digoxin a marked positive inotropic effect is observed on this preparation with concentrations of 1 in 10^7 to 2 in 10^7 but with the irradiated solution no effect at all could be detected when the concentration (equivalent to the digoxin originally present) was increased to 1 in 10^5. It is therefore considered that the irradiation products were cardiotonic.

Attempts were made to isolate the reaction products and to characterise them as crystalline substances but great difficulty was encountered. Upon working up the irradiated solution a waxy material was obtained which was partly soluble in water and could not be obtained in crystalline form by repeated solution in different solvents. Column chromatography on diatomaceous earth also failed to yield a crystallisable substance but from certain strongly polar fractions of the eluate a white noncrystalline powder was obtained which was homogenous and very nearly pure when tested by paper chromatography. This corresponded to the material DIB.

This substance on analysis gave an empirical figure which corresponded to digoxin with one molecule of water added. Since this material has not been obtained crystalline the melting point was indefinite and we would not regard it as a pure compound.

To simplify the problem the aglycones digoxigenin and digitoxigenin were also irradiated as it was considered that the absence of the sugar moiety might make the production of crystallisable materials possible.

The common feature of the irradiation of all the three substances was the production of compounds showing increased polarity, the loss of pharmacological activity and the loss of a positive reaction to the Raymond test indicating that the unsaturated lactone ring is no longer present after irradiation.

In the case of digitoxigenin three compounds were formed which were all more polar than the starting material and these were designated TGA, TGB and TGC in order of ascending R_F values. The intensity of the spots TGA and TGC increased until all the digitoxigenin was transformed (4 to 5 hours) whereas TGB reached a maximum after $1\frac{1}{2}$ to 2 hours irradiation and then decreased quickly indicating that this material is itself sensitive to ultra-violet irradiation and may be an intermediate product.

Efforts to isolate these reaction products in pure form by fractional crystallisation or partition column chromatography have not so far been successful and only the two most polar substances have been separated from the remaining reaction mixture.

The behaviour of digoxigenin upon irradiation which is, in general, characteristic of all the three cardenolides studied is illustrated in Figure 1.

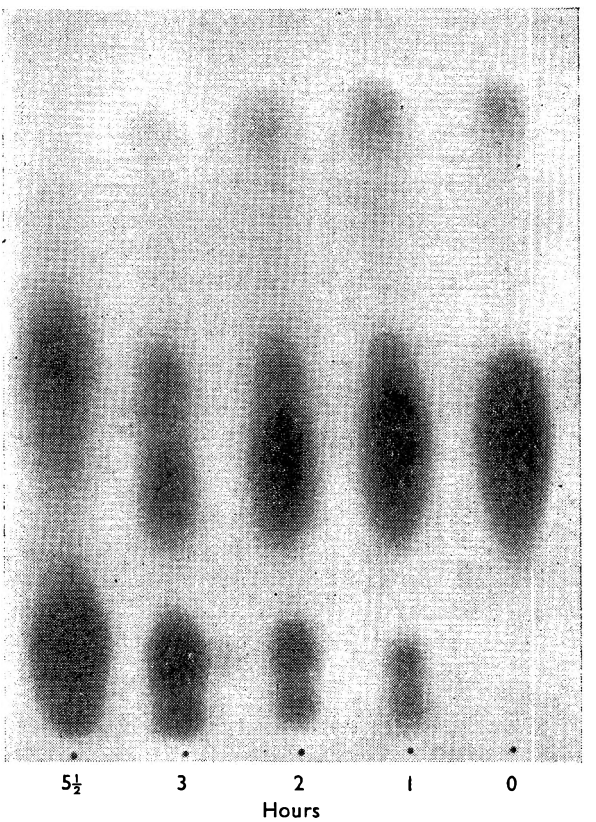

FIG. 1. Ultra-violet fluorescence chromatogram showing the change at various times in composition of digoxigenin solution by irradiation.

Three compounds are formed as the result of the irradiation, two of which are more polar than digoxigenin and stay near the starting line. The third travels slightly ahead but is seen clearly only when the digoxigenin spot is greatly reduced, after 3 or 5½ hours in the figure. It will be seen that practically none of the original material remains after 5½ hours.

The separation of the reaction products of digoxigenin have met with partial success. A total of 750 mg. was subjected to irradiation and the reaction materials collected. This material was separated by repeated chromatography on partition columns followed by several recrystallisations and finally a substance corresponding to compound B was

obtained. This crystallised well from chloroform-ethyl acetate and approximately 30 mg. was obtained with a melting point 238 to 240°. Analysis corresponded to $C_{23}H_{36}O_6$ and indicated a molecule of digoxigenin with an added molecule of water. The Raymond test was negative so that we might presume this product of irradiation was formed either by opening the lactone ring coupled perhaps with isomerisation at the double bond or by the addition of the elements of water across the double bond of the unsaturated lactone ring.

We are at present engaged in a further characterisation of this compound and in the isolation of the other reaction products of the irradiated mixture.

Experimental

A solution of the cardenolide dissolved in 90 per cent ethanol, purified for spectrophotometry, was irradiated.

The alcoholic solution was placed in a cylindrical vessel approximately 48 mm. in diameter and 150 mm. high. The vessel was surrounded with coolant maintained at $-5°$ and the alcoholic solution stirred and kept free of oxygen by bubbling through it a slow stream of nitrogen, previously washed by passage through Fieser's solution, and then a wash bottle containing 90 per cent ethanol. The nitrogen was allowed to flow for an hour to rid the solution of dissolved oxygen before irradiation was commenced. The lamp was a U-shaped Hanovia low pressure quartz lamp approximately 25 mm. wide and 150 mm. high, running at 300 V. 30 m.A. This was vertically mounted and immersed in the solution. After introducing the solution the opening of the container was tightly plugged with cotton wool to prevent the diffusion of air into the container which was kept under a slight positive pressure by the constant stream of nitrogen. No difficulty was experienced in keeping the temperature of the solution within the limits $\pm 2°$ by adjusting the bath temperature to approximately 5° below the temperature of the irradiated solution.

Digoxin

The first experiments were made with 100 ml. of a solution of 50 mg. digoxin in 90 per cent ethanol irradiated for 12 to 18 hours. In later experiments the concentration of digoxin was increased to 100 mg./100 ml. solvent and the irradiation time reduced to 6 to 7 hours at 0° to $+4°$. To isolate the reaction product the alcoholic solution was treated with 2 to 3 ml. of a concentrated solution of sodium bicarbonate and the solvent was removed under reduced pressure at 40 to 45°. Upon repeated trituration with small quantities of water the product turned brittle and powdery and could be separated by filtration or centrifuging. The composition of this material differed very little from the original irradiation mixture. Efforts to purify it by extraction or crystallisation from various solvents were not successful. The material was very soluble in ethanol and methanol, and partly soluble in ether. A further quantity of material was found to have dissolved in the aqueous extract mentioned above from which it could be largely recovered by salting out with NaCl; this fraction also failed to yield a crystalline compound.

Encouraged by the good separations achieved on paper, the material obtained in the several previous irradiation experiments (620 mg.) was mixed and submitted to partition chromatography using a column 26 mm. in diameter and packed to a height of 150 mm. with 35g. of diatomaceous earth (purified "Hyflo") mixed with an equal weight of water saturated with benzene. The solvents used for elution and the fractions obtained are those shown in Table I.

TABLE I

FRACTIONATION OF IRRADIATED DIGOXIN BY PARTITION COLUMN CHROMATOGRAPHY

Fraction No.	Solvent used (100 ml.)	mg. recovered	Composition by paper chromatogram
1	Benzene	47	mixture
2	"	20	"
3	Benzene : chloroform		
	9 : 1	22	1 spot
4	8 : 2	21	
5	7 : 3	18	1 spot different from 3
6	6 : 4	27	2 spots
7	1 : 1	36	1 spot
8	3 : 7	60	"
9	2 : 8	54	2 spots
10	Benzene : chloroform : ethyl acetate		the main corresponds to
	2 : 75 : 0·5	64	" the spot of fraction 5
11	2 : 7 : 1	44	"
12	2 : 6 : 2	15	"
13	2 : 6 : 3	8	
14	1 : 6 : 3	6	
15	Chloroform : ethyl acetate		
	7·5 : 2·5	6	
16	Chloroform : ethyl acetate : methanol		
	7·5 : 2·5 : 0·5	21	3 spots

Some more material was recovered by eluting the diatomaceous earth with hot chloroform—methanol mixture 1 : 1. The material of fraction 8 was purified by reprecipitation from a solution in chloroform by light petroleum. It represented then a white fine non-crystalline powder which melted over a range of 165 to 170° with previous sintering at approximately 155°. This substance gave a positive Keller-Kiliani and negative Raymond test. It was homogenous when chromatographed on paper and analysed C, 61·9; H, 8·7 per cent, calculated for $C_{41}H_{66}O_{15}$ C, 61·6; H, 8·3.

Fraction 7 when purified as above gave an amorphous powder melting in a range 165 to 170° with preceding sintering at approximately 150°. On paper chromatography it showed the presence of a trace of a more polar component. It gave the following analytical figures: C, 62·4; H, 8·8 per cent.

The material from fractions 9 to 12 was treated together by reprecipitation from methanol and water but no homogenous compounds were isolated from the different fractions.

Digoxigenin

In a preliminary experiment, using 100 mg. digoxigenin in 100 ml. ethanol (90 per cent) (purified and freed from acids and aldehydes) it was shown that after an irradiation of 6 hours practically all the starting material had been converted into secondary products which comprised three main components. Based on this experience 669 mg. of digoxigenin

dissolved in 140 ml. ethanol 90 per cent were irradiated for 6 hours and the solution was evaporated under reduced pressure at a temperature below 45°. The residual viscous oil was combined with the corresponding material from the pilot experiment and dissolved in ethanol; this solution was taken up into 4 g. of diatomaceous earth. After removing the solvent the dry mixture in the diatomaceous earth was placed on a column (28 mm. diameter) packed with 35 g. of diatomaceous earth mixed with 35 ml. of water previously saturated with benzene. The column was eluted with 100 ml. portions of solvent and the fractions collected as shown in Table II.

TABLE II

FRACTIONATION OF IRRADIATED DIGOXIGENIN BY PARTITION COLUMN CHROMATOGRAPHY

Fraction No.	Solvent composition	mg.	Substance composition by paper chromatography
1	Benzene	3·5	3 spots
2	"	6·0	"
3	Benzene : chloroform		
	9·5 : 0·5	7·0	"
4	9 : 1	9·5	"
5	8 : 2	16·3	2 main spots and 1 smaller one
6	7 : 3	16·1	" "
7	1 : 1	35·0	" "
8	2 : 8	118·1	2 main spots and trace of a third
9	Chloroform	131·5	1 main spot, 1 small and a trace of a third
10	Chloroform : ethyl acetate		
	9·25 : 0·25	79·8	1 main spot, 1 very small and a trace of a third
11	9 : 1	166·9	1 main spot and trace of a second
12	8 : 2	104·7	" "
13	6 : 4	38·7	1 main spot and a small second
14	Chloroform : ethyl acetate + methanol		
	6 : 4 ½ per cent	18·9	
15	6 : 4 1 "	11·6	
16	6 : 4 3 "	7·1	
17	6 : 4 5 "	4·4	
18	6 : 4 5 "	4·7	

The material from fractions 11 and 12 together totalling 266 mg. was rechromatographed on a column as described before and 22 fractions using 50 ml. solvent of increased polarity were collected.

Fractions 15 to 19 eluted with chloroform containing increasing proportions of ethyl acetate (1 to 5 per cent) carried the bulk of material (140 mg.) and showed on the chromatogram that they consisted mainly of one compound contaminated by a slight admixture of two other components.

This material was rechromatographed as before using 30 ml. of similar solvents for each fraction.

Three corresponding fractions eluted with chloroform containing 1 to 3 per cent ethyl acetate yielded 37·5, 28·8 and 19·2 mg. of material. The material from these fractions was twice recrystallised separately from a mixture of chloroform and ethyl acetate. From the middle fraction a crystalline compound showing a sharp m.p. 238 to 240° was recovered and gave the following analytical figures: C, 66·65, 66·8; H, 8·8, 8·8 per cent, for $C_{23}H_{36}O_6$ required C, 67·3; H, 8·9 per cent. The specific rotation $[\alpha]_D = +22{\cdot}1°$ (*c*, 1·14 per cent in ethanol).

From the third fraction a compound was recovered which melted less sharply at 238 to 241° with previous sintering at approximately 234°. It analysed for C, 66·2; H, 8·9 per cent.

All these compounds gave a negative Raymond test but a blue fluorescent spot with trichloroacetic acid in the reaction of Svendsen and Jensen.

References

1. U.S. Pharmacopeia XV.
2. Cattell and Gold, *J. Pharmacol.*, 1938, **62**, 116.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Veratrum album*, Alkaloids of.** H. R. Hegi and H. Flück. (*Pharm. Acta Helvet.*, 1956, **31**, 428.) The alkaloids of the leaves of *Veratrum album* L. have received little attention. The authors have extracted the alkaloids from the leaves, and separated and identified them chromatographically, full details being given of the chromatographic methods used. The alkaloidal mixture from the leaves differed considerably from that of the root. The latter contained protoveratrin (A and B), rubijervin, jervin and veratrobasin, which could not be detected in the leaves of the same plants, although in some other samples of leaves traces of some of these alkaloids were observed. In all the samples of leaves which were examined there was a dominant alkaloid which could amount to one half of the total alkaloids and which was never found in extracts of subterranean organs. Provisionally this was called alkaloid X. In addition there were found on the paper chromatographs from the leaf extracts, in all, 6 to 13 indications of alkaloids which could not be identified with any of the pure alkaloids available. It is to be assumed that at least some of these would be identical with already known alkaloids from the subterranean parts of the plant. G. M.

ANALYTICAL

Apomorphine Hydrochloide, Titration of, in Non-aqueous Media. A. Paulsen. (*Medd. Norsk Farm. Sels.*, 1956, **18**, 145.) With mercuric acetate, apomorphine hydrochloride gives a blue colour, due to reaction of a phenol group; it is therefore not possible to titrate the hydrochloride directly with perchloric acid in the usual way. It was found, however, that on titration with perchloric acid the blue colour changes to red at the end point. If on the other hand the solution of apomorphine hydrochloride in glacial acetic acid is cooled to below 15° before the addition of mercuric acetate, it remains colourless and the titration may be carried out with perchloric acid in the usual way using crystal violet as indicator. Two methods of titration are therefore recommended. In the first, 0·03 g. of apomorphine hydrochloride is dissolved in 30 ml. of glacial acetic acid, the solution is cooled to 30° and 10 ml. of 5 per cent mercuric acetate is added. The solution is then titrated with 0·1N perchloric acid in glacial acetic acid until the colour changes from blue to a bright red. In the second method the solution in glacial acetic acid, as above, is cooled to 10 to 15° before the addition of dioxan and mercuric acetate, and the titration is done with crystal violet as indicator. G. M.

Barbiturates, Toxicological Detection of. J. W. Huisman. (*Pharm. Weekbl.*, 1956, **91**, 505.) In cases of barbiturate poisoning it is important to detect with certainty the presence of the compound in the gastric contents or rinsings. A simple method is to dilute 1 part of the rinsings with 9 parts of water, and filter: 5 ml. of the filtrate is treated with 5 ml. of N sodium hydroxide and the absorption is measured between 225 and 260 mμ against a blank composed of

5 ml. of the filtrate diluted with 5 ml. of water. If no distinct maximum and minimum are obtained then a lesser dilution should be examined. Under these conditions barbiturates show a maximum at 250 to 255 mμ, and a minimum at 230 to 235 mμ. *N*-Substituted barbiturates show an absorption curve different from the other barbiturates, although in fact they are rapidly decomposed in the alkaline solution. The method may also be applied to urine, diluting with 99 parts of water, but in all cases the minimum is displaced towards greater wavelengths and may be found at 240 to 245 mμ. In such cases a preliminary extraction with chloroform is recommended. G. M.

Cocaine, Procaine and Amethocaine, Spectrophotometric Determination of. M. J. Pro, R. A. Nelson, W. P. Butler and A. P. Mathers. (*J. Ass. off. agric. Chem.*, 1956, **39**, 957.) In order to detect procaine and amethocaine as diluents in samples of cocaine, and to perform a simultaneous assay of these in a mixture using small samples, e.g., in solutions containing about 10 to 20 parts per million of each substance, it was found that wider separation of ultra-violet absorption peaks was obtained if the sample was hydrolysed in alkaline solution to give the sodium salts of respective aromatic acids, viz., benzoic, *p*-amino-benzoic and *p*-butylaminobenzoic acids resulting from the hydrolysis of cocaine, procaine and amethocaine respectively. Experimental details are given together with equations for calculating the concentrations of each substance from the intensity of absorption at the three respective peaks involved. Moreover, infra-red spectra are shown to be of use in qualitative identification of the pure and mixed compounds. D. B. C.

Gelatin, Detection of Micro Quantities of. P. Davis. (*J. appl. Chem.*, 1956, 6, 413.) This method was devised for the detection of very small amounts of gelatin inside the silver halide grains of photographic emulsions. It depends upon the ability of gelatin to prevent the colour change from red to blue of a gold sol under standard conditions and is adapted for use in the presence of large concentrations of electrolyte, e.g., 0·4M sodium thiosulphate containing silver halide. Some twenty to thirty-fold concentration of the gelatin is achieved by a foaming procedure, details of which are given; 80 to 90 per cent recovery could be achieved. The ability of the concentrate to retard change of colour of the gold sol is then compared under standard conditions, against a blank containing no gelatin, with the effect produced by known amounts of gelatin. Using the foaming technique, the method is sensitive to as little as 4×10^{-8} g./ml. of gelatin. The method can be made semi-quantitative and is probably applicable to many other substances. D. B. C.

Menthol in Peppermint Oil, Determination of, by Chromatographic Analysis. S. K. Hamarneh, M. I. Blake and C. E. Miller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 713.) Solutions of menthol in chloroform give a red colour when treated with *p*-dimethylaminobenzaldehyde reagent, and this is the basis of a quantitative determination. A standard curve is prepared by treating known quantities of menthol in chloroform solution with the reagent, and measuring the colour after 2 hours, using a photoelectric colorimeter with a filter transmitting in the 500 to 570 mμ region. Using 1 ml. of chloroform solution with 5 ml. of reagent, Beer's law is obeyed for quantities of 0·01 to 0·13 mg. of menthol. In the determination of menthol in peppermint oil a preliminary separation is necessary to remove menthyl acetate, which undergoes the same colour reaction as free menthol. A sample of oil is placed on a silicic acid column and washed through with chloroform. The free menthol

appears in the early fractions of the eluate and may be determined as above. Menthyl acetate appears in the later fractions and so does not interfere in the determination. G. B.

Narcotine, Papaverine, Codeine, Strychnine and Brucine, Semimicro Determination of. B. Buděšínský. (*Československ. Farm.*, 1956, **5**, 579.) The alkaloids narcotine, papaverine, codeine, strychnine and brucine form iodobismuthate complexes in acid solution with a reagent containing bismuth ethylenediaminetetra-acetate (0·028M) and potassium iodide (0·112M), an equivalent amount of ethylenediaminetetra-acetic acid being liberated; the liberated acid is determined by titration with zinc sulphate solution. The reagent is prepared by dissolving 10 g. of crystalline sodium sulphite, 19 g. of potassium iodide and 10·6 g. of disodium ethylenediaminetetra-acetate (Complexone III) in 800 ml. of water, adding 9 g. of bismuth chloride, filtering the resulting solution and making the volume of the filtrate up to one litre. 5 ml. of reagent is added to a solution containing 20 to 40 mg. of alkaloid in 0·5N hydrochloric acid. The mixture is centrifuged and a 7-ml. aliquot of the supernatant liquid is pipetted into a flask containing 30 ml. of borax buffer solution (pH 9·1). The liberated ethylenediaminetetra-acetic acid is titrated against 0·01M zinc sulphate solution with Eriochrome Black T as indicator. A blank experiment is also carried out. The method can be used to determine the alkaloids in mixtures provided that tertiary amines, quaternary ammonium salts and the corresponding sulphonium, phosphonium and arsonium compounds are absent. E. H.

Reserpine, Contribution to the Analysis of. J. Reichelt. (*Československ. Farm.*, 1956, **5**, 516.) Reserpine forms a 1 : 1 addition compound with methyl orange and this reaction can be used for the colorimetric determination of reserpine in tablets. A weighed powdered sample containing 0·5 mg. of reserpine is shaken with 10 ml. of water in a separator; 10 ml. of sodium phosphate ($Na_2HPO_4.12H_2O$) solution (25 per cent) is added and the solution is extracted with four 25-ml. quantities of chloroform, the extracts being filtered through cotton wool. The volume of the combined extracts is made up to 100 ml. A 25-ml. aliquot of this solution is shaken for 3 minutes with 5 ml. of a freshly prepared solution containing 0·1 per cent of methyl orange and 4 per cent of boric acid. The chloroform layer is separated and dried with sodium sulphate. To 5 ml. of the dry solution 1 ml. of ethanolic sulphuric acid (prepared by adding 1 ml. of concentrated H_2SO_4 to 40 ml. of ethanol and diluting the cooled solution to 50 ml. with ethanol) is added. The intensity of the colour produced is measured in a Pulfrich photometer with filter S53. The reserpine content of the tablets is determined from a calibration curve constructed from results obtained on solutions of pure reserpine. Reserpine can be detected in tablets by paper chromatography on Whatman No. 1 paper with formamide-benzene as a solvent system. The reserpine spots can be observed in ultra-violet light. E. H.

Sodium Tetraphenylboron as an Alkaloid Precipitant. O. Aklin and J. Dürst. (*Pharm. Acta Helvet.*, 1956, **31**, 457.) This reagent gives a precipitate with the majority of aliphatic amines, quaternary ammonium bases and heterocyclic bases. For use in the quantitative gravimetric determination of alkaloids, the most important factors are thorough washing of the precipitate, and drying, the latter at a temperature not exceeding 80°. Satisfactory results were obtained in the assay of papaverine by precipitation (at pH 5 and 70°) and of strychnine (at pH 5·5 and 70°). G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Narcotic Drugs, Enzymatic *N*-Demethylation of. J. Axelrod. (*J. Pharmacol.*, 1956, **117**, 322.) Enzyme systems in rat liver homogenates which *N*-demethylate morphine and its congeners, methadone and pethidine were studied. Tissue homogenates from kidney, brain, muscle and spleen of the rat were also examined, but these had no demethylating activity. In the liver the enzymes are located in the liver microsomes and require reduced triphosphopyridine nucleotide, oxygen and other cofactors for activity. Rat whole liver homogenates had relatively little activity, but when these were differentially centrifuged and the nuclei and mitochondria removed, considerable demethylating activity was observed in the remaining fractions. The inhibitory factor present in nuclei and mitochondria was heat-labile. Rabbit whole liver homogenates, in contrast to those of the rat, had the same demethylating activity as the isolated microsome and supernatant fractions, indicating the absence of inhibitory factors in the nuclei and mitochondria. There was also some species difference in enzyme specificity: rabbit liver demethylated (−)-methadone and pethidine about twice as effectively as rat liver, whereas rat liver was the more active on morphine. Only small amounts of enzyme activity were demonstrable in guinea pig's liver and none in mouse liver. There was also a marked sex difference: liver from male rats had ten times the activity of those from females, using morphine as substrate. After oestradiol administration to male rats, there was a marked fall in enzyme activity. Similarly, in testosterone-treated females, enzyme activity rose. G P.

BIOCHEMICAL ANALYSIS

Chlorpromazine in Biological Material, Estimation of. N. P. Salzman and B. B. Brodie. (*J. Pharmacol.*, 1956, **118**, 46.) A method is described suitable for the estimation of the plasma levels of chlorpromazine in animals receiving large doses, but not sufficiently sensitive for assay in humans receiving therapeutic doses. To 1 to 4 ml. of urine or plasma add 1 ml. of 10 per cent sodium hydroxide and 25 ml. of heptane containing 1·5 per cent of *iso*amyl alcohol. Shake for 30 minutes and transfer 20 ml. of the heptane phase to a stoppered bottle containing 8 ml. of 0·1M acetate buffer, pH 5·6. Shake 5 minutes and seperate the two phases by centrifuging. Transfer a 15 ml. aliquot of the organic phase to another glass stoppered bottle containing 5 ml. of 0·1M hydrochloric acid, shake for 5 minutes, separate by centrifuging and remove the organic phase. Transfer about 3 ml. of the aqueous phase to a quartz cuvette and determine the optical density at 255 and 270 mμ. The biological "blank" is also measured at both wavelengths and the optical densities subtracted from the unknown sample. Standards are prepared by dissolving the compound in water and preparing final dilutions in 0·1N hydrochloric acid. The amount of chlorpromazine, in μg., in the sample is $\frac{U_a - U_b}{S_a - S_b} \times C \times 5 \times \frac{5}{3}$ where U_a and U_b are the optical densities of the unknown at 255 and 270 mμ respectively after correction for blank. S_a and S_b are the optical densities of the standards at these wavelengths. C is the concentration of chlorpromazine in the standard in μg./ml. For tissues use homogenates in 0·1N hydrochloric acid. For the estimation of chlorpromazine sulphoxide, extract from the biological material

and seperate from chlorpromazine by shaking the heptane phase with 0·1M acetate buffer as described in the chlorpromazine method. A 5 ml. aliquot of the buffer is acidified with 1 ml. of 1N hydrochloric acid and the optical density of the solution determined at 275 mμ. A study of the metabolism of chlorpromazine in the dog showed it to be metabolised almost completely, but slowly because of its extensive localisation in various tissues. A major metabolite of chlorpromazine was found to be the sulphoxide and this was shown to have a sedative action on dog and man. G. F. S.

PHARMACOGNOSY

***Atropa belladonna*, Formation of Alkaloids in.** D. Daleff, N. Stojanoff, B. Awramowa, G. Deltscheff and I. Drenowska. (*Pharm. Zentralh.*, 1956, **95**, 437.) The development of alkaloids in *Atropa belladonna* was investigated at five stages of growth. The highest content (over 1 per cent) was found in the roots during the period of bud formation; in the aerial parts the maximum was found during bud formation and at the commencement of flowering. A considerable alkaloidal content was found in the thin roots, the root stumps, the upper stem parts and ripe fruits. These have not in the past been used as galenicals, but it is suggested that they should be considered. The content of wild plants from different regions showed considerable differences, and in one case an alkaloidal content of 1·27 per cent in the roots and 0·74 per cent in the leaves was observed. This appears to indicate that the selection of plant material to give a high concentration of alkaloids would be worth while. G. M.

***Atropa belladonna*, Lyophilised, Extraction of Constituents of.** E. B. Sommers and E. P. Guth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 55.) Leaves were collected and dried in an oven at 50° for 36 hours, and other samples were packed in solid carbon dioxide immediately after collection, and subsequently freeze-dried. All the dried samples were comminuted to No. 20, 40 or 60 powder, and examined for moisture content, total extractive, total alkaloids, chlorophyll and sugar. The total alkaloidal content and rate of extraction were similar for oven- and freeze-dried samples, but freeze-dried samples contained more chlorophyll, particularly chlorophyll A. Oven-dried samples yielded more extractive to a mixture of 3 volumes of ethanol and 1 volume of water, partly accounted for by increased nitrogenous extractive and carbohydrate content. G. B.

Digitalis, Potency of, at Different Stages of Growth. D. H. E. Tattje. (*Pharm. Weekbl.*, 1956, **91**, 541.) The assay method of Tattje and van Os was extended by a determination of the gitoxigenin content by the Tattje reagent, the aglycones being obtained by boiling with N hydrochloric acid in 25 per cent ethanol followed by extraction with chloroform. The total aglycones were then determined by the Baljet reaction, and the gitoxigenin by the Tattje reaction. The molar extinctions of digitoxin and gitoxin (Baljet reaction), after boiling with acid, are 17,600 and 15,600 respectively: that of digitoxigenin is increased by treatment with acid to from 17,200 to 18,200: that of gitoxigenin from 13,500 to 14,800. These values are used for the assay of the drug. Assays of young and full grown leaves of one year digitalis plants, collected at different seasons, showed that the total aglycone content of full grown leaves showed a

maximum at the beginning of July (4½ to 5 months after sowing). Following a drop, a second somewhat lower maximum occurs in October. With young leaves the total aglycone content is highest at the end of August, that is a month later than with full grown leaves. A striking feature is the marked rise in the ratio gitoxigenin: total aglycones, from 17 to 35 per cent. The content of crude fibre, calculated on the dry material, ranged from 6·8 to 13·8 per cent, being highest in August. The true ash content (sand-free) varied from 6·6 to 10·7 per cent: the Dutch official limit of 10 per cent is thus too strict. G. M.

Vitamin K and Naphthaleneacetic acid, Effect of, on *Datura stramonium*. B. Lowén. (*Svensk farm. Tidskrift*, 1956, **32**, 737.) Young plants of *Datura stramonium inermis* "Caspers" were sprayed with a water-soluble vitamin K analogue, stated to be 2-methyl-1 : 4-naphthaquinone sodium disulphate or with "naphthaleneacetic acid", or a mixture of the two. Some plants were sprayed once only with vitamin K analogue, 50, 150 or 300 p.p.m., while others were sprayed twice with K analogue, 50 p.p.m., naphthaleneacetic acid 50 p.p.m. or 50 p.p.m. of each. Three weeks after each spraying, leaves were collected and assayed for alkaloids. At the first collection a single spraying of K analogue 50 p.p.m. was found to increase the fresh weight, dried weight and alkaloidal content per leaf. In plants treated with 150 and 300 p.p.m. the effect was substantially greater, and the effect persisted longer when the larger amount was applied. Of the plants sprayed twice, the greatest increase was found in those plants treated with K analogue, a smaller increase occurred in those treated with K analogue and naphthaleneacetic acid and no increase in those treated with naphthaleneacetic acid only. The same effect was obtained by spraying once with a high concentration or several times with a low concentration of K analogue. G. B.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline Release from the Cat Adrenal Gland. K. R. Butterworth and M. Mann. (*Nature, Lond.*, 1956, **178**, 1234.) The adrenal glands of atropinised cats were depleted of their amine content by repeated doses of acetylcholine. In all experiments one gland of the animal was used as a control and the depleted gland compared with it. It was found that there was an equal percentage loss of adrenaline and of noradrenaline and that this was irrespective of the degree of depletion, which ranged from 7 to 86 per cent. Correlating the amines lost from the depleted gland with the amines in the plasma obtained from the adrenal venous effluent, it was found that the percentages of noradrenaline in both the control gland and the depleted gland and in the plasma were the same. The total amount of amine in the plasma was equal to or a little less than the amines lost from the depleted gland. Thus there was no resynthesis of the amines while the gland was being stimulated. Making recovery experiments following the depletion, it was found to take 6 to 7 days for the total amine content of the gland to be replaced. However, by this time the amines were not present in the same proportion as they were before depletion, there being at the end of this recovery period a considerably higher proportion of noradrenaline. These experiments show that adrenaline is synthesised at a much slower rate than noradrenaline and it is inferred that it is produced from noradrenaline and not independently. M. M.

Antacids, Comparative *In Vivo* Study of. E. W. Packman, M. E. Goldberg and J. W. E. Harrisson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 735.) Dogs with a Pavlov simple gastric fistula were given 1 mg. of histamine phosphate subcutaneously to stimulate gastric flow. After 5 minutes a sample of gastric juice was collected and a solution or suspension of the antacid under investigation was introduced into the stomach. Gastric samples were taken every 15 minutes up to 75 minutes, the acidity of each sample being determined by titration with 0·1N sodium hydroxide using bromphenol blue as indicator. The reaction of each sample was determined by pH meter. Tests were carried out using dihydroxyaluminium sodium carbonate, aluminium hydroxide, sodium bicarbonate and calcium carbonate. Using a test dose of 5 g., all these antacids reduced the titratable acidity to a low level, and raised the pH above the desired range of 3 to 5·5. Using doses of 0·5 and 1 g., only dihydroxyaluminium sodium carbonate maintained the reaction within the limits pH 3 to 5 over the whole period of the test. Results are compared with those of the U.S. Pharmacopeia *in vitro* method. G. B.

Antacids, Comparative *In Vivo* Study of. J. W. E. Harrisson, E. W. Packman, B. Trabin and M. E. Goldberg. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 738.) Guinea pigs were given 50 mg. of histamine phosphate in an oil/beeswax depot preparation intramuscularly each day. Daily doses of a suspension of the antacid under test were given by oral intubation. After 10 days, the animals were killed and the stomach and duodenum examined for ulceration, the degree of mucosal damage being assessed on an arbitrary scale. Dihydroxyaluminium sodium carbonate, aluminium hydroxide and calcium carbonate were found to provide partial protection against histamine-induced mucosal damage, due largely to the increased secretion of acid gastric juice. Compression of the antacids into tablets did not appear to lower their antacid action. Of the 3 commercial tablets examined the most effective was dihydroxyaluminium sodium carbonate, aluminium hydroxide was less effective, and a mixture of calcium carbonate with magnesium carbonate and magnesium trisilicate least effective. The advantages of *in vivo* tests for antacids are discussed. G. B.

Carbetapentane (Toclase); Value in Suppressing Cough Reflex. C. H. Carter and M. C. Maley. (*Amer. J. med. Sci.*, 1957, **233**, 77.) This study was undertaken to test the clinical effectiveness of carbetapentane citrate (Toclase) in relieving the cough of respiratory disorders. 557 patients were treated with carbetapentane and 134 patients were given placebo tablets. The medicament was administered either as tablets, syrup (carbetapentane 7·25 mg./5 ml.), or expectorant compounds (7·25 mg. carbetapentane and 16·67 mg. terpin hydrate/5 ml.). The amount of carbetapentane given ranged from 7 to 25 mg. per dose, and up to 150 mg. per day. Therapy usually lasted about 5 days or until the patient was free of cough. Carbetapentane was effective in reducing or eliminating the cough reflex in 91 per cent of the 557 patients, whereas the placebo was effective in only 5 per cent of the 134 cases. All dosage forms were well accepted by the patients, but the syrup and the expectorant compound were both shown to be more effective than the tablets. No side-effects occurred sufficient to warrant discontinuance of the drug. Minor undesirable effects occurred in 25 patients. These included dryness of the mouth or throat, a feeling of tightness in the chest, and a slight degree of respiratory depression. No evidence of habituation or dependence was encountered. The optimal dose was suggested to be about 7 to 10 mg. in children and 25 mg. in adults.
S. L. W.

9-[(2-Chlorethyl) ethylaminomethyl] Anthracene Hydrochloride, Pharmacology of. H. Minatoya and F. P. Luduena. (*Arch. int. pharmacodyn.*, 1956, **108**, 102.) This new orally effective adrenolytic drug was studied in comparison with phenoxybenzamine hydrochloride (dibenzyline) for its adrenolytic and sympatholytic activity in anaesthetised dogs and cats, its protective action against (—)-adrenaline toxicity in mice and its effect on the blood pressure of renal hypertensive rats. It was found that the anthracene derivative in doses of 0·25 to 1 mg./kg. given intravenously, reversed the pressor effect of adrenaline and that larger doses blocked the effect of noradrenaline. It was about 5 times more active than dibenzyline in this respect. Following oral administration of the substance, it was found to have a fairly rapid onset of adrenolytic action and a long duration of effect—about 52 hours. It was effective in depressing the carotid occlusion pressor effect and the pressor effect resulting from vagal stimulation. The contraction of the nictitating membrane in response to stimulation of the cervical sympathetic was completely abolished by 0·5 mg./kg. of the drug. It reduced the toxicity of adrenaline in mice, being a little more potent than dibenzyline. In renal hypertensive rats, a dose of 0·125 mg./kg. given orally reduced the blood pressure by as much as did 2 mg./kg. of dibenzyline. The duration of hypotension was prolonged. It was found to be one-half as toxic as dibenzyline when given intravenously and two-thirds as toxic orally. In a three week sub-acute toxicity test in rats, it was well tolerated in oral doses of up to 100 mg./kg., administered 18 times. The growth rate was depressed but there were no significant haematological or pathological changes. It would therefore be of interest to study this drug clinically in the treatment of peripheral vascular diseases. M. M.

Colchamine, Pharmacology of. I. M. Sharapov. (*Farmakologiya i Toksikologiya*, 1956, **19**, No. 2, 33.) Results of a pharmacological study of colchamine are reported. This alkaloid, which is *N*-methyldesacetylcolchicine, was isolated in 1950 from *Colchicum speciosum* and is a white or yellowish crystalline powder with m.p. 181° to 182° and $[\alpha]_D^{18} = 136{\cdot}1°$ (ethanol). It is very soluble in chloroform, ethanol and methanol, insoluble in ether and soluble to the extent of 20 per cent in water. Tests on white mice, rats and rabbits showed that the general physiological action of colchamine is similar to that of colchicine. In toxic doses it produces general and respiratory depression, insensitivity to pain, loss of appetite and diarrhoea; the toxic effects are cumulative. Colchamine is, however, much less toxic than colchicine. The subcutaneous LD100 for white mice is 75 mg./kg. while that of colchicine is 5 mg./kg.; the corresponding doses for rabbits are 20 to 25 mg./kg. and 3 to 5 mg./kg. Single doses of 5, 10, or 20 mg./kg., or repeated doses of 1, 3 or 5 mg./kg. given daily for 10 days, produced anaemia and leucopenia in rats. E. H.

Dextromethorphan Hydrobromide and other Antitussives, Comparison of. L. J. Cass and W. S. Frederik. (*J. Lab. clin. Med.*, 1956, **48**, 879.) A controlled study was carried out on 63 patients with chronic cough to compare the antitussive effectiveness of dextromethorphan hydrobromide (Romilar) at two dose levels (10 mg. and 20 mg.), codeine sulphate (15 mg.), caramiphen ethanedisulphonate (Toryn) (10 mg.), and a placebo. The five materials were supplied as tablets of identical appearance, and the tablets were given 4 times a day for 10 days each, each period of drug administration being followed by 3 days of placebo medication. The cough-suppressing activity of the drugs and the placebo was recorded 3 times daily by means of a numerical scale based on 4 degrees of severity of coughing. All the drugs were shown to have antitussive

properties and to be significantly more effective for this purpose than the placebo. Ten mg. of caramiphen ethanedisulphonate was less effective than 10 or 20 mg. of dextromethorphan hydrobromide or 15 mg. of codeine sulphate. For all practical purposes it was shown that dextromethorphan hydrobromide and codeine sulphate are of equal effectiveness as antitussives on a weight basis.

S. L. W.

Dipipanone Hydrochloride in Severe Pain. R. O. Gillhespy, E. Cope and P. O. Jones. (*Brit. med. J.*, 1956, **2**, 1094.) Dipipanone, or DL-6-piperidino-4:4-diphenyl-heptan-3-one, is closely related chemically to methadone. In experimental animals the pain threshold is raised by an effective dose of dipipanone to an extent comparable with that obtained with morphine. The respiratory depressant action is stated to be less than that of an equivalent analgesic dose of morphine. The acute toxicity is about the same as that of methadone. Signs of overdosage with dogs closely resemble those occurring with morphine (constriction of the pupil, salivation, defaecation and vomiting). The mechanism of action on the bowel is probably the same as that of other morphine-like analgesics. While its main toxic action is on respiration, intravenous doses of 5 mg./kg. reduces the tone of cardiac muscle. It has some atropine-like properties. Nalorphine antagonizes the analgesic and respiratory actions of dipipanone; the effective dose is approximately the same as that required to antagonize an equi-analgesic dose of morphine. The results of administration of dipipanone hydrochloride to 100 cases of pain due to a variety of acute and chronic medical conditions, and to 100 cases of post-operative pain after major gynaecological surgery are given. Only 3 of the 200 cases failed to obtain any relief from the drug. The optimal dose was found to be 20 mg. subcutaneously in the medical cases and 25 mg. in the post-operative cases. The onset of analgesia occurred within 10 minutes and maximal relief was obtained in about 20 minutes in most cases. The effect lasted for 5 or 6 hours. There was no obvious depression of respiration or tendency to drowsiness, nor was there any local reaction or pain at the site of injection. Side-effects occurred in 4 to 5 per cent of patients, and consisted of nausea, vomiting, sweating, and giddiness.

S. L. W.

Histamine, Inhibition of Release by Sodium Salicylate and Other Compounds. C. G. Haining. (*Brit. J. Pharmacol.*, 1956, **11**, 357.) In rabbits sensitised with a modified Freund type antigen, anaphylactic histamine release in the blood was reduced by a lowering of plasma pH and by suitable concentrations of sodium chloride, potassium chloride, sodium benzoate, sodium salicylate and 3-hydroxy-2-phenylcinchoninic acid. The latter was the most effective compound being approximately eight times as active as sodium salicylate and sodium benzoate. Sodium salicylate, heparin and dextran sulphate of low molecular weight inhibited in normal blood the histamine released due to incubation with washed antigen-antibody precipitate. Sodium salicylate was effective against release due to plasma activated by antigen-antibody precipitate. Both sodium salicylate and 3-hydroxy-2-phenyl cinchoninic acid did not prevent the formation of anaphylatoxin, but inhibited its action on isolated guinea pig ileum, which was not due to histamine antagonism. Both drugs appeared to exert their inhibiting action by an extracellular mechanism and it may be that anaphylatoxin is not able to initiate the release mechanism because some factor in serum is inactivated or prevented from reaching the cell site. The possibility of a transient intracellular action cannot be ruled out. G. F. S.

Methyprylone, Central Depressant Effects of. W. Schallek, A. Kuehn and D. K. Seppelin. (*J. Pharmacol.*, 1956, **118**, 139.) Methyprylone (3:3-diethyl-5-methyl-2:4-piperidinedione, Noludar), is a new non-barbiturate sedative hypnotic. Its effects have been compared with chlorpromazine, meprobamate, and pentobarbitone. A subcutaneous dose of 45 mg./kg. was the ED50 for reducing locomotor activity in rats compared with 1·9 for chlorpromazine, 240 for meprobamate and 35 for pentobarbitone. Against induced electroshock convulsions in mice the ED50 by mouth was 150 mg./kg. compared with 150 for chlorpromazine, 200 for meprobamate, 75 for pentobarbitone and 20 for phenobarbitone. In protecting mice against leptazol convulsions the ED50 by mouth was 45 mg./kg. compared with >400 for chlorpromazine, 133 for meprobamate, 42 for pentobarbitone and 33 for phenobarbitone. The LD50 by mouth was 890 mg./kg. compared with 530 for chlorpromazine, 2000 for meprobamate, 170 for pentobarbitone and 240 for phenobarbitone. It protected dogs against vomiting induced by apomorphine in doses which caused ataxia (48 mg./kg. s.c.). Here chlorpromazine was most active, the ED50 being 0·1 mg./kg. which did not cause ataxia, meprobamate was inactive. In trained dogs it produced, like meprobamate and pentobarbitone, sleep and depression of the response to a sharp handclap. Chlorpromazine produced sedation without sleep. On the basis of doses causing equivalent duration of sleep, methyprylone was a quarter and meprobamate was one eighth as active as pentobarbitone in the dog. All the drugs produced large, slow waves in the EEG of dogs, which were greatest for pentobarbitone followed by methyprylone, meprobamate and chlorpromazine. G. F. S.

Methylphenidate Hydrochloride Parenteral Solution. J. T. Ferguson, F. V. Z. Linn, J. A. Sheets and M. M. Nickels. (*J. Amer. med. Ass.*, 1956, **162**, 1303.) Methylphenidate hydrochloride (Ritalin) was administered by intravenous injection of a 1 per cent solution in 10 mg. doses to 164 hospitalised mental patients who manifested sleepiness, lethargy, tremors, drooling, nasal congestion and Parkinson-like gait following overdosage with reserpine, promazine or chlorpromazine hydrochloride. One hundred and six of the patients showed improvement of their mental alertness and a decrease in their side-reactions within 5 to 90 minutes after the first injection, 38 responded after a second injection of 10 mg., 16 required 30 mg. before a clinical change was recorded and 4 showed no change even after 90 mg. After administering 10 to 30 mg. intravenously 3 times daily for periods ranging from 24 to 72 hours it was possible to maintain the improved condition of 151 of the 160 patients with a comparable dose of methylphenidate given orally 3 times daily. No gross change in blood pressure, pulse or respiration was recorded following the injections in 160 of the patients; 8 became apprehensive and fearful and 8 overactive. Subsequently a group of 11 chronic, regressed, underactive patients were treated with intravenous injections of the drug. Three injections of 10 mg. sufficed in every case to cause marked clinical improvement, with increased activity, sudden awareness of surroundings, and other marked changes of behaviour. These changes were obtained repeatedly, appeared promptly, were of limited duration, and were not seen after injections of a placebo. Two patients showed dramatic improvement in behaviour after 21 and 17 years respectively of extreme inactivity. Six out of 7 patients in whom the drug was continued orally after initial injections continued to show a slow, steady improvement; in the seventh patient the improvement could only be maintained by parenteral injections. S. L. W.

Morphine-Nalorphine Mixtures, Effect of, on Psychomotor Performance. R. O. Bauer and R. G. Pearson. (*J. Pharmacol.*, 1956, **117**, 258.) The effects of combining nalorphine and morphine on perceptual-motor performance were examined in 96 normal male subjects. The test used was one of compensation for random movements of four dials, by manipulation of foot and hand controls. Drug combinations used were: normal saline (placebo); morphine control, 8 mg.; morphine-nalorphine mixtures in the following proportions 8 mg.: 1 mg.; 8 mg.: 2 mg.; 8 mg.: 4 mg.; and nalorphine 4 mg. given alone; all were administered intravenously over a two-minute period. The performance of the group given morphine was no poorer than that of the group given saline. However, with morphine-nalorphine mixtures and nalorphine alone, performance in the test decreased, with increasing dose of nalorphine. Nalorphine appeared to have a "soporific effect" in these instances. The incidence of toxic side effects of morphine was greater when nalorphine was also present. The 4 mg. dose of nalorphine given alone also caused a high incidence of side actions. G. P.

Ointments, A New Simplified Method for the Determination of Percutaneous Absorption of. H. Nogami, J. Hasegawa and M. Hanano. (*Pharm. Bull. Japan*, 1956, **4**, 347.) A method is described for studying the absorption of medicaments from ointment bases in human volunteers. The medicaments were salicylic acid and sodium salicylate in soft paraffin, a hydrophilic base, simple ointment and an absorption ointment. A weighed amount of the ointment was applied on a film 4 cm. square to the skin in the femoral region. The amount of percutaneous absorption was determined over eight or sixteen hours by (*a*) by measuring the decrease of salicylate in the ointment, or (*b*) by measuring the urinary excretion of salicylate. The results showed that salicylic acid was absorbed very well through the skin, but sodium salicylate was absorbed only slightly. The influence of the ointment base was not great, but with salicylic acid, absorption from hydrophilic ointment was less than from the other ointment bases. G. F. S.

Phenolphthalein, Carbon-14 labelled, Studies on the Fate of. W. J. Visek, W. C. Liu and L. J. Roth. (*J. Pharmacol.*, 1956, **117**, 347.) Carbon-14 labelled phenolphthalein was administered orally or intravenously to mice and dogs. Mice excreted 96 per cent of the administered radioactivity in the faeces and urine within 48 hours. No $^{14}CO_2$ was found in the expired air of the mice indicating that the phenolphthalein was not being broken down. In the dog with oral administration about 51 per cent of the radioactivity administered was present in the faeces and about 36 per cent in the urine; when the drug was injected intravenously the figures were slightly higher. When excretion in the bile was measured, after oral doses the excretion values were: faeces, 30·8; urine, 37·5; and bile 22·2 per cent. Corresponding values for intravenous administration were 11·2, 35·2 and 43·2 per cent. The drug was not concentrated in any of the organs studied. Radioactivity was found in all segments of the gastrointestinal tract after intravenous injection, suggesting that the drug was being excreted to some extent by this route. In mice, phenolphthalein crossed the placental barrier in both directions. There was no evidence of retention in the maternal tissue or the foetus. G. P.

Poliomyelitis Vaccine, Antigenic Potency of. J. E. Salk. (*J. Amer. med. Ass.*, 1956, **162**, 1451.) Groups of children who had been given different doses of a poliomyelitis vaccine in the spring of 1955 were all reinoculated in the

spring of 1956 with the same dosage of a vaccine of average potency. Quantitative studies showed both the persistence of the antibody over the course of a year and the increase in titre in response to the third dose given at this time. It was shown that to secure an optimum response to the booster injections it was necessary to give an adequate dose in the primary antigenic stimulation. After the booster injections the antibody titres equalled or exceeded those found in a group of convalescents from recent type 1 paralytic poliomyelitis; the non-living, non-multiplying virus antigen reproduced the serologic effects of naturally acquired infection-immunity. In 4617 subjects who received 3 inoculations only 224 had antibody at a level of 1:64 for all 3 types before inoculation, but 4548 had antibody at this level for all 3 types after the third inoculation. Six batches of commercially prepared vaccine were used in a study of 214 subjects as to the advantages of a second inoculation; the rise in titre produced by the second inoculation was clearly demonstrated for each batch, a low proportion of response to the first dose being converted to a high proportion of response by the second dose. It is clear that the amount of virus antigen injected is the chief determinant of the intensity of the immune response; this is reflected either in level of antibody induced or in degree of immunologic hyperreactivity effected. It has also been shown that the amount of virus antigen required to induce a maximum immulogic effect is substantially less if multiple injections are given with adequate spacing between inoculations. The two primary doses should be spaced not less than 2 weeks apart and during periods of potentially high prevalance 2 doses should be given within this short interval; in periods of low prevalance a preferable spacing would be 4 to 6 weeks or longer. The third dose, in either case, should be given about 7 months or longer after the second dose but prior to the ensuing seasonal prevalence. The indications are that immunity to paralysis is effected not only through existing antibody in the circulating blood but through the mechanism of immulogic hyperreactivity that calls forth antibody sufficiently rapidly, after exposure, to intercept invasion of the central nervous system even though pharyngeal or intestinal infection may have occurred. It is therefore conceivable that hyperimmunisation, such as tends to occur after secondary stimulation (booster), will not only limit or prevent the establishment of infection but will also limit or reduce the reservoir of infection by reducing the number of carriers in the population. Thus, poliomyelitis vaccination may bring about a sharper reduction in the amount of paralytic poliomyelitis than could be expected merely on the basis of the number of individuals vaccinated. There is indeed evidence that this is already occurring. S. L. W.

Sulfamethoxypyridazine; a Long-acting Sulphonamide. W. P. Boger, C. S. Strickland and J. M. Gylfe. (*Antibiotic Med.*, 1956, **3**, 378.) Sulfamethoxypyridazine is an antibacterial sulphonamide, 3-sulphanilamido-6-methoxypyridazine (Kynex) that is readily absorbed from the gastro-intestinal tract but is excreted from the body at a very slow rate. The drug has been studied in 67 patients and administered to an additional 35 patients in doses ranging from 1 to 4 g. Following the oral administration of single doses of 1 or 2 g. doses, therapeutically significant plasma concentractions are promptly achieved and are maintained for many hours; the drug can be measured in the plasma for as long as 168 hours following a single 2 g. dose. Sulfamethoxypyridazine is slowly excreted into the urine both as free and acetylated compound; the insolubility of the acetylated form and its appearance in quantity in the urine will call for care in the use of the drug. The diffusion of the drug into the cerebrospinal fluid occurs to a greater extent than with other commonly

(ABSTRACTS *continued on p. 496.*)

BOOK REVIEWS

SEMIMICRO QUALITATIVE ORGANIC ANALYSIS: The Systematic Identification of Organic Compounds. Second Edition, 1957. By Nicholas D. Cheronis and John B. Entrikin. Pp. xiv + 774 (including index). Interscience Publishers, London, 72s. New York, $9.00.

Ten years ago Professors Cheronis and Entrikin in the first edition of their book made a praiseworthy attempt to use semi-micro techniques for systematic organic analysis. Brought up to date in a second edition it now contains approximately 250 pages of new material while following closely the pattern of the first edition.

Part I is devoted to techniques of organic analysis and deals with equipment and procedures for weighing, measuring, crystallisation, distillation, sublimation, extraction, chromatography and determination of physical constants. Although much commercially available apparatus is described the authors frequently indicate how suitable equipment may be made by anybody possessing modest glassblowing ability.

Part II deals with procedures for the tentative identification of an unknown substance and seven chapters describe preliminary tests, classification by solubility, use of indicators, general tests for functional groups, specific class tests, separation of mixtures and tentative identification.

For carrying out the final identification 13 chapters are included in Part III. The analytically useful properties of all the main classes of organic compounds are described together with the preparation of suitable derivatives. Full experimental details are given and the beginner should have no difficulty in preparing any of the compounds recommended for identification purposes.

Part IV, the final section, consists of an extensive collection of tables giving physical constants of classified organic compounds and their derivatives. An appendix gives a list of apparatus, chemicals and reagents required by those using the book and there is a comprehensive index.

The book is intended both for students and industrial workers and for this purpose full references to the literature are given at the end of each chapter and in the text. Notes on laboratory accidents have been incorporated so as to appear as soon as the book is opened. This is an excellent feature as so many students are quite unaware of laboratory hazards until involved in an accident. The tables in Part IV occupy nearly 200 pages and contain too much information for the average student and probably too little for the industrial worker.

The book is well printed and produced and American spelling is used throughout; some words like "derivatization" will seem strange to British readers. Although not free from typographical errors the proof reading has been well done in view of the size of the book, which can be thoroughly recommended to students but most industrial workers would require a more comprehensive work for reference.

G. E. FOSTER.

A TEXTBOOK OF FORENSIC PHARMACY. By Thomas Dewar. Fourth Edition. Pp. xvi + 288 (including Index). Edward Arnold (Publishers) Ltd., London. 1957. 24s.

The first edition of this book appeared eleven years ago with the avowed object of presenting in a single volume all the forensic pharmacy which is ordinarily taught to students preparing for the qualifying examinations of the Pharmaceutical Society. Its success may be gauged by the fact that it has now reached a fourth edition and perhaps also from the relatively high proportion of passes achieved in this subject. The present edition follows the form previously adopted and all the changes in legislation, since the third edition appeared, are included. As the Society's examination appears to be largely based on what the retail pharmacist should know, it naturally follows that the book must be of considerable value as a work of reference for the practising pharmacist. Doubt, however, might be expressed on the value of including some schedules to the National Health Service Regulations. In one instance, the detail is insufficient for use in the busy dispensing department where the Drug Tariff would necessarily be consulted, and in another, even a hospital pharmacist could not be expected to produce any information on the charges for wigs or the soling and heeling of surgical boots. The students' appreciation of the revision questions might be enhanced if some of those set by the examiners during the past twelve years were indicated with the appropriate date. More cross references in the index might profitably be inserted, e.g., the Pharmacutical Society's coat of arms appears only under the main heading of "Titles and Descriptions", and to determine the conditions under which a poison may be sent through the post it is necessary to search for the entry which is "Poisons, Sale of—by Post". This book will continue to be the industrious student's sure guide to examination success and an authoritative exposition of the law.

J. ANDERSON STEWART.

(ABSTRACTS *continued from p. 494.*)

employed sulphonamides. In this series of patients 2 complained of headache following administration of the drug, 3 had mild nausea and anorexia during the first 2 days of administration, and one patient developed drug fever and became acutely ill 7 days after administration of a single 2 g. dose. S. L. W.

APPLIED BACTERIOLOGY

Acrylic Film for Surgical Dressings, Physical and Bacteriological Investigations of. B. T. Ekenstam, B. H. F. von Fieandt, F. Henn and K. B. Olow. (*Scand. J. clin. lab. Invest.*, 1956, **8**, 278.) The preparations investigated consisted of polymerised methacrylic esters dissolved in ethyl acetate (Nobecutan). Films prepared from the ethyl acetate solution were tested for tensile strength, elasticity, fatigue on folding and permeability to water and saline. Films of the polymerised butyl ester of methacrylic acid were stronger than those of the 2-ethoxyethyl ester, but the ethoxyethyl was better than the butyl ester in the elasticity and fatigue on folding tests, and was more permeable to water vapour. Solutions of the polymers in ethyl acetate were found to be sterile and to have a weak antibacterial activity. Films prepared from these solutions were initially sterile but had no antibacterial properties. Tetramethylthiuram disulphide was found to be an active antimicrobial agent, effective under aerobic and anaerobic conditions and in the presence of protein. It is soluble in the plastic solution, and the addition of 0·25 per cent yielded films active against Gram-positive and Gram-negative bacteria and fungi. G. B.