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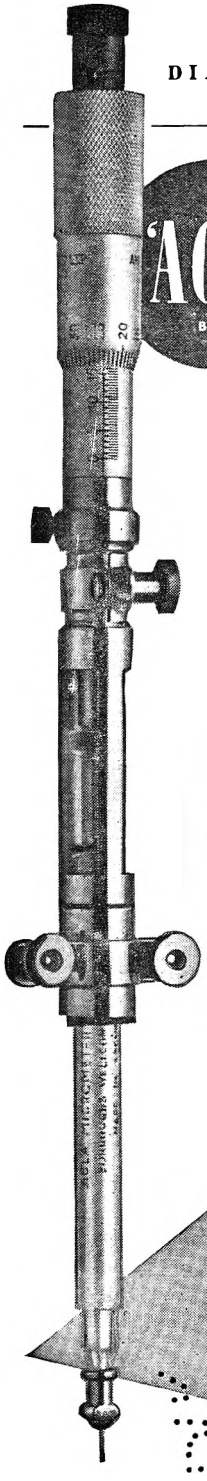

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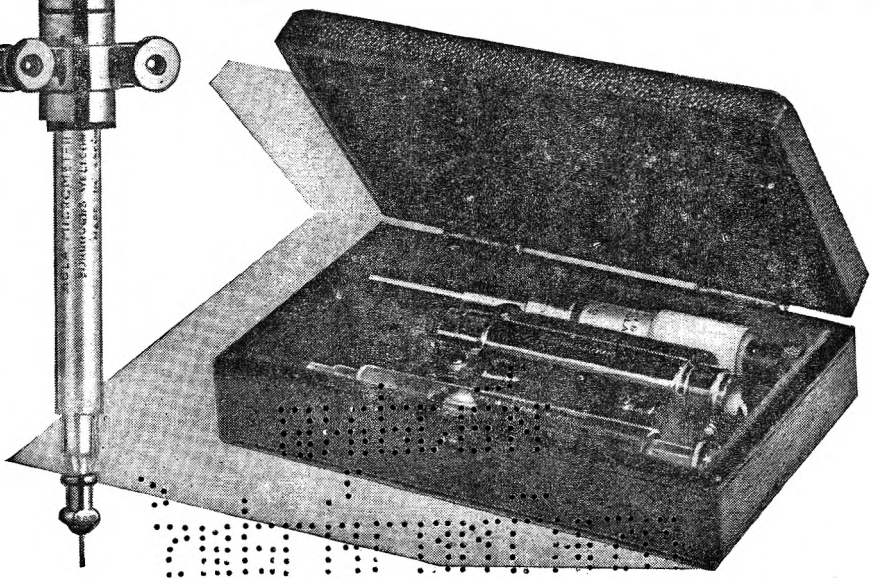

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Vol. VII. No. 9 **September, 1955**

CONTENTS

[*Continued on page ii*

CONTENTS

PAGE

Research Papers—(*continued*)

Abstracts of Scientific Literature

REVIEW ARTICLE

DISINFECTION

A Critical Review of Methods and Mechanisms

By G. Sykes, M.Sc., F.R.I.C.

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A recent correspondent to the *Pharmaceutical Journal*[1] asked the pertinent question why neither the British Pharmacopœia nor the British Pharmaceutical Codex specifies any biological tests in monographs for disinfectants and antiseptics. There are good reasons in favour of such specifications, for we know that variations in the constituents of some disinfectant preparations can affect significantly their germicidal properties. A good example is the influence of fatty acids on the phenol coefficient of lysol. There are equally good reasons why at present a range of tests should not be included. First, because the various techniques available require a certain skill acquired only by constant practice before reproducible results can be obtained. Secondly, having obtained the results, there is still a good deal of uncertainty about how to translate the findings into practical usage. When the wide variety of disinfectant preparations which are available is considered and the diversity of application is appreciated, the complexity of the problem will be realised.

With any disinfectant, the primary questions to be answered are (1) is it active against all types of organisms, (2) is its activity influenced by organic matter, (3) is its action rapid or slow, (4) does temperature and concentration affect it, and (5) has it undesirable corrosive or irritant properties? Finally, there is the economic aspect to be considered. Some of these questions will be discussed in the following pages.

The Process of Disinfection

The Role of Bacterial Enzymes

The only criterion by which we can judge whether a microbial cell is alive or dead is by its ability to reproduce and proliferate. Reproduction is a natural outcome of the metabolic life cycle of the cell which is constituted, in effect, of a complex chain of enzyme-catalysed reactions. These reactions involve assimilating nutrient substances from the surrounding medium and building it up into protein and similar cellular material; they determine, therefore, the growth characteristics and other properties of the cell. Karström[2] has classed the bacterial enzymes into two groups: (*a*) the constitutive essential enzymes, fundamental to the life of the cell and formed independently of growth conditions, and (*b*) the adaptive enzymes, produced only as required according to prevailing cultural conditions. Clearly the most important in terms of disinfection are those comprising the first group, although adaptations resulting in mutant forms of the original culture can be significant; thus, Berger and Wyss[3] believe

the high resistances to phenol of some cells in a bacterial culture to be due to mutants with certain adaptive abilities.

The nature and properties of bacterial enzymes have been discussed at length by Gale[4]. Like other enzymes, they have complex protein structures and they are specific in catalysing one particular reaction or, in some cases, a particular type of reaction. Because of this specificity it follows that the bacterial cell must carry a multiplicity of enzymes to complete the chain of reactions necessary to fulfil its growth and reproductive cycle. If the chain is broken the cell becomes moribund and dies. Some disinfectants act on a whole series of the enzymes, whilst others are specific against one enzyme only. The disinfecting action of phenols, alcohols, acids and the salts of the heavy metals can be attributed to their denaturing action on the protein moiety of the enzymes generally, and examples of specific interferences are found with the acridines, which are thought to combine with the bacterial coenzymes[4]; with the sulphonamides, which inhibit the enzyme responsible for metabolising *p*-aminobenzoic acid, an essential growth factor[5], and with the esters of *p*-hydroxybenzoic acid which are also said to block an essential enzyme system[6].

Clearly, there must be considerable differences in the enzyme make-up of the various types of bacteria, for through these their individual characteristics are determined. Changes must also take place in the enzyme balance of the cell during its life cycle. Moreover, it is known that the enzymes are sited in different parts of the organism, some in the protoplasm, others in the surface membrane. From these facts may be deduced some explanation for the differences in resistance under adverse conditions which occur not only between types and species of organisms, but also between individual cells of a single population.

Although enzyme interference appears to be the effective mode of action of disinfectants in the majority of cases—interference arising from coagulation, denaturation or other breakdown of the protein moiety of the particular enzymes concerned—it must not be assumed that it represents the action of all forms of disinfection. Several investigators (see Rahn[7]) have claimed that disinfectant action is not due to enzyme inactivation but to reactions within the cell mechanisms concerned with reproduction, but whether these two opinions are in fact different is a matter of conjecture. However, in certain instances, disinfection must be disassociated with enzyme interference. Thus, it has been shown by electron microscopic studies that quaternary ammonium compounds cause release of cell constituents[8] and produce lysis[9] at least at lower concentrations, the action being attributed to lipoprotein complexes being split[10]; penicillin prevents diffusion of the essential metabolite, glutamic acid, into the cells of *Staphylococcus aureus*[11], and cell disruption can be brought about by various physical means.

The Dynamics of Disinfection

The death rate of a bacterial population under the influence of any disinfecting agent, chemical or physical, is dependent on the temperature of treatment and the concentration of the disinfectant as well as on the

resistance characteristics of the particular organism concerned. The former are fixed characteristics but the latter is a biological variable. Investigations into the rate of death of bacteria during disinfection have been made by several workers, amongst whom one of the earliest was Chick[12]. She originally postulated that under a given set of conditions, the course of disinfection follows that of a unimolecular reaction and so yields a straight line response when the log. survivors are plotted against time. However, Chick, and others, recorded numerous exceptions in which a sigmoid curve was generally obtained. Henderson Smith[13] was able to demonstrate a change from the sigmoid to an exponential form by increasing the rate of disinfection, and thus was able to reconcile the discrepancies recorded earlier. He believed the change to be due to the initial lag phase being completed so rapidly that it cannot be detected experimentally, and so concluded that a false impression is given of the initial mode of response which makes the emergence of the exponential form more apparent than real. This opinion was supported by Jordan and Jacobs[14]. Withell[15] investigating the action of a number of germicidal substances, obtained three types of response: (*a*) those which give sigmoid time-survivor curves, (*b*) those which give exponential curves, and (*c*) those which give a lag phase followed by an exponential curve. Not infrequently the same organism under ostensibly the same conditions gave varying types of curve when the experiments were repeated. These observations led him to the main conclusion that "the different rates of destruction of bacteria under the influence of a bactericide is determined essentially by differences in the manner in which the resistances of the organisms are distributed." In phenol coefficient tests, where the end-point is that of a complete kill, these observations are significant.

Because of the variations in death rates, Withell was unable to accept the suggestion made much earlier by Phelps[16] that bactericides could be evaluated by comparing the values of the constant k in the expression:—

$$k = \frac{1}{t_1 - t_2} \log \frac{n_1}{n_2}$$

where n_1 = number of viable organisms at time t_1, and n_2 = number of viable organisms at time t_2. Phelps himself did not find k to be constant for the whole disinfection process, and suggested that a mean of several estimations might be used for calculating a "coefficient." Withell rejected this suggestion in favour of using a probit–log. survivor-time curve, which gives a straight line when there is a normal distribution of resistance of the cells.

In an extensive study of the effect of phenol on *Bacterium coli*, Jordan and Jacobs[14] did not agree that this was universally applicable, and quoted several examples where there was a change of slope in the probit-log survival-time line. Berry and Michaels[17] supported this view and concluded the probit-log time relationship over the whole range of mortalities is sigmoid, but over the range of probits 4 to 6 believed from their own experiments that linearity might reasonably be assumed. Since, however,

they agreed that this could not be taken as a generalisation, and in their own experiments with ethylene glycol and its mono-alkyl ethers they were unable to obtain parallel regressions, they considered that bactericidal efficiencies could not be compared by this means.

Jordan and Jacobs[14] realised that many of the difficulties in assessing the factors influencing rate of kill arise from variables such as fluctuation in resistance of the culture and errors due to sampling, and they devised an elaborate cultural and testing technique designed to eliminate these as far as possible. Under these conditions they obtained responses indicating an initial lag phase followed by a slow but increasing death rate which merged into a second phase of constant death rate. This continued until towards the end of the disinfection period when there was a slight decline in the death rate. The decline was thought to be partly due to difficulties in obtaining reliable survivor counts when the mortality exceeded 95 per cent. Because of this and of other cogent reasons, Jordan and Jacobs were of the opinion that, after the initial lag, death rate could be considered to remain constant to the virtual end of the disinfection.

This approach may be satisfactory for the purpose of determining factors such as the concentration and temperature coefficients of germicides, but, as Jordan and Jacobs inferred, in disinfectant testing it serves only to expose the fallacies of the present phenol coefficient methods which use virtual sterilisation as the end-point. Such an end-point is manifestly unsuitable because from the foregoing observations it is most susceptible to variations. Moreover, it is well known that routine laboratory cultures do not have a fixed normal distribution of resistance; also the hazards of picking up the odd surviving cell in a small sample increases as the disinfection approaches completion. These points will be discussed later.

The Effect of Temperature and Concentration on Disinfectant Activity

Clearly it is of considerable practical importance to know the effects of temperature and of concentration on the rate of disinfection. Many attempts have been made in the past to calculate by formulæ the temperature coefficient and the concentration exponent, or dilution coefficient, but according to McCulloch[18] "such formulæ have not proved entirely reliable, probably because of the complicated nature of the phenomenon of disinfection, including as it does the result of many diverse influences not readily expressed by simple equations." Nevertheless, some knowledge of these performance characteristics is essential in order to assess the practical value of a germicide. A single assessment at one temperature and with one end-point is not adequate. Ideally, the rate of kill at two concentrations and at two temperatures should be ascertained, from which it is possible to assess the two coefficients; these should then be confirmed by experimental data at other points. Rahn[19] has suggested that the death times at two or three concentrations at least should be determined.

In general, the activity of a germicide increases as the temperature rises, but exceptions have been noted[7,19,20]. According to Cooper and Haines[21] high coefficients are associated with oxidizing reactions and low coefficients

with reducing reactions. The temperature coefficient is an exponential function, and it appears to be related to the concentration exponent. Each substance has its own temperature coefficient, which is subject to variation according to the range of temperatures being considered and often with the test organism. These points were well demonstrated by Tilley[22] with a series of phenols and alcohols.

Although several different formulæ have been proposed for calculating the temperature coefficient, Jordan and Jacobs[23], like McCulloch[18], were of the opinion that none was really adequate. They found the graph of log. (killing time -10) against temperature to fit the Pearl-Verlhaust equation[24], and therefore suggested this as the most suitable means of calculating the coefficient. In practice, the value seems to be used only occasionally, probably because most germicides are required for use at one or two temperatures only, usually at 37° C. or at about 20° C., to which interest is confined.

The dilution coefficient is a somewhat more important factor. It is exponential and again varies with the type of disinfectant. The value is subject to a large experimental error, as shown by Tilley[25], so that numerous replicates are necessary to obtain the correct value. The most generally accepted formula for calculating the concentration exponent is,

$$\frac{\text{log. initial no. organisms}}{\text{log. survivors}} = Ktc^n$$

where K = the reaction velocity constant; t = time of disinfection; c = the concentration of the disinfectant and n = the concentration exponent. With a fixed end-point such as that of a complete kill, this can be simplified to,

$$tc^n = \text{a constant } \textit{or}$$
$$n \log c + \log t = \text{a constant.}$$

According to Jordan and Jacobs[26] this expression fits observed values when t is the *virtual sterilisation time*, but different values of n are obtained when 99·9 per cent. or 90 per cent. mortality times are used.

The significance of the concentration exponent is that when it is high the germicidal activity of a substance, and consequently its disinfecting time, is markedly affected by small changes in concentration. On this basis, therefore, it is essential to measure the death-rate of a disinfectant at not less than two concentrations; information obtained from a single test with an end-point determined at a fixed interval of time is incomplete, and can be misleading.

Bacteriostasis

Because of the differences in virility of the individual cells of a bacterial population, it is not difficult to appreciate that, with certain disinfectant treatments, the less resistant cells are killed easily, others may be partially damaged or inhibited, whilst the most resistant ones may be completely unaffected. The proportions of cells falling into these categories depend on the conditions of disinfection, and cessation of viability in a population

must be a progressive phenomenon. There can be no sharp distinction and no sudden transition between bacteriostatic and bactericidal conditions. In this connection, Dubos[27] wrote "the difference between bacteriostatic and bactericidal effect is often of a quantitative rather than of a qualitative nature," and Price[28] was of the same opinion stating that "sensitivity to inhibition and sensitivity to death cannot be separated. . . . Indeed, it may be questioned whether one often sees inhibition free from death, except in so far as one preceeds the other." These findings were supported on a quantitative basis by Cook[29] as a result of his evaluations of the bacteriostatic activities of phenol against a variety of organisms. On the other hand, Rahn and Van Esseltine[30] believed bactericidal and bacteriostatic actions to be fundamentally different, the latter being determined entirely by the reversibility of enzyme and other reactions. But in a complex and delicately balanced system such as that of the bacterial cell, the necessary reversibility may easily be upset resulting in the ultimate death of the cell.

Under conditions of prolonged bacteriostasis, it is said that certain changes can take place which ultimately render the cell incapable of reproduction. Clearly, the cell must either adapt itself to proliferate in its new surroundings or it becomes moribund and so must be considered dead. This is of importance in disinfectant testing, for, unless due care is taken to eliminate bacteriostasis, misleadingly high results in disinfectant tests can be obtained. In the majority of instances provision of a culture medium in adequate volume for the surviving bacteria to proliferate is sufficient, but in others a more positive approach is necessary because the disinfectants are strongly adsorbed to the bacterial surface. Thus the mercurials, which act on the thiol receptors of bacteria[31], are most effectively neutralised by adding excess of a thiol compound, such as thioglycollic acid, to the medium; the quaternaries, being cationic surface active agents, are neutralised by certain anionic or non-ionic compounds, such as "Lubrol W"[32] and lecithin in "Tween 80"[33] or in "Lissapol N"[34].

Standard Methods of Testing

The methods of estimating the activities of germicides all stem from the original work of Koch[35] and of Kronig and Paul[36] towards the end of the last century. Progress in the development of testing techniques, somewhat slow at first but rapid during the last two decades, has proceeded in two main directions: (*a*) methods applicable to substances used for the disinfection of inanimate objects, and (*b*) methods applicable to substances intended for use on living tissues, including wound surfaces. The procedures followed in the two types of test are fundamentally different. For preparations included in the first group, commonly called "disinfectants," the principal basis of assessment is by phenol coefficient tests, although other tests are also used which take cognisance of the effect of organic matter either in solution or in suspension, of the types of organism to be treated whether they are sporing or non-sporing, and of the physical state of the organisms whether they are in suspension or on surfaces. For germicides intended for disinfecting living tissues, that is those used

clinically in surgery or in personal hygiene and conveniently called "antiseptics," a more diverse series of tests are available depending on whether the preparation is to be used in liquid form or compounded in solids, pastes or ointments for disinfecting the skin, for oral application or for treatment of wounds. The type of infection to be dealt with by this group must be taken into account. Host tissue cell toxicities are also important, but they are outside the scope of this present discussion; so are also the recently discovered chemotherapeutic agents and antibiotics, except in so far as the studies of the latter has revealed much valuable information on the mechanism of disinfection generally[5,12,37].

Phenol Coefficient Tests

The principal methods of determining the germicidal activities of disinfectants are by one or more of the phenol coefficient tests, of which the two official British methods are the Rideal-Walker[38] and the Chick-Martin tests[39]; the Food and Drug Administration (F.D.A.)[40] and more recently the Association of Official Agricultural Chemists (A.O.A.C.)[41] methods are used in the United States. They all use the same basic principles, namely, phenol is the reference standard; the test solutions are simple dilutions in water, with the exception of the Chick-Martin test which employs yeast as added organic matter; the test organisms are selected strains of *Salmonella typhi* or of *Staphylococcus aureus*; the killing time is relatively short, being measured in minutes; the end-point is that of a virtual complete kill, and germicidal activity is expressed as a coefficient related to the lethal effect of phenol.

In this country, the Chick-Martin coefficient serves under the Diseases of Animals Act, 1950, as a basis for calculating dilution awards for phenolic disinfectants; it is also recommended as a means for selecting suitable disinfectants for certain hospital uses[42]. In the United States, a dilution award of 20 times the phenol coefficient is usually given for a disinfectant, provided it is sustained in the recently introduced "Use-Dilution Confirmation" test.

Full details of all of the techniques will not be discussed here but it is desirable to mention some of their salient features and then to draw attention to some of their disadvantages. The Rideal-Walker method was the first phenol coefficient test to be devised. It was originally published in 1903, since when it has undergone several modifications leading to its present form. All other tests are, in effect, modifications and improvements (*sic*) on the original method. The Rideal-Walker test uses a selected strain of *Salm. typhi* (*N.C.T.C.* 786) grown in a medium containing 2 per cent. of peptone (Allen and Hanbury's Eupeptone No. 1), 1 per cent. of Lab-Lemco and 1 per cent. of salt. Serial dilutions of the disinfectant and of phenol at 17 to 18° C. are inoculated with a 24 hour culture, and subcultures are made into the standard broth at $2\frac{1}{2}$ minute intervals. The phenol coefficient is obtained by dividing the lowest concentration of the disinfectant which kills the culture in $7\frac{1}{2}$ but not in 5 minutes by the lowest concentration of phenol which gives the same response. The U.S.F.D.A. method uses a somewhat different medium

containing 1 per cent. of Armour's peptone, 0·5 per cent. of Liebig's beef extract and 0·5 per cent. of salt. The Hopkin's strain of *Salm. typhi* is used and the end-point of the test is the lowest concentration of disinfectant and phenol killing in 10 minutes but not in 5 minutes at 20° C. The culture medium used in the F.D.A. test gives organisms of rather higher resistance than those grown in Rideal-Walker medium, and this is said to result in somewhat lower phenol coefficients. The A.O.A.C. method is a modification of the F.D.A. method, the principal point being that one of a number of subculture media may be used depending on the nature of the disinfectant substance under consideration; thus, a thioglycollate medium is used for dealing with mercurial disinfectants and a lecithin-Tween broth for cationic surface-active substances.

It is of interest to note that quite recently the "Use-Dilution Confirmation" test was introduced[43] as a supplement to the A.O.A.C. phenol coefficient method. The purpose of this test is to confirm that a dilution award on a disinfectant of 20 times the phenol coefficient is, in fact, satisfactory. In this test, small metal "penicillin assay" cylinders, 10 in number, are infected with a test organism, either *Salmonella choleraesuis* or a *Staph. aureus*, dried for a short period and then immersed for 10 minutes in the chosen disinfectant dilution. Each cylinder is then transferred to a nutrient broth and incubated. A satisfactory test requires complete absence of growth. In the event of any growth, the disinfectant dilution must be adjusted appropriately.

Both this and the Chick-Martin technique are more realistic in terms of practical usage. The Use-Dilution test takes into account the effect of organisms dried on a surface, and the Chick-Martin test includes organic matter in the form of yeast cells in the disinfectant dilutions. Both are important, as dried organisms may be more difficult to sterilise, and it is well known that organic matter generally depresses the activity of most disinfectants.

Disadvantages of Phenol Coefficient Tests

Phenol is the chosen standard for most disinfectant tests because (*a*) its disinfecting properties are well established, (*b*) it is a compound of known stability and purity, and (*c*) it is desirable in all tests of this type to have a standard reference material as a control. Phenol is not, however, an ideal standard, for it has a high dilution coefficient and killing rate, and its physical characteristics in solution are often quite unlike those of the disinfectant fluids with which it is being compared. Thus, as pointed out by Berry[44], all phenol coefficient tests contravene a fundamental requirement of all biological tests, namely, that like should be compared with like. There is as yet no satisfactory solution to the problem. *p*-Chloro-*m*-cresol has been tried with no greater success[45].

The result of a phenol coefficient test simply gives the information that under certain conditions a certain dilution of the disinfectant in water will kill a selected strain of *Salm. typhi* or *Staph. aureus* in a given short time. These conditions can give only limited information on the actual value of the disinfectant in practice, and, therefore, the tests can be treated only as

minimum performance tests or as a means of batch standardisation. Moreover, if the germicides are markedly different chemically from phenol, the results may be misleading. They give no information on the effect of time, concentration and temperature, of the influence of organic matter and of the significance of other bacteria. It is only when the A.O.A.C. "Use-Dilution Confirmation" or the Chick-Martin technique is employed that a phenol coefficient test begins to approach practicality.

All phenol coefficient tests suffer the technical disadvantage that their results depend on the assessment of an apparent absolute kill of a given test inoculum. This choice of end-point is unfortunate because it does not necessarily represent sterility but merely "absence of viable organisms in the sample removed and diluted with broth. This sample is small, and a varying percentage of viable organisms may still be alive when the broth yields a negative result."[46] This point had earlier been mentioned by Thaysen[45] who, by actual plate counts, showed that the apparent end-point of total kill is entirely dependent on the amount of sample taken. Vastly different points were obtained with, for example, sub-culture volumes of one loopful and 2·8 ml., the former giving a "kill" in 15 minutes, whereas the latter required 45 minutes. In addition, Thaysen drew attention to the hazards of chance survival of badly damaged cells in the medium selected. The nutritive properties of the culture medium undoubtedly plays an important part in this respect. This is well illustrated by results obtained by Hampil[47] on a series of alkyl resorcinols using media made with two different peptones. For the *iso*hexyl compound she recorded a coefficient of 40·9 in one medium and 75 in the other, and for the heptyl compound coefficients of 49 and 127 respectively. Beef extract can also cause fluctuations in resistance, to such an extent that, according to Goetchius[48], day to day variations in the phenol coefficient can oscillate between 155 and 500. It has also been noted that marked changes in resistance of a culture can be produced by altering the temperature of incubation by as little as 1° C.

The spacings of the dilutions of the phenol and of the disinfectant, and the permitted range of responses of the phenol consequent on the variations in resistance of the cultures, all militate against the precision of results obtained by any phenol coefficient test. Ortenzio *et al.*[49] have recorded variations in resistance of *Salm. typhi* to phenol at dilutions between 1 in 65 and 1 in 100 when grown and tested under standard conditions. The effects of these fluctuations on the values obtained by the F.D.A. method were studied by Rahn[7]. According to his calculations, the death-rate constant for phenol can fluctuate by a factor of 2·4 and still remain within the acceptable lethal limits of the test. Likewise the constant for the disinfectant can fluctuate by a factor of 1·5, giving a total fluctuation for the test of $2{\cdot}4 \times 1{\cdot}5 = 3{\cdot}6$. Rahn stated that a phenol coefficient test cannot be more accurate than the death-rate constants, and so was led to the conclusion that "this [360 per cent.] is the error which the specified conditions present and to which must be added the personal error of the experimenter" to which he added the sweeping statement "They appear to be more accurate because the larger deviations

are not published." The same argument applies to the Rideal-Walker test, although to a rather less extent, because the conditions of the test and the spacings of the dilutions are more closely specified, hence the errors are not likely to be so great. It must also be borne in mind that the deviations quoted are maximal; in practice, the cultural and test conditions in any one laboratory are sufficiently constant to produce lower deviations, but this might not be true of *conditions* between laboratories.

Newer Approaches to Testing

Several new tests, or improvements on existing ones, have been proposed from time to time, all devised with a view to overcoming the difficulties of the present standard methods. The most important points to be dealt with are those concerning the definition of the end-point, namely, the choice of percentage kills up to 100 per cent., and the amount of sample required to assess this. Thaysen[45] was amongst the first to criticise the existing phenol coefficient tests on these grounds, and later Withell[46] proposed determining the time for a 50 per cent. kill and he suggested that this be done with different concentrations of the disinfectant, at different temperatures and with various organisms. Whilst agreeing that an end-point of less than a virtual total kill was desirable, Jordan and Jacobs[26] criticised adversely the 50 per cent. end-point, because the concentration exponent at this mortality level is not constant. In addition, there are obvious technical difficulties in determining a 50 per cent. survival from a large bacterial population. Jordan and Jacobs were of the opinion that the 99 or 99·9 per cent. mortality times would prove more satisfactory, as both give a linear response between log. concentration and log. time. These levels have the further advantage that they can be more easily determined by plate counts.

Needham[50] proposed a nephelometric method in which survivor levels of approximately 3, 2 and 0·75 per cent. are estimated. He chose a sub-culture volume of 0·5 ml. in order to overcome the inherent sampling error of the usual single loopful and he employed a simple peptone medium which he claimed gave more constant and reproducible results than do other more complex media. The end-point is obtained by incubating the sub-culture broth for exactly five hours at 37° C., after which the opacity developed from the surviving proliferating cells is measured nephelometrically and compared with the appropriate dilutions of the standard culture incubated under identical conditions. The mortality curves in relation to concentration for different disinfectants do not always run parallel, but where a comparison of activity between two preparations is required, it is suggested that the means of the concentrations giving the 3, 2 and 0·75 per cent. survivals should be used.

A method devised by Bean and Berry[51] particularly for testing disinfectants in soap solutions reverts to a virtual complete kill, but it uses a multiple drop technique to assess accurately the end-point. The scheme of the test is that immediately after the serial dilutions of the disinfectant have been inoculated with the test organism, six uniform drops are delivered into each of a series of sterile tubes at 20° C. After a measured

time, broth is added to each tube which is then incubated and the growths recorded. They later showed[52] that the use of extensive replication and of constant sampling volumes withdrawn immediately after inoculating the bactericide, combined with suitable short sampling intervals, give estimates of mean extinction times which are comparable with those obtained by any other technique. Using a somewhat similar technique, Cook and Wills[53] established a correlation of the extinction method and the percentage survivors at shorter contact times as a means of estimating bactericidal activity. In these opinions and those of Needham, Thaysen, Withell and others we see expressed diametrically opposed ideas about what constitutes the most precise and acceptable end-point.

A number of other proposed testing techniques are worthy of mention, not so much because of their immediate value but to illustrate some of the newer approaches to the problem of assessing germicidal activities. Several investigators have used methods based on the inhibition of specific enzyme activities within the cell. Thus, Roberts and Rahn[54] observed complete enzyme inactivation at bactericidal levels but not with bacteriostatic doses; Sykes[55] suggested that inhibition of succinic acid dehydrogenase activity might be used for this purpose; Sevag and Shelburn[56] correlated closely the retardation of respiration and of growth of streptococci when treated with sulphonamides; Knox *et al.*[57] showed that the death of *Bact. coli* treated with cationic detergents parallels the inhibition of the lactic acid oxidase, and Robertson and Oliver[58] similarly correlated loss of decarboxylase activity with loss of viability in certain organisms after treatment with heat or chemical disinfectants. It is unlikely that any of these methods could become generally applicable mainly because of the extreme divergencies in the modes of action of germicides of different types.

Manometric methods have been suggested[59,60] in which the effect of disinfectants on the oxygen uptake of bacteria is said to parallel their influence on viability. Bronfenbrenner *et al.*[59] found this particularly true for *Staph. aureus* and *Bact. coli* when the respiratory end-point is taken as a reduction of 50 per cent. in oxygen uptake between the fifteenth and twentieth minutes. Hugo[61] reviewed several of the proposed manometric methods and came to the conclusion that such attempts at evaluating disinfectants may lead to false conclusions because their effect on different enzyme systems are not necessarily the same, and the reaction does not necessarily parallel cell viability.

Maurice[62] made the somewhat novel observation that suspensions of bacteria treated with basic dyes increase in turbidity, and the rate of this increase is enhanced by adding various phenols and other compounds. The increase is related to the concentration of antibacterial agent, and so it was found possible to relate the "phenol equivalent activity" obtained from the test with the actual lethal activity of the substance. According to Mandels and Darby[63], microbial cells freshly inoculated into a nutrient medium increase in volume and the increase is related to the viability of the cell population. On this basis, the authors devised a test primarily for testing fungicidal agents but which they claimed could be adapted to

disinfectant testing. Finally, Fischer and Larose[64,65] devised a method for assessing antibacterial activity in terms of affinity for wool. The basis of this test is that there is a common α-keratin structure in wool and in the bacterial cytoplasmic membrane which renders wool and bacteria similar in their responses to disinfectants.

Although each of these tests has its merits and it cannot be denied that the correlations cited must exist under certain circumstances they obviously are of no value for determining the general antibacterial properties of a compound; they neither give any measure of its relative bactericidal and bacteriostatic activities, nor do they give any indication of its selective action against different types or species of bacteria.

Other Testing Techniques

In addition to the standard phenol coefficient tests, a number of other tests have been devised to assess the germicidal activities of certain types of antibacterial preparations intended for specific uses. Because of the interest centring round these tests, they justify some separate consideration. They include the testing of preparations for surface disinfection and for skin and wound disinfection as well as the whole range of creams, ointments and other pharmaceutical preparations used in the treatment of various bacterial and fungal infections.

Tests of this type are all characterised by the facts that they are more realistic than phenol coefficient tests in that the conditions in terms of the menstruum, type of organism, time of exposure, etc., more nearly simulate the actual conditions of usage, and they generally do not use a total kill as the end-point.

Surface Disinfection Tests

Tests for assessing the activities of disinfectants on inanimate surfaces were first devised by Koch[35] and by Kronig and Paul[36], but they have only come into prominence in the last twenty years because of the increasing importance of disinfection in the food and canning industries. Weber and Black[66] were not convinced of the necessity of including deliberately infected surfaces in such assessments, having obtained identical results by a suspension method, but this opinion does not seem to be shared by the majority. The reasons for this are probably twofold: first, most preparations intended for surface disinfection contain a detergent, which obviously plays a significant part in any washing process, and, secondly, organic matter adhering to a surface may exert a marked protective effect on the bacteria.

Jensen and Jensen[67] used a technique in which a test culture is dried on cover slips for a short period after which they are immersed for two minutes in the disinfectant dilutions and then cultured in broth. Mallman and Haines' modification[68] uses infected glass cylinders (this technique was subsequently adopted as the basis for the United States "Use Dilution Confirmation Test"), and Stedman, Kravitz and Bell's modification[69,70] employs small squares of a selected test material—metal, glass, linoleum, etc.—and allows a disinfecting period of ten minutes; a kill of

99·9 to 99·99 per cent. is considered a satisfactory end-point. In later publications Stedman, Kravitz and Bell[71] discuss the significance of detergency and the influence of porous surfaces on the activities of disinfectants.

Because of the importance of surface disinfection in dairying a number of tests specific for this purpose have been devised[72–76]. All include milk solids and they differ only in the type of test material used—glass slides, rubber strips, metal strips or metal cans—in the time of disinfection and in the method of assessing the end-point. In general a kill of the order of 99·9 per cent. is accepted.

Testing Quaternary Ammonium Compounds

The most numerous and controversial of these tests are concerned with the surface active cationic group of substances, the quaternary ammonium compounds, and an extensive literature has accumulated around them. The compounds and their properties have been well described by Lawrence[77] and their particular application in the disinfection, or "sanitisation," of food and beverage utensils have been discussed by Resuggan[78]. Difficulties were first encountered in testing these compounds because of their high surface activities and the consequent bacteriostatic carry-over on subculture which led to indeterminate and false-high values. The position was rectified, however, by the discovery of a number of antagonists or inactivating agents, amongst which are phospholipids[79], lecithin with "Tween 80"[33] or with "Lissapol N"[34], suramin sodium[80], agar and milk[81]: nutrient broth alone is not an inactivator[82,83], and this explains the reason for the initial testing difficulties.

The false-negative results frequently obtained were at first thought to be due to the test organisms being massed on the walls of the tube by the action of the quaternary compound, and to overcome this Klarmann and Wright[84] devised a semi-micro test method in which the whole of the test solutions could be cultured. As was to be anticipated, the results obtained were lower, due not so much to the elimination of sampling errors than to the use of a much greater subculture volume. Davies[32] believed the discordant results to be due mainly to the bacteria being clumped and he proposed a testing technique which (*a*) allows a sufficiently large sample to be subcultured to ensure including some bacterial clumps, (*b*) provides means for breaking up the clumps, and (*c*) gives an actual count of surviving bacteria at chosen time intervals. He used a 1 per cent. solution of "Lubrol W" as the inactivating agent. The method is similar to that proposed by Weber and Black[66]. They used two test organisms, *Staph. aureus* and *Bact. coli*, and obtained the end-point by plating the disinfectant mixture, after quenching with lecithin-Tween, at intervals between 15 and 300 seconds contact. They claimed that the only satisfactory end-point is that of a total kill, on the argument that the variable death rates of bacteria under the influence of different disinfectants render other end-points unacceptable. They also claimed that their method gives a reliable practical dilution value if the end-point is assessed after 30 seconds exposure. Cousins[34] employed a somewhat different technique, including

milk solids as organic matter in the disinfectant dilutions. She used only a two minute disinfection period employed lecithin in "Lissapol N" as the inactivator and plated decimal dilutions to obtain the end-point. This method is more realistic in that it takes into account the effect of organic matter, but the choice of only two minutes contact, although simulating the average immersion time of utensils for washing up, is rather short for experimental observations.

Skin Disinfection

In considering skin disinfection, it should be remembered that, owing to its particular structure, it is not possible ever to achieve complete sterilisation of the skin, and so "skin antisepsis" is probably a more appropriate term. Any germicide applied to the skin will only deal with those micro-organisms with which it comes in contact, that is, the transient types in or near the surface; it cannot touch those resident deep in the pores of the skin. For this reason, many workers believe that a type of germicide should be employed which will retain its activity on the skin for some time, and a test on these lines was recently described by Powell and Culbertson[85]. It is similar to one used in the author's laboratory for some years. Briefly the technique consists of applying known dilutions of the germicide to small marked areas of the skin and then, at selected time intervals up to several hours, infecting these areas with a culture of *Staph. aureus* and assessing survivors after ten minute contact by swabbing and plating.

A practical *in vivo* method of assessing the value of skin disinfectants, particularly for those in which soap or other detergents are employed, is that devised by Price[86] or one of the several modifications subsequently suggested[87,88,89]. All of these tests employ some variation of a multiple hand-basin washing technique in which the hands are first washed for a fixed time under controlled conditions with the given germicide dilution and then rinsed in several basins of sterile water which are subsequently plated to count surviving bacteria. Other types of test have been suggested[90–94], but Price[95] has expressed the opinion that "the serial-basin hand-washing test is the only one proposed so far which is able to measure reliably the skin disinfectant action of mechanical cleansing or chemical germicides." The method is, however, cumbersome and requires a large number of test subjects in order to obtain reliable results.

Many skin disinfectants are made with a phenolic germicide in a soap or other detergent base, a typical example being Solution of Chloroxylenol B.P. Varying results on the effects of soaps on the activities of phenolic compounds have been reported; Hampil[96], for example, found that sodium oleate depresses the activities of phenol, cresol and hexylresorcinol, whilst others have recorded enhanced activities. Frobisher[97] and Cade[98] observed variable effects according to the particular phenol used and the concentration of soap in the solution. These differences can be accounted for on the basis that small concentrations of soaps reduce the activities of phenols, but above certain concentrations the soaps give rise to micelle formations and these act as centres for solubilising substances which are

otherwise relatively insoluble. The subject was studied in detail by Bean and Berry[99] using chloroxylenol and benzylchlorophenol in potassium laurate solutions. They showed that the bactericidal properties of these solutions are related to the concentration of the phenols in the micelles and not to the overall concentration in the system as a whole.

Testing Antiseptics

For the large group of preparations popularly known as "antiseptics," a number of tests have been devised according to the purposes for which the preparations are recommended. Thus, with some preparations the nature of the organic matter, serum, pus or saliva, and the types of bacteria to be dealt with may be important, and so tests are devised with strains of staphylococci, streptococci, *Bact. coli*, *Pseudomonas pyocyanea* and *Proteus vulgaris* in the presence of serum or whole blood. Speed of action may also be important, depending on whether the germicide can be left in contact for only a short period of seconds or minutes or for a longer duration of several hours. It is usual to make such assessments at blood heat rather than at normal room temperature, and it may be desirable to have some comparison of the bactericidal and bacteriostatic activities.

With semi-solid pharmaceutical preparations such as ointments and creams, two types of test are generally used, (*a*) one of the many variants of the agar plate diffusion test, such as that quoted by Ruehle and Brewer[40], to assess penetrability, and (*b*) a lethal test to assess the killing properties of the preparation. One of the simplest forms of the latter is to inoculate the surface of a serum agar plate with the test organism, incubate for a few hours to establish growth, smear the semi-solid germicide over the surface and then at selected intervals cut out small discs of the treated agar and culture to determine survivors. Somewhat different methods are given by Foter and Nisonger[100] and by Walters[101].

Several *in vitro-in vivo* type tests have also been devised involving tissue toxicities[59,102], tests for toxicity to leucocytes[103,104] and tests in egg membranes[105] as well as those in which treated infected materials are introduced into experimental animals[106,107,108]. Of the last group of tests, the one most generally accepted is that of Nungester and Kempf[109], a revival of the method first proposed by Christiansen[110], in which the tip of the tail of mice is infected with a selected organism, treated for a short period with the disinfectant and then amputated and inserted in the peritoneal cavity of the animal. The limitation of the test is that a mouse-pathogenic organism must be used, but the method is said to give consistent results.

It has not been possible in this review to consider anti-fungal preparations. Because of their importance both medically and industrially a great deal of attention has been paid to them, but it must suffice here to state that in general fungal spores show considerable variations in resistance between the different genera and species, and they are more resistant than most bacteria. Thus, an effective antibacterial preparation is not necessarily active against moulds. The tests devised for assessing activity

against moulds are many and varied, but they do not appear to be as reliable as those used for bacteria.

Conclusions

The study of disinfection has progressed in two main directions, (*a*) investigations into the mode of action of disinfectants on the bacterial cell, and (*b*) the development of methods of testing their efficiencies under diverse conditions. The first line of investigation has been followed mainly in connection with the antibiotics and other chemotherapeutic agents, as exemplified in the extensive work of Gale[4] and his colleagues[11,111–113], of Albert *et al.*[114,115] and of McIlwain[116], and in several reviews and symposia, e.g.[37,117–120]. It is natural that such investigations should have been concentrated primarily on the groups of substances likely to be of chemotherapeutic value because of their special interest in medicine and the desire to produce even more effective agents. Nevertheless, they also give valuable information on the likely mode of action of disinfectants and germicides generally.

Also embraced in this type of investigation is the fundamental work on the variation in resistance of the cells of a bacterial population consequent on its cultural condition and the development of mutants or variants. Because of the fluctuations observed in routine daily cultures and in cultures grown in different laboratories this aspect is of considerable importance in devising any type of test, phenol coefficient or otherwise, in which the resistance of the test organism may be involved.

Turning to the second group of investigations, that is, those concerned with the development of testing methods, it is clear that there is a strong trend away from the classical phenol coefficient tests to methods which are more directly related to conditions of usage. Whilst it must be agreed that the former have proved of value in the past, and still continue to do so if used in their right context, it cannot be denied that on certain points discussed earlier they are unsound. The methods are acceptable if they are confined to standardising phenolic disinfectants or if the results obtained are considered only to be a means of determining minimum performances.

One of the main questions with any disinfectant test is that of deciding what is the most suitable end-point. Opinions are divided; it sounds better to report in terms of a total kill, but there are cogent arguments against this. This is a matter of fundamental importance and much more work is necessary before an *ex cathedra* opinion can be expressed.

Of the more recent testing techniques proposed, nearly all dispense with any reference standard, and the conditions more nearly represent those encountered in actual use, in the way of the menstruum and test materials employed, the time of contact and temperature of disinfection. This applies particularly to the large and varied groups of germicidal preparations used in surgery and in personal hygiene. In this connection, Reddish[121] has proposed a "panel of methods for testing antiseptics" from which the potentialities of any preparation can be determined. Whilst serving as a useful guide, it should not be assumed that these are

the most suitable and the only tests to apply under all circumstances. It is now accepted that no single test can be devised to give all the essential information.

There seems to be little doubt that, with the expansion in the range of germicidal substances now in use and the many diverse uses to which they are put, the testing of disinfectants and antiseptics, rather lightly dismissed in the past, is assuming a more important role in pharmaceutics and in microbiology generally.

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RESEARCH PAPERS

ORAL AND BRONCHIAL FLUIDS IN POISONING WITH ANTICHOLINESTERASES

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PROFUSE secretion of fluid from mouth, nose and eyes is a frequently observed sign in acute poisoning with anticholinesterases, and the accumulation in the airway of this fluid may aggravate the respiratory distress caused.

The present investigation reports preliminary studies on the origin of this secretion. In order to obtain quantitative information, fluids flowing from the cannulated trachea and also from the mouth (and nose) of anæsthetised rabbits, cats and monkeys were collected before and after the administration of anticholinesterase or parasympathomimetic agents.

Only small increases of the normally very low flow of bronchial secretion were found after *iso*propylmethylphosphorofluoridate (Sarin), *N-p*-chlorophenyl-*N*-methyl carbamate of *m*-hydroxy phenyltrimethylammonium bromide (Nu 1250), and di*iso*propylphosphorofluoridate (dyflos); pilocarpine increased the bronchial fluid flow considerably; 5-(2 methyl-1 : 3-dioxa*cyclo*pentane) choline (2268 F) had no effect at all. Salivary secretion was significantly increased after large doses of the anticholinesterases and abundant after therapeutic doses of parasympathomimetics.

METHODS

Nineteen rabbits, 7 cats, and 2 monkeys were used. The anæsthetics were varied; pentobarbitone, urethane or chloralose were employed. The animals were held in the prone position in a V-shaped elevated trough inclined towards the head end at 15 to 20° to the horizontal. The legs could be fastened to the vertical stands of the trough.

Collection of Bronchial Fluid

To facilitate the free flow of bronchial fluid the animal must breathe warm moist air but rebreathing with accumulation of CO_2 has to be avoided. A free modification of the apparatus employed by Perry and Boyd[1] was used to collect the bronchial fluid (Fig. 1). One limb of a short 4-way cruciform cannula was inserted in the trachea just below the larynx. The animal was then transferred to the trough and a current of air at 41° C. and saturated with water vapour at this temperature was passed through the now horizontal limbs of the cannula. The animal could breathe freely from this current of air while all expired air was swept away in the current so that rebreathing artefacts were avoided. The lower part of the vertical limb, carrying droplets of bronchial fluid

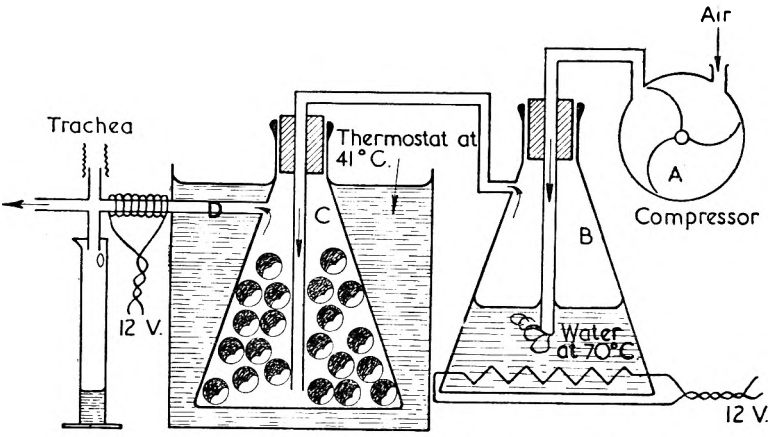

FIG. 1. Apparatus for the collection of bronchial fluid.

and some spurious condensate, was fitted into a small measuring cylinder. A diagram of the arrangement is shown in Fig. 1. The current of air (4 to 5 l./min.) was provided by the small compressor (A) and was bubbled through water in flask (B). The water in this flask was maintained at 60° C. by means of a low-voltage immersion heater. The outgoing air, partially saturated with water vapour at 60° C. was led into vessel (C). This vessel was immersed in a water bath which was thermostatically controlled and kept at a temperature of 41° C. The vessel was filled with pieces of broken glass and here the excess water vapour could condense and the air reach a temperature of 41° C. while it was fully saturated with water at this temperature. The tube (D) leading from this vessel to the trachea of the animal was lagged with an electrically heated flex and kept at 42 to 45° C. In this way all condensation in the tube was avoided.

Collection of Fluid from Mouth and Nose

Copious secretions from these sites occur frequently and collection was simple. The hanging head of the animal was resting in a glass funnel and the stem of the funnel was placed in a measuring cylinder. In some cases it was necessary to keep the mouth open by an inserted wire ring.

Readings of the accumulated volume of fluid were taken at hourly or shorter intervals. From these data fluid flow rates were calculated as ml./hr./kg. To assess the effect of a treatment the maximum flow rate obtained during or after the treatment was related to the mean pre-treatment flow rate of the same animal.

RESULTS

Pre-Treatment Flow-rates

Before commencing any treatment, flowrates were observed for a period of two hours in order to obtain a "normal baseline" for each animal. Flowrates of oral and bronchial fluid are approximately 0·1 to 0·3 ml./hr./kg. and are similar for rabbit, cat and monkey. In the cat and the

rabbit the oral fluid flow is two to three times greater than the bronchial flow. Rabbits produce slightly more fluid than cats. Most of the variation is due to difference between animals; the flowrates of each individual animal do not change greatly over a period of one to four hours. Bronchial fluid in rabbits is thin, almost colourless and only slightly opalescent. In cats, globules of thick green mucus are not uncommon.

Sarin

Four cats were injected subcutaneously with 12 to 25 μg./kg. doses of sarin, repeated at half hourly intervals, until general symptoms appeared. In three out of the four cats slight convulsions, dyspnœa or arrest of respiration occurred after a total dose of 50 to 60 μg./kg. of sarin had been given. (In the fourth cat this happened after 25 μg./kg.) An increased oral fluid flow occurred only when signs of sarin poisoning were evident and the rate of flow was roughly proportional to the severity of the symptoms. Thus the onset of the increased flow was always accompanied by extensive skin twitching and reached its height during the muscular fasciculations and general convulsions. (To prolong the life of the animals, positive pressure respiration was given at the first signs of an arrest of respiration.) When these general symptoms had appeared the oral fluid flow rate was increased to a maximum of 3·7 ml./hr./kg., i.e., approximately 18 times the pre-treatment rate. The bronchial fluid flow was only slightly increased to a maximum of 0·08 ml./hr./kg., i.e., to not quite twice the pre-treatment rate.

Rabbits were found to yield smaller volumes of fluid than cats after sarin. Repeated subcutaneous doses of between 10–20 μg./kg. were given to four rabbits. After a mean dose of 34 μg./kg. general symptoms developed and the oral fluid rate increased to a maximum of 0·46 ml./hr./kg., i.e., to 2·4 times the pre-treatment value. The corresponding value for the bronchial fluid was 0·13 ml./hr./kg. (three times the pre-treatment mean). One monkey had a subcutaneous dose of 70 μg./kg. followed by 17 μg./kg. repeated four times at half hourly intervals. After a total dose of 138 μg./kg. general muscular twitching developed and simultaneously the oral fluid rate increased to a maximum of 9 ml./hr./kg. (300 times the pre-treatment mean). The bronchial fluid reached a maximum of 0·24 ml./hr./kg. (nearly twice the pre-treatment mean). A further dose of 17 μg./kg. of sarin at this stage prolonged the increased flow but did not augment it.

Sarin Mixed with Acetylcholine

In sarin poisoning the twitching and fasciculations of the muscles seemed to be the necessary preliminary for the commencement of an increased flow. It could be assumed that the acetylcholine formed in the course of this muscular activity builds up a gradually increasing blood level which reinforces the acetylcholine formed locally in the salivary gland[2].

To simulate a fast build-up of acetylcholine, mixtures of sarin and acetylcholine were administered in small doses repeated at regular intervals. Considerably increased naso-oral flow rates are obtained in

this way. The onset is very gradual at first but after 6 or 7 preliminary doses the flow suddenly increases to a relatively high rate. Six rabbits received 8 half-hourly doses of 7·5 μg./kg. sarin mixed with 60 μg./kg. acetylcholine. The maximum effect on oral flow rate occurred after a total of 50 μg./kg. of sarin had been given; this maximum was 7·5 ml./hr./kg. (29 times the pre-treatment rate). The bronchial flow rate was only slightly affected with a maximum of 0·18 ml./hr./kg. (1·5 times the pre-treatment rate). One cat, after 3 half-hourly doses of 10 μg./kg. sarin mixed with 100 μg./kg. acetylcholine produced a maximal oral flow of 21 ml./hr./kg. (269 times the pre-treatment rate). The maximal bronchial flow was 0·6 ml./hr./kg. (19 times the pre-treatment rate). One monkey treated with 2 half-hourly subcutaneous doses of a mixture of sarin (6 μg./kg.) and acetylcholine (60 μg./kg.) developed a maximal oral flow of 9·8 ml./hr./kg. (82 times the pre-treatment rate) and a bronchial flow maximum of 0·12 ml./hr./kg. or twice the pre-treatment rate. Three further doses of 6 μg./kg. of sarin + 60 μg./kg. of acetylcholine at half-hourly intervals prolonged the response without further augmenting it.

Nu 1250

This substance inhibits the "true" cholinesterase predominantly. It has been shown to produce regularly a copious spontaneous secretion of saliva after close-arterial injection into the submaxillary gland[2]. In one rabbit, after a single subcutaneous dose of 0·2 mg./kg. of Nu 1250 a maximal oral fluid flow of 42 ml./hr./kg. (127 times the pre-treatment rate) developed during the violent terminal convulsions lasting 5 minutes. Simultaneously the bronchial fluid flow increased to 1·2 ml./hr./kg. (10 times the pre-treatment rate). The second rabbit, after a dose of 0·5 mg./kg. of Nu 1250, did not convulse and maximal fluid flow rates were much lower, viz., 1·3 ml./hr./kg. for the oral, 0·12 ml./hr./kg. for the bronchial flow, i.e., 26 times and twice the pre-treatment rates respectively.

Dyflos and Dyflos Mixed with Acetylcholine

One rabbit received seven doses of 0·1 mg./kg. dyflos at half-hourly intervals. Oral and bronchial fluid flows remained approximately at pre-treatment rates. A second rabbit, after two doses of 0·5 mg./kg. dyflos showed an increase in oral fluid to 2·5 ml./hr./kg. (8·6 times the pre-treatment rate) while the bronchial flow decreased to half the pre-treatment rate.

A mixture of dyflos and acetylcholine was tried in one cat. After six doses of 0·1 mg./kg. dyflos + 50 μg. of acetylcholine followed by 4 doses of 0·2 mg./kg. dyflos + 0·1 mg./kg. of acetylcholine the oral fluid flow reached a maximum of 5·1 ml./hr./kg. (34 times the pre-treatment rate) whereas the bronchial fluid maximum was only 0·14 ml./hr./kg. (twice the pre-treatment rate).

Pilocarpine and 2268 *F*

These parasympathomimetic agents produce by far the most dramatic effects on oral fluid flow at doses which are not close to the LD50, but

whereas pilocarpine increases the bronchial flow substantially, 2268 F does not produce an increase. Pilocarpine in a single dose of 6·2 mg./kg. to a cat produced a sudden increase of oral flow to a maximum of 24 ml./hr./kg. with a simultaneous sharp rise of bronchial fluid flow to a maximum of 0·7 ml./hr./kg.

2268 F in a single dose of 200 μg./kg. to a rabbit gave, within a few minutes, a maximum oral flow of 43 ml./hr./kg. but had no effect at all on the bronchial fluid. Another rabbit had three subcutaneous doses of 20 μg. of 2268 F at half-hourly intervals. A maximum oral fluid flow of 49 ml./hr./kg. was reached and within the next 3 hours a total of 240 ml. of oral fluid was collected. Again there was no effect at all on the bronchial fluid flow.

Acetylcholine

Repeated doses of acetylcholine by itself were given subcutaneously to two rabbits as a control to the experiments with mixtures of sarin and acetylcholine. Seven half-hourly doses of 50 μg. of acetylcholine per kg. did not alter the pre-treatment rate of flow of either oral or bronchial fluids. Seven 500 μg. doses of acetylcholine given at half-hourly intervals produced erratic increases to a maximum of 0·7 ml./hr./kg. for the oral and 0·2 ml./hr./kg. for the bronchial flow.

Discussion

Two essential points are borne out by the results. Firstly, all the anticholinesterases and parasympathomimetics that were tried had a much greater effect on the oral, than on the bronchial flow. Secondly, in poisoning with anticholinesterases fluid flow rates increased in parallel with the severity of the general signs of the poisoning. If the dose was not large enough to cause at least skin twitching, flow rates remained at pre-treatment levels. Sarin administered subcutaneously to conscious

TABLE I

SUMMARY OF EFFECT OF VARIOUS ANTICHOLINESTERASES ON ORAL AND BRONCHIAL FLUID FLOW

Species and No. used	Agent	Mean total dose in μg.	Oral fluid	Bronchial fluid
			(The figures are ratios of flowrates before and after treatment)	
4 Rabbits	Sarin	34	2·8	3·0
4 Cats	Sarin	47	18·3	1·4
1 Monkey	Sarin	138	30·7	1·9
6 Rabbits	Sarin Acetylcholine	51 442	29·0	1·5
1 Cat	Sarin Acetylcholine	30 300	296·0	19·0
1 Monkey	Sarin Acetylcholine	12 120	81·7	2·0
1 Rabbit	Acetylcholine	350	1·0	1·0
1 Rabbit	Acetylcholine	3500	2·3	1·8
2 Rabbits	Dyflos	850	4·5	1·0
1 Cat	Dyflos Acetylcholine	1400 700	34·0	2·0
2 Rabbits	Nu 1250	350	76·8	5·0
2 Rabbits	2268 F	130	88·0	0·8
1 Rabbit	Pilocarpine	6·4	66·2	3·1
1 Cat	Pilocarpine	6·2	100·0	14·4

dogs provokes intense salivary secretion usually only at doses which cause general convulsions. A slightly increased flow of saliva appears shortly before the onset of the convulsions, but the animal can usually swallow this secretion. As the convulsions become more intense, copious saliva escapes from the animal's mouth (Spencer, oral communication).

It is interesting to note the difference on bronchial flow between 2268 F and pilocarpine. Both agents have a powerful action on salivary secretion, the smooth muscles of stomach, intestine and urinary bladder, and a similar miotic action[3]; yet only pilocarpine increases the bronchial fluid flow[4]; 2268 F leaves it unaffected.

A summary of the mean experimental results is shown in Table I.

Summary

1. An attempt has been made to obtain quantitative data on oral and bronchial fluid flow in poisoning with anticholinesterases and parasympathomimetics.

2. All anticholinesterases and parasympathomimetics tried had a much greater effect on the oral than on the bronchial fluid flow.

3. In poisoning with anticholinesterase fluid flow rates increased in parallel with the severity of the general signs of the poisoning.

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THE SCOPE OF PHENOL-CHLOROFORM-ACETONITRILE AS A SOLVENT SYSTEM IN NONAQUEOUS TITRIMETRY

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Glacial acetic acid has been considered by many workers[1–5] to be the solvent of choice for the nonaqueous titration of salts of organic bases. Markunas and Riddick[1] reported representative groups of over 400 compounds which they tested, while Pifer and Wollish[2] published a report which mainly dealt with the determination of halide salts of organic bases, using glacial acetic acid as the solvent.

Recently a method was devised in this laboratory[6] which facilitated the quantitative determination of codeine phosphate when combined with acetylsalicylic acid, phenacetin and caffeine, by using a phenol-chloroform-acetonitrile solvent system. From the results obtained during that investigation, it was believed this solvent system may be used to determine the salts of other organic bases.

Experimental

Reagents. (*a*) Perchloric acid 70–72 per cent.; (*b*) dioxane, Eastman white label; (*c*) glacial acetic acid, A.C.S. grade; (*d*) phenol, reagent grade; (*e*) chloroform, A.C.S. grade; (*f*) acetonitrile, repurified by shaking with amberlite IRC-50 as previously reported by this laboratory[7]; (*g*) 0·05N acetous perchloric acid standardised against potassium acid phthalate, A.C.S. grade; (*h*) 0·05N perchloric acid in dioxane, standardised as above; (*i*) methyl red 0·25 per cent. in 2 g. of phenol and 100 ml. of chloroform.

Apparatus. A Fisher titrimeter, complete with glass-calomel electrode combination and semimicro burette.

Procedure. A sample of the organic salt, sufficient to permit a titration of 2 to 3 ml., was weighed into beaker, 5 g. of phenol and 10 to 20 ml. of chloroform were added. The mixture was stirred electromagnetically to complete solution, then 50 ml. of acetonitrile and 1 to 2 drops of methyl red indicator were added. As the end-point is approached, the indicator passes through several stages of colour from peach to red-violet. The final change is usually very sharp. In the potentiometric titrations, millivolt readings were recorded for every 0·02 ml. of titrant in the vicinity of the end-point, which was taken at the millivolt reading where the ratio dE/dV was a maximum.

Experimental Results and Discussion

The results obtained when aliquots of pure salts of amines and heterocyclic nitrogen compounds, dissolved in phenol-chloroform-acetonitrile solvent system and titrated with 0·05N perchloric acid in dioxane, are recorded in Table I. Water content of the compounds was determined

TABLE I

TITRATION OF PURE SALTS

Salt	Potentiometric		Visual		Per cent. water (Karl Fischer)
	Mg. taken	Mg. recovered	Mg. taken	Mg. recovered	
Amphetamine sulphate	52·0	52·5	52·0	52·5	
	50·6	50·4	36·0	36·0	0
	42·0	42·3	36·9	36·6	
Dexamphetamine sulphate	54·4	54·3	42·1	42·3	
	44·9	44·5	38·8	38·7	0
	45·3	44·8	54·4	54·3	
Ephedrine sulphate ..	43·6	43·4	45·7	45·4	
	42·4	42·4	45·0	44·6	0
	41·7	41·9	45·3	45·0	
Morphine sulphate ..	88·6	87·4	88·6	87·4	
	84·8	83·8	89·1	89·6	9·03
	87·6	87·0	84·8	83·8	
Codeine sulphate ..	83·3	84·3	77·5	76·9	
	70·9	71·9	35·5	36·0	6·39
			70·9	71·9	
Butacaine sulphate ..	37·4	37·4			
	77·8	77·4	Indicator	unsuitable	—
	76·0	75·9			
Quinine sulphate ..	63·4	63·6			
	66·8	67·0	Indicator	unsuitable	4·3
	66·9	67·1			
Cinchonine sulphate ..	26·2	25·9			
	27·1	26·6	Indicator	unsuitable	2·77
	27·7	27·8			
Cinchonidine sulphate	25·7	25·5			
	29·7	28·9	Indicator	unsuitable	3·89
	25·3	24·7			
Strychnine sulphate ..	93·7	93·7	79·0	78·5	
	83·9	83·5	93·7	93·7	10·5
	87·6	87·1	87·6	87·1	
Physostigmine sulphate	66·3	66·5	66·3	66·5	
	74·2	74·7	66·9	67·2	2·3
	65·3	65·0	68·1	68·4	
Physostigmine salicylate	52·0	51·1	52·0	51·7	
	50·1	49·7	50·1	50·1	0·4
	50·8	50·3	50·8	50·7	
Phenindamine tartrate	45·3	44·6	45·6	44·6	
	55·8	54·6	45·3	44·6	
	50·9	49·8	55·8	54·6	
Pilocarpine nitrate ..	30·3	30·3			
	36·7	36·9	Indicator	unsuitable	—
	34·6	36·1			
Codeine phosphate ..	Previously reported[6]		43·4	43·6	
			46·2	46·6	5·26
			44·3	44·6	
Morphine acetate ..	46·8	45·7	48·0	46·9	
	53·5	51·9	53·5	52·3	9·6
	49·2	47·6	46·8	45·7	
Dihydrocodeinone bitartrate	57·1	58·1	64·3	64·6	
	56·4	56·8	66·3	65·8	8·35
	65·6	65·6	65·6	66·0	
Dioxyline phosphate ..	41·9	41·7	43·3	43·6	
	62·7	62·8	41·9	41·1	2·14
	58·6	58·1	58·6	58·1	

by Karl Fischer titrations and adjustments were made in the molecular weights where necessary. Where a change in molecular weight occurred due to the water content, the new molecular weight was employed to calculate the recovery of the drug. Comparative assays were made with glacial acetic acid as a solvent and 0·05N acetous perchloric acid as titrant. Thus, it was found that neither potentiometric nor visual titrations with crystal violet could be performed for butacaine sulphate, physostigmine sulphate and physostigmine salicylate in the latter system whereas excellent potentiometric changes occurred for all three salts in phenol-chloroform-acetonitrile and very sharp visual end-points were noted for the two physostigmine salts. With the exception of the sulphates of cinchonine and cinchonidine, markedly improved potentiometric end-points occurred for all salts in Table I. Where methyl red could be used, the indicator change was, in almost every instance, much sharper than that obtained for the same salt using crystal violet in glacial acetic acid.

Riddick[8,9] reported that there was considerable evidence that mixed solvents may be superior to a single solvent in general solvent power and in sharpness of the colour change of indicators or in the potentiometric break. Pifer and Wollish[2] found that the addition of dioxane to glacial acetic acid enhanced the potentiometric break, while Fritz and Fulda[10] demonstrated the advantages of a mixed solvent system in their work on the titration of certain weak bases.

A comparison of the potentiometric curves obtained for ephedrine sulphate, in the two respective solvent systems, is given in Figures 1 and 2.

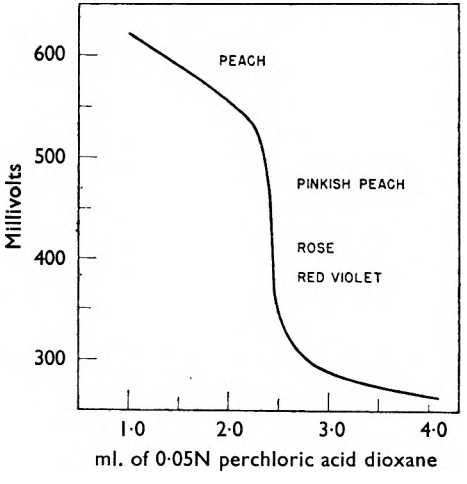

FIG. 1. Titration of ephedrine sulphate in phenol–chloroform–acetonitrile solvent system.

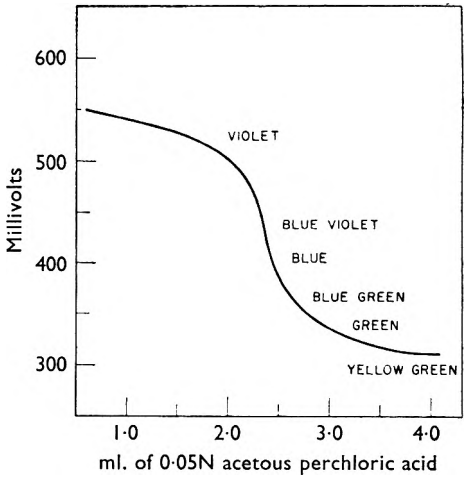

FIG. 2. Titration of ephedrine sulphate in glacial acetic acid.

It is seen that the change in potential at the end-point, in phenol-chloroform-acetonitrile, is considerably larger than that obtained for the same compound in glacial acetic acid. In fact, the dE/dV for ephedrine sulphate, in the former solvent system, ranges from 2500 to 3000, whereas

the dE/dV for the same drug in the latter system is only 500 to 600. In addition, it was noted that the dE/dV for all salts in Table I, with the exception of the cinchona alkaloidal sulphates and pilocarpine nitrate were equal to or greater than 2000 in the phenol-chloroform-acetonitrile solvent system whereas, in glacial acetic acid, only the dE/dV of phenindamine tartrate and of dioxyline phosphate exceeded 800. For most of the other compounds it was much less.

Saunders and Srivastava[11] utilised an aqueous-alcoholic solvent system to titrate a number of salts of organic bases. Although they make no mention of the dE/dV at the end-points, it would seem from the appearance of their curves that generally they were considerably smaller than those obtained in phenol-chloroform-acetonitrile and in no instance have they been able to perform a visual titration.

Gautier and Pellerin[12], in reporting the assay of sulphates of organic bases, found it was essential to precipitate the sulphate by the addition of benzidine. Higuchi and Concha[13] pointed out that considerable difficulty was encountered in the titration of sulphates because of their low solubility in glacial acetic acid. While not all salts of organic bases are soluble in phenol-chloroform, those reported in Table I possess a high solubility in that system. Although amphetamine and dexamphetamine sulphates are readily soluble in phenol-chloroform, the addition of acetonitrile turned the solution milky. This phenomenon had no effect upon the quantitative recoveries as the solution cleared during the titration before the end-point was reached. The phenol-chloroform combination possess the solubilising properties but acetonitrile must be added for its stabilising effect in potentiometric titrations[6]. In fact, methyl red will not function as an indicator if phenol-chloroform is used alone but requires the presence of acetonitrile.

However, phenol-chloroform-acetonitrile possesses certain limitations as a solvent system. The solubilities of morphine tartrate, morphine meconate, chlorothen citrate, the sodium barbiturates as well as the alkali-metal salts of carboxylic acids and of the antibiotics were so small as to render the system valueless for them. Each compound should be tested individually before a decision about its solubility can be reached.

Halogen salts cannot be titrated as a side reaction occurs with the solvent system when mercuric acetate is dissolved in it. In addition, for reasons unexplained, no visual or potentiometric end-point could be detected for pyrilamine maleate and the recovery of doxylamine succinate was not quantitative.

Summary

1. Phenol-chloroform-acetonitrile, as a nonaqueous solvent system, has been applied to a number of salts of organic bases and found to offer the advantages of increased solubility and increased ease of detection of end-points.

This finding appears to agree with the results of other workers who have employed mixed solvents in nonaqueous titrimetry.

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CHRONIC TOXICITY STUDIES ON FOOD COLOURS

PART I. OBSERVATIONS ON THE TOXICITY OF FD&C YELLOW NO. 3 (OIL YELLOW AB) AND FD&C YELLOW NO. 4 (OIL YELLOW OB) IN RATS

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BADGER *et al.*[1] and Sugiura[2] reported that tumours were not produced in mice or rats when Yellow AB and OB colours were applied to the skin or incorporated in the diet for periods of treatment longer than one year in some instances. Where relatively large doses were administered the mortality was high, but no mention was made of the possible cause of death. In a recent report issued by the British Ministry of Food it was mentioned that these colours and some others are suspected to have harmful effects[3].

The experiments here reported were made in order to determine the effects of the oral administration of Oil Yellow AB and Oil Yellow OB on growth, food consumption, food efficiency, blood values, and on the pathology of a number of the organs.

METHODS

The food colours were incorporated in the laboratory diet in the following concentrations: 0·03, 1·5, and 3·0 per cent. To the basic diet (Fox Breeder Meal, Toronto Elevators, Ltd.) was added a supplement, which contained ground Fox Breeder Meal 38·4 per cent., Haliver oil with viosterol (60,000 I.U. Vitamin A and 10,000 I.U. Vitamin D per g.) 1·6 per cent. and crude casein 60·0 per cent., in the following proportions: ground Fox Breeder Meal 92·0 per cent., supplement 5·0 per cent. and alphacel plus colour 3·0 per cent. Each colour was added to the basic diet in the dry form and incorporated by means of a blender. The rats, in groups of 25 males and 25 females, were approximately 5 to 6 weeks of age at the beginning of the experiment. The three concentrations of each food colour were assigned at random. The animals were kept in groups of 12 or 13 rats to a cage and were given free access to their respective diets and water. Their weights and food consumption were recorded weekly. For a more accurate evaluation of food consumption it would have been preferable to put one rat only in a cage, but this was not possible. Post-mortem examinations were made on the rats which died but, in many cases due to advanced autolytic changes it was not possible to determine the cause of death. All other surviving animals were killed at the end of the experiments and post-mortem examinations made.

RESULTS AND DISCUSSION

The effect on Growth Rate, Food Consumption and Food Efficiency. Growth, food consumption and food efficiency curves of the experimental

groups receiving 0·03 per cent. of the colours in the diet and the control groups are shown in Figures 1 and 2. The growth curves for the experimental groups did not deviate appreciably from the controls during the

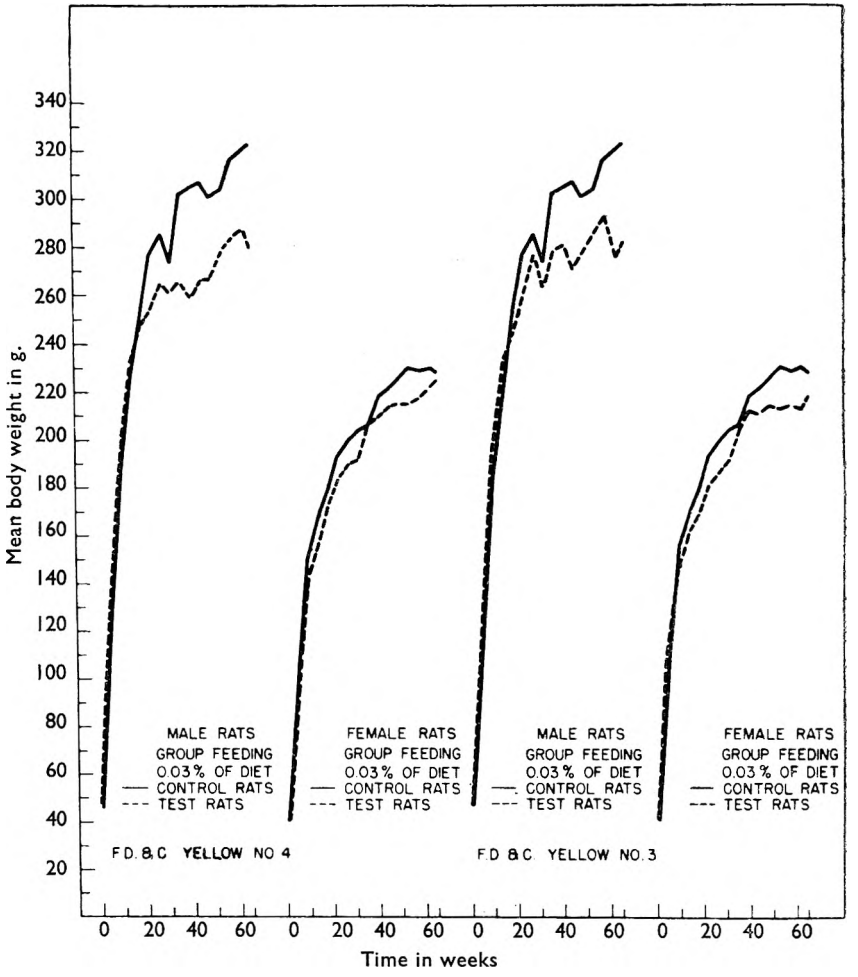

FIG. 1. Growth rate curves for groups of control rats and those receiving the colours.

first 20 weeks, but from the 20th week until the 65th week the test rats were retarded in their growth. However, when the test was terminated after 65 weeks the weights of the remaining rats on test were not significantly different from the controls. On the 1·5 and 3·0 per cent. diets all the rats died within 10 weeks. There was a gradual retardation of growth until death occurred. No significant differences in food consumption were found in the groups of female rats receiving 0·03 per cent. F.D.&C. Yellows No. 3 and 4 for 65 weeks compared with the controls. The amounts consumed were 12·3, 12·1 and 12·5 g. per rat per day for

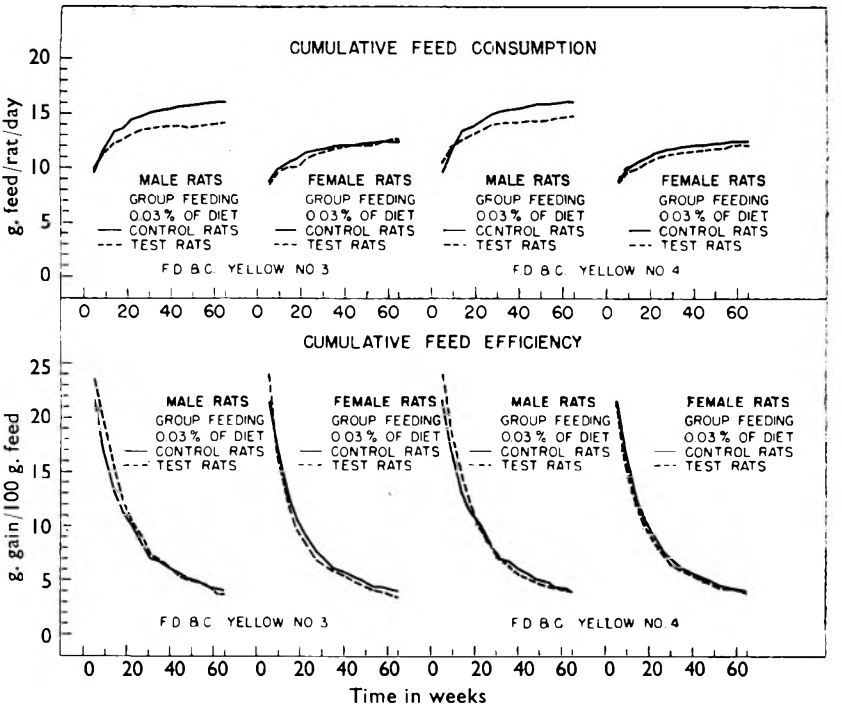

FIG. 2. Food consumption and food efficiency curves for control groups of rats and those receiving the colours.

the groups receiving the control diet and 0·03 per cent. concentrations of FD&C Yellows No. 3 and 4 respectively. The food consumption of the male rats on the same concentration of the colours was 14·7 and 14·1 g. per rat per day as compared to 16·0 g. per rat per day for the controls. The control male rats ate more than the males on test. Whether this lesser food consumption on the part of the males fed these colours in the diet is due to a dislike to the diet-colour mixture or to the toxic effects of the colours cannot be ascertained from these group feeding experiments. Another experiment in which groups of rats were force fed 5 days a week, for 20 weeks, on doses equivalent to 200 and 400 mg./kg. respectively of both colours, showed that the greatest effect on food consumption was caused by the 400 mg./kg. dose, and the food consumption of both males and females was affected. The other groups were affected to a lesser extent. It would appear then, that the food consumption is affected to some extent when the colour is consumed in the food or is given by means of stomach tube. The extent of the effect on food consumption will depend on the dose of the colour. A summary of the data on food consumption and food efficiency obtained in this experiment is shown in Table I. The weights of the rats at the termination of this experiment are recorded in Table III. On some of these doses the effect on growth could be attributed to a lower food consumption, and in others to the utilisation of the food eaten.

TABLE I

SUMMARY OF DATA ON MORTALITY, FOOD CONSUMPTION AND FOOD EFFICIENCY WHEN FOOD COLOURS WERE GIVEN BY STOMACH TUBE FOR 20 WEEKS

Dose	Sex	Mortality	Food consumption g./rat/day	Food efficiency g. gain/g. food consumed × 100
Control	M	1	13·2	5·2
	F	0	11·1	4·7
200 mg./kg./orally/daily	M	0	13·2	4·5
FD&C Yellow No. 4	F	0	10·8	3·3
400 mg./kg./orally/daily	M	3	12·0	1·3
FD&C Yellow No. 4	F	1	11·3	3·1
200 mg./kg./orally/daily	M	1	12·4	5·7
FD&C Yellow No. 3	F	0	11·1	3·7
400 mg./kg./orally/daily	M		11·2	2·6
FD&C Yellow No. 3	F	0	9·9	3·0

The cumulative feed efficiency data for the group feeding experiment is shown in Figure 2. These data when plotted in a log relationship with time and straight lines fitted to the points by the method of least squares did not show a significant difference between the lines, thus indicating that the food efficiency is not affected by these levels of the colours in the diet.

The Effect on Mortality. During the 65 weeks the rats were on test a considerable mortality from respiratory infections resulted. For example the total mortality on the controls amounted to 54·0 per cent. The total mortality of the rats on 0·03 per cent. dietary level of FD&C Yellow No. 4 amounted to 52·0 per cent. and for the same level of FD&C Yellow No. 3 it was 20 per cent. Although it was not possible to properly diagnose the cause of death because of advanced autolysis in a number of cases, it is believed that most of these deaths were due to respiratory conditions. The rats on the 3·0 per cent. level of both colours all died by the end of the 3rd week and with the exception of one rat all the rats died on the 1·5 per cent. level of both colours by the end of the 5th week. In those rats which died on the 1·5 and 3·0 per cent. dosage levels, and on which it was possible to make autopsies soon after death, it was observed that all the tissues and organs were stained with the colour. There was an acute catarrhal gastro-enteritis; the kidneys were soft, dark and swollen. The spleen was greatly enlarged and dark. The picture was one of an acute toxæmia. Bacteriological examination was negative, that is, no pathogenic organisms were recovered from the gastro-intestinal tract, and death was attributed to the acute toxic effects of the colour.

The deaths could not be attributed to lack of food as the food consumption of the rats on the 1·5 and 3·0 per cent. levels was only slightly less than the control during their survival period. The cumulative number of deaths are shown in Table II.

The Effect on Organ Weights. In Table III are recorded the mean weights, in mg./g. of total body weight, of a number of the organs removed from the animals at the time the experiments were terminated. Although the mean weights for a number of organs have been shown to be significantly different from the control weights the differences were small in

most cases. On the 0·03 per cent. dose of FD&C Yellow No. 4 significant increases in weight of heart and liver were found in the males, while on the same dose of FD&C Yellow No. 3 in the males an increase in liver and kidney weights and a decrease in weight of testes were found. In females, with the exception of an increase in the weight of the liver, there was no appreciable change in the organ weights on the 0·03 per cent. dietary levels for both colours. For the higher doses shown in Table III, namely 200 and 400 mg./kg./rat per day, significant weight changes from the controls on a number of the organs examined were found. The rats on these dosages were killed at the end of 20 weeks.

TABLE II

CUMULATIVE NUMBER OF DEATHS

Conc. of colour in diet	Sex	No. rats on test	Time in weeks on test														
			1	3	5	10	15	20	25	30	35	40	45	50	55	60	65
FD&C Yellow No. 4																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	0	1	2	3	5	5	5	5	7	10	13	13	13
	F	25	0	0	0	2	3	4	4	5	8	8	8	11	12	13	13
1·5 per cent.	M	25	7	20	24	25											
	F	25	11	24	25												
3·0 per cent.	M	25	13	25													
	F	25	16	25													
FD&C Yellow No. 3																	
Control	M	25	1	6	7	8	10	11	12	13	14	14	14	14	14	16	17
	F	25	0	1	1	2	3	3	3	6	6	7	8	8	8	8	10
0·03 per cent.	M	25	0	0	1	1	1	1	1	1	1	1	1	1	1	3	3
	F	25	0	0	0	1	1	3	3	5	5	5	5	7	7	7	7
1·5 per cent.	M	25	4	24	25												
	F	25	4	24	25												
3·0 per cent.	M	25	16	25													
	F	25	18	25													

Hæmatology. Hæmoglobin determinations were made at weekly intervals on groups of male and female rats, 10 rats in a group, given daily oral doses of 200 mg./kg. and 400 mg./kg. respectively of FD&C Yellow colours No. 3 and 4 for 20 weeks. A slight modification of the pyridine-hæmochromogen method of Rimington[4] was used. The combined results of these determinations on both sexes are shown graphically in Figure 3 and the mean values of the final determinations are shown in Table III. The combined blood hæmoglobin values for both sexes shows a significant decline in all the groups on both colours. However, when these values for both sexes were examined separately only the values for the males were found to be different from the controls on both dosage levels of the two colours, while in the females only the 200 and 400 mg./kg. doses of FD&C Yellow No. 3 were significantly different

TABLE III

COMPREHENSIVE SUMMARY OF OBSERVATIONS ON RATS FED FD&C YELLOW NO. 3 AND FD&C YELLOW NO. 4

Product	Dosage	No. weeks on test	No. rats surviving / No. rats on test	Mean body weight g. ± s.e.		Mean Hb (g. per) cent) ± s.e.†	Mean organ weight, mg./g. rat ± s.e.					
				Initial	Final		Heart	Liver	Kidneys	Adrenals	Spleen	Testes
Males												
Control		65	8/25	45·6 ± 2·7	323·4 ± 20·0	17·2 ± 0·35	3·2 ± 0·11	30·3 ± 1·01	6·2 ± 0·19	0·08 ± 0·008	2·7 ± 0·26	8·6 ± 0·40
FD&C Yellow No. 4	0·03 per cent of diet	65	12/25	45·6 ± 2·6	280·4 ± 8·9	16·9 ± 0·42	3·6 ± 0·11*	36·5 ± 1·81*	7·4 ± 0·47	0·09 ± 0·004	2·4 ± 0·08	7·4 ± 0·42
FD&C Yellow No. 3	0·03 per cent. of diet	65	22/25	45·7 ± 2·7	282·1 ± 10·3	16·3 ± 0·22*	3·4 ± 0·07	35·5 ± 1·04*	6·8 ± 0·13*	0·08 ± 0·004	2·5 ± 0·07	5·1 ± 0·28*
Control		20	4/10	118·7 ± 5·7	251·0 ± 11·0	17·3 ± 0·40	3·6 ± 0·05	34·9 ± 1·55	7·6 ± 0·25	0·09 ± 0·005	2·5 ± 0·28	9·7 ± 0·38
FD&C Yellow No. 4	Oral 200 mg./kg./day	20	7/10	118·9 ± 5·0	211·6 ± 9·9*	15·3 ± 0·29*	4·3 ± 0·16*	37·8 ± 1·30	7·5 ± 0·72	0·09 ± 0·010	2·7 ± 0·02	11·1 ± 0·46
FD&C Yellow No. 4	Oral 400 mg./kg./day	20	2/10	114·2 ± 5·1	163·5 ± 1·5*	11·9 ± 0·35*	4·7 ± 0·30*	48·6 ± 0·40*	8·8 ± 0·10*	0·13 ± 0·005*	4·1 ± 0·25*	6·3 ± 2·60
FD&C Yellow No. 3	Oral 200 mg./kg./day	20	4/10	112·7 ± 3·5	221·3 ± 13·6	13·7 ± 0·24*	3·8 ± 0·09*	45·0 ± 1·16*	7·8 ± 0·15	0·10 ± 0·014	5·2 ± 0·04*	10·6 ± 0·63
FD&C Yellow No. 3	Oral 400 mg./kg./day	20	4/10	105·8 ± 6·7	152·5 ± 19·3	13·6 ± 0·35*	5·4 ± 0·44*	58·7 ± 2·91*	9·5 ± 0·65*	0·16 ± 0·024*	8·9 ± 1·08*	7·0 ± 1·76
Females												
Control		65	15/25	39·6 ± 2·4	227·9 ± 3·9	16·0 ± 0·19	4·1 ± 0·07	35·4 ± 0·84	7·0 ± 0·18	0·22 ± 0·008	3·6 ± 0·13	
FD&C Yellow No. 4	0·03 per cent. of diet	65	12/25	39·2 ± 2·2	225·2 ± 7·6	15·6 ± 0·18	4·0 ± 0·07	35·3 ± 0·70	6·8 ± 0·19	0·23 ± 0·011	3·6 ± 0·17	
FD&C Yellow No. 3	0·03 per cent. of diet	65	18/25	39·4 ± 2·2	217·9 ± 8·1	15·8 ± 0·21	4·1 ± 0·11	30·1 ± 1·02*	7·1 ± 0·24	0·19 ± 0·011	3·7 ± 0·20	
Control		20	9/10	93·0 ± 3·9	166·9 ± 6·5	15·7 ± 0·65	4·5 ± 0·41	37·5 ± 1·38	8·2 ± 0·45	0·26 ± 0·031	3·1 ± 0·33	
FD&C Yellow No. 4	Oral 200 mg./kg./day	20	5/10	91·6 ± 4·4	163·4 ± 12·8	14·9 ± 0·64	4·2 ± 0·18	40·9 ± 2·00	7·3 ± 0·22	0·19 ± 0·019	3·3 ± 0·38	
FD&C Yellow No. 4	Oral 400 mg./kg./day	20	4/10	92·2 ± 3·4	148·8 ± 1·8*	15·1 ± 0·50	4·4 ± 0·07	50·3 ± 1·26*	8·6 ± 0·21	0·19 ± 0·009	4·2 ± 0·33	
FD&C Yellow No. 3	Oral 200 mg./kg./day	20	7/10	89·6 ± 4·3	145·4 ± 5·8*	14·5 ± 0·54*	4·6 ± 0·10	47·6 ± 1·93*	8·3 ± 0·22	0·19 ± 0·012	6·9 ± 0·31*	
FD&C Yellow No. 3	Oral 400 mg./kg./day	20	7/10	94·3 ± 6·3	138·0 ± 5·2*	11·6 ± 0·70*	5·5 ± 0·20	56·0 ± 1·82	9·6 ± 0·82	0·23 ± 0·016	12·0 ± 1·01*	

* Significant at $P = 0·05$

† Determination done on 5 rats.

from the corresponding female controls. Blood hæmoglobin values were also determined on the surviving rats from the group feeding experiment. These results are also shown in Table III. With the exception of the male rats fed FD&C Yellow No. 3, 0·03 per cent. in the diet, which showed blood hæmoglobin values statistically different from the controls, the others were about the same as the controls.

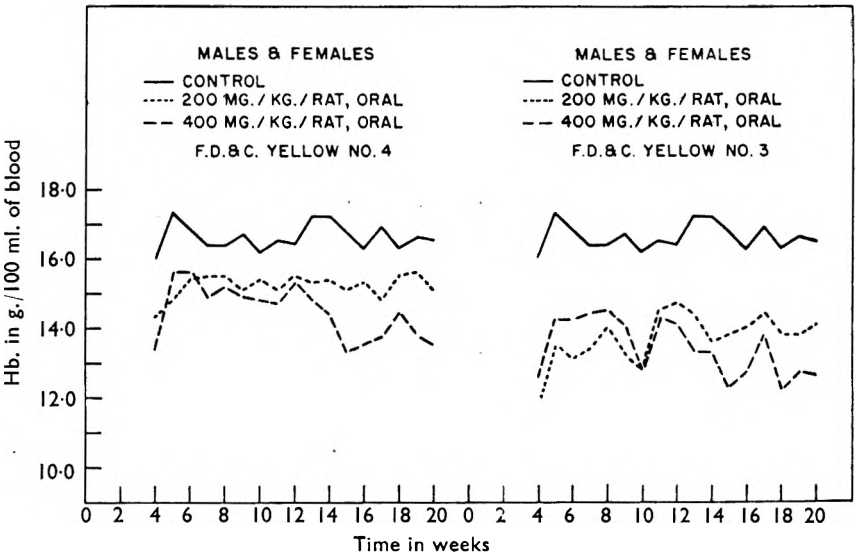

FIG. 3. Combined results of hæmoglobin determinations on both sexes of control rats and those given the two colours at different doses.

In order to obtain more information on the effect of FD&C Yellow No. 3 on the blood picture, a few rats of both sexes were given relatively high daily oral doses of this colour and determinations were made at weekly intervals for hæmoglobin, erythrocytes, hæmatocrit, reticulocytes, and total leucocytes. A summary of the mean values for the surviving rats at the conclusion of the experiment are shown in Table IV. The hæmoglobin of some of the rats before death was as low as 3 g. per 100 ml. of blood. The total erythrocyte count in some cases was below one million per cu. mm. The reticulocytes were greatly increased from a normal of 2 to 4 per cent. in control rats to 16 to 50 per cent. in the colour treated rats. The hæmatocrit values were lower in the test animals. The mean corpuscular volume in the control rats was 54·1 cubic microns. Some of the test rats had mean corpuscular volumes as high as 130 cubic microns with a mean of 85·7 and 90·0 cubic microns for the respective groups on test. The mean corpuscular hæmoglobin concentration, however, was reduced. The blood picture of these rats was therefore suggestive of a macrocytic hypochromic anæmia.

Further hæmatological findings are outlined as follows: There were poikilocytosis, anisocytosis and polychromatophilia. The occasional oxyphilic normoblast with structureless nucleus was noted. Howell

TABLE IV

SUMMARY OF HÆMATOLOGICAL FINDINGS

No. of rats	Age in weeks at start of test	Weeks on test	Dose of FD&C Yellow No. 3	Mean blood Hb g./100 ml. blood	Range	Mean RBC count millions/ mm^3 blood	Range	Mean hæmatocrit ml./100 ml. blood	Range	Mean reticulocyte per cent. of total RBC	Range	Mean corpuscular volume (μ^3)	Range
5	8	12	Control	17·8	17·2–18·2	8·5	8·0–9·0	52·0	50–54	2·6	2–4	54·1	50–57
6	8	12	90 mg./orally daily	10·2	8·3–12·8	3·8	2·8–4·5	37·0	31–43	26·0	18–40	85·7	61–108
6	8	7	0·9 per cent in diet	6·8	2·3–9·8	2·5	0·9–5·6	27·0	17–42	34·0	16–50	90·0	68–130

Jolly bodies were present but not numerous. The blood serum was of a brown colour suggesting the presence of some foreign substance. However, it was not possible to detect unaltered colour on chemical examination of the serum, but the spectrophotometric curve of the serum from the treated rats was similar in many respects to a control serum which contained the added Oil Yellow AB colour, indicating that the colour is very likely present in an altered form. Giemsa and Wright stains showed the bone marrow to be megaloblastic. The total and differential white cell counts were within normal limits.

Histopathology. Detailed examination was made on the hæmatoxylin-eosin stained paraffin sections of a number of the organs including the lung, heart, liver, spleen, thyroid, pancreas, stomach, small intestine, kidney, urinary bladder, adrenal, testes, ovaries and thymus. The number of organs in which important changes were found are shown in Table V. A brief description of the more important histological changes which were observed in those organs examined, is as follows:

FD&C Yellow No. 3, 0·03 *per cent. in the diet*, 65 *weeks*

Testes. Testicular changes were essentially of a degenerative and atrophic nature involving principally the spermatogenic cells. The testes were divided into 3 more or less arbitrary groups according to the tubular pathology. Five testes exhibited marked changes, 26 exhibited moderate changes, 11 exhibited slight changes, and 2 were normal.

In the testes in which the changes were described as marked, the majority of tubules were utterly devoid of spermatogenic cells. Sertoli cells were present but were irregular in outline, their cytoplasm appearing to merge into the cytoplasm of the walls of the tubules. The outline of the wall of the tubule was indefinite, with thin strands of cytoplasm or fibrin-like material projecting irregularly from the inner periphery towards the lumen of the tubules. Other tubules, in which degenerative changes were not as advanced, contained a lacy

TABLE V

SUMMARY OF HISTOPATHOLOGICAL FINDINGS

	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	FD&C Yellow No. 4	FD&C Yellow No. 3	Control	FD&C Yellow No. 4	FD&C Yellow No. 3	FD&C Yellow No. 4	FD&C Yellow No. 3
Sex Dose	Male 0·03 per cent in diet			Female 0·03 per cent in diet			Male 400 mg./kg./day			Male 200 mg./kg./day		Female 400 mg./kg./day			Female 200 mg./kg./day	
Number of rats on test	25	25	25	25	25	25	10	10	10	10	10	10	10	10	10	10
Number of rats examined	8	12	22	15	12	18	4	3	5	4	4	8	4	6	5	7
Heart, cloudy swelling		1	1			1				1						
Interstitial œdema								1		1					1	
Pericarditis		1														
Lung, inflammation	6	10	17	12	11	16	4	2	5	3	4	6	2	6	5	7
Gastrointestinal, neoplasm			1													
enteritis-chronic fibrosing				1												
Liver, degeneration			1						3				1	5		1
Kidney, total no. showing change		3	8	5	3	10			4	1				5		1
A. Inflammations																
(i) Glomerulitis		1	8	3	3	6			4	1		1		2		
(ii) Pyelonephritis									1							
(iii) Interstitial nephritis				1		3			1	1						
B. Degenerations																
(i) Nephrosis (proximal)		1	2			1			3	1		1		2		
(distal)										1		1		2		
(ii) Hydronephrosis				2		4								3		1
C. Congenital cystic kidney		1														
D. Bladder parasites		5	10	2	4	8	2			2	2	3			2	3
Testes, testicular degenerations		3	22					2	3							
Adrenal																
A. Neoplasm (phæochromocytoma)						1										
B. Inflammation						1										
Spleen																
A. Hyperplasia, hyperchromic nuclei			7			4		2	4		4			6		5
B. Excessive red blood cells			5			3			2		1			6		4

fibrin-like web in which were nestled degenerating spermatogenic cells. The majority of these appeared to be undifferentiated germ cells although some were undoubtedly primary spermatocytes. In the testes described as showing moderate changes, tubules showing the above pathology were noted, but in the majority of the tubules secondary spermatocytes were present. Most of these were undergoing varying degrees of degeneration. In other tubules spermatogenesis was complete although a number of spermatozoa were undergoing degeneration. In the group in which changes were described as slight, the majority of the tubules showed complete spermatogenesis but there was a reduction in the normal number of spermatozoa. General tubular morphology appeared normal in this group although there was slight tubular atrophy. The Leydig cells in the test animals were essentially normal, although the nuclei were slightly denser and took on a more intense stain. They were somewhat more flattened than the nuclei

seen in the control animals and appeared more abundant in the testes showing moderate changes (Figs. 4, 5 and 6).

It is understandable that overlapping would occur in the three groups, that is tubules showing the features described in group 1 did occur in group 2 and tubules described as typical of group 3 did occur in group 2. It might be argued that in the tubules showing partial spermatogenesis with no cells undergoing degeneration, the processes would be one of arrest. The number of tubules exhibiting this pathology are very much in the minority and it is felt that the process is essentially a degenerative lesion of the spermatogenic cells due to a specific toxic action of the colour.

Kidney. The principal changes in the kidney involved the glomerulus. These changes were generally not marked. In most instances there was an increase in the amount of cytoplasm of the epithelium and endothelium of the glomerulus with a slight cellular increase, resulting in an increase in the density of the tuft. Even in these animals normal appearing glomeruli were relatively abundant. Hyaline change was observed in the glomerulus in two animals and was accompanied by nephrosis. In three animals the condition was one of a mild subacute glomerulitis.

Spleen. There was a hyperplasia of the white pulp, the cells of which were hyperchromatic. The white pulp did not display the reaction centre encountered in some toxic conditions. In the red pulp there was an increased number of phagocytes containing disintegrated red blood cells. The interiors of the red pulp cords were engorged with red blood cells. In general the splenic changes appeared to be of a proliferative compensatory nature.

Lungs. Chronic inflammatory changes with some degree of emphysema, atelectasis and bronchiectasis were present in the lungs of most of the test and control animals.

Neoplasm. A reticulum cell sarcoma involving principally the mesentery and infiltrating the muscular coats of the ileum and cæcum was noted in one animal and an adenoma was noted in the adrenal gland of one animal.

FD&C Yellow No. 4, 0·03 *per cent. in diet*, 65 *weeks*

There was no constant or specific change that could be attributed to the toxic effect of the colour. The testes, kidneys and spleen were essentially normal.

FD&C Yellow No. 3, 200 *mg./kg./day*, 20 *weeks*

Spleen. The histological picture was similar to that observed in the organs of rats on the FD&C Yellow No. 3 on the lower doses, which was a hyperplasia of the white pulp, an intense staining of the cells of the pulp, an increased phagocytosis of disintegrated red blood cells and an engorgement of the red pulp with red blood cells.

FD & C Yellow No. 3, 400 *mg./kg./day*, 20 *weeks*

Testes. Of the animals examined 2 were normal. The testes of 3 animals showed alterations similar to those observed in the testes of rats on the 0·03 per cent. dietary level of FD&C Yellow No. 3.

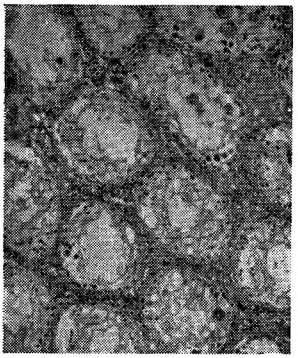

FIG. 4. Dietary level 0·03 per cent. FD & C Yellow No. 3. 65 weeks. Testes. Advanced degenerative change. Complete absence of spermatogenesis in most tubules. Tubular atrophy marked. × 210.

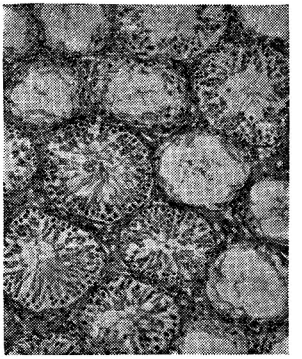

FIG. 5. Dietary level 0·03 per cent. FD & C Yellow No. 3. 65 weeks. Testes. Slight change. Some tubules show complete but reduced spermatogenesis. Occasional tubule shows complete absence of spermatogenesis. × 210.

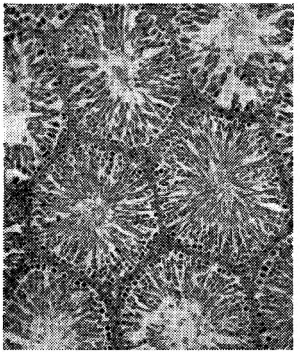

FIG. 6. Control 65 weeks. Testes. Testes normal. × 210.

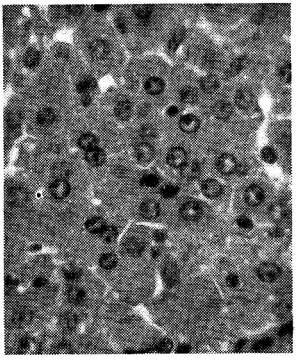

FIG. 7. Dietary level 3 per cent. FD & C Yellow No. 3. Liver. Partial compensation —partial failure—regeneration with increase nuclear ratio in a given field. Many binucleated cells, nucleoli hypertrophied and hyperchromatic. Degeneration with nuclear distortion, pyknosis, some necrosis. ×330.

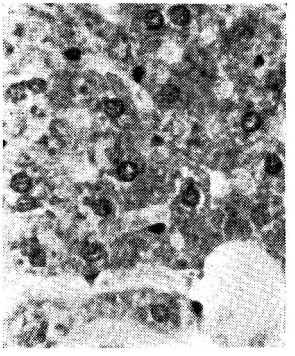

FIG. 8. Control. Liver. Liver normal. × 330.

Spleen. The Prussian blue positive pigment was excessive. There was engorgement of the red pulp with red blood cells.

Kidneys. Mild tubular alterations were present in 4 animals. These were in the nature of a proximal and distal nephron nephrosis; the tubules were swollen and smudgy with a loss of epithelial elements to the lumen. There was an accompanying glomerulitis in 2 animals. Prussian blue positive pigment was present in the cytoplasm of kidney tubular epithelium.

Liver. Liver change was present in 8 animals. This was in the nature of a cloudy swelling. There was an accompanying fatty change in one animal and marked necrosis was observed in one instance. The nuclear pattern was disrupted in the affected animals with a loss of uniformity in nuclear size and increase in nuclear ratio in a given field, most cells being binucleated, that is there appeared to be some regeneration. Kupffer cells were abundant and contained large amounts of Prussian blue positive material

FD&C Yellow No. 4, 200 *mg./kg./day*, 20 *weeks*

There were no marked alterations from the normal which could be attributed to the toxic effects of the colour.

FD&C Yellow No. 4, 400 *mg/kg./day*, 20 *weeks*

Testes. Only the testes of 3 males were examined, in 1 animal the testes were normal and in the other 2 the degenerative changes were moderate. It was not possible, due to the advanced autolytic changes, to do a histopathological examination of most of the animals which died on the higher doses of the 1·5 and 3·0 per cent. dietary levels. A number of animals were killed and the findings were as follows:

Liver. The findings in the liver were similar to that found in the livers of rats on the FD&C Yellow No. 3 on the 400 mg./kg. daily dose, except the necrosis found in most livers was more marked (Figs. 7 and 8).

Thymus. The thymus generally exhibited changes of a marked involution. Medulla and cortex were indistinct. The majority of the lymphocytes were pyknotic; macrophages were prominent and phagocytosis of the degenerating lymphocytes by the macrophages was evident, but reticular network was quite distinct.

Adrenals. Congestion and necrosis of the cortex occurred in a few animals. Cloudy swelling in the zona fasciculata was noted in one instance.

Pancreas. Many glands showed a slight loss of basal basophilia with an increase in the refractile granules in the supra nuclear portion of the acini.

Kidney. The findings in the kidney were similar to that found in the kidneys of rats on the FD&C Yellow No. 3 on the 400 mg./kg. daily dose except the various conditions noted were more marked.

Spleen. The white pulp displayed toxic reaction centres. Many pyknotic nuclei were noted in the white pulp.

SUMMARY

1. FD&C Yellow colours No. 3 and 4 in concentrations of 0·03 per cent. in the diet were found to reduce the food consumption of male, but not female rats. Heart and liver weights were increased for male rats on the 0·03 per cent. level of FD&C Yellow No. 4 and kidney and liver weights were found to be slightly increased for both male and female rats on the same level of FD&C Yellow No. 3. The weights of the testes on the 0·03 per cent. dietary level of FD&C Yellow No. 3 were found to be less than the controls.

2. Daily oral doses of 200 mg./kg. and 400 mg./kg. of FD&C Yellow colours No. 3 and 4 in males caused a significant fall in hæmoglobin values, but in females only the oral doses of 200 mg./kg. and 400 mg./kg./day of FD&C Yellow No. 3 caused a significant fall in blood hæmoglobin concentration after 20 weeks. Significant weight changes in a number of organs on these levels were found at the termination of the experiment.

3. Daily oral doses of 90 mg./day/rat of FD&C Yellow No. 3 for seven weeks caused a marked decline in blood hæmoglobin concentration, total erythrocyte count, and hæmatocrit value, and an increase in reticulocyte counts. The total leucocyte and differential leucocyte counts were within normal ranges. Similar results were obtained on a dietary level of 0·9 per cent. FD&C Yellow No. 3.

4. Tissue histology of those rats surviving for 65 weeks on the 0·03 per cent. dietary levels of FD&C Yellow No. 3 revealed marked atrophy and degeneration in the testes, mild inflammatory changes in the kidney and slight hypertrophic changes in the spleen. There were no significant histological changes in the organs examined from surviving rats on FD&C Yellow No. 4 on the same dietary level. Dietary levels of 1·5 and 3·0 per cent. of both colours caused some tissue changes in the liver, thymus, adrenal, pancreas and kidney. Daily oral doses of 400 mg./kg. of FD&C Yellow No. 3 for 20 weeks caused changes in testes, spleen, kidney and liver. The only changes of significance on the 200 mg./kg. daily dose of FD&C Yellow No. 3 were in the spleen of males. The 400 mg./kg. daily dose of FD&C Yellow No. 4 caused testicular changes in 20 weeks and the 200 mg./kg. daily dose caused no changes of significance.

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A NOTE ON THE PAPER CHROMATOGRAPHIC SEPARATION OF PHENOBARBITONE METABOLITES

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Barbiturates are the most common poisonous drugs in Britain at the present time. Death frequently involves a long period of coma and consequently little barbiturate remains in the body for the chemical toxicologist to identify. Any method which gives a lead to the particular barbiturate ingested is therefore of value. The identification of pentobarbitone from its urinary metabolites has been reported[1] and I wish to report a paper chromatographic pattern which has been frequently seen in this laboratory in the urinary barbiturate extract only in acute phenobarbitone poisoning. Figure 1 illustrates the pattern. The butanol: ammonia solvent system of Algeri and Walker[2] on Whatman No. 1 paper followed by their mercuric sulphate: diphenylcarbazone method of development has been used. The tungstic acid protein precipitation method used by Valov[3] followed by a sodium bicarbonate wash to remove acids has in all instances been used to extract the barbiturates. They have been then extracted from the ether solution by 0·5N sodium hydroxide when acidification and re-extraction into ether has given the crude barbiturate extract for chromatography.

Fig. 1.

(i) Urinary extract.
(ii) Amylobarbitone 75 μg.
(iii) Phenobarbitone 50 μg.

Apart from a recent report of the isolation of *p*-hydroxy phenobarbitone[4] in the urine of dogs after ingestion of phenobarbitone no other metabolites have as yet to my knowledge been reported. The slower running spot $R_F = 0{\cdot}17$ has been extracted from the paper and shows ultra-violet absorption spectra at *p*H 13, 10 and 2 characteristic of the barbiturate ring. However, there is a slight variation. At *p*H 13 $\lambda_{min.} = 232{\cdot}5$ mμ and $\lambda_{max.} = 249{\cdot}5$ mμ as distinct from $\lambda_{min.} = 233$ mμ and $\lambda_{max.} = 255$ mμ for phenobarbitone. Otherwise the spectra, and the change in spectra with *p*H, follow those generally observed for all barbiturates. This laboratory investigates many instances of barbiturate poisoning, but we have not seen this pattern reproduced by any barbiturate other than phenobarbitone, nor does it normally appear in the blood, brain liver or

cerebrospinal fluid extracts. In only two instances of phenobarbitone poisoning has a slower running spot appeared in other than urine extracts. In both of these the liver extracts also showed a spot which agreed in R_F with the much larger quantity of slower running spot seen in the urine.

The paper chromatographic separation offers an easy method for the isolation of this metabolite and work is prceeding with its identification.

Thanks are accorded to Dr. F. G. Tryhorn for advice.

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A NOTE ON THE DETERMINATION OF ASCARIDOLE IN OIL OF CHENOPODIUM

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In a previous communication[1] describing results in which specially purified ascaridole[2] was used, it was pointed out that the factor used in the B.P. 1953 for the conversion of volume of titrant into a weight of ascaridole was incorrect, e.g., a sample of Oil of Chenopodium containing 54 per cent. w/w of ascaridole gave a result of 65 per cent. w/w by the B.P. method. Furthermore, because the amount of iodine liberated is not directly proportional to the weight of ascaridole, analysis of samples of low grade oils gave proportionately higher errors in the results than those obtained for high grade oils; an invariant factor is therefore inadequate.

A quadratic expression was proposed, which, when applied to the observed measurements using the B.P. procedure, gave correct figures for the ascaridole content of the oils provided that the volumes of titrant were within a specified range. Unfortunately, the use of the expression is time-consuming.

TABLE I

Equivalent (in g.) of $C_{10}H_{16}O_2$ (Ascaridole) for Values of "*n*" Between 20 and 40 ml. 0·1 N $Na_2S_2O_3$

ml.	0	1	2	3	4	5	6	7	8	9	Mean Differences 1 2 3	4 5 6	7 8 9
20	0·1088	1094	1100	1106	1112	1118	1124	1130	1136	1142	1 1 2	2 3 4	4 5 5
21	0·1148	1154	1160	1166	1172	1178	1184	1191	1197	1203	1 1 2	2 3 4	4 5 5
22	0·1209	1215	1221	1227	1233	1239	1246	1252	1258	1264	1 1 2	2 3 4	4 5 6
23	0·1270	1276	1282	1289	1295	1301	1307	1313	1320	1326	1 1 2	2 3 4	4 5 6
24	0·1332	1338	1344	1351	1357	1363	1369	1376	1382	1388	1 1 2	2 3 4	4 5 6
25	0·1394	1401	1407	1413	1419	1426	1432	1438	1445	1451	1 1 2	3 3 4	4 5 6
26	0·1457	1464	1470	1476	1483	1489	1495	1502	1508	1514	1 1 2	3 3 4	4 5 6
27	0·1521	1527	1534	1540	1546	1553	1559	1565	1572	1578	1 1 2	3 3 4	4 5 6
28	0·1585	1591	1598	1604	1611	1617	1623	1630	1636	1643	1 1 2	3 3 4	5 5 6
29	0·1649	1656	1662	1669	1675	1682	1688	1695	1701	1708	1 1 2	3 3 4	5 5 6
30	0·1714	1721	1728	1734	1741	1747	1754	1760	1767	1774	1 1 2	3 3 4	5 5 6
31	0·1780	1787	1793	1800	1807	1813	1820	1826	1833	1840	1 1 2	3 3 4	5 5 6
32	0·1846	1853	1860	1866	1873	1880	1886	1893	1900	1906	1 1 2	3 3 4	5 5 6
33	0·1913	1920	1927	1933	1940	1947	1953	1960	1967	1974	1 1 2	3 3 4	5 5 6
34	0·1980	1987	1994	2001	2008	2014	2021	2028	2035	2041	1 1 2	3 3 4	5 5 6
35	0·2048	2055	2062	2069	2076	2082	2089	2096	2103	2110	1 1 2	3 3 4	5 5 6
36	0·2117	2124	2131	2137	2144	2151	2158	2165	2172	2179	1 1 2	3 3 4	5 6 6
37	0·2186	2193	2200	2207	2214	2220	2227	2234	2241	2248	1 1 2	3 3 4	5 6 6
38	0·2255	2262	2269	2276	2283	2290	2297	2304	2311	2318	1 1 2	3 4 4	5 6 6
39	0·2325	2332	2339	2346	2354	2361	2368	2375	2382	2389	1 1 2	3 4 4	5 6 6

The statement in a recent review,[3] concerning the lack of a more adequate procedure than the official method for a routine test for the commercial

evaluation of oils of chenopodium, prompts us to present a Table of equivalents obtained by the use of the previously reported quadratic expression.[1] The application of the iodimetric method in conjunction with the Table gives a correct evaluation of the ascaridole content of chenopodium oils.

Method for the Determination of Ascaridole in Oils of Chenopodium

(1) Perform the determination as described under Chenopodium Oil B.P. 1953.

(2) If the number of ml. (*n*) of 0·1 N sodium thiosulphate required (after deduction of the blank titration) is within the limits 20 to 40, read the equivalent of ascaridole (in g.) from the Table (used similarly to logarithmic tables).

(3) If the titration is outside the stated limits, repeat the determination using more or less than the stated 5 ml. of the acetic acid solution of the oil, to give a titration between 20 and 40 ml. of 0·1 N sodium thiosulphate.

References

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Aloes, Composition and Assay of. H. Auterhoff and B. Ball. (*Arzneimitt.-Forsch.*, 1954, **4**, 725.) Pharmacological assay of aloes preparations on mice is unsatisfactory, as the dose required is relatively very much greater than with man. Trials were therefore made by using 45 volunteers, in order to test the comparative effect of different preparations. For the chemical determination of aloin, 20 mg. of the material was refluxed for 3 hours with 1 ml. of ferric chloride solution (25 per cent.) and 10 ml. of acetone and the solution was then evaporated to dryness. The residue was warmed with 20 ml. of *n*-butanol, and the solution was transferred to a separating funnel, with the aid of a little water, the flask being rinsed successively with 15 ml. of boiling hydrochloric acid (5 per cent.) and 15 ml. of boiling butanol. After shaking, the aqueous layer was rejected, the butanol phase being washed with 15 ml. of dilute hydrochloric acid and then filtered through cotton wool. The butanol solution was treated with 30 ml. of methanol, and made up to 100 ml. with a solution containing 5 per cent. of sodium hydroxide and 2 per cent. of ammonia. After standing for 15 minutes, 5 ml. was diluted with the above alkali to 100 ml. and the extinction was determined using a filter VG9 (owing to variations in shade, more accurate methods of photometry are undesirable.) The conclusions drawn were as follows: the aloin content, determined as above, is a measure of the laxative action on human subjects, although the results of trials with mice show a different picture. The action of preparations is determined by the aloin content. Aqueous and acetone extracts are comparable in action with the drug itself. Side effects are apparently less frequent with the aqueous extract than with the drug, but the view that this is also the case with the acetone extract is not confirmed. G. M.

Chloramphenicol, Colorimetric Determination of. W. Döll. (*Arzneimitt.-Forsch.*, 1955, **5**, 97.) 1 ml. of a solution of chloramphenicol and 4 ml. of sodium hydroxide (40 per cent.) is brought to the boil, resulting in a yellow colour apparently due to the sodium salt of *p*-nitrophenol. This colour is determined photometrically. The method may be applied directly to serum or blood, after the removal of protein with trichloroacetic acid. G. M.

Dihydrostreptomycin, Chemical Assay of. H. Vogt. (*Arch. Pharm. Berl.*, 1955, **288**, 20.) Dihydrostreptomycin is quantitatively precipitated by ammonium reineckate, 1 molecule of the dihydrostreptomycin combining with 3 molecules of reinecke acid. For the assay 5 ml. of a solution (containing about 10 mg. of dihydrostreptomycin sulphate) is treated with 5 ml. of a freshly prepared 2 per cent. solution of ammonium reineckate. After standing for 30 minutes in ice, the mixture is centrifuged: 5 ml. of the clear supernatant liquid is removed, diluted with 40 ml. of water and 2 ml. of Fehling's solution (No. 2), and hydrolysed on the water bath. The mixture is acidified with nitric acid, treated with a definite excess of 0·1 *N* silver nitrate solution, and titrated back with ammonium thiocyanate. A blank is done with 5 ml.

of water and 5 ml. of the ammonium reineckate solution. 1 ml. of 0·1 *N* solution is equivalent to 6·09 mg. of dihydrostreptomycin sulphate. In presence of procaine-penicillin the solution is first extracted with chloroform for 1 hour, chloroform being removed from the resulting solution by a current of air.

G. M.

Opium Alkaloids, Spectrophotometric Determination of. M. S. Dyer and A. J. McBay. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 156.) Ultra-violet absorption spectra were determined for the principal alkaloids of opium from 240 to 330 mμ in solutions of varying *p*H. Absorption maxima suitable for quantitative analytical use were found in each case. Thebaine appeared to be unstable in the presence of hydrochloric acid but a solution in N sulphuric acid was stable and had a suitable spectrum. For the assay of papaveretum and similar products a method of analysis was evolved depending on solvent extraction to separate the alkaloids partially so as to obtain a series of solutions each containing two alkaloids, except that the narcotine was separated from all the others. The codeine/thebaine solution was analysed by measurements at 265 and 285 mμ, the morphine/narceine solution at 278 and 300 mμ, the papaverine/thebaine solution at 285 and 312 mμ, and narcotine at 312 mμ. From these results the total quantity of each alkaloid was calculated. G. B.

Parathion, Identification and Determination of. A. I. Biggs. (*Analyst*, 1955, **80**, 279.) The ultra-violet absorption spectra of parathion and its hydrolysis product *p*-nitrophenol have been studied. Parathion shows a well defined peak in 95 per cent. ethanol solution at 276 mμ, ϵ = 9630; and in *n*-hexane at 268 mμ, ϵ = 10,350, the spectrum being unaffected by acid or alkali. *p*-Nitrophenol shows, in ethanolic hydrogen chloride (0·001 N) a maximum at 314 mμ (ϵ = 10,750) and in ethanolic potassium hydroxide (0·01 N) a peak at 408 mμ (ϵ = 20,830). On heating 95 per cent. ethanolic solutions of parathion with 0·01 N potassium hydroxide in sealed ampoules at 100° C. for several hours, the maximum at 276 mμ disappeared and was replaced by the maximum at 408 mμ due to the alkaline form of *p*-nitrophenol. Experiments on the analysis of viscera showed that a clean extraction of parathion could be made with *n*-hexane as solvent, the parathion present being determined spectrophotometrically, using the peak at 276 mμ; hydrolysis to *p*-nitrophenol and measurement of the light absorption at 408 mμ gave an added identification and check on the concentration present. R. E. S.

Zinc in Copper Sulphate, Detection of. M. Langejan and J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1955, **90**, 333.) Two new methods are given for the detection of small quantities of zinc in copper sulphate. By the first, 100 mg. of copper sulphate is dissolved in 10 ml. of hot water, treated with 2 ml. of 4 N sodium hydroxide solution, and boiled. After cooling and filtering, the filtrate is acidified with acetic acid and 2 drops of potassium ferrocyanide solution are added. After 30 minutes the solution should not be turbid. This method will detect 0·25 mg. of zinc sulphate. In the second method, 100 mg. of copper sulphate dissolved in 1 ml. of water is treated with 0·25 ml. of dilute acetic acid and 300 mg. of sheet aluminium. The mixture is heated on the water bath for 5 minutes. The solution is decanted, treated with 1 drop of hydrogen peroxide (3 per cent.), and brought to the boil. After cooling, 3 drops of ammonium mercurithiocyanate solution and 1 drop of phosphoric acid is added. After rubbing with a glass rod a precipitate results which may be greenish yellow, but not violet or dark coloured. This test will detect 0·1 to 0·2 per cent. of zinc sulphate. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Prothrombin and the One-stage Prothrombin Time. A. J. Quick and C. V. Hussey. (*Brit. med. J.*, 1955, **1**, 934.) Two accessory factors are known to affect the prothrombin time as determined in the one-stage test and the authors report experimental and clinical observations on the effects of these factors in relation to a re-evaluation of the test. One factor (the "labile factor") disappears from human plasma during storage; the second factor is distinguished as the "stable factor". The blood used in the investigation was obtained from 3 patients with hypoprothrombinæmia, each having a normal concentration of labile factor and a prolonged prothrombin time not shortened by adding stable factor. A further patient having a prothrombin time varying from 26 to 55 seconds, due to stable factor deficiency, was also used. By determining the effect of adding one or other of the two factors to normal and deficient plasma, it was shown that the constancy of the prothrombin time of normal fresh plasma can be explained by assuming constancy in the prothrombin level. Experiments on stored plasma showed that changes in the prothrombin time of stored blood are due to loss of labile factor and to either an increase of the prothrombin content or to generation of an accelerator independent of the two known accessory factors. The prothrombin time of serum from hæmophilic or thrombocytopenic blood is shorter than that from the corresponding plasma because almost no prothrombin is consumed and in addition either more prothrombin is formed or an accelerator is generated. The prothrombin content of the blood of newborn infants is only about 30 per cent. of that of adults but the normal prothrombin time is the same, namely 12 seconds. It has been shown that both accessory factors are deficient in newborn infants. The authors find that the prothrombin time of the plasma of newborn infants does not decrease on storage and that the time for the serum from platelet-poor newborn plasma is the same as that of the original plasma but higher than that for serum from platelet-poor adult plasma. The authors suggest that their observations can be explained on the hypothesis that adult blood, but not newborn blood, contains a prothrombin precursor, "prothrombinogen", kept inactive by an inhibitor. On storage or clotting, the inhibitor disappears and prothrombin is liberated. The one-stage prothrombin test measures active prothrombin whereas the two-stage method includes also the prothrombinogen. As the two methods measure different things, one cannot be used to assess the reliability of the other. The one-stage method remains the simplest and most reliable method of determining prothrombin. H. T. B.

BIOCHEMICAL ANALYSIS

***p*-Aminosalicylic Acid and Isoniazid Blood Levels, Determinations of, with Vanillin.** E. N. Deeb and G. R. Vitagliano. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 182.) A simple method depends on the formation of yellow compounds by reaction with vanillin. The following method is recommended for the determination of *p*-aminosalicylic acid. To 2 ml. of plasma add water to produce 12 ml. followed by 4 ml. of a 20 per cent. solution of trichloroacetic acid. Centrifuge, filter and to 8 ml. of clear filtrate add 1 ml. of 10 per cent. solution of vanillin in ethanol (50 per cent.). Allow to stand for 10 minutes and determine the light absorption at 410 mμ. The result is calculated by comparison with the light absorption of solutions prepared by adding known quantities of *p*-aminosalicylic acid to normal plasma and treating as above. A

similar procedure may be employed for the determination of isoniazid, using 4-ml. samples of plasma and a 2 per cent. solution of vanillin in N sulphuric acid, the absorption measurements being carried out at 400 mμ. The precision of the method is ±0·9 per cent. for *p*-aminosalicylic acid and ±0·4 per cent. for isoniazid. G. B.

Boric Acid in Biological Materials, Determination of. W. C. Smith, A. J. Goudie and J. N. Sivertson. (*Analyt. Chem.*, 1955, **27**, 295.) The determination of traces of boric acid in blood, urine and animal tissues is accomplished by a colorimetric method using carminic acid. Organic matter is destroyed by fusing the sample with lithium carbonate and dissolving the residue in hydrochloric acid. Sulphuric acid is then added followed by a solution of carminic acid; the resulting colour is measured spectrophotometrically at 575 mμ after 5 minutes. Both carmine N.F. and carminic acid produced the colour satisfactorily but the colour developed much more rapidly with carminic acid. Inorganic materials that are normally found in blood, urine, and animal tissue did not interfere appreciably. The method was applied satisfactorily to the determination of boron in blood, urine, lung, heart, thymus, liver, spleen, stomach, duodenum, kidney, prostate or uterus, brain, voluntary muscle, skin, urinary bladder, jejunum, cæcum, and terminal colon, as well as fæcal matter and gastric contents. R. E. S.

Calcium in Serum, Estimation of. S. Natelson and R. Penniall. (*Analyt. Chem.*, 1955, **27**, 434.) A colorimetric method is described for the determination of calcium by extraction of the calcium-alizarin complex in *n*-octanol. Practical or technical grades of triethanolamine are used to adjust the *p*H to avoid turbidity in the alcohol phase; pure grades of triethanolamine were unsatisfactory. The serum sample (fresh) is diluted with water, and aqueous triethanolamine and alizarin solution in *n*-octanol are added from burettes. After shaking for 20 minutes the mixture is centrifuged and the colour is measured absorptiometrically at 560 mμ after dilution with *n*-octanol. Using inorganic alkali, iron interferes; a number of satisfactory grades of triethanolamine are listed. For human serum a factor of 7 per cent. for magnesium interference has to be deducted. When relatively large amounts of serum are available it is preferable first to precipitate the calcium as oxalate. R. E. S.

Iron in Plasma, Determination of. T. H. Bothwell and B. Mallett. (*Biochem. J.*, 1955, **59**, 599.) A method is presented for the determination of iron in plasma or serum in which hydrochloric acid is added to the sample and the plasma proteins are precipitated with trichloroacetic acid. After centrifuging, the supernatant fluid is decanted and is added to a mixture of thioglycollic acid, 2:2′-dipyridyl (in glacial acetic acid) and a saturated solution of sodium acetate. The mixture is shaken and the colour intensity is measured absorptiometrically, the concentration of iron being obtained by comparison with a standard curve prepared using known amounts of iron; a reagent blank is also measured. A table is given showing that complete recoveries were obtained in 101 estimations. By giving tracer doses of ^{59}Fe parenterally or orally, radioactive plasma samples with the transport iron attached to β_1-globulin were obtained; this iron was found to be completely separated from its protein attachment after thorough mixing with dilute hydrochloric and trichloroacetic acids. R. E. S,

Salicylic Acid and Two Metabolites in Plasma and Urine, Determination of, using Fluorimetry. E. B. Truitt, Jr., A. M. Morgan and J. M. Little. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 142.) Determinations of free salicylic acid in plasma and urine were carried out by extraction with carbon tetrachloride, followed by measurement of the colour produced by reaction with ferric nitrate. On account of the relatively high solubility of salicylic acid in the solvent, and the weak colour produced by salicyluric acid, the test was 50 times more sensitive to salicylic than salicyluric acid and corrections were necessary only when large quantities of the latter were present, for example in human urine. Salicyluric acid was determined by extraction of plasma or urine with ethylene dichloride and measurement of the fluorescence at *p*H 10. Salicylic acid gave a relatively weak fluorescence under these conditions but a small correction was usually necessary. The quantity of salicylglycuronides was estimated by submitting diluted urine samples to hydrolysis with concentrated hydrochloric acid in a boiling water bath for 3 hours, during which time the whole of the salicylglycuronides and 62 per cent. of the salicyluric acid were hydrolysed to salicylic acid. The solution was extracted with ethylene dichloride and total salicylate determined by the ferric nitrate method. A reference curve was made using urine containing known quantities of salicylic acid treated under the same conditions, since hydrochloric acid affects the efficiency of the extraction procedure. A synthetic sample of *O*-(β-D-glucuronosido)salicylic acid gave no colour with ferric nitrate until hydrolysed with hydrochloric acid and did not contribute to the fluorescence of ethylene dichloride extracts. G. B.

CHEMOTHERAPY

***N*-(2-Hydroxy-5-chlorobenzylidine)anilines and *N*-(2-Hydroxy-5-chlorobenzyl) anilines.** D. B. Reisner and P. M. Borick. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 148.) A number of *N*-(2-hydroxy-5-chlorobenzylidine)anilines were prepared by reaction of 5-chlorosalicylaldehyde with the required aromatic amine in alcoholic solution. The corresponding *N*-(2-hydroxy-5-chlorobenzyl) anilines were prepared by catalytic reduction of the corresponding Schiff's bases in the presence of platinum oxide and acetic acid. Compounds of both series were tested for bacteriostatic and fungistatic activity against *Escherichia coli*, *Micrococcus pyogenes* var. *aureus*, *Trichophyton interdigitale*, *Mycophyton audouini* and *Candida albicans* by a serial dilution method. The compounds showed a high degree of activity, the most active substances studied being the *N*-(2-hydroxy-5-chlorobenzyl)chloroanilines and *N*-(2-hydroxy-5-chlorobenzyl) 1-naphthylamine. G. B.

***iso*Nicotinoylsalicylidene Hydrazine as a Tuberculostatic.** T. Cănback, N. Diding, P. Lundgren, P. Ekeblad, O. Alm, K. Erne and S. Linde. (*Svensk farm. Tidskr.*, 1955, **24**, 1.) Tests are reported on the nicotinylhydrazide of salicylic aldehyde ("Acozide"). Compared with isoniazid in tests *in vitro* on *Myco. tuberculosis*, its activity is only about one tenth. On the other hand, the subcutaneous toxicity of the new compound is less than one twentieth of that of isoniazid, while comparison of oral toxicities is even more favourable. Acozide may be determined photometrically by measuring the extinction at 288 mμ at *p*H 6. The shape of the extinction curve alters with the *p*H. In presence of isoniazid or salicylaldehyde (hydrolysis products) it is necessary to make measurements also at 251, 255·5 or 262 mμ, and to compare the figures with that at 288 mμ. G. M.

PHARMACY

NOTES AND FORMULÆ

Antacids, A Study of. J. K. Dale and R. E. Booth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 170.) A series of ingredients of antacid mixtures, some experimental formulæ and 24 commercial products were examined, two tests being employed. Either the *p*H was determined after the addition of varying amounts of antacid to 100 ml. of 0·1N hydrochloric acid, or 1 dose of antacid was added to 50 ml. of 0·1N hydrochloric acid, 2 ml. of N hydrochloric acid being added every 10 minutes and the *p*H determined at intervals. Mixtures of aluminium hydroxide and magnesium trisilicate were the best antacids at *p*H 4 or below, whereas a mixture of 12 parts of calcium carbonate, 2 of magnesium trisilicate and 1 of magnesium carbonate was better below *p*H 6·8. For the Sippy treatment, requiring neutralisation to *p*H 7 or below, a mixture of equal quantities of magnesium carbonate and sodium bicarbonate was most effective. In these experiments bentonite, kaolin, aluminium silicate, silica gel, sodium cellulose sulphate, veegum, methylcellulose and guar gum had no buffering action. Only slight buffering action was obtained with sodium carboxymethylcellulose, bismuth subcarbonate, calcium gluconate and calcium glycerophosphate. Effective buffers were prepared from aluminium phosphate, bone phosphates, glycine, polyamine methylene resin, calcium citrate, dicalcium phosphate, aluminium hydroxide, magnesium trisilicate, colloidal magnesium silicate, calcium carbonate, sodium bicarbonate, magnesium carbonate and magnesium oxide. Variations in buffering power were observed in examining samples of pharmacopœial quality and it is suggested that further tests should be added to the monographs. G. B.

Cycrimine Hydrochloride (Pagitane Hydrochloride). (*New and Nonofficial Remedies*; *J. Amer. med. Ass.*, 1955, **157**, 510.) Cycrimine hydrochloride is *cyclo*pentylphenyl-3-(1-piperidyl)-1-propanol hydrochloride and occurs as a white, odourless, bitter substance, m. pt. 241° to 244° C. (with decomposition), soluble, at 25° C., in 150 parts of water, in 50 parts of ethanol, and 33 parts of chloroform, and practically insoluble in benzene and ether. A 0·5 per cent. solution in water has *p*H 4·9 to 5·4, and when made alkaline with sodium hydroxide and cooled overnight yields a precipitate of cycrimine, which melts at 90° to 96° C., after washing with a little cold water and drying in a vacuum over phosphorus pentoxide for 5 hours. A 0·07 per cent. solution in water exhibits ultra-violet absorption maxima at about 251, 257 and 263 mμ ($E^{1\%}_{1\,cm.}$, about 5·4, 6·2 and 5·0), minima at about 230, 254 and 261 mμ, and an inflection point at about 247 mμ. The ratio of the absorptions at 251 and 257 mμ is 1·0 to 1·3. Cycrimine hydrochloride loses not more than 1·0 per cent. of its weight when dried at 105° C. for 5 hours, and yields not more than 0·1 per cent. of sulphated ash; the limit of heavy metals is 20 parts per million. It is assayed in solution in glacial acetic acid, by the addition of a 6 per cent. solution of mercuric acetate and titration with 0·1 N perchloric acid, using crystal violet as indicator; it contains 97 to 103 per cent. of cycrimine hydrochloride. Tablets are identified by extracting with chloroform and applying the identity tests for chloride and melting point of the free base to the residue obtained on evaporation. They contain 92·5 to 107·5 per cent. of the labelled amount of cycrimine hydrochloride, and are assayed by extracting the base from an alkaline solution with ether, extracting the ether with sulphuric acid,

and titrating the excess of acid with sodium hydroxide, using methyl red as indicator. Cycrimine hydrochloride is used in the treatment of paralysis agitans. G. R. K.

Dihydrostreptomycin, Stability of Aqueous Solutions of. H. Vogt. (*Arch. Pharm. Berl.*, 1955, **288**, 26.) Aqueous solutions of dihydrostreptomycin sulphate lose 8 to 9 per cent. of their activity after 6 weeks in a refrigerator, and 11 to 12 per cent. at room temperature. If the solutions are first sterilised at 120° C. then the losses are about 15 per cent. in both cases. A buffered solution (pH = 3·6) only decomposes to about one half the extent of an unbuffered one. Ointments containing dihydrostreptomycin with yellow soft paraffin lose strength at about the same rate as the aqueous solutions, i.e., about 11 to 12 per cent. after 5 weeks. Thus the stability of dihydrostreptomycin is appreciably greater than that of penicillin. G. M.

Pyrogens, Testing Injections for. A. Engelund and P. Terp. (*Arch. pharm. chemi*, 1955, **62**, 1.) In testing for pyrogens, the injection of 10 ml. of a solution into a rabbit may sometimes result in toxic effects. This applies to a 10 per cent. solution of calcium gluconate, and in this case it is recommended that 10 ml. of a 2 per cent. solution should be used for the test. The method of precipitating the calcium with sodium carbonate before injection is not advised. For a 2 per cent. solution of sodium citrate, it is necessary either to dilute with water or saline to a concentration of 1 per cent., of which 10 ml. is injected; or calcium equivalent to the citrate must be added. Injections containing potassium may be injected without trouble. G. M.

PHARMACOGNOSY

Alkaloidal Ontogenesis in *Lupinus luteus*. A. van der Kuy. (*Pharm. Weekbl.*, 1955, **90**, 65.) The three chief alkaloids of *Lupinus luteus* may be precipitated by flavianic acid in ether solution. Lupinine flavianate is separated by precipitation in chloroform, in which the flavianates of the other two are insoluble. Sparteine and lupanine are easily separated, since only the former is volatile in steam. Studies on the plant at different stages showed the following picture. Lupinine is the chief alkaloid of the seed, but disappears soon after germination, reappearing during flowering and fruiting. It is never found in the root. Sparteine is a major constituent at all times and in all organs. In addition to sparteine and lupinine, other alkaloids are present in all vegetative parts of the plant. At certain stages these are present in greater amount than sparteine. G. M.

***Datura* Species, Hybridisation Experiments with.** E. Steinegger. (*Pharm. Acta Helvet.*, 1954, **29**, 378.) Hybridisation of *D. stramonium* (tetraploid). *D. tatula* and *D. tatula* var. inermis generally gives satisfactory results, but attempts to cross *D. innoxia* with the above species were unsuccessful, since very few of the seeds could be germinated. Certain abnormalities were observed on 4n mother plants: fruit formation without seeds; fruit with very small seeds which would not germinate; fruit with seeds producing diploid offspring similar to the mother plant but with lower chromosome number; and tetraploid offspring identical with the mother plants. The 4n offspring showed no alteration in alkaloidal content, whereas the 2n offspring from 4n mother plants had a considerably reduced alkaloidal content. This is a confirmation of the effect of polyploidy in increasing the content. G. M.

***Rheum palmatum*, Anthraglycoside Content of.** E. Schratz and H. Tombergs. (*Arzneimitt.-Forsch.*, 1954, **4**, 678.) For the assay of rhubarb root, free anthraquinones are determined by extraction of the root with anhydrous ether, the ethereal solution being then shaken out with a solution containing 5 per cent. of sodium hydroxide and 2 per cent. of ammonia. The extinction is then measured. For combined anthraquinones and reduced antharacene derivatives, 50 mg. is refluxed with 7·5 ml. of glacial acetic acid, then exhausted with ether. The solution is neutralised with soda (taking care to avoid any rise of temperature), and the anthraquinone fraction is shaken out into the above alkaline solution. It is then determined photometrically, using pure 1:8-dihydroxyanthraquinone as standard. The content of reduced anthraquinone compounds is determined similarly after oxidation by heating for two hours on the water bath. It is not possible to avoid a certain amount of oxidation of the anthranols during the alkaline treatment, and for this reason it is essential to cool the acid mixture during neutralisation, and to cut down the time of exposure to alkali to a minimum. The colorimetric determinations should be carried out as soon as possible. Mean results of a large number of determinations are summarised in the tables below (calculated as percentage of dry weight of the root).

Total content		Anthranol content		Anthraquinone content	
Summer	Winter	Summer	Winter	Summer	Winter
3·09	3·01	1·10	1·165	2·02	1·76

Percentage of total content:			
Anthranols		Free anthraquinones	
Summer	Winter	Summer	Winter
35·1	34·8	26·7	34·1

These tests were carried out on about 300 individual plants of *Rheum palmatum*. The total content of anthracene derivatives varied greatly: the range of variation being from about 1·5 to 6·0 per cent. No significant difference could be detected between the plants at the height of the vegetative period (August) and at mid-winter (December). G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline, Potentiation of, by Local Anæsthetics. H. E. D'Amato and A. P. Truant. (*Arch. int. Pharmacodyn.*, 1955, **100**, 113.) In the unanæsthetised dog local anæsthetics—procaine, lidocaine, butethamine, metabutethamine and pravocaine—potentiate, like cocaine, the pressor response to adrenaline. Diastolic pressure is potentiated to a greater extent than systolic pressure. Anæsthetised dogs do not always show the potentiation, depth of anæsthesia and type of anæsthetic affecting the response. Potentiation with synthetic local anæsthetics, unlike cocaine which may last for over an hour, is relatively short lived. Atropinisation, ganglionic or adrenolytic blockade do not abolish or diminish potentiation by cocaine or procaine. The depressor response to small doses of adrenaline is converted to a pressor response by cocaine and the depressor response after adrenergic blockade is also potentiated. The pressor response of noradrenaline is also potentiated and there is a greater degree of potentiation of noradrenaline than adrenaline with cocaine. Local anæsthetics do not modify the pressor response to occlusion of the carotid arteries. G. F. S.

Alkyl Tin Compounds, Studies on the Toxicity of. H. B. Stoner, J. M. Barnes and J. I. Duff. (*Brit. J. Pharmacol.*, 1955, **10**. 16.) A series of mono-, di-, tri and tetra- alkyl tin compounds and some other organic tin compounds were studied in rats, rabbits, guinea-pigs and fowls. In acute experiments on rabbits, triethyl tin, the most active compound, produced muscular weakness, followed by an apparent partial recovery and then by muscular tremors, which led to convulsions and death. This general pattern was common to all of the compounds and species studied, with slight variation. Other actions observed were vasodilatation of the blood vessels of the rabbits' ear, fall in rectal temperature in the rat and rabbit, a vasopressor action in the cat and neuromuscular blockade in the rabbit and rat. Chronic administration of the tin compounds caused in all species muscular weakness. The main site of action appeared to be the central nervous system, although there was no evidence of concentration of the compounds in any particular organ. In the case of diethyl tin, the mode of action may involve reaction with sulphhydryl groups as its toxic effects were antagonized by dimercaprol. Dimercaprol had no effect on the toxicity of triethyl tin. The effects of tetraethyl tin resembled those of triethyl tin closely after an initial latent period, which suggests *in vivo* conversion of the tetra- to the tri-alkyl derivative. Since the delayed effects of triethyl tin poisoning could be reproduced by large doses of sodium tin tartrate, the same relationship might exist between triethyl tin and other forms of tin, as exists between lead, mercury and antimony and their respective alkyl derivatives. G. P.

Anthelmintic Activity, *In Vitro* Tests for, on *Ascaris lumbricoides* and *Fasciola hepatica*. A. Mackie, G. M. Stewart, A. A. Cutler and A. L. Misra. (*Brit. J. Pharmacol.*, 1955, **10**, 7.) The anthelminthic activity of derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, phenothiazine and rhodanine, and of other miscellaneous compounds against *Fasciola hepatica* (liver fluke), and the anterior preparation of *Ascaris lumbricoides* ("roundworm"), was assessed *in vitro* by kymographic technique. The 2:3-dihydro-3-ketobenzo-1:4-thiazine derivatives, where active, showed only depressant effects against *A. lumbricoides*, although some activity against the liver fluke was observed in some of these compounds, particularly the 6-bromo compound. Increase in side-chain length usually decreased the activity against the liver fluke. Some of the amino-acetylphenothiazines were active against the liver fluke; β-10-phenothiazinyl propionic acid was lethal. Of the rhodanine derivatives, only 5-*iso*nitroso-3-allyl-rhodanine paralysed *Ascaris*, but some, especially the benzylidene compounds killed the liver fluke. Among the miscellaneous compounds the following were very active: allyl iodide and sodium azide, against *Ascaris*; carbon tetrabromide, benzene hexachloride, allyl iodide, allyl *iso*thiocyanate, mercuric chloride, ethyl mercuric chloride, ethoxyethyl mercuric chloride, diphenylamine and *p*-nitrophenol, against *Fasciola*. G. P.

Chlorpromazine, Effect of Temperature on the Toxicity of. T. Berti and L. Cima. (*Arzneimitt.-Forsch.*, 1955, **5**, 73.) The influence of temperature on the toxicity or chlorpromazine to mice was investigated. The effect of temperature is very marked. Doses of 100 mg./kg., which are certainly fatal at 18 to 20° C. and also at 33 to 38° C., do not kill at 28 to 29° C. The toxicity between 25·5 and 30·5° C. is 30 times less than that at lower temperatures. At the lower temperatures the predominant symptoms are of central depression; as the temperature rises excitation symptoms become more clearly marked. At 30° C. death occurs from violent convulsions. G. M.

Cholinesterase Inhibition and Increase in Muscle Tone in Rabbit Duodenum, a Correlation Between. H. Shelley. (*Brit. J. Pharmacol.*, 1955, **10**, 26.) The increase in tone caused by eserine and dyflos in isolated strips of longitudinal muscle from rabbit duodenum and in the isolated intact duodenum was closely correlated with the inhibition by these drugs of the true cholinesterase in the two tissues. Increase in tone first appeared with eserine concentration of $2{\cdot}7 \times 10^{-8}$ M or with dyflos concentration of 10^{-7} M. As the anticholinesterase concentration was increased, tone also increased to a maximum with $2{\cdot}7 \times 10^{-6}$ M eserine or 10^{-5} M dyflos. Above these concentrations tone decreased. The distribution of true- and pseudo- cholinesterases in the different layers of the duodenum was determined manometrically. Highest activity of both enzymes was found in the longitudinal muscle layer, to which most of the cells and fibres of Auerbach's plexus were found to adhere on separation. The true- and pseudo- cholinesterases of these longitudinal muscle strips were equally inhibited by eserine, but with dyflos the pseudocholinesterase was much more readily inhibited. In estimating the degree of inhibition of the enzymes in the longitudinal muscle strips the muscle was treated with dyflos and then homogenized. This procedure gave results similar to that where the muscle was homogenized before treatment with the dyflos, showing adequate penetration of the dyflos into the tissue. G. P.

Compound 48/80, the Effect of, on Ganglionic Transmission. S. B. Gertner. (*Brit. J. Pharmacol.*, 1955, **10**, 103.) Both compound 48/80 and propamidine blocked transmission through the cat superior cervical ganglion, perfused with Locke solution, contraction of the nictitating membrane being used as an indication of transmission. Injected into the ganglionic circulation 48/80 had no ganglion-stimulating action in doses up to 100 μg., but regularly produced relaxation of the nictitating membrane during continuous preganglionic stimulation. Acetylcholine release during ganglion block by 48/80 was estimated on the eserinised dorsal muscle of the leech and did not differ from ACh release during normal transmission. The sensitivity of the ganglion to stimulation by injected ACh was decreased by 48/80; the block could be overcome partially by increasing the dose of ACh. The release of histamine from the ganglion could not account for the block by 48/80 since; (*a*) ganglionic transmission is not impaired by histamine; (*b*) the degree of block is not increased by histamine; (*c*) the amounts of histamine released decreased with successive injections of 48/80 whereas the block became more pronounced, so that even when no histamine was being liberated block was still obtained. This effect could explain the finding that in rats depleted of their tissue histamine by repeated injections of 48/80 there was still a residual vasodepressor effect with subsequent injections of the liberator (Nasmyth, *Brit. J. Pharmacol.*, 1955, **10**, 51). (*see* p. 618). G. P.

β-Diethylaminoethyl Diphenylpropylacetate Hydrochloride, Enhancement of the Action of Analgesic Drugs by. L. Cook, G. Navis and E. J. Fellows. (*J. Pharmacol.*, 1954, **112**, 473.) In the rat, β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF525-A) prolonged the analgesic action of morphine, methadone, pethidine, codeine and methorphinan. The LD50 estimates of morphine and pethidine were not significantly altered nor was the respiratory depressant action of morphine increased by the drug. In rats tolerant to morphine, SKF525-A increased the effects of further doses of the analgesic; however, tolerance was just as easily induced by morphine–SKF525-A combinations as with morphine alone. SKF525-A, alone, had a slight analgesic effect, but only in doses much larger than those used to potentiate the analgesics. G. P.

Ferritin, Study of the Pharmacology of. T. J. Haley and J. L. Leitch. (*Arch. int. Pharmacodyn.*, 1954, **100**, 120.) Ferritin is the form in which iron is transported from the intestine and stored in the liver. Ferritin extracted from dog liver, spleen and horse spleen is shown to have little effect on the isolated guinea-pig heart, on the pressor and respiratory effects of adrenaline in the rabbit, cat or dog and on the response of the cat nictitating membrane to adrenaline and to preganglionic stimulation. It also did not alter the blood flow in the hind limb of the dog or the response of the isolated guinea-pig seminal vesicle to adrenaline. G. F. S.

Heterocyclic Bis-quaternary Compounds, Particularly of a Pyrrolidinium Series, the Actions of. D. F. J. Mason and R. Wien. (*Brit. J. Pharmacol.*, 1955, **10**, 124.) The actions of three series of bis-quaternary salts having the general formula $A.(CH_2)_n.A$, where A is a heterocyclic nucleus (1-methylpiperidino-, 4-methylmorpholino-, or 1-methylpyrrolidino-) were studied on transmission through the superior cervical, vagal and ciliary ganglia of the cat, on salivary flow in the cat, on the peristaltic reflex of the guinea-pig ileum and on neuromuscular transmission in the cat and rabbit. Anticholinesterase activity was measured manometrically on horse erythrocyte cholinesterase. Where the quaternizing groups on the nitrogen atoms were methyl, compounds of the piperidinium series where $n = 4$, 5 and 6 and of the morpholinium series where $n = 5$ and 6, all had ganglion-blocking activity similar to that of hexamethonium. In the piperidinium series the decane members (and where the quaternizing groups were either methyl or ethyl) had about one-fifth the activity of tubocurarine at the neuromuscular junction, but the morpholinium series had negligible activity at this site. The pentane member of the pyrrolidinium series (with methyl quaternizing groups) had peak ganglion-blocking activity of all the compounds and was about five times as active as hexamethonium on the superior cervical ganglion of the cat. The mode of action of this compound, called "pentapyrrolidinium" [pentamethylene-1 : 5 bis-(1-methyl-pyrrolidinium)] was similar to that of hexamethonium. G. P.

Histamine Release and the "Stress" Phenomenon. P. A. Nasmyth. (*Brit. J. Pharmacol.*, 1955, **10**, 51.) The histamine-liberator 48/80 precipitated the stress phenomenon on subcutaneous injection into normal rats, in a dose of 0·5 mg./100 g. body weight. At this dose level there was a marked depletion of the adrenal ascorbic acid content. In rats where the tissue histamine had previously been depleted by 48/80 administration, a further 0·5 mg./100 g. of the liberator had less action on the adrenal ascorbic acid than in the normal animal. Where the adrenals had been demedullated 36 to 56 days previously the ascorbic acid depletion was more prolonged than in the normal animal, after 48/80 administration, but where six months had elapsed between operation and 48/80 administration there was no significant difference between operated and normal. The reason for this may be that the adrenal cortical tissue had not regenerated adequately within 36 to 56 days of the operation. 0·5 mg./kg. of 48/80 injected intravenously had much less effect on the blood pressure of the histamine-depleted than on the normal rats, due to a decreased release of histamine. It was not possible to say how far this accounted for the reduced effect on the adrenal ascorbic acid in these animals. In demedullated rats the blood pressure after intravenous injection of 48/80 followed the same pattern as in the normal and histamine-depleted animals, but the fall was more precipitous and death invariably occurred between 20 and 30 minutes after the dose. It was concluded that released histamine plays some part in the response of the adrenal cortex to 48/80. G. P.

Histamine Release in Rabbit Blood by Dextran and Dextran Sulphate. C. G. Haining. (*Brit. J. Pharmacol.*, 1955, **10**, 87.) The cellular histamine of rabbit blood is known to be released by a number of compounds causing anaphylaxis and anaphylactoid reactions *in vivo*. Dextran and dextran sulphate, which produce these reactions in the rat, guinea-pig and man, were also found to cause histamine release on incubation with rabbit blood for 30 minutes at 37° C. The liberated histamine was extracted and assayed on the guinea-pig ileum. Histamine release by dextran depended upon the concentration and molecular weight, samples of mol. wt. between 22,000 and 1,000,000 being effective, but not those below 14,000. Similarly, with dextran sulphate, samples of mol. wt. below 10,000 were inactive, the effect being graded for samples between 40,000 and 440,000. Dextran sulphate differed from dextran in that there was an optimum concentration necessary for maximum histamine release. Dextran sulphate of high mol. wt. had a dual action, behaving as an activator of the histamine release mechanism at low concentrations and as an inhibitor at high concentrations. Histamine release by the dextran sulphate of mol. wt. 440,000 was inhibited by sodium oxalate, heparin, maltotriose sulphate and low mol. wt. dextran sulphate. Low mol. wt. dextran was ineffective as an inhibitor, suggesting the importance of the sulphate ester group. G. P.

5-Hydroxytryptamine, Effects of, on the Nictitating Membrane of the Cat. J. Lecomte. (*Arch. int. Pharmacodyn.*, 1955, **100**, 457.) The actions of intravenous and intra-arterial injections of 5-hydroxytryptamine in conjunction with serotonin and antihistamine compounds have been studied on the blood pressure and nictitating membrane of the anæsthetised cat. 5-Hydroxytryptamine had a direct contracting action on the nictitating membrane which was potentiated by the local instillation of tutocaine. The response was related to the dose but tachyphylaxis occurred. The response of the nictitating membrane to adrenaline was potentiated. Intra-arterial injections into the aorta, close to the suprarenal arteries, caused a smaller and slower contraction. Administration of 10 to 30 mg./kg. of mepyramine intravenously increased the contractions of the nictitating membrane to intravenous injections of adrenaline and serotonin, but abolished the response to intra-arterial injections of serotonin. Promethazine, 17·5 mg. per kg. abolished the contractions to adrenaline and serotonin. Local instillation of a 1 per cent. solution of chlorpromazine also abolished the responses. G. F. S.

Mephenesin, the Effect of, on Barbiturate Anæsthesia. F. M. Berger and T. E. Lynes. (*Arch. int. Pharmacodyn.*, 1955, **100**, 401.) Experiments carried out in mice show that mephenesin has a synergistic action with hexobarbitone and butabarbital and that mephenesin suppresses prehypnotic excitement. The combined action of mephenesin and pentobarbitone is merely additive, but the combined effect of butabarbital and mephenesin varies with the experimental design from incomplete addition to potentiation. It is suggested that the modifying effect of mephenesin on barbiturate anæsthesia will be of considerable value in therapeutics. G. F. S.

Meratran, New Blocking Agent against LSD 25 Psychosis. H. D. Fabing. (*Science*, 1955, **121**, 208.) Meratran (α-(4-piperidyl) benzhydrol hydrochloride) blocked the psychotic states precipitated in normal healthy subjects by the oral administration of 100 μg. of lysergic acid diethylamide (LSD25). The visceral effects of LSD25 (nausea, dryness of the mouth, sweating and numbness in the limbs) were unaffected. Blockade of the psychosis was achieved either by an

oral dose schedule before ingestion of the LSD25 or by intravenous injection during the psychosis. The blocking agent alone produced no subjective or objective reactions during the premedication period. Preliminary experiments with mescaline sulphate suggest that the psychosis induced with this drug is also abolished by the blocking agent. G. P.

***N*-(4-Methoxybenzyl)-*iso*quinolinium Chloride, Pharmacology of.** J. Dipalma. (*J. Pharmacol.*, 1955, **113**, 125.) This drug is an aromatic quarternary ammonium compound. Its main action is to increase the rate and force of the heart beat. This has been shown on the isolated atrium of the cat, in intact dogs and in humans. It is more potent than quinidine in raising the threshold of electrically induced atrial fibrillation in the cat, but is unlike quinidine in that it does not protect against adrenaline-hydrocarbon induced arrhythmias. It has weak ganglionic and neuromuscular blocking actions. It also causes local anæsthesia when injected intradermally, having the same order of potency as procaine. The drug is well tolerated in man in doses up to 5 mg./kg., the only side-effects being a transient dilation of the pupils, flushing and occasionally vertigo. It is now undergoing clinical trial. M. M.

Myleran in Chronic Myeloid Leukæmia. D. A. G. Galton and M. Till. (*Lancet*, 1955, **268**, 425.) Of 11 patients suffering from chronic myeloid leukæmia treated with myleran alone for periods varying from 1 to 4 years, 7 are still living; only 2 have been observed for more than 3 years from diagnosis which is about the median survival from diagnosis in patients treated by external radiation and with radioactive phosphorus. It is therefore too early to compare the survival-rates of patients treated with myleran alone with those treated by radiotherapy alone. Thus, while myleran cannot at present be advocated as a first line of treatment, it appears to be a satisfactory substitute for radiotherapy and can justifiably be used as such if radiotherapy is unavailable, impracticable or contra-indicated. Administration was by means of tablets containing 0·5 or 2 mg., the standard dose for an adult being 0·06 mg./kg. of bodyweight daily (about 4 mg.). Treatment was stopped when the clinical and hæmatological improvement seemed to justify it or when the leucocyte count was thought to be falling too steeply. With the daily standard dosage the treatment lasted from 3 to 7 months. Subsequent treatment was deferred until symptoms returned; symptoms reappeared in from 5 to 18 months. Remissions following second and third courses of myleran were usually shorter, none exceeding 1 year. The dose required for maintenance varied considerably, the smallest dose used being 0·5 mg. daily and the largest 6 mg. daily. No patient has yet received continuous therapy for longer than 23 months, but the development of specific resistance to myleran in 3 patients suggests that it will not be effective indefinitely. The general response to myleran therapy, previously observed, was confirmed. Symptoms were rapidly relieved; the Hb level rose steadily; splenic regression, though somewhat slower than with radiotherapy, was equal in extent; and absolute and differential leucocyte counts approached normal. There were no side-effects, and thrombocytopenia, formerly thought to be an occasional hazard with therapeutic dosage, developed only once after avoidance of doses exceeding 0·06 mg./kg. of bodyweight daily. (Details are also included of 20 patients treated with myleran after previous courses of radiotherapy, radioactive phosphorus, urethane or nitrogen mustard.) S. L. W.

Nalorphine, the Effect of, on the Antidiuretic Action of Morphine in Rats and Man. H. Schnieden and E. K. Blackmore. (*Brit. J. Pharmacol.*, 1955, **10**, 45.) The antidiuretic effect of morphine injected subcutaneously into "water-loaded" rats was decreased by the simultaneous injection of a dose of nalorphine which alone had no antidiuretic action. The analgesic effects of morphine in rats were also reduced by nalorphine, which by itself had slight analgesic action. The antidiuretic effect of morphine in rats was partly due to a prolongation of gastric emptying time, and partly to a reduction in water excretion after it had been absorbed. Both effects were decreased by nalorphine. The water content of the brain of rats to which water had been administered was not significantly higher after morphine than after saline injection. In hydrated normal healthy men the marked antidiuretic effects of morphine were not affected by simultaneous injection of nalorphine. In some subjects nalorphine given alone produced an antidiuresis. G. P.

Phenobarbitone and Diphenylhydantoin Sodium, Anticonvulsant Properties of. G. Chen and C. R. Ensor. (*Arch. int. Pharmacodyn.*, 1954, **100**, 234.) The anticonvulsant properties of phenobarbitone, diphenylhydantoin sodium, pentobarbitone and barbitone and their combinations were investigated in mice by their depression of leptazol- and electroshock- induced convulsions. The anti-electroshock activity was greatest with phenobarbitone and diphenylhydantoin, being high at non-hypnotic dose levels, whereas both pentobarbitone and barbitone were only effective at minimal anæsthetic dosage. For both phenobarbitone and diphenylhydantoin the time course of action was the same, being slow to reach peak effect. In the combination of drugs it was found that pentobarbitone and barbitone were alike in their action, diphenylhydantoin and barbitone had different modes of action, but were additive, and with combinations of phenobarbitone and diphenylhydantoin there was a mutual potentiating effect. However, no potentiation beyond an additive effect was found for the LD 50 estimates in mice for phenobarbitone-diphenylhydantoin combinations. The anti-electroshock activity was suggested as a possible mechanism for the enhanced anti-epileptic efficacy of diphenylhydantoin-phenobarbitone mixtures. G. P.

Pitressin, Modifications in the Determination of the Antidiuretic Activity of. J. Tripod, C. Bruni and R. Meier. (*Arch. int. Pharmacodyn.*, 1955, **100**, 1.) For the determination of the antidiuretic activity of pitressin in the rat an initial water load of 50 ml./kg. by mouth gives the greatest sensitivity and is the simplest method for routine assays. Determination of the time of maximal excretion as described by Burn, or the determination of the urinary retention over 3 hours in relation to controls is a satisfactory and easy procedure. G. F. S.

Protoveratrine, Hypotensive Mechanism of. E. Fernandez and A. Cerletti. (*Arch. int. Pharmacodyn.*, 1955, **100**, 425.) In the intact cat, under chloralose-urethane anæsthesia, 1·5 to 2·5 μg. of protoveratrine causes a maximal fall in blood pressure of 26 per cent., which is reduced to 14 per cent. in the atropinised animal. After bilateral vagotomy the fall in blood pressure is very slow, a maximal effect taking 20 minutes. In animals with bilateral denervation of the carotid sinus, but with the vagus nerves intact, the blood pressure fall is more pronounced, and is reduced by prior administration of atropine. In vagotomised and carotid denervated cats protoveratrine has no hypotensive action, and in most cases the blood pressure increases. The results show that the vagus nerve plays the main role in the immediate hypotensive effect of protoveratrine and the carotid sinus has an important buffer action, diminishing the initial rapid

fall of blood pressure. A direct effect on the central nervous system does not play any important part in the hypotensive action of small doses of protoveratrine. G. F. S.

Quinoline Derivatives, Cardiovascular and Oxytocic Actions of a Series of. K. Kamijo and G. B. Koelle. (*J. Pharmacol.*, 1954, **112**, 444.) Three groups of quinoline derivatives were investigated for cardiovascular actions on the blood pressure of the dog and for oxytocic action on the isolated guinea-pig uterus and rabbit ileum. Group A, 3-quinolinecarboxamide derivatives had little activity of either type. Group B, tetrahydro-3-quinolinecarboxamides had slight hypotensive activity, but were relatively strong oxytocics. The mode of action appeared to differ from that of ergometrine. The 3-carbamylquinolinium halides (group C) were relatively strong hypotensive agents, but had no oxytocic activity. One member of this group, the most active, 1-methyl-3-[*N*-(1-carboethoxyethylcarbamyl)]quinolinium iodide, designated McN-259-15, was investigated in detail. Five mg./kg. intravenously in dogs caused a rapid, prolonged fall in blood pressure, accompanied by a brief tachycardia and apnœa. The hypotension lasted for from 30 minutes to 2 hours, but circulatory reflexes (response to carotid occlusion; stimulation of the central vagus) were depressed for longer periods. The initial rapid vasodepressor response appeared to be initiated from some structure in the head or neck, since intracarotid injections of small doses elicited this response and spinal cord section at C-3 effectively abolished it. Antihistamines had no effect on this initial action, but abolished the prolonged hypotension which followed. This prolonged phase appeared to be due to histamine liberation since it showed tachyphylaxis, considerable individual variation and was accompanied by increased peripheral blood flow. Ganglionic blockade, section of the vagi above or below the nodose ganglion, or spinal transection did not affect this response. Perfusion of the drug through the carotid sinus–carotid body circulation had no effect. The drug also did not inhibit cholinesterase, mono- or di-amine oxidase in pharmacological concentrations. Assays for histamine-like activity on the guinea-pig uterus showed an increased blood–histamine-equivalent during the hypotensive action of McN-259, which paralleled the degree of hypotension. Responses to McN-259 were considerably smaller in cats and rabbits than in dogs. The actions of the drug were discussed in comparison with those of other histamine liberators. G. P.

Reserpine in the Treatment of Hypertension. W. M. Hughes, E. Dennis and J. H. Moyer. (*Amer. J. med. Sci.*, 1955, **229**, 121.) This study was conducted on a group of 73 out-patients with mild to severe hypertension. Of this number, 26 received only reserpine, in a daily dose of 2 mg.; 6 of the patients so treated became normotensive. There was no evidence of tolerance to reserpine, and patients who were normotensive after 3 months of treatment have continued so for a year or more. In a further 15 patients, 14 of whom had previously been treated with reserpine alone without becoming normotensive, reserpine was used in combination with hydrallazine; 87 per cent. of the patients obtained a significant reduction in blood pressure on the combined therapy, and 33 per cent. became normotensive. The average daily dose of hydrallazine when combined with reserpine was 331 mg. in the responsive patients. In the remaining 32 patients, 27 of whom had failed to become normotensive under reserpine therapy alone, reserpine was used in combination with oral hexamethonium; 84 per cent. were responsive to the combined therapy and 47 per cent. became normotensive. The average daily dose of hexamethonium in

combination with reserpine for the responsive patients was 1·44 g. It was observed that the severity of side reactions of both hydrallazine and hexamethonium was reduced in the combined treatments as compared with their use alone. When reserpine was used concurrently with hexamethonium the dosage requirement of the latter was reduced significantly and the blood pressure response was more stable in comparison with that following the use of hexamethonium alone. Observations on renal hæmodynamics indicate that glomerular filtration and renal blood flow were not increased with any one of the three therapeutic programmes, which indicates that the clinical improvement on these patients was probably a direct result of blood pressure reduction. S. L. W.

Sorbitol, The Diuretic Effect of. A. Leimdorfer. (*Arch. int. Pharmacodyn.*, 1954, **100**, 161.) Sorbitol injected intravenously into hydrated anæsthetised dogs induced a diuresis lasting about an hour. Urinary output of sodium and chloride were raised and potassium output unchanged. Combined administration of mersalyl-theophylline or thiomerin with the sorbitol produced a much greater diuresis than did sorbitol alone, even where the dose of the mercurial given alone, under the same conditions, had little or no effect. During sorbitol administration there was a likelihood of accumulation of NPN in the blood and depletion of chloride. It was suggested that sorbitol might be of value in oliguric conditions. G. P.

Sulphones and Sulphoxides, the Therapeutic Activity of, in Experimental Tuberculosis of Guinea-pigs. S. K. Gupta and R. N. Chakravarti. (*Brit. J. Pharmacol.*, 1955, **10**, 113.) On the assumption that a lipophilic drug would be capable of reaching lipid-rich mycobacteria and harbouring tissues, a series of unsymmetrical sulphides, sulphoxides and sulphones, carrying an alkylamino group at one end and a free or potentially free amino group at the other, have been synthesized. The drugs were investigated for antituberculous activity in guinea-pigs infected with the H37Rv strain of *Myco. tuberculosis*, comparing the activity with dihydrostreptomycin, isoniazid and *pp'*-diaminodiphenylsulphone. Two of the series, SN 44 (*p*-ethylamino-*p'*-aminodiphenylsulphone) and SN 47 (*p-iso*butylamino-*p'*-aminodiphenylsulphone), had activity comparable with dihydrostreptomycin sulphate and isoniazid. None of the drugs produced any toxic symptoms in the animals and the appearance of the tissues of the lung, liver, spleen and kidney showed no damage attributable to the drugs.

G. P.

Surface-active Polyoxyethylene Ethers, Antituberculous Effects of. J. W. Cornforth, P. D'A. Hart, G. A. Nicholls, R. J. W. Rees and J. A. Stock. (*Brit. J. Pharmacol.*, 1955, **10**, 73.) The preparation and separation of linear condensation products of *p*-octylphenol with formaldehyde, including homogeneous dicyclic and tricyclic compounds, are described, together with the preparation of macrocyclic condensation products of formaldehyde with *p-tert.*-butylphenol and *p-tert.*-octylphenol. Condensation of each product was then effected with ethylene oxide. The final compounds were investigated for *in vitro* tuberculostatic activity against the H37Rv strain of *Myco. tuberculosis* and for *in vivo* activity in mice infected with the same strain. Some members of the series showed high *in vivo* activity comparable with that of streptomycin. None of those compounds having *in vivo* activity had any tuberculostatic activity *in vitro*, even in high concentration, nor was any tuberculostatic substance detected in the blood or tissue fluids from treated animals. It was suggested

that the action of these compounds *in vivo* was indirect, through the host, rather than by a direct antibacterial action. The ethylene oxide chain length was critical in determining the *in vivo* activity; peak activity was found with compounds having chain lengths of 15 to 20 units. The type of activity also changed with increasing chain length from antituberculous ⟶ inactive ⟶ "protuberculous" (where the average length was 45 units or more, the infection was enhanced by the agent). This change was accompanied by a decrease in the lipophilic/hydrophilic ratio. The site of activity may be the monocytes, which have been shown to be altered in some way by one of these agents (Mackaness, *Amer. Rev. Tuberc.*, 1954, **69**, 690). This is further supported by the observation that the compounds enter the monocyte *in vivo*. It seems likely that the chemotherapeutic activity of the compounds is related in some way to their surface-active properties. G. P.

Teridax, Absorption and Excretion of. P. L. Perlman, R. E. Kosinski and D. Sutter. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 69.) When a single oral dose of 3 g. of a new cholecystographic agent, teridax (2:4:6-triiodo-3-hydroxyphenylpropionic acid) was administered to fasting male adults, iodine equivalent to 17–23 per cent. of the dose was recovered from the urine in the following 48 hours. Blood iodine levels reached a maximum after 6 to 8 hours and then decreased rapidly although traces of iodine could be detected up to 98 days. When iopanoic acid was administered in a similar dose, 22–29 per cent. was eliminated in the stools and very small amounts in the urine. Blood iodine levels reached a maximum after about 10 hours and then declined slowly. Both teridax and iopanoic acid were better retained when given to fasting patients. In dogs and rats, teridax was mainly excreted in the urine, only traces being found in the stools. Tissue analyses in dogs indicated that the remaining iodine was stored in the liver, skin, fat and muscle.

G. B.

Thiobenzilic Acid Esters and Related Compounds, Spasmolytic Properties of. M. W. Parkes. (*Brit. J. Pharmacol.*, 1955, **10**, 95.) A series of esters of thiobenzilic acid of the general structure $Ph_2C(OH)CO.S.(CH_2)_nNRR'$, together with some benzilic acid esters, and ethers and thioethers of the type $Ph_2C(OH)CH_2.O.(or.S.)(CH_2)_nNRR'$, were tested for spasmolytic activity on the isolated ileum of the guinea-pig, against contractions induced by acetylcholine, histamine, barium and potassium chlorides, and nicotine. Many of the compounds had a much greater spasmolytic action against barium, potassium and nicotine than had papaverine. In the majority of cases this effect was specific, and anti-acetylcholine and antihistamine activity were low. This specificity suggests a mode of action different from that of papaverine and is probably at the ganglionic level. This was borne out by the activities of the compounds on other tissues; on the isolated intestine of the rabbit and the rat they were much less active and no compound equalled papaverine in spasmolytic activity on the rat uterus, guinea-pig and rabbit tracheal muscle and on the coronary vessels of the isolated rabbit heart. A marked discrepancy was found when comparing the spasmolytic potencies of the series against barium and nicotine on the ileum of different strains of guinea-pig, although the potency of papaverine varied only slightly, as did the response of the tissue to the stimulant drugs. This may imply differences in the extent to which the nervous network of the intestine is concerned in the response to barium and nicotine. The antagonism of barium as a criterion for papaverine-like spasmolytic activity seems questionable on the above grounds. G. P.

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