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J. Pharm. Pharmacol., 1965, **17**, 65–82

Review Article

The physical chemistry of some non-ionic detergents*

P. H. ELWORTHY, B.Pharm., Ph.D., M.P.S., A.R.I.C. AND C. B. MACFARLANE, B.Sc., Ph.D., M.P.S.

THE physical chemistry of non-ionic detergents, in particular their surface activity and micellar properties in aqueous solution, has received much attention. In this review the physical chemistry of polyoxyethylene-ether detergents of the type $Me{\cdot}[CH_2]_y{\cdot}[OCH_2{\cdot}CH_2]_x{\cdot}OH$ is outlined. For convenience, the formulae are abbreviated in the style thus: Dodecyl-n_6 for $Me{\cdot}[CH_2]_{11}{\cdot}[OCH_2{\cdot}CH_2]_6{\cdot}OH$, n representing the ethylene oxide unit.

Non-ionic detergents do not ionise in aqueous solution, and thus have many advantages both in detergent properties and for chemical studies. Furthermore, a range of compounds with a constant hydrophobic portion, but a varying hydrophilic moiety, can be obtained without fundamentally altering the chemical structure of the detergent, enabling a fuller and more comprehensive study to be made than is possible in an ionic series where the hydrophilic portion can be altered only by changing the ion.

Although polymers of ethylene oxide of up to six units were first synthesised over 100 years ago, it was not until around 1930 that their commercial potential, and particularly that of their derivatives, was realised. Since then wide ranges of compounds incorporating the polyoxyethylene glycols have been produced for a multitude of purposes, ranging from aids in drilling to pharmaceutical preparations, where their wetting, foaming, dispersing, or emulsifying properties are utilised.

In the study of non-ionic detergents the most important experimental methods used have been those described below.

LIGHT SCATTERING

Measurement of the turbidity, τ, as a function of concentration, c, gives the micellar weight, M, from

$$\frac{Hc}{\tau} = \frac{1}{M} + 2Bc \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where H, is the optical constant (dependent on the wavelength of light used, the specific refractive index increment); c and τ are previously corrected by subtraction of the appropriate values for the concentration and the turbidity of the solution at the critical micellar concentration. B, the second virial coefficient, is a function of the size and shape of the micelles.

In equation (1) it is assumed that no micellar dimension exceeds $\lambda/20$ ($\approx$250 Å). For larger particles, this equation is modified to include the particle scattering factor, $P(\theta)$.

From the Department of Pharmacy, University of Strathclyde, Glasgow, C.1.

* The first of two articles discussing this topic.

VISCOSITY

Measurement of the specific viscosity, η_{sp}, as a function of concentration enables the intrinsic viscosity, $[\eta]$, to be determined.

$$\mathop{\mathcal{L}}_{c \to 0} \frac{\eta_{sp}}{c} = [\eta] = \nu(\bar{V}_2 + w_1 V_1^{\circ}) \quad .. \quad .. \quad .. \quad (2)$$

where ν, is a factor depending on the shape of the particles (Simha, 1945); $\bar{V}_2$, the partial specific volume of the solute; V_1°, the specific volume of the solvent; and w_1, the solvation expressed as solvent/g solute. The measured intrinsic viscosity therefore depends on both the shape and the solvation of the micelles.

DIFFUSION

For a spherical, unsolvated micelle the diffusion coefficient, D, after extrapolation to zero concentration, is related to the micellar weight and the viscosity of the medium, η, by:

$$D = \frac{RT}{6\pi\eta N\left(\frac{3M\bar{V}_2}{4\pi N}\right)^{\frac{1}{3}}} \quad .. \quad .. \quad (3)$$

where N, is Avogadro's Number; R and T have their usual significance. Corrections for deviations from a spherical shape or for the presence of solvation may be determined using viscosity or sedimentation velocity results.

The combination of diffusion, viscosity, and sedimentation data in the manner suggested by Scheraga & Mandelkern (1953), can lead to the independent determination of particle shape and hydration. The use of these relationships implies that the micelle is treated as a reasonably rigid particle.

SEDIMENTATION VELOCITY

From the measurements of the sedimentation coefficient, s, extrapolated to zero concentration, the micellar weight can be obtained from:

$$M = \frac{RTs}{D(1 - \bar{V}_2\rho)} \quad .. \quad .. \quad .. \quad (4)$$

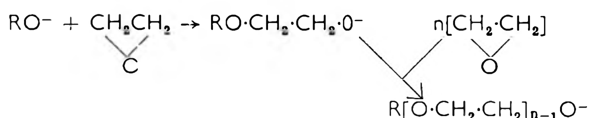
where ρ is the density of the solvent. The micellar weight obtained from this last equation is independent of particle shape and solvation.

Preparation of ethylene oxide adducts

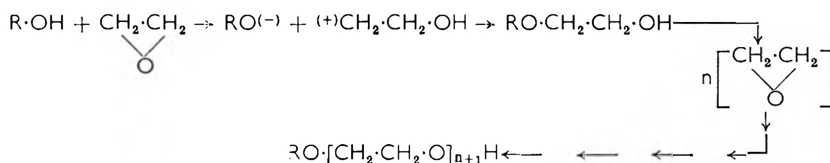
Ethylene oxide is a colourless gas with an ether-like odour, prepared on a small scale by heating 2-chloroethyl acetate with potassium hydroxide and on a commercial scale by the chlorohydrin process, or by direct oxidation of ethylene. Highly inflammable, liable to undergo exothermic reaction with itself, and of a toxicity similar to carbon monoxide, it is however the fundamental unit in the production of polyoxyethylene

polymers. Although early polyoxyethylene glycols were made as by-products of diethylene glycol syntheses (Laurenco, 1859, 1863; Wurtz, 1859, 1863), all ethylene oxide adducts made on a commercial basis today are prepared by the polymerisation of ethylene oxide in the presence of a suitable catalyst (Cohen, 1952; Vaughan, Jackson & Lunsted, 1949, 1952; Stevens, 1957; Wrigley, Smith & Stirton, 1957; Fine, 1958).

Various schemes have been suggested for the polymerisation mechanism; several of which appear to be influenced by the type of catalyst used. Gee, Higginson & Merral (1959) suggested the following reaction sequence when an alkoxide ion e.g. sodium methoxide is used as initiator:



Various other catalysts, alkaline and acid, have been mentioned in the literature; as a general route for the polymerisation Fine (1958) proposed that the epoxide ring of the ethylene oxide opened, combined with an active hydrogen atom contained in a functional group of the second reactant, then combined with the latter to form its hydroxyethyl derivative.



The active hydrogen of the hydroxyethyl group was then available for reaction with an additional epoxide group, and, by repetition of this process, the polyoxyethylene compound was built up; the degree of polymerisation being controlled by the amount of ethylene oxide made available.

Alcohols, phenols, carboxylic acids, amines, amides, alkyl and aryl sugar derivatives, and mercaptans are among the compounds most commonly combined with a polyoxyethylene chain; the properties and stability of the resulting compounds being influenced by the choice of hydrophobe, the type of linkage, and the extent of polymerisation.

When polyoxyethylene chains are built up ideally, from a fixed number of propagating units by a sequence of kinetically identical additions of monomer, the size distribution of chain lengths has been shown (Flory, 1940) to be described by Poisson's formula (Molina, 1942).

Although the conditions producing a Poisson distribution are found in the reaction of ethylene glycol with ethylene oxide (Weibull & Nycander, 1954), complications are encountered when the rate of the initial step of the reaction is different from that of further steps, or the subsequent additions themselves vary in rate as the chain lengthens. Complex formulae have been derived for reactions where all steps are kinetically different (Natta & Mantica, 1952), but are cumbersome to use in practice.

As a compromise, Weibull & Nycander (1954) produced a simplified formula based on the assumption that the rate of ethylene oxide additions for each step was equal, but differed from that of the initial reaction step of ethylene oxide with the parent hydrophobe.

Fractional distillation of compounds prepared by Wrigley, Howard & Stirton (1960) showed a distribution of chain lengths which agreed with Weibull & Nycander's equation, rather than with the Poisson distribution. This work, however, was limited to long chain fatty alcohol adducts. On the other hand, the Flory-Poisson distribution has been found valid for the oxyethylation of ethylene glycol (Weibull & Nycander, 1954), phenols (Miller, Bann, & Thrower, 1950; Mayhew & Hyatt, 1952), and stearic acid (Birkmeier & Brander, 1958); in the last instance presumably because of preferential combination of the parent compound with ethylene oxide before significant further reaction of the ether alcohols took place (Schechter & Wynstra, 1956; Wrigley & others, 1957).

Industrially an alkaline catalyst seems to be preferred although the reaction may also be catalysed by acid, or even proceed uncatalysed (Chitwood & Freure, 1946). Acid catalysts may be used if low temperature conditions are required (Staudinger & Schweitzer, 1929). The choice of catalyst and the purity of the starting materials have a striking effect on the end product. For obvious reasons manufacturers do not disclose their particular routes, but work by Drew & Schaeffer (1958) illustrates this point. In their experiments they found acid-catalysed reactions gave rise to byproducts, the amount of which increased with the degree of polymerisation, and the presence of traces of water (either by contamination or by formation during the reaction) resulted in the formation of glycols. The concentration and type of catalyst, and the reaction conditions affected the distribution of chain lengths (Gee, Higginson, & Merral, 1959; Tischbirek, 1960; Ginn, Church & Harris, 1961), and in practice, these factors are often varied to obtain the most suitable product for a particular purpose.

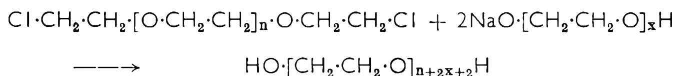
Thus there is a great variation in the possible distribution of the ethylene oxide chain lengths in a glycol or detergent; the properties identified with the various commercial products are influenced by this distribution as well as by the average molecular ratio of ethylene oxide to hydrophobe (Mayhew & Hyatt, 1952; Raphael 1958).

In an attempt to obtain a clearer view of the properties of this type of compound, various means have been tried to obtain a homogeneous preparation, i.e. one which did not contain a distribution of chain lengths. Fractionation of the polymerised products by distillation (Mayhew & Hyatt, 1952) and chromatography (Gallo, 1953; Kelly & Greenwald, 1958) have been tried, but as yet have only succeeded in narrowing the chain length distribution. Alternative routes of building up the chains have also been investigated. As mentioned previously, short length polyoxyethylene glycols were first described by Laurenco (1859), who prepared them by reacting ethylene glycol with either ethylene dibromide or ethylene bromohydrin, and by Wurtz (1859), who reacted ethylene oxide with ethylene glycol or by hydration of ethylene oxide, whereas

Mohr (1866a,b) heated ethylene glycol monoacetate with the monosodium derivative of ethylene glycol.

Although certain early workers suggested a cyclic structure (Roithner, 1894) for the polyoxyethylene glycols, much of the data supporting this was of a negative nature, and the detection of hydroxyl groups was taken as evidence of an open chain (Carothers, 1931; Fordyce, Lovell & Hibbert, 1939).

The first direct method of synthesising a single polyoxyethylene glycol, devised by Perry & Hibbert (1936), used a Williamson type ether reaction of the $\alpha\omega$-dichloro-derivative of a pure lower glycol with two molecules of the monosodium salt of another or the same glycol.



$$Cl{\cdot}CH_2{\cdot}CH_2{\cdot}[O{\cdot}CH_2{\cdot}CH_2]_n{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}Cl + 2NaO{\cdot}[CH_2{\cdot}CH_2{\cdot}O]_xH \longrightarrow HO{\cdot}[CH_2{\cdot}CH_2{\cdot}O]_{n+2x+2}H$$

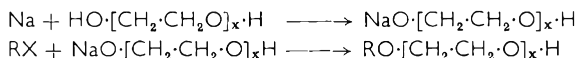
Using this procedure, Fordyce & others (1939) synthesised glycols containing up to 186 ethylene oxide units. The compounds containing up to six ethylene oxide units were distilled, but the longer ones had to be purified by extraction and crystallisation; the final purity being assessed by time/temperature cooling curves. Examination of these synthesised polyoxyethylene glycols by X-ray diffraction (Barnes & Ross, 1936) showed, that as far as could be detected by that technique, there was no essential difference between them and the products of ethylene oxide polymerisation, substantiating the claims of other workers about the identity of the two end products (Staudinger & Schweitzer, 1929; Carothers, 1931).

Synthesis of homogeneous glycol derivatives presents an even more formidable problem, particularly when a compound with a hydrophobic radical at one end only is required. Certain workers have tried to evade this problem by using a short sodium alkoxide (Nakagawa, Kuriyama & Inoue, 1960; Kuriyama, 1962a), e.g., sodium methoxide, to initiate the polymerisation reaction, obtaining a methoxypolyoxyethylene glycol, fractionating this, and converting the required fraction to its sodium salt. Thence, by the Williamson reaction with the hydrophobe halide, they obtained the desired compound. Such a compound is based on the product of the polymerisation reaction, and hence a uniform chain length is extremely difficult to obtain (Mayhew & Hyatt, 1952; Kelly & Greenwald, 1958). Moreover the compound must be used as its methoxy derivative; removal of this group without breaking the hydrophobe-glycol link is virtually impossible.

A few reports have appeared in the literature describing synthesised compounds (Turner, Saunders & Willaman, 1951; Goto, Sugano & Koizumi, 1953; Gingras & Bayley, 1957, 1958; Wrigley, Howard & Stirton, 1960), but most have contained short polyoxyethylene chains of up to six ethylene oxide units (Mulley, 1958, 1961; Corkill, Goodman & Ottewill, 1961).

For detergents, the synthetic route used has also been via Williamson ether synthesis type reactions, two ways being available:

(*a*) Building up the glycol by the method of Perry & Hibbert (1936) converting it to its monosodium salt, and reacting this with the hydrophobe halide, RX



$$Na + HO{\cdot}[CH_2{\cdot}CH_2O]_x{\cdot}H \longrightarrow NaO{\cdot}[CH_2{\cdot}CH_2{\cdot}O]_x{\cdot}H$$
$$RX + NaO{\cdot}[CH_2{\cdot}CH_2{\cdot}O]_xH \longrightarrow RO{\cdot}[CH_2{\cdot}CH_2{\cdot}O]_x{\cdot}H$$

(*b*) By preparing a detergent containing a small number of ethylene oxide units by route (*a*), chlorinating the terminal hydroxyl group with thionyl chloride, then reacting this compound with a monosodium derivative of a glycol.

Yields by either route are not particularly good and side reactions such as dehydrohalogenation of the chloro-compound have been reported (Corkill & others 1961). The largest homogeneous detergents reported in the literature, a range of hexadecyl monoethers containing six to twenty-one ethylene units respectively, have been successfully synthesised by these routes (Elworthy & Macfarlane, 1962a, 1963); similarly, some branched hydrocarbon chain compounds have recently been obtained by these methods (Elworthy & Florence, 1964).

Alkyl *p*-tolylsulphonates have been described as intermediates (Shirley, Zeitz, & Reedy, 1953), and the stepwise synthesis of the monodecyl ethers of mono- to tetra-oxyethylene glycols by acid-catalysed addition of ethylene oxide has been reported (Chakhovskoy, Martin & Van Nechel, 1956).

The difficulties encountered in the purification and separation of the end products from the reaction mixture have been one of the main causes of delay in the syntheses of longer chain compounds. Traces of glycols are one of the most common contaminants and crystallisation and other purification techniques are hampered by the similarity of the properties of these trace glycols to those of the adduct, particularly when the glycol chains become large. Further complications arise because of the surface-active properties of the derivatives which cause emulsion formation when partition techniques are used. To overcome this, partition between ethyl acetate and saturated sodium chloride solution (Weibull, 1960) has been used, so has heating an aqueous solution of the mixture to about 100° and separating the detergent-rich phase (Nakagawa & others, 1960); in both instances the glycol and other water soluble products of the reaction were retained in the aqueous phase. A relatively short oxyethylene chain compound, such as the monohexadecyl ether of hexaoxyethylene glycol, is about the longest which will withstand normal distillation, and, even under vacuum, the conditions required are rather rigorous for an organic compound of this nature (b.p. $235°/10^{-3}$ mm). Neither do the actual distillation procedures used seem to produce an entirely satisfactory compound, as oxidised byproducts have been reported (Gingras & Bayley, 1957; Corkill & others, 1961) in the distillate, and chromatography on silica was required to remove them.

A chromatography step would appear to be all important in the purification of these compounds, as tests have shown (Macfarlane, unpublished) that experimental conditions can be adjusted so that traces of

contaminating glycols are held very firmly on the column, while impurities (containing peaks at ca. 1740–1750 cm^{-1} in their infrared spectra) are removed in the first stages of the elution. The desired detergent follows these impurities down the column. Furthermore, on an alumina column, by careful control of the elution scheme, $Me{\cdot}[CH_2]_{15}{\cdot}[O{\cdot}CH_2{\cdot}CH_2]_9{\cdot}Cl$ has been separated from $Me{\cdot}[CH_2]_{15}[O{\cdot}CH_2{\cdot}CH_2]_{15}{\cdot}OH$ in the working up of the reaction mixture in the synthesis of the latter compound. For compounds containing short hydrocarbon chains, silica gel appears to be a better agent for chromatography than alumina.

Analysis

In estimating the purity of synthesised compounds, it has been shown that although standard analyses (percentages of C, H, and O) gave figures indicating satisfactory purity, such analyses were not sufficiently sensitive to detect in this type of compound impurities which caused minima in the surface tension/concentration curves (Corkill & others, 1961). Estimation of the hydroxyl group by acetylation or other techniques also loses its sensitivity when only small samples are available and the hydroxyl group is equivalent only to some 1–2% of the molecular weight.

Various assays have been developed to estimate the ethylene oxide content of the adducts. Most of these have been dependent on degradation of the chain, or on complex formation; the complexes being formed by reacting the ethylene oxide derivatives with heteropoly inorganic acids, such as silico-tungstic (Schaeffer & Critchfield, 1947; Wurzschmitt, 1950; Schonfeldt, 1955), or phosphomolybdic (Oliver & Preston, 1949; Stevenson, 1954) acids, or with tannins, potassium ferrocyanide or other suitable reagents (Haakh, von Candie & Mobus, 1951; McAllister & Lisk, 1951; Coppini & Cameron, 1953; Brown & Hayes, 1955). These complexes were then quantitatively estimated either by gravimetric, volumetric, or absorptiometric techniques, and a direct relationship could be established with the particular detergent being determined.

Dichromate oxidation has been advocated by Edkins, Storlazzi & Hammond (1942), Werner & Mitchell (1943) and others, but this lacks the desired specificity for ethylene oxide units. Morgan (1946), using a modified alkoxyl apparatus, decomposed the ethylene oxide residue to ethylene iodide and ethylene with constant boiling hydriodic acid. The latter method has been developed by Siggia, Starke, Garis & Stahl (1958), who refluxed the polyoxyethylene adduct with excess hydriodic acid under an atmosphere of carbon dioxide, and titrated the liberated iodine with sodium thiosulphate. This reaction has the advantage over the other chemical methods that it is an absolute method; no standard curve need be prepared.

All the above methods estimate the total amounts of ethylene oxide present, thus the presence of glycols will affect the result. Several methods of estimating the amount of glycol present have been devised

(Nakagawa & Nakata, 1956; Weibull, 1960; Ginn & others, 1961), generally these involve the separation of the glycol from the detergent, but recently, correlation of total ethylene oxide content obtained by the hydriodic acid analysis with the percentage of hydroxyl groups found by acetylation, has been used to calculate the quantity of glycol in the adduct (Elworthy, 1963).

Properties in aqueous solution

FORMATION OF MICELLES

In contrast with the copious work on ionic detergents (Pethica, 1960), the number of papers on the micellar structure of non-ionic detergents in aqueous solution is relatively small. Using the Scatchard method (Scatchard, Jones & Prentice, 1932) of determining freezing-point depressions, Gonick & McBain (1947) found that results obtained for both Triton X 100, a commercial octyl-phenyl oxyethylene adduct, and a monolaurate of nonaoxyethylene glycol, in aqueous solution, bore a striking resemblance to those obtained for colloidal electrolytes. Correlating this work with previous results on solubilisation of benzene and various dyes by the lauryl adduct (Gonick, 1946), and X-ray diffraction (McBain & Marsden, 1947), they concluded that non-ionic detergents also formed micelles. This deduction has since been confirmed by other workers using X-ray and solubilisation techniques, and by viscosity and turbidimetric data (McBain & Marsden, 1948; Schulman, Matalon & Cohen, 1951; Goto, Sugano & Koizumi, 1954; Greenwald & Brown, 1954; Kushner & Hubbard, 1954; Kuroiwa, 1956).

Many theories have been put forward for the formation of detergent micelles (Pethica, 1960; Veis & Hoerr, 1960; Mukerjee, 1962; Aranow, 1963). In one of the simpler theories for an ionic detergent, Debye (1949) proposed that as the number of long chain ions aggregated to form a droplet or core of hydrophobic tails, the number of charges per aggregate increased. The charge density at the periphery rose and with it the electrostatic free energy of the growing aggregate. An equilibrium was reached when the drop in energy due to aggregation of hydrophobic groups was balanced by the rise in energy due to electrostatic repulsion. Criticism has been levelled at Debye's treatment as it was based on minimising the free energy of the micelle rather than the entire system (Oosika, 1954; Reich, 1956).

It is evident from knowledge of the non-ionic detergents that other factors must also be at work in the process of micelle formation; the necessity of looking for these additional factors which could balance the drop in energy due to aggregation of hydrophobic groups, and thus the need to look at the behaviour of all other components has been stressed by Reich (1956), who calculated the equilibrium micellar size from energy and entropy changes taking place during micelle formation. His calculation was based on a simple model of coalescence of hydrocarbon tails to a liquid droplet, and fitting the ethylene oxide chains over their surfaces. On the basis of his theory, Reich also postulated that micelle formation

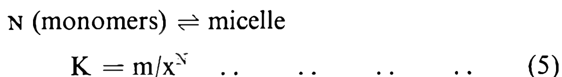
of non-ionic detergents occurred at a distinct critical micellar concentration (CMC), that the CMC decreased with increasing hydrocarbon chain length, and increased with increasing ethylene oxide chain length.

Kushner, Hubbard & Doan (1957) have pointed out that the sharpness of the micelle appearance forecast by Reich was dependent on the values assigned to the entropy and energy change associated with micelle formation; his assignment of a rather high negative value for the entropy change was probably unwarranted since it was based primarily on considerations of the heat of vaporisation of dodecane, and neglected the positive contribution to the total entropy change which results from the uncurling of the hydrocarbon portion of the molecule when it enters the micelle. Choice of a smaller value for ΔS would have reduced the predicted sharpness of micelle formation.

Hoeve & Benson (1957), in an extensive statistical mechanical treatment, extended Reich's approach and also pointed out that dehydration phenomena had to be taken into account. The simple shielding of hydrocarbon tails from water by ethylene oxide used in Reich's calculations accounted for only "complete micelles" i.e., where the hydrocarbon core was entirely covered by ethylene oxide chains. It has also been pointed out that, unfortunately, undefined parameters in Hoeve and Benson's theory makes comparison with practical results somewhat difficult (Debye & Prins, 1958; Schick, Atlas & Eirich, 1962). Aranow (1963) has reanalysed Reich's model from the two phase approach and developed a theory of micellar statistical mechanics from the extended theory of dilute solutions (Fowler & Guggenheim, 1956), the dielectric continuum model of the solvent, and the statistical mechanical treatment of physical clusters at constant pressure (Hill, 1955). Aranow has also formulated a more general theory by elimination of the first two approaches.

A theory postulated by Nakagawa & Kuriyama (1957a), considered that the main factors preventing association are the heat of hydration and the configurational entropy terms of the flexible hydrophilic chain. It gave quantitative predictions for the CMC, micellar weight and other properties in solution, but could not reasonably explain temperature effects and clouding phenomena.

Illustrated in terms of the monomer concentration, the two main approaches to miscellisation are shown in Fig. 1. The mass action approach considers the equilibrium between monomers and micelles (molar concentrations x and m respectively), in terms of an equilibrium constant K, and an aggregation number N



$$\text{N (monomers)} \rightleftharpoons \text{micelle}$$

$$K = m/x^{N} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

Concentrations have been written for activities in (5), and the activity of water entering into the equilibrium has been neglected, perhaps in an unwarranted manner for low N values, but the point at issue here (Fig. 1) is that the concentration of monomers increases above the CMC, as a

result of the mass action approach. The increase is more pronounced at low aggregation numbers than at high ones.

The pseudophase approach, which suggests that all micellised solute forms a separate but soluble phase, would give the picture shown by the broken lines in Fig. 1; as the concentration is increased above CMC, all added solute micellises, and there is no further increase in monomer concentration.

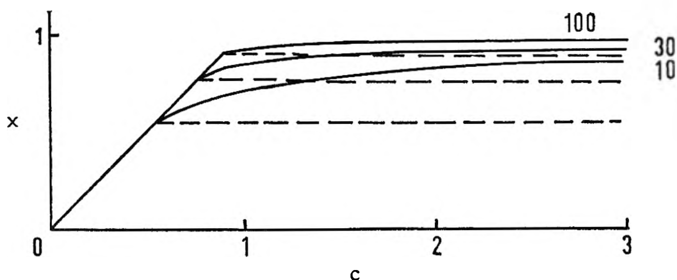


FIG. 1. Plot of concentrations of monomers, x, against total detergent concentration c, evaluated from equation (5). In all instances K = 1 (unbroken lines). The broken lines indicate the monomer concentration above the CMC as expected from the pseudo-phase theory of micellisation. Figures on the curves are aggregation numbers.

Practically, if the aggregation number is greater than 20, then either model gives the same thermodynamic properties of micellisation, within experimental error. Fundamentally, it is necessary to know which approach is correct; at the present time there are several *experimental* inconsistencies.

Gibbs' equation relates the change in surface tension, $d\gamma$, to the surface excess, Γ_2, and the solute activity, a_2.

$$-d\gamma = \Gamma_2 RT \, d \ln a_2 \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

Provided Γ_2 is constant, as it appears to be in the post CMC region, any change in solute activity should give rise to a change in surface tension. Using the mass-action law and Gibbs' equation, rough calculations of the surface tension variation between one and ten times the CMC can be made, giving a few tenths of a dyne/cm at an aggregation number of 100, and around 1 dyne/cm at N = 20. Examination of most surface tension-concentration curves reported in the literature shows a constant surface tension in the post CMC region (the exception are the results of Hudson & Pethica, 1964). At first sight this is support for the pseudo-phase model of micellisation, even at low micellar aggregation numbers. These results are inconsistent with vapour pressure-concentration curves of Corkill, Goodman & Harrold (1964) on C_8n_6, or Corkill, Goodman & Tate (1964) on C_6n_6, and of Elworthy & Florence (unpublished) on C_4n_6 and $Me_2CH{\cdot}CH_2n_6$. The vapour pressure studies clearly show a change of solute activity above the CMC, which would be expected if the mass action approach applied to micellisation.

These inconsistencies are within (not between) the results of two groups of workers, and it is difficult to see why they have arisen, unless the synthesised compounds are satisfactory for the study of bulk properties, but not sufficiently pure for surface chemistry. It is pertinent to report unpublished work of Mysels & Elworthy, who have found that a clear picture of the surface tension of sodium dodecyl sulphate solutions at concentrations greater than the CMC could be obtained only if the detergent was purified by foaming immediately before use, and all measurements were made in essentially a closed system. The variation of surface tension with concentration was then apparent, indicating that the mass action approach was valid for this system.

A recent paper by Mukerjee (1962) has come down heavily in favour of the mass action approach and attempted to point out some of the difficulties and inaccuracies of the two phase theory.

Some concern about the thermodynamic picture being built up may be necessary, as the CMC values from which these properties are calculated are largely determined from surface tension measurements. There is need for a second precise method of determining CMC, preferably based on bulk properties: unfortunately dye solubilisation or interferometry do not appear to be precise enough.

Using the pseudo-phase approach, the temperature dependence of the CMC can be used to evaluate the heat of micellisation, ΔH_m

$$\Delta H_m = -RT^2\left(\frac{\partial \ln \text{CMC}}{\partial T}\right)_p \quad \ldots \quad \ldots \quad \ldots \quad (7)$$

and the entropy of micellisation, ΔS_m

$$\Delta S_m = \Delta H_m/T \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (8)$$

ΔS_m is positive for a variety of synthetic detergents, and micellisation is governed to a large extent by the gain in entropy associated with the transfer of a monomer into a micelle. This seems to be due to the loss of the "iceberg" structure of water from around the hydrocarbon chain during this step (Corkill, Goodman & Ottewill, 1961; Schick, 1963a; Elworthy & Florence 1963, 1964; Corkill, Goodman & Harrold, 1964; Corkill, Goodman & Tate, 1964). Wishnia (1963) has shown that on transferring n-butane from an aqueous solution into a hydrocarbon phase, the entropy change is 23 cal mole^{-1} deg.$^{-1}$. Similar data for the series of normal hydrocarbons usually found in non-ionic detergents would be a very useful starting point for the discussion of the thermodynamics of micellisation. Other factors pertinent to the micellisation process are configuration entropy changes of hydrocarbon and polyoxyethylene chains, and possible changes of the solvation of the polyoxyethylene chain when micellisation takes place. The useful approach of direct measurement of heats of micellisation by calorimetry has recently been made by Corkill, Goodman & Tate (1964), showing an increase in ΔS_m with increasing hydrocarbon chain length for monoethers of hexaoxyethylene glycol.

It is somewhat of a disadvantage to know so little of the properties of monomers of non-ionic detergents in solution. However, preliminary studies of synthetic glycols and of detergents with short hydrocarbon chains indicate a fairly high degree of hydration of the polyoxyethylene chains in solution (Elworthy & Florence, unpublished).

CRITICAL MICELLAR CONCENTRATION

The formation of micelles causes abrupt changes in many of the physical properties of a solution of a surfactant (Grindley & Bury, 1929) provided that the aggregation number is fairly large, and measurement of the concentration at which these changes take place is generally taken as indicative of the coalescence of the monomers. Phillips (1955) has proposed that the CMC could conveniently be defined mathematically as the total concentration (c) at which some colligative property ϕ gave $d\phi^3/dc^3 = 0$ but the choice of the colligative property for defining the CMC generally depended on factors of convenience. The exact location of the CMC is thus to some extent subjective, but can be recorded with sufficient meaning in most instances.

Due to the absence of electrostatic repulsion, non-ionic detergents tend to form micelles at much lower concentrations (Bury & Browning, 1953; Cohen, 1952) than their ionic counterparts (Nakagawa, Kuriyama, Inaba & Tori, 1956; Weil & Stirton, 1956), thus other common methods are not sufficiently sensitive. Techniques have therefore been developed in an effort to overcome these difficulties.

Ross & Olivier (1959) have reported a photometric method for the determination of the CMC of polyoxyethylene adducts in both aqueous and nonaqueous solutions, based on the formation of a molecular complex between iodine and the non-ionic micelle which showed an absorption maximum at 360 mμ and Becher (1959) has shown good agreement between the results of this method and values obtained from light scattering with a range of commercial non-ionic detergents. Elworthy (1960a) followed up this work using cetomacrogol, a commercial polyoxyethylene monocetyl ether containing an average of 22 ethylene oxide units, and obtained consistent results for three methods: the iodine method, solubilisation of a dye (dimethyl yellow), and a surface tension technique. Japanese workers (Nakagawa, Kuriyama, Inaba & Tori, 1956; Nakagawa, Kuriyama & Tori, 1956; Nakagawa & Kuriyama, 1957b), as well as using conventional surface tension and solubilisation methods, have suggested polarographic and other techniques. Surface potential measurements (Schick, 1963b), showing similar results to those obtained from surface tension (Schick, 1962), have also been described. Donbrow & Jan (1963) reported the use of interferometry for determining the CMC of some synthetic compounds.

As already mentioned, the absence of electrostatic repulsive forces results in the formation of micelles at very low concentrations, and figures down to 10^{-6} molar appear in the literature (Corkill & others, 1961; Elworthy & Macfarlane, 1962b). The CMC, as might be expected, has been interpreted as a function of the hydrophobe-hydrophile balance of

the molecule, and Reich's suggestion (Reich, 1956), that, for a given hydrophobic moiety, the CMC will increase with increasing ethylene oxide chain length, has been demonstrated by several workers (Nakagawa & Kuriyama, 1957b; Lange, 1960; Schick, 1963b). Formulae for their relationship, on lines parallel to that of Klevens (1953) for a homologous series of ionic detergents, have been published (Hsaio, Dunning & Lorenz, 1956; Becher, 1959; Elworthy & Macfarlane, 1962b) i.e.,

$$\log \text{CMC} = A + Bx$$

where A and B are constants for a given hydrophobe and x is the number of ethylene units per molecule. These equations may only hold over a limited range of ethylene oxide chain lengths (see Table 1), as a comparison of octadecyl ethers containing fourteen and one hundred ethylene oxide units per molecule respectively, have shown an unpredictably lower CMC values for the latter (Schick, 1962) while for some short hydrocarbon chain compounds, Mulley & Metcalf (1962) have claimed that increasing the glycol chain lengths had little effect on the CMC (up to six ethylene units per molecule).

As far as alteration of the hydrophobic group was concerned the number of carbon atoms, even in a saturated hydrocarbon chain, did not necessarily directly influence the hydrophobic weighting of that moiety as far as CMC was concerned. The overall number of carbon atoms in a straight chain appeared to be the main factor, as comparison of the CMC of branched tridecyl and n-dodecyl adducts (Schick, 1962), with the same number of ethylene oxide units, showed the latter to be approximately half that of the former. Experiments with synthesised (Corkill & others, 1961), and commercial compounds (Schick, 1962; Hsaio & others, 1956), have shown that for a given ethylene oxide chain the CMC decreased with increased length of the hydrophobic moiety. Schick (1962), however, found little difference in the CMC of fractionated octadecyl and dodecyl monoesters containing fourteen ethylene oxide units, and Lange (1960) found the generally accepted behaviour was invalid with polyoxyethylene ethers of paraffin chain alcohols greater than dodecyl.

Attempts at correlation between the values of CMC for different commercial alcohol adducts (Becher, 1959) showed the slopes of graphs of log CMC vs. ethylene oxide content decreased with increase of the number of carbon atoms in the straight chain of the hydrophobic group, and the series had a common intercept at a hypothetical zero ethylene oxide content. Unlike most published CMC results, concentrations in this work were based on % w/v; conversion of these results to the more common moles/litre designation of concentration have shown that the CMC for the lauryl and stearyl adducts decreased with ethylene oxide chain length, although the CMC for the (oxo-process) tridecyl alcohol adducts still increased. Despite this, for a given ethylene oxide chain, the CMC (moles/litre) decreased with lengthening of the aliphatic chain. Such results further stress the unsatisfactory nature of working with heterogeneous compounds.

The presence of a sharply defined CMC has been assumed in most of

TABLE 1. CRITICAL MICELLE CONCENTRATIONS OF SYNTHETIC NON-IONIC DETERGENTS

Abbreviations: n-butyl = C_4, n-octyl = C_8, etc., branched chain hydrocarbon structures written in full; $n_6 = -(OCH_2CH_2)_6OH$, etc., * is mean value of determinations by several methods.

Compound		CMC Moles/litre (upper line) at ° C (lower line)					Reference
C_4n_6		0·80	0·76		0·71		Elworthy & Florence, 1964
		20	30		40		
$Me_2CH{\cdot}CH_2n_6$		0·91	0·88		0·85		*Ibid.*
		20	30		40		
C_6n_3	$(\times 10^{-2})$	10·7	10·0		7·8		Corkill, Goodman & Harrold,
		15	25		35		1964
C_6n_4	$(\times 10^{-2})$	9·0					Donbrow & Jan, 1963
		20					
C_6n_5	$(\times 10^{-2})$	9·2					*Ibid.*
		20					
C_6n_6	$(\times 10^{-2})$	7·4	6·5		5·2		Elworthy & Florence, 1964
		20	30		40		
$Et_2CH{\cdot}CH_2n_6$	$(\times 10^{-2})$	10·0	9·3		8·7		*Ibid.*
		20	30		40		
C_8n_3	$(\times 10^{-3}$	9·3	7·5				Corkill, Goodman & Harrold,
		15	25				1964
C_8n_6	$(\times 10^{-3})$	11·9	9·9	7·7		6·7	*Ibid.*
		15	25	35		45	
	$(\times 10^{-3})$	9·8					Corkill & others, 1961
		25					
	$(\times 10^{-3})$	11·3	8·9		7·2		Balmbra & others, 1964
		18	30		40		
$Pr_2CH{\cdot}CH_2n_6$	$(\times 10^{-3})$	23	20				Elworthy & Florence, 1964
		20	30				
C_8n_9	$(\times 10^{-3})$	16	13		11		Corkill, Goodman & Harrold,
		15	25		35		1964
$C_{10}n_3$	$(\times 10^{-4})$	7·3	6·0		5·6		*Ibid.*
		15	25		35		
$C_{10}n_4$	$(\times 10^{-4})$	14·5	11·2	9·8		9·0	Hudson & Pethica, 1964
		1	6·8	10		15	
	$(\times 10^{-4})$	7·3	6·8				
		20	25				
	$(\times 10^{-4})$	0·53*					Carless, Challis & Mulley, 1964
		16					
$C_{10}n_5$	$(\times 10^{-4})$	14·1	11·8	9·7		9·0	Hudson & Pethica, 1964
		5	10	15		20	
	$(\times 10^{-4})$	8·1	7·6	7·2		6·8	
		25	30	35		40	
	$(\times 10^{-4})$	8·6*					Donbrow & Jan, 1963
		20					
	$(\times 10^{-4})$	9·3*					Carless & others, 1964
		20					
$C_{10}n_6$	$(\times 10^{-4})$	11·4	9·0	6·6		6·4	Corkill, Goodman & Harrold,
		15	25	35		45	1964
	$(\times 10^{-4})$	9·0	6·6		4·3		Balmbra & others, 1964
		25	35		45		
	$(\times 10^{-4})$	9·4*					Donbrow & Jan, 1963
		20					
$Bu_2CH{\cdot}CH_2n_6$	$(\times 10^{-4})$	34	31		28		Elworthy & Florence, 1964
		15	20		25		
$C_{10}n_8$	$(\times 10^{-4})$	21	17·2	14		11	Hudson & Pethica, 1964
		5	10	15		20	
		9	7·3				
		25	35				
$C_{10}n_9$	$(\times 10^{-4})$	14	13		11		Corkill, Goodman & Harrold
		15	25		35		1964
$Bu_2CH{\cdot}CH_2n_9$	$(\times 10^{-4})$	32	28			24	Elworthy & Florence, 1964
		20	30			40	
$C_{12}n_8$	$(\times 10^{-5})$	4·2*					Donbrow & Jan, 1963
		20					
	$(\times 10^{-5})$	5·7					Lange, 1960
		23					
	$(\times 10^{-5})$	3·8*					Carless & others, 1964
		20					
$C_{12}n_6$	$(\times 10^{-5})$	10·8	8·7			7·2	Corkill & others, 1961
		15	25			35	Corkill, Goodman & Harrold, 1964
	$(\times 10^{-5})$	8·7*					Donbrow & Jan, 1963
		20					
	$(\times 10^{-5})$	7·9*					Carless & others, 1964
		20					

TABLE 1—*continued*

Compound		CMC Moles/litre (upper line) at ° C (lower line)					Reference
$C_{12}n_7$	$(\times 10^{-5})$	8·0 23					Lange, 1960
$C_{12}n_9$	$(\times 10^{-5})$	10 23					*Ibid.*
$C_{12}n_{12}$	$(\times 10^{-5})$	14 23					*Ibid.*
$C_{13}n_8$	$(\times 10^{-5})$	11 25					Becher, 1964
$C_{13}n_{10}$	$\times 10^{-5})$	12 25					*Ibid.*
$C_{13}n_{12}$		11 25					*Ibid.*
$C_{14}n_6$	$(\times 10^{-6})$	10 25					Balmbra & others, 1964
$C_{16}n_6$	$(\times 10^{-6})$	1 35					*Ibid.*
	$(\times 10^{-6})$	1 25					Corkill & others, 1961
$C_{16}n_6$	$(\times 10^{-6})$	1·7 25					Elworthy & Macfarlane, 1963
	$(\times 10^{-3})$	97* 27					Carless & others, 1964
$C_{16}n_7$	$(\times 10^{-6})$	1·7 25					Elworthy & Macfarlane, 1963b
$C_{16}n_9$	$(\times 10^{-6})$	2·1 25					*Ibid.*
	$(\times 10^{-3})$	38* 20					Donbrow & Jan, 1963
	$(\times 10^{-3})$	36 20					Carless & others, 1964
$C_{16}n_{12}$	$(\times 10^{-6})$	2·3 25					Elworthy & Macfarlane, 1962b
$C_{16}n_{15}$	$(\times 10^{-6})$	3·1 25					*Ibid.*
$C_{16}n_{21}$	$\times 10^{-6})$	3·9 25					*Ibid.*

the previously mentioned work on this subject, but Kushner & Hubbard (1954) claimed, from turbidimetric results on a sample of Triton X 100, that there was no well defined CMC but a gradual increase in the fraction of added detergent forming micelles up to a concentration of about 0·3 g/100 ml, where virtually all of each increment added to the solution became micellar. The authors suggested that this may have been caused by the effects of the chain length distribution; the shorter ethylene oxide adducts forming micelles at lower concentrations. Later work (Kushner, Hubbard & Doan, 1957) on molecularly distilled fractions still showed no sharp CMC. The concentration where the concentration/turbidity vs. concentration graph settled to a straight line was lower in the case of the fraction with the shorter mean ethylene oxide chain length. When considering these latter results it must be borne in mind that molecular distillation does not give a series of clear cut chemical entities, but at the best merely increases the incidence of molecules having an ethylene oxide content close to that of the mean. It has been suggested that this monomer saturation concentration is an artifact arising from non-equilibrated solutions (Becher & Clifton, 1959), but this time dependence of micellar breakdown has in turn been attributed to the presence of impurities (Corkill & others, 1961), and did not occur when chemically pure synthetic compounds were studied.

The determination of CMC of a series of homogeneous *p*-t-octylphenoxy-ethoxyethanols (containing from 1–10 ethylene oxide units) by Crook, Fordyce & Trebbi (1963), showed the general trend of increased CMC with polyoxyethylene chain lengthening.

Recent work on a synthetic dodecyl ether of hexaoxyethylene glycol (Balmbra, Clunie, Corkill & Goodman, 1962) gives another interesting aspect. Comparison of the CMC obtained from surface tension plots (c_0) with that apparent from turbidity (c_1) showed the latter to be much higher. The constant value for the surface tension observed at concentrations greater than c_0 was taken to indicate that the activity of the monomers in equilibrium with the surface phase remained constant, c_1 being caused by the micellar units between c_0 and c_1 having such low aggregation numbers that the solution turbidities are too small to be distinguished experimentally from the solvent turbidity, and only at concentrations greater than c_1 does the micellar weight become appreciable and constant.

Furthermore, in a study of the light scattering of monohexadecyl-n_6 and monohexadecyl-n_7, an association of small into large micelles has been detected (Elworthy & Macfarlane, 1962a); bearing out this idea of a second association concentration higher than the first CMC. The values of this second association concentration appear to decrease with increasing ethylene oxide chain length and may account for some of the apparently anomalous results obtained in studying these CMC values by different methods.

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Research Papers

Salicylate and aminotransferases

B. J. GOULD* AND M. J. H. SMITH

Enzymic transfer of the amino-group of each of 20 amino-acids to α-oxoglutarate has been studied using lyophilised extracts of rat heart, liver, brain, kidney and skeletal muscle as enzyme source. The glutamate produced was estimated from the carbon dioxide liberated by a bacterial decarboxylase in an autoanalyser. Only asparagine was completely inactive in the transfer. Aminotransferase activity was otherwise unevenly distributed in the tissues. Salicylate (10 mM) was generally inhibitory but it activated the L-tryptophan-α-oxoglutarate aminotransferase.

SALICYLATE has been found to interfere with several important pathways involving the metabolism of glutamate in animal tissue extracts. The drug inhibits the synthesis of glutamine (Messer, 1958), the incorporation of glutamate into protein (Manchester, Randle & Smith, 1958), the conversion of the amino-acid to proline (Bellamy, Huggins & Smith, 1963) and the enzymes responsible for the dehydrogenation and decarboxylation of glutamate (Gould, Huggins & Smith, 1963). An important pathway for glutamate metabolism is controlled by the amino-transferases. These enzymes catalyse the interaction of amino- and α-oxo-acids leading to the interconversion of many amino-acids. Glutamate appears to be a key amino-acid, acting as a donor of amino-groups for the α-oxo-acids corresponding to a number of other amino-acids. Salicylate inhibits alanine and aspartate aminotransferase activities in rat serum and in extracts of rat tissues (Huggins, Smith & Moses, 1961; Yoshida, Metcoff & Kaiser, 1961). These particular enzyme activities have been the most intensively studied but the work of Cammarata & Cohen (1950), Awapara & Seale (1952) and Rowsell (1956) showed that multiple aminotransferase activities, involving glutamate as one of the reactants, occur in mammalian tissues. The purpose of the present work was to study the distribution of these aminotransferases in five rat tissues and to investigate the effect of salicylate on their activities.

Experimental

MATERIALS

γ-Aminobutyric acid, L-ornithine hydrochloride and glutamate decarboxylase (acetone dried powder of *Escherichia coli*) were obtained from the Sigma Chemical Co., St. Louis, Mo., U.S.A. The other L-amino-acids and glycine were obtained from B.D.H. Ltd.; the pyridoxal phosphate from L. Light & Co., Ltd. and all other chemicals were of analytical grade.

From the Arthritis and Rheumatism Council Research Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

* Smith and Nephew Research Fellow.

RAT TISSUE PREPARATIONS

Wistar rats, weighing between 200 and 300 g, maintained on M.R.C. cube diet No. 41, were killed by cervical fracture. The five tissues, liver, kidney, brain, heart and skeletal muscle (quadriceps femoris) from 10 animals, were removed and the bulked specimens of each whole tissue homogenised in twice their weight of ice-cold distilled water, using a Waring blendor followed by a Potter homogeniser. The water homogenates were dialysed for 48 hr at 2° against distilled water and finally freeze-dried. Each batch of freeze-dried preparations sufficed for the investigation of six different aminotransferase activities. Larger samples, prepared from more animals, were not used because it has been shown (Cammarata & Cohen, 1950) that the enzyme activities decrease on storage. Before use the freeze-dried preparations were homogenised in the appropriate media with a Potter homogeniser.

Measurement of aminotransferase activities. All solutions were prepared in 0·1 M potassium phosphate buffer, pH 8·0 Reaction mixtures (0·5 ml) containing the freeze-dried preparation (5 mg) and pyridoxal phosphate (50 μg) were incubated for 15 min at 0° before being added to 0·5 ml of buffer containing 20 μmoles of α-oxoglutarate, 20 μmoles of the appropriate amino-acid and salicylate, when present, to give a final concentration of 10 mM. The mixtures were incubated in stoppered tubes at 37° for 4 hr with constant shaking and the reaction was stopped by placing the tubes in boiling water for 3 min. If necessary, the tubes were stored at −15° before analysis.

The glutamate formed was measured as CO_2 after reaction with glutamate decarboxylase. Each sample was mixed with buffer and the enzyme and the evolved CO_2 automatically estimated. The whole process was carried out with a Technicon Autoanalyser (Technicon Instrument Co., London) which permitted continuous analyses. The method was tested by analysing four samples of each of a series of glutamate solutions of known concentration, ranging from 0·2–10 μmoles/ml. The standard deviation from the mean of the results at each concentration of amino-acid was less than 2% of the total glutamate present. A minimum of four determinations were made for each aminotransferase activity measured with or without salicylate present. The possibility that the constituents of the reaction mixtures may have interfered with the final colour development was excluded by analysing appropriate mixtures in which the various constituents were added to known concentrations of glutamate. Although salicylate inhibits glutamate decarboxylase activity (Gould & others, 1963), the concentrations of the drug in the reaction mixtures after dilution with the various reagents were such that they did not affect the enzyme activity.

Paper chromatographic separations and identifications of the amino- and α-oxo-acids were made and the total α-oxo-acids, total amino-acids and α-oxoglutarate plus glutamate were measured by the methods of Krebs (1950), Rosen (1957) and Meister (1952), before and after the reactions. These procedures were made to ensure that each activity resulted from a true aminotransferase reaction (*cf.* Meister, 1955).

Results

The results (Table 1) show that amino-groups were transferred to α-oxoglutarate from 19 amino-acids in the presence of extracts of one or more of the five rat tissues. These are unequivocal aminotransferase activities because each reaction system satisfies the strict criteria specified by Meister (1955).

L-Glutamine:α-oxoglutarate aminotransferase activity was not unequivocally detected in brain, kidney and skeletal muscle. With brain and kidney extracts, large amounts of glutamate were formed in the reaction mixtures but this was due to the presence of glutaminase which hydrolysed glutamine to glutamate. This reaction obscured any aminotransferase activity which may have been present. Three other L-amino acids studied, lysine, cystine and tyrosine, reacted with the glutamate decarboxylase preparation used for the estimation of the glutamate. It was therefore not possible to determine if aminotransferase activities involving them and α-oxoglutarate occur in the rat tissues. No detectable reaction between asparagine and α-oxoglutarate was found.

Table 1 also shows that 10 mM salicylate, with one exception, significantly inhibits each aminotransferase activity in at least one tissue. There appeared to be no correlation between the degree of inhibition and other factors such as the tissue, the relative activity of the aminotransferase or the chemical type of the amino-acid reacting with the α-oxoglutarate. The exception to this general inhibitory effect of salicylate was the aminotransferase catalysing the reaction between L-tryptophan and the α-oxoglutarate. The enzyme was detected in liver, kidney and heart and in each instance its activity was increased in the presence of salicylate; this effect was statistically significant for the liver and heart.

Discussion

The present results confirm and extend the earlier work of Awapara & Seale (1952) and of Rowsell (1956) in showing that rat tissues possess multiple aminotransferase activities involving glutamate as one of the substrates. Awapara & Seale (1952) used unwashed whole homogenates prepared from eight rat organs and reported the presence of aminotransferase activities involving α-oxoglutarate and the following amino-acids: L-aspartate, L-alanine, L-leucine, L-methionine, L-proline, DL-isoleucine, DL-valine, DL-phenylalanine, DL-threonine and glycine. Rowsell (1956) considered that the use of such homogenates, while providing information about overall amino transfers, was not satisfactory for the recognition of individual aminotransferase reactions because of the presence of endogenous amino- and α-oxo-acids in fresh tissues. This author used washed particles of rat kidney and liver and reported the presence of aminotransferases catalysing the reaction between α-oxoglutarate and the following amino-acids: L-phenylalanine, L-tyrosine, L-tryptophan, L-leucine, L-valine, L-methionine, L-alanine, L-aspartate, DL-norleucine and DL-isoleucine. The present work used lyophilised

ห้องสมุด กรมวิทยาศาสตร์

TABLE 1. EFFECTS OF 10mM SALICYLATE ON AMINOTRANSFERASE ACTIVITIES IN RAT TISSUES

Control values are the mean of four determinations and are expressed as μ moles of glutamate formed in 4 hr. The salicylate figures are given as percentage inhibitions of the corresponding control value; the differences between the control and salicylate results have been analysed by the t-test, Bessel's correction for small samples being used, and values of P are included in brackets. The minimal acceptable level of significance has been taken as $P = 0{\cdot}05$.

Amino acid reacting with α-oxoglutarate	Liver		Kidney		Brain		Heart		Skeletal muscle	
	Control	Salicylate	Control	Salicylate	Control	Salicylate	Control	Salicylate	Control	Salicylate
Glycine	0		0		0·3	16 (0·1)	0		0·5	70 (0·05)
L-Alanine	5·1	34 (0·001)	1·6	36 (0·01)	2·5	45 (0·001)	2·1	25 (0·01)	2·9	35 (0·01)
L-Isoleucine	0		0·9	23 (0·05)	1·4	15 (0·2)	2·4	18 (0·01)	0·6	48 (0·05)
L-Leucine	0		1·4	49 (0·05)	1·6	30 (0·1)	1·7	12 (0·05)	2·0	80 (0·001)
L-Valine	0		0		2·9	13 (0·01)	0·6	55 (0·01)	0	
γ-Aminobutyrate	0·6	55 (0·001)	0		1·2	26 (0·05)	0		0	
L-Serine	0		1·8	21 (0·01)	0		0·4	46 (0·01)	0	
L-Threonine	0·3	31 (0·01)	1·4	11 (0·2)	0		0·3	47 (0·05)	0	
L-Aspartate	8·8	0	9·1	4 (0·05)	11·9	7 (0·05)	10·8	6 (0·05)	11·0	0
L-Glutamine	0·8	49 (0·05)	0		0		0·4	47 (0·05)	0	
L-Asparagine	0		0		0		0		0	
L-Ornithine	10·9	10 (0·05)	20·0	0	1·5	10 (0·2)	1·9	8 (0·2)	0	
L-Arginine	2·8	22 (0·01)	0		0		0		0	
L-Cysteine	1·5	30 (0·01)	1·2	19 (0·01)	0·4	23 (0·001)	2·4	51 (0·001)	0·9	27 (0·05)
L-Methionine	0		0		1·0	34 (0·001)	0		0	
L-Phenylalanine	0		0·6	70 (0·001)	1·3	20 (0·1)	1·5	14 (0·3)	1·1	78 (0·01)
L-Tryptophan	1·0	52* (0·01)	1·4	11* (0·3)	0		1·7	38* (0·01)	0	
L-Histidine	0		1·0	10 (0·3)	0		0		0·8	66 (0·05)
L-Proline	0		0·9	6 (0·5)	0		0		0	
L-Hydroxyproline	0		0·7	8 (0·1)	0		0		0	

* Indicates stimulation of activity by salicylate.

preparations from five rat tissues and shows that seventeen L-amino-acids as well as glycine and γ-aminobutyrate, participate in aminotransferase reactions with α-oxoglutarate. There is therefore abundant evidence that aminotransferase reactions involving glutamate, are widely distributed in rat tissues. The multiplicity of these reactions and the relatively high activity of many of the enzymes show that the aminotransferases play an important role in the metabolism of glutamate and of many other amino-acids *in vivo.*

Salicylate possesses a general inhibitory effect on the rat tissue aminotransferases. The salicylate concentration (10 mM) used was high but inhibitions of 50% or more were frequently observed (Table 1) and inhibition could occur *in vivo* with the lower salicylate concentrations (2 to 3 mM) attained and maintained during the therapy of rheumatic disorders in man. It is also relevant that the mechanism of inhibition may involve competition with the α-oxo-acid and amino-acid substrates since this has been shown to be so for the inhibitory action of the drug on alanine and aspartate aminotransferases *in vitro* (Gould, 1964). If this mechanism of inhibition is operative for the other aminotransferases then the degree of inhibition would not depend solely on the salicylate concentration reached in the tissues, but also on the endogenous concentrations of the reactants of the aminotransferase. Salicylate could therefore produce widespread and also differential inhibitory actions on aminotransferase reactions *in vivo* depending on the particular tissue salicylate concentration and on the amounts of α-oxo- and amino-acids present in individual tissues. This should in turn affect the interconversion of many amino-acids and alter the pool sizes of the tissue amino-acids. There is some experimental evidence supporting this hypothesis because Yoshida, Metcoff & Kaiser (1961) and Huggins & Smith (1963) found that the injection of salicylate in the intact rat caused changes in the levels of glutamate and alanine in the liver and brain. A further implication of these effects is that there may be abnormal peptide and protein synthesis due to either a relative deficiency or excess of one or more of the parent amino-acids.

The only exception to the general inhibitory action of salicylate on aminotransferases found during the present work was the enzyme catalysing the reaction between L-tryptophan and α-oxoglutarate. Salicylate increased the activity of this enzyme in the three rat tissues (liver, kidney and heart) where it occurred. The mechanism of this effect of the drug remains to be established.

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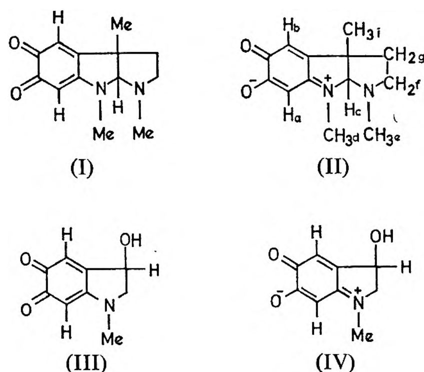
The structure of rubreserine, a decomposition product of physostigmine*

B. ROBINSON

Confirmation of the structure of rubreserine as the resonance hybrid (I)$\leftarrow\!\!\rightarrow$(II) has been obtained by comparison of its ultraviolet, infrared, and proton magnetic resonance spectra with those of adrenochrome (III)$\leftarrow\!\!\rightarrow$(IV).

RUBRESERINE, the red oxidation product of physostigmine, has been assigned structure (II) (Coyne & Paterson, 1961). By comparing the ultraviolet, infrared and nuclear magnetic resonance spectra for adrenochrome, known to have the resonance hybrid structure (III) $\leftarrow\!\!\rightarrow$ (IV) in which the zwitterionic mesomeric structure (IV) makes the major contribution (Harley-Mason, 1948), with the corresponding spectra of rubreserine, support for the resonance hybrid structure (I) $\leftarrow\!\!\rightarrow$ (II) for rubreserine has now been obtained.

Ultraviolet–visible spectra. A comparison of the spectrum of rubreserine (Coyne & Paterson, 1961) and adrenochrome (Beaudet, 1951; Beaudet, Debot, Lambot & Toussaint, 1951; Marquardt & Carl, 1952; Sabotka & Austin, 1951), both recorded quantitatively, shows that the two spectra are almost superimposable, thus suggesting that the same chromophore is present in both molecules.



Infrared spectra. Both compounds show similar spectra (recorded in Nujol) in the 1700–1550 cm^{-1} region. Rubreserine, ν_{max} 1671 m, 1651 m (shoulder), 1628 m (shoulder), 1614 m (shoulder) and 1600 s cm^{-1}. Adrenochrome, ν_{max} 1671 m, 1661 m, 1635 w, 1616 m and 1590 s cm^{-1} (all frequency measurements are ± 3 cm^{-1}). For adrenochrome, assignments involving C=O, C—O, C=C and C=N stretching have been given to these absorption bands (Heacock & Mahan, 1958). The

From the Department of Pharmacy, The University, Manchester, 13.

* For the preceding paper in this series see Robinson & Spiteller (1964).

spectrum of adrenochrome had a broad absorption band at 3280 ± 10 cm^{-1} (m) (O—H stretching) but that of rubreserine, which had been kept *in vacuo* over phosphorus pentoxide for 24 hr before recording the spectrum, showed no absorption in the 4000–3100 cm^{-1} region, contrary to previous observations (Coyne & Paterson, 1961).

Proton magnetic resonance spectra. The spectrum of rubreserine in deuterochloroform had singlets at τ = 8·50, 7·30, 6·90 and 5·64, with intensities 3, 3, 3 and 1 respectively, which are assigned and compared with the corresponding protons in physostigmine (Robinson, 1964) in Table 1. The protons of the two methylene groups in rubreserine form an ABXY system, as do the corresponding protons in physostigmine

TABLE 1. PROTON MAGNETIC RESONANCE DATA

Rubreserine τ	Intensity	Multiplicity	Assignment	Physostigmine τ
3·68 3·52*	1	Singlet	H_a	
4·56 4·64*	1	Singlet	H_b	
5·64	1	Singlet	H_c	5·77
6·90	3	Singlet	H_d	7·00
7·30	3	Singlet	H_e	7·36
6·83–7·24	2	Multiplet half of ABXY system	H_f	7·01–7·30
7·78–8·07	2	Multiplet half of ABXY system	H_g	7·81–8·09
8·50	3	Singlet	H_i	8·53

* Solvent: dimethyl sulphoxide.

(Robinson, 1964), which give rise to two multiplets, each of intensity 2, these are also compared with those for physostigmine and assigned in Table 1. The remaining two protons of rubreserine give rise to two singlets, each of intensity 1, at τ = 4·56 and 3·68. To compare these latter two τ values with those of the corresponding protons in adrenochrome it was necessary to record the spectra in dimethyl sulphoxide, since adrenochrome is only slightly soluble in deuterochloroform. In this solvent the two protons give rise to singlets of equal intensities at τ = 4·64 and 3·52, the corresponding two protons of adrenochrome giving rise to singlets of equal intensities at τ = 4·53 and 3·50.

Experimental

Proton magnetic resonance spectra were recorded on a Varian A.60 spectrometer operating at 60 Mc/sec; tetramethylsilane was used as internal standard and intensities were measured using a planimeter. Infrared spectra were recorded on a Unicam SP.200 spectrophotometer.

Adrenochrome (III) ←→ (IV). This was prepared by oxidation of adrenaline with silver oxide by one of the methods described in the literature (Sabotka & Austin, 1951) [see also (Heacock, Nerenberg & Payza, 1958)].

Rubreserine (I) ←—〉〉〉 (II). Physostigmine (1·00 g) was added to 5% aqueous sodium hydroxide solution (70 ml) in a 250-ml separating funnel. After shaking gently for 10 min some of the physostigmine still remained undissolved and a deep-red colour had developed in the alkaline solution. Chloroform (70 ml) was then added (immediately the remaining solid physostigmine dissolved). After shaking for a further 5 min, the deep-red chloroform layer was separated off; the basic layer was then shaken with further quantities of chloroform (2 × 70 ml) and the combined deep-red chloroform extracts were evaporated under vacuum at room temperature to about 5 ml. Careful addition of light petroleum (b.p. 40–60°) (15–20 ml) dropwise with shaking then effected the crystallisation of rubreserine as bright-red needles (164·7 mg; 12·5%), which after standing over P_2O_5 under vacuum for 24 hr had m.p. 142–145° (reported m.p. 144–145°, Ellis, 1943; 145–146°, Coyne & Paterson, 1961). One recrystallisation from chloroform/light petroleum (40–60°) followed by drying as above, gave bright-red needles, m.p. 145–146°.

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Histamine gastric ulceration in the guinea-pig. Some observations on a new method

W. ANDERSON AND P. D. SOMAN

Consistent gastric ulceration can be produced in suitable strains of guinea-pig, after high duodenal ligation under pentobarbitone anaesthesia, by the subcutaneous injection of a relatively low dose of aqueous histamine acid phosphate. The ulceration coincides with a dose of histamine which produces sub-maximal secretion volume and which is greater than that producing the maximum secretion. The method does not require antihistamine cover, and it is shown that antihistamines may complicate the true histamine response by the stomach. The results provide evidence that this type of ulceration follows the action of gastric juice on a functionally impaired mucosa.

SINCE Code and his colleagues (Code & Varco, 1940, Hay, Varco, Code & Wangensteen, 1942) first studied the use of a long-acting non-aqueous intramuscular injection of a suspension of histamine dihydrochloride as an ulcerogenic agent, several workers (Crane, 1947; Ambrus, 1951, 1953; Harrison, 1955) using oily suspensions of histamine, have used the guinea-pig in this type of experiment for studies in peptic ulceration. The animal is sensitive to histamine which induces a copious gastric secretion readily obtainable by stomach tube without surgery (Watt, 1955); and it is possible to produce ulceration without causing death.

Halpern & Martin (1946) and Zaidi & Mukerji (1958) on the other hand, used a relatively large dose of the faster-acting aqueous solution of histamine hydrochloride given intraperitoneally, at the same time protecting the animals with the antihistamine promethazine given by the same route.

Both methods have limitations: they normally require the use of high doses of histamine which necessitate protection of the animals either by delaying the release rate by using a wax-oil base or by concurrent use of antihistamine drugs or both. Antihistamines may modify the gastric effects of histamine to some extent, although it has been widely assumed that the ulcerative and secretory effects of histamine on the stomach are, in general, unimpaired, the drugs merely eliminating the fatal systemic effects of histamine otherwise liable to occur with such dosage.

The Shay method (Shay, Komarov, Fels, Meranze, Gruenstein & Siplet, 1945), which uses the rat, is based on the fact that gastric juice retained in the stomach as a result of pyloric ligation is a principal aetiological factor in this type of experimental gastric ulceration. These authors stated that gastric ulcers may also be produced in the guinea-pig by the method described for the rat. However, experimental details for the guinea-pig were not given and furthermore, the method for the rat required about 18 hr of unanaesthetized ligation. In the method to be described we have used the guinea-pig for studying experimental peptic ulceration. This is produced by high duodenal ligation with subcutaneous injection of aqueous histamine acid phosphate in a dose small enough of

From the Department of Pharmacy, University of Strathclyde, Glasgow, C.1.

itself to permit indefinite survival of the animal without resort to antihistamines, which, although frequently used in this type of study, complicate the picture of histamine action on the stomach of the guinea-pig.

Experimental and results

Male albino guinea-pigs, 250–700 g body weight, were fasted for 18–24 hr in conditions preventing coprophagy; water was allowed *ad lib.* They were anaesthetised by pentobarbitone sodium, 30 mg/kg intraperitoneally. A single mid-line abdominal incision (2–3 cm) was made, carefully avoiding damaging the stomach when entering the peritoneal cavity. The pyloric end of the stomach was located and the duodenum exteriorised without disturbing the stomach. An artery clip was placed on the duodenum above the opening of the bile duct avoiding interruption of blood supply. This constriction ensures retention of the whole gastric secretion; it also prevents regurgitation of duodenal contents which might interfere with the effect of the acid secretion. The peritoneal cavity was closed around the protruding artery clip.

Aqueous solution of histamine acid phosphate (2·5–7·5 mg/kg depending on the strain used) was injected subcutaneously, the control animals receiving the same volume of normal saline after undergoing the same operative procedure. The animals were left undisturbed in a supine position on a warm table and 1 hr after the injection of histamine they were killed with ether. The stomach was carefully removed with the clip in position, washed, and the gastric secretion collected, centrifuged, its volume measured and free and total acidities titrated with Topfer's reagent and phenolphthalein respectively. Volume of juice was reported as ml/kg body weight. The stomach was then distended with tap water injected through the cardiac orifice and examined against a good light for ulceration. The severity of ulceration may be graded according to the following scale: +, a few small ulcers (up to four); 2+, several small ulcers (five to eight); 3+, many small ulcers (9–16) and a few large ulcers; 4+, large areas of ulceration with confluence or more than 16 small ulcers, or impending perforation. Average group scores were calculated.

Ulcers were observed singly, in groups, or as diffuse ulcerated regions. Although ulcers are found in the antral region and less frequently on the lesser curvature, they usually appear in the fundic region and along the line of greater curvature indicating that the glandular portion of the stomach, which possesses the highest secretory activity, appears to be most susceptible. Two types of ulcers were observed; diffuse ulcerated regions and small round punched out areas, both types occurring in all ulcer-bearing regions. The latter type had a clean transparent floor surrounded by a whitish lining of mucus. Haemorrhage was not a common feature and only occurred when ulceration was severe. Non-ulcerated regions occasionally showed a superficially eroded mucosa but in most instances, these areas and tissues peripheral to an ulcer were apparently healthy.

The dose of histamine acid phosphate (which varied from 2·5–7·5 mg/kg) giving a consistent average ulcer picture of 3+ to 4+ was ascertained for

each strain after the animals had been maintained exclusively on diet 18 and water for at least one week immediately before the experiment. The diet was therefore relatively low in ascorbic acid; nevertheless, the animals appeared healthy and showed no signs of scurvy.

Control animals did not show ulceration, although secretions were acid. Differences in volume and in free and total acidity between control and test groups were significant (Table 1).

TABLE 1. HISTAMINE GASTRIC ULCERATION AND SECRETION IN DIFFERENT STRAINS OF GUINEA-PIG

Strain	No. of animals	Dose of histamine acid phosphate mg/kg s.c.	Average ulceration	Average volume of secretion (ml/kg)	Average acidity of secretion (m-equiv./litre)	
					Free	Total
P	5	2·5	1·4+	40	114	120
T	6	2·5	1·6+	35	118	131
P	12	5·0	3·2+	30	98	105
T	6	5·0	1·7+	25	100	108
P	5	Control	0	15	54	63
T	7	Control	0	17	59	67

T = resistant strain; P = susceptible strain. Significance of differences between controls and histamine-treated: volumes $P<0.02$; acidities $P = 0.001$–0.05.

EFFECT OF ANAESTHETIC

Pentobarbitone sodium, 30 mg/kg intraperitoneally, was chosen in preference to urethane because when urethane, 1·25 g/kg intraperitoneally, was used with histamine, 2·5 mg/kg subcutaneously, ulceration appeared to be much more severe. The 10 animals given pentobarbitone had average ulceration of 0·2+; average secretion 35 ml/kg and average acidity of secretion (m-equiv./litre) free 109, total 117. Corresponding figures for the 8 animals given urethane were 2·4+; 30; 114 and 121. An ulcer-resistant strain which consistently failed to show ulceration with 2·5 mg/kg histamine acid phosphate when pentobarbitone was used, did so with urethane, with the same dose of histamine (and even in the absence of histamine in certain preliminary control experiments).

STRAIN DIFFERENCE

Differences in response by different strains of guinea-pig were observed. In the more susceptible (P) strain, 2·5 mg/kg histamine acid phosphate increased the volume and free and total acidity of gastric secretions, and produced low degree of ulceration, while at 5·0 mg/kg significant increase in ulceration associated with a slight decrease in volume and acidity was observed (Table 1). The resistant (T) strain behaved similarly except that the high dose did not increase ulceration. Table 1 further shows that for both resistant and susceptible strains, the volume of gastric juice and its acidity are not maximal when the dose of histamine is high enough to cause ulceration.

ROLE OF FREE ACID IN STOMACH CONTENTS

In the same type of experiment using the susceptible (P) strain, aluminium hydroxide gel B.P. (2 ml) was introduced into the stomach by

tube after ligation of the duodenum and subcutaneous injection of histamine acid phosphate 5 mg/kg. Control animals received water (2 ml). Acidity of gastric secretion was eliminated, the 11 animals receiving gel having average ulceration of 0·4+ ; average volume of secretion 27 ml/kg; average acidity of secretion (m-equiv./litre) free 0, total 0. Corresponding figures for the 5 controls were 4+ ; 31 ; 92 and 100.

EFFECT OF ANTIHISTAMINES

In attempting to intensify ulceration in the resistant (T) strain, a higher dose of histamine (7·5 mg/kg) was employed with the antihistamine promethazine hydrochloride, 1 mg/kg intramuscularly, 30 min before. The promethazine gave this strain complete protection against gastric ulceration. The susceptible strain, receiving 5 mg/kg histamine acid phosphate, was also protected by the drug ($P<0{\cdot}001$). Antihistamine protection by promethazine is apparently not associated with significant change in total volume or acidity (Table 2). Similarly, the specific anti-

TABLE 2. EFFECT OF ANTIHISTAMINES ON HISTAMINE ULCERATION AND SECRETION IN AN ULCER-RESISTANT AND AN ULCER-SUSCEPTIBLE STRAIN OF GUINEA-PIG

Strain	No. of animals	Dose of histamine acid phosphate (mg/kg s.c.)	Average ulceration		Average volume of secretion (ml/kg)	Average acidity of secretion (m-equiv./litre)	
			With promethazine	Without 1 mg/kg i.m.		Free	Total
T	6	7·5	0	–	33	119	124
P	15	5	+	–	30	108	116
			Mepyramine maleate 5 mg/kg i.m.				
T	7	7·5	+	–	47	116	125
P	16	5	1·6+	–	41	119	126
T	6	7·5	–	3·3+	30	87	97
P	12	5	–	3·2+	30	98	105

T = resistant strain; P = susceptible strain.

histamine mepyramine maleate (5·0 mg/kg intramuscularly 30 min before histamine) reduces ulceration by 50% ($P<0{\cdot}01$), the only change in the secretory pattern being an increase ($P<0{\cdot}01$) in the volume of gastric juice secreted.

Discussion

The technique described will yield consistent gastric ulceration in a susceptible strain of guinea-pig. Histamine is required in relatively low dosage of aqueous solution injected subcutaneously and this contrasts with the larger doses of longer acting non-aqueous suspensions used in earlier methods. By this present method, the gastric response is likely to be more uniform, since several complicating factors are eliminated. Where delayed absorption is obtained by intramuscular injection of suspensions containing relatively high doses of histamine and of varying physico-chemical properties, the different responses obtained, for example by Olovson (1950) and Williams (1951), probably indicate varying release rates from the sites of injection. By our technique these factors are

minimised. An aqueous solution of histamine acid phosphate is more likely, after relatively rapid absorption, to yield a threshold histamine level more quickly and more certainly than an oily suspension which after slower absorption would give a lower, more prolonged but less consistent level; and it may well be that the ulceration and secretory effects here measured after 1 hr, follow such a level more consistently. This may explain one of our unreported findings that the severity of gastric ulceration was variable when such a suspension was given by the intramuscular route but not so with the present technique.

The finding that urethane was not a suitable anaesthetic bears out Schachter's (1949) findings that urethane stimulated secretion of high acidity in dogs after a delay of 2 hr. These effects may be referable to a stimulating effect of urethane on the secretion of endogenous histamine.

The difference in resistance between the two strains of guinea-pig therefore appears to be to ulceration rather than to changes in secretory pattern, which are broadly similar; whether this is an intrinsic difference between strains in terms of gastric response to histamine or whether there is some other factor is not yet clear. This uniformity of secretion accompanying a varying ulcer-proneness is of direct interest when considering the relationship between results obtained using this animal and their relevance to the disease in man where ulcer-proneness is an accepted though not a clearly understood concept.

In the guinea-pig, histamine ulceration has been variously attributed either to the secreted gastric juice (Hay & others, 1942; Halpern & Martin, 1946; Zaidi & Mukerji, 1958) or to the angiotoxic effect of histamine (Merkel, 1942; Williams, 1951; Kowalewski, 1954) and recently Watt (1959) has suggested that the ulcerative process is initiated in the first instance by an effect of the acid gastric juice on the mucosal vessels. The present results indicate that, in the guinea-pig, whether or not the particular strain is ulcer-resistant, an increase in histamine dosage beyond a certain level results in an output of gastric juice lower than that produced by doses not producing ulceration, an observation made under different experimental conditions by Ivy, Grossman & Bachrach (1951). These results are interpreted as indicating that at the higher (ulcerogenic and above) dose of histamine, a depressant effect on the cellular function of the secreting mucosa sets in as evidenced by failure of secretion volume to increase with dose, and this could be indirectly referable to the angiotoxic effects of histamine, whereas below this ulcerogenic threshold, the action of histamine on the secretory function appears to be one of increasing stimulation.

In the ulcer-prone strain, this is sufficient to allow the juice secreted in lower volume to take its part in the ulcerative process. The necessity of secreted acid is borne out by the results of the experiments with aluminium hydroxide where complete protection against ulceration is found with a complete neutralisation of free acidity of the gastric secretion. The results of the experiments are therefore compatible with the concept that both the angiotoxic effects as well as irritant action of hydrochloric acid are concerned in ulcerogenesis under these conditions.

Antihistamines have frequently been used in studies on the experimental ulcer ever since Halpern & Martin (1946) made it known that a potent antihistamine will protect the guinea-pig against systemic effects of histamine given in a dosage sufficient to produce peptic ulceration at the same time. We find that the potent antihistamine promethazine does modify the ulceration picture when a sub-lethal dose of histamine is used as the ulceration stimulus.

Unlike mepyramine maleate, the anti-ulcer property of promethazine appears not to be associated with increase in volume of secretion. The differences in the effects of the different antihistamines is not surprising since pharmacological experience reveals that specificity of action is relative and not absolute, absolute specificity being rare or practically unattainable (Loew, 1947). Promethazine, besides being an antihistamine, exhibits atropine-like and ganglion blocking action and with these actions secretion volume is unchanged, and ulceration fails to occur. Mepyramine maleate, while being more specific, is a weaker antihistamine than promethazine, and its ulcer-protective effect is accompanied by an increase in volume of secretion. Wood (1949) also observed an increase in volume of secretion after mepyramine in histamine-stimulated cats. The increased volume of secretion and the diminution of ulceration may well be due to improved circulation in the gastric vascular bed, as a result of histamine antagonism.

This work, while providing a method of producing gastric ulcers in the guinea-pig, also supports the view that histamine gastric ulceration in the guinea-pig is apparently the result of the action of gastric juice on a mucosa functionally disturbed by histamine.

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Pharmacological properties of some West Indian medicinal plants

PATRICIA A. HOOPER AND B. E. LEONARD*

Aqueous extracts of 47 West Indian medicinal plants have been tested for their pharmacological activity. Most of the extracts had slight activity only. Some extracts had more pronounced pharmacological properties and are discussed in greater detail.

A COMPREHENSIVE study of West Indian medicinal plants was undertaken to determine whether these plants contain substances of therapeutic or toxicological importance. Many are still used by certain groups of West Indians. The plants are usually ingested as beverages ("bush teas") prepared by steeping either the leaves or the whole plant in hot water and their prolonged use may play a part in the aetiology of some diseases which are relatively common in the West Indies, such as veno-occlusive disease of the liver (Bras, Berry & György, 1957).

The present study was done in collaboration with the University of the West Indies. The review of 250 Jamaican medicinal plants by Asprey & Thornton (1953, 1954, 1955) was used as a guide in the selection of plants to be tested. A report on the pharmacological properties of 55 plants has already been published by Feng, Haynes, Magnus, Plimmer & Sherratt (1962). The results of the pharmacological testing of a further 47 plants are herein described. Thirty-two of these plants have previously been examined by Feng, Haynes, Magnus & Plimmer (1964), using a different screening programme.

Methods

PREPARATION OF PLANT EXTRACTS

The plants were identified by the Botany Department of the University of the West Indies and the aqueous extracts prepared by the Chemistry Department of that University, using the method of Feng & others (1962). Usually the aqueous extract from which high molecular weight material had been precipitated with ethanol was used in the pharmacological tests. However, the crude aqueous extracts of a few plants were used before precipitation with ethanol and these plants are marked with an asterisk in the tables of results. One ml of the final aqueous extract was equivalent to 1 g of fresh plant tissue. When necessary extracts were neutralised before testing.

PHARMACOLOGICAL TESTING

The following pharmacological tests were made.

Acute toxicity. Intraperitoneal injection. The plant extracts were injected intraperitoneally (i.p.) into albino mice weighing 20–30 g; 5 mice

From the Department of Medical Biochemistry and Pharmacology, Medical School, Birmingham, 15.

* Present address: Department of Pharmacy, University Park, Nottingham.

were used at each dose level. Initially the dose was usually 10 ml/kg. Depending on the effects observed further doses in a logarithmic series ranging from 2·5–20 ml/kg were injected into other groups of mice. The animals were observed for at least 15 min after injection and a record was made of the effect of the extract on general behaviour, respiration, reflexes (pinna, corneal and righting), grip, performance on an inclined plane at 45° and on a rotating rod. Surviving animals were examined at 24 hr intervals for one week and any deaths were recorded.

Intravenous injection. Extracts were only injected intravenously (i.v.) if they had a pronounced effect on intraperitoneal injection. Injections were made into the lateral tail vein of groups of 5 albino mice (20–30 g) and the same observations made as for the intraperitoneal injections. The initial dose was usually 5 ml/kg but lower doses were injected if this dose killed all the mice in the group.

Effect on isolated organ preparations. Guinea-pig ileum. A portion of terminal ileum (3–4 cm long) was set up in a 15 ml bath of oxygenated Tyrode solution at 37°. The extract (0·007–0·013 ml/ml bath fluid) was added to the bath and left in contact with the ileum for 25 sec. If the extract possessed marked spasmogenic properties an attempt was made to determine the site of action of the active principle by adding antagonists to known spasmogens to the Tyrode solution. The antagonists used were atropine sulphate (0·005 μg/ml bath fluid), mepyramine maleate (0·1 μg/ml bath fluid) and 2-bromolysergic acid diethylamide (BOL; 13 μg/ml bath fluid). These doses were sufficient to block the submaximal responses of the ileum to acetylcholine, histamine and 5-hydroxytryptamine (5-HT) respectively. Submaximal contractions of the ileum were obtained by the consecutive addition of 2 known spasmogens and the extract to the bath. The antagonist to one of the known spasmogens was then added to the Tyrode solution and the 3 spasmogens tested in the same order as before.

All the plant extracts were also tested for their effect on submaximal contractions of the ileum induced by acetylcholine, histamine and barium chloride. Depending on the sensitivity of the preparation, the doses used of these spasmogens were approximately 0·007–0·02 μg/ml bath fluid of acetylcholine and histamine and 70 μg/ml bath fluid of barium chloride. The dose of extract was 0·007–0·013 ml/ml bath fluid.

Rabbit duodenum. A portion of duodenum (approximately 4 cm long) was set up in a 50 ml bath of Tyrode solution at 37°, through which passed a constant stream of 95% oxygen, 5% carbon dioxide. Up to 0·017 ml extract per ml bath fluid was added to observe the effect of the extract on the pendular movement and muscle tone of the duodenum.

Rat phrenic nerve-diaphragm. The preparation was set up in a 100 ml bath of oxygenated Tyrode solution at 37° as described by Bulbring (1946). The effect of the extract on the response to nerve stimulation was observed. The dose of extract was 0·001–0·005 ml/ml bath fluid.

Rabbit heart. The Langendorff preparation of the rabbit isolated heart was perfused with oxygenated Ringer's solution at 35°. The effect

of the extract on the amplitude of contraction was noted after adding up to 0·2 ml of the extract to the perfusing fluid.

Effect on cat blood pressure. Some of the extracts that had an effect on the rabbit isolated heart were tested for their effect on the cat blood pressure. Cats (2–3 kg) were anaesthetised with chloralose (80 mg/kg intraperitoneally). The blood pressure was recorded from the common carotid artery. Heparin (1,000 units/kg) was given intravenously. The extracts (0·1 ml/kg) were injected into the femoral vein.

Effect on the barbiturate sleeping time of mice. Only those plant extracts were tested that caused convulsions, excitement or sedation on intravenous injection into mice. Hexobarbitone sodium (100–125 mg/kg) was injected intraperitoneally into groups of 6 albino mice. This produced a sleeping time of 10–20 min in the control group, the members of which also received an intraperitoneal injection of 0·9% saline (10 ml/kg). The experimental group was injected intraperitoneally with up to 10 ml/kg of extract; the dose of extract used was always below the minimal lethal dose. The sleeping time was regarded as the period between the loss and regaining of the righting reflex.

The significance of the results was assessed by Student's *t*-test.

Results and discussion

The results are described in Tables 1–5. None of the plant extracts had any effect on the rat phrenic nerve-diaphragm preparation and the results of this test have therefore not been tabulated.

The extracts tested had a number of different pharmacological activities which frequently could not be related either to known constituents of the plants or to their medicinal uses in the West Indies and elsewhere. However, it is possible to draw certain conclusions from the results obtained.

Some plants, e.g., *Mangifera indica*, *Cordia brownei*, *Poinciana regia* and *Desmodium axillare*, caused depression, frequently accompanied by writhing and ataxia, on intraperitoneal injection but had little or no effect on intravenous injection. This reaction was probably due to pain and with *M. indica* might have been due to the tannins known to be present in the leaves of this plant, which are used in Africa and the East for their astringent properties (Asprey & Thornton, 1953). In most instances, however, the active principles are unknown.

A few plant extracts were found to contain substances with either acetylcholine or histamine-like activities. Thus *Triumfetta hispida* and *Cissus sicyoides* were found to contain acetylcholine or a related compound, and histamine was identified pharmacologically in extracts of *Ervatamia divaricata*, *Opuntia tuna*, *Pedilanthus jamaicensis* and *Sida rhombifolia*. The presence of these substances in "bush teas" would not be of any medicinal value, since they are both rapidly inactivated in the gastrointestinal tract.

Various *Cassia* species are widely used as purgatives and anthelmintics. Senna is the name given to the dried leaves or pods of *C. acutifolia* Delile

TABLE 1. ACUTE TOXICITY TESTS ON MICE

Plant		Route of admin.	Dose ml/kg	Effects on groups of 5 mice
Family	Botanical name			
Apocynaceae	*Ervatamia divaricata* (L.) Burkh.	i.p.	10–20	Depression and slight tachypnoea. Mice normal after 24 hr
		i.v.	5	Transient excitation and tachypnoea
Asclepiadaceae	*Calotropis procera* R.Br.	i.p.	10	Depression. After 24 hr 4 mice normal; 1 mouse depressed; dead after 72 hr
		i.v.	5	Depression and hypopnoea. Mice normal after 24 hr
Bignoniaceae	*Catalpa longissima* Dum.-Cours.	i.p.	10	Slight depression, ataxia and writhing. 2 mice dead after 72 hr. Remainder normal after 1 week
		i.v.	5	Depression and hypopnoea. Mice normal after 24 hr
	Crescentia cujete L.	i.p.	20	No immediate effect, but 2 mice dead after 48 hr. Remainder normal after 1 week
Boraginaceae	**Heliotropium angiospermum* Murray	i.p.	10–20	Depression and slight tachypnoea. Mice normal after 24 hr
		i.v.	5	Transient slight tremor and apparent increase in aggressiveness
Caesalpiniaceae	*Cassia emarginata* L.	i.p.	2·5	Depression, ataxia and hyperpnoea. Mice became more sensitive to sound; clonic convulsions within 15 min. 3 died; remainder normal after 24 hr
		i.v.	0·5	Depression, ataxia and hyperpnoea. Mice showed tremor; 2 had clonic convulsions. 1 died; remainder normal after 24 hr
	Cassia fistula L.	i.p.	10–20	Depression, ataxia and hypopnoea. Mice normal after 24 hr
		i.v.	5	Depression and hypopnoea. Mice normal after 24 hr
	**Poinciana regia* Boj.	i.p.	10	Severe depression, writhing and hypopnoea. Mice normal after 24 hr
		i.v.	5	No immediate effect. After 24 hr 1 mouse dead; remainder depressed and ataxic but normal after 48 hr
Combretaceae	*Terminalia catappa* L.	i.p.	5	Depression, slight ataxia and hypopnoea. After 24 hr mice depressed and constipated; 5 dead after 72 hr
		i.v.	5	Depression, ataxia and hypopnoea. After 24 hr 4 mice normal; 1 dead
Compositae	*Tithonia diversifolia* Gray	i.p.	10–20	Depression, ataxia and hyperpnoea. Mice normal after 24 hr
		i.v.	5	No apparent effect on 4 mice; 1 had clonic convulsions, but recovered rapidly
Cucurbitaceae	*Cucumis anguria* L.	i.p.	20	Depression, twitching and hypopnoea. After 24 hr 1 mouse dead; remainder severely depressed; dead after 48 hr
		i.v.	5	Slight twitching and tachypnoea. Mice normal after 24 hr
Iridaceae	*Aristea compressa* Buch.	i.p.	10	Depression and hypopnoea. After 24 hr 4 mice dead; survivor severely depressed; dead after 48 hr
		i.v.	5	Slight excitation and apparent increase in aggressiveness. After 24 hr 3 mice depressed; 3 dead after 48 hr. Remainder normal after 1 week

* Crude aqueous extracts.

TABLE 1—*continued*

Plant		Route of admin.	Dose ml/kg	Effects on groups of 5 mice
Family	Botanical name			
Meliaceae ..	*Trichilia hirta* L.	i.p.	10	Depression and transient writhing. Mice normal after 24 hr
		i.v.	5	2 mice had clonic convulsions. 1 died. Remainder normal after 24 hr
Mimosaceae ..	*Acacia lutea* Hitch.	i.p.	10	Depression and slight dyspnoea. After 24 hr 1 mouse dead; 4 apparently normal. After 48 hr 3 mice dead; survivor normal after 1 week
		i.v.	5	Depression and hypopnoea. After 24 hr 1 mouse dead; remainder normal
Nyctaginaceae ..	*Mirabilis jalapa* L.	i.p.	5	Depression, tachypnoea and reduced grip. The cornea was opaque and corneal and pinna reflexes were reduced. After 24 hr 1 mouse dead; 2 normal and 2 depressed. After 48 hr 2 mice dead; remainder normal after 1 week
Polygonaceae ..	*Polygonum chinense* L.	i.p.	10	Depression and ataxia. Mice normal after 24 hr
		i.v.	5	Depression, ataxia, hyperpnoea and slight head tremor. After 24 hr 1 mouse dead; remainder normal
Solanaceae ..	*Capsicum frutescens* L.	i.p.	10	Depression and slight ataxia. Mice normal after 24 hr
		i.v.	2·5	Depression, dyspnoea and clonic convulsions. 1 mouse died after convulsions; remainder normal after 24 hr
	Solanum verbascifolium L.	i.p.	10	Depression, ataxia and tachypnoea. 5 mice dead after 2 hr
		i.v.	2·5	Depression, ataxia and hyperpnoea. 2 mice had clonic convulsions and died. Remainder normal after 24 hr
Verbenaceae ..	**Lantana camara* L.	i.p.	10	Depression and tachypnoea. After 24 hr mice slightly depressed. After 48 hr 2 mice dead; remainder normal
		i.v.	5	Depression, hypopnoea, fine head tremor and twitching. Mice normal after 24 hr
Vitaceae	*Cissus sicyoides* L.	i.p.	10	Depression. Mice normal after 24 hr
		i.v.	5	Slight excitation. Mice normal after 24 hr
Zygophyllaceae ..	**Kallstroemia maxima* Torr. et Gr.	i.p.	10	Depression, ataxia and tachypnoea. After 24 hr 4 mice dead; survivor severely depressed, dying after 48 hr
		i.v.	5	Depression. After 24 hr 1 mouse dead; remainder depressed; normal after 48 hr

The following extracts had no effect in a dose of 20 ml/kg i.p.

Annonaceae *Annona squamosa* L. Asclepiadaceae *Asclepias curassavica* L. Bignoniaceae *Tecomaria capensis* Fenzl. Cactaceae *Opuntia tuna* Mill. Convolvulaceae *Argyreia speciosa* Sweet. Cucurbitaceae *Luffa cylindrica* M.Roem. Phytolaccaceae *Petiveria alliacea* L.

On i.p. injection (10–20 ml/kg) the following extracts caused some of the symptoms of peritoneal irritation, e.g., tachypnoea, ataxia, writhing and depression, sometimes followed by death. However, they had no effect on i.v. injection.

Acanthaceae *Thunbergia grandiflora* Roxb. Anacardiaceae *Mangifera indica* L. var. "Black". Apocynaceae **Echites umbellata* Jacq. Balsaminaceae *Impatiens sultani* Hook. Boraginaceae *Cordia brownei* (Friesen) Johnston. Euphorbiaceae **Pedilanthus jamaicensis* Millsp. & Britton. Malvaceae **Hibiscus rosa-sinensis* L.; *Sida acuta* Burm.; *Sida rhombifolia* L. Mimosaceae *Albizia lebbeck* Benth. Moraceae *Ficus benjamina* L. Nyctaginaceae *Bougainvillea* sp. Papilionaceae *Desmodium axillare* DC. Rhamnaceae **Ziziphus mauritiana* Lam. Sapindaceae *Meliococca bijuga* L. Sapotaceae **Chrysophyllum cainito* L. Solanaceae *Cestrum diurnum* L. var. *venenatum* (Mill.)O.E.Sch. Tiliaceae *Triumfetta hispida* A.Rich. Verbenaceae *Verbena bonariensis* L.

TABLE 2. EFFECTS ON SMOOTH MUSCLE

Botanical name	Guinea-pig ileum		Rabbit duodenum	
	Dose ml/ml bath fluid	Effect	Dose ml/ml bath fluid	Effect
Annona squamosa L. . .	0·013	ACh, histamine and $BaCl_2$ contractions reduced by 90%, 80% and 50% respectively	0·002	Decrease in tone
Ervatamia divaricata (L.) Burkh. . .	0·007	Contraction equivalent to 85% maximal ACh contraction antagonised by mepyramine	0·017	None
Calotropis procera R. Br.	0·013	Contraction equivalent to 25% maximal ACh contraction	0·002	Gradual increase in tone, followed by irreversible spasm
Catalpa longissima Dum.-Cours. . .	0·013	ACh contraction reduced by 60%	0·007	Decrease in size of contractions
Cordia brownei (Friesen) Johnston	0·013	ACh and histamine contractions reduced by 20% and 25% respectively	0·007	Rapid decrease in tone
Opuntia tuna Mill. . .	0·007	Contraction equivalent to 75% maximal ACh contraction antagonised by mepyramine	0·017	Slight increase in tone
Cassia emarginata L.	0·013	ACh contraction reduced by 50%	0·007	Slight decrease in tone
Cassia fistula L. . .	0·007	Contraction equivalent to 70% maximal ACh contraction antagonised by BOL	0·005	Increase in tone
Tithonia diversifolia Grey	0·013	None	0·005	Decrease in size of contractions
Cucumis anguria L. . .	0·013	ACh contraction reduced by 40%; histamine contraction increased by 25%	0·004	Slight decrease in tone
Luffa cylindrica M. Roem.	0·013	ACh and histamine contractions reduced by 75% and 20% respectively	0·017	None
**Pedilanthus jamaicensis* Millsp. & Britton	0·007	Contraction equivalent to 55% maximal ACh contraction antagonised by mepyramine	0·017	None
Sida rhombifolia L. . .	0·013	Contraction equivalent to 90% maximal ACh contraction antagonised by mepyramine	0·017	None
Trichilia hirta L. . .	0·013	None	0·002	Large transient decrease in tone
Acacia lutea Hitch. . .	0·013	ACh contraction reduced by 20%	0·017	Slight increase in tone
Mirabilis jalapa L. . .	0·013	None	0·002	Sudden large decrease in tone and in size of contractions
Desmodium axillare DC.	0·013	ACh contraction reduced by 60%	0·007	Gradual increase in tone
Petiveria alliacea L. . .	0·013	ACh contraction reduced by 30%	0·017	None

TABLE 2—*continued*

Botanical name	Guinea-pig ileum		Rabbit duodenum	
	Dose ml/ml bath fluid	Effect	Dose ml/ml bath fluid	Effect
Polygonum chinense L.	0·013	Contraction equivalent to 20% maximal ACh contraction	0·004	Slight increase in tone
Capsicum frutescens L.	0·013	ACh and histamine contractions reduced by 50% and 30% respectively	0·017	Slight decrease in tone
Solanum verbascifolium L.	0·013	Contraction equivalent to 65% maximal ACh contraction partially antagonised by both atropine and BOL. ACh, histamine and $BaCl_2$ contractions reduced by 60%, 60% and 30% respectively	0·005	Increase in tone, followed by spasm
Triumfetta hispida A. Rich	0·007	Contraction equivalent to 90% maximal ACh contraction antagonised by atropine	0·002	Rapid increase in tone
Cissus sicyoides L.	0·013	Contraction equivalent to 15% maximal ACh contraction antagonised by atropine. ACh contraction reduced by 40%	0·017	Slight decrease in tone
**Kallstroemia maxima* Torr. et Gr.	0·013	None	0·006	Slight decrease in tone, followed by an increase and prolonged spasm

All extracts were tested on guinea-pig ileum and rabbit duodenum but only those extracts which had an effect are listed in this table.

or *C. angustifolia* Vahl and *C. fistula* is a well-known source of the purgative cassia pod and pulp. The purgative principles in this genus are anthraquinones, whose irritant properties may partly account for the depression seen in mice after intraperitoneal injection of *C. fistula* and *C. emarginata*. *C. fistula* was also found to contain 5-HT, which would explain the stimulating effect on smooth muscle and the increase in barbiturate sleeping time obtained with this extract. Some *Cassia* species are known to contain hydrocyanic acid (Watt & Breyer-Brandwijk, 1962) and this substance may be the cause of the clonic convulsions occurring after intraperitoneal and intravenous injections of an extract of *C. emarginata*. The presence of this compound in an extract of *C. emarginata* would also account for its depressant effect on rabbit isolated heart and duodenum and for the increase in the barbiturate sleeping time.

The presence of hydrocyanic acid has also been reported in *Tithonia diversifolia* (Watt & Breyer-Brandwijk, 1962). This may have been the cause of the clonic convulsions occurring in one mouse after intravenous injection of the extract, the increase in the barbiturate sleeping time and the depressant effect on rabbit isolated duodenum. No reference to the medicinal uses of this plant has been found.

Many species of *Trichilia* are used in Africa as purgatives, and the roots of some species are also used as an emetic in rheumatism, dropsy and heart disease. This genus is known to contain saponins, tannins,

TABLE 3. EFFECTS ON RABBIT ISOLATED HEART

Botanical name	Dose ml	Effect
Mangifera indica L. var. "Black" ..	0·05	Transient decrease in amplitude
Annona squamosa L.	0·05	Increase in rate and amplitude, followed by recovery
**Echites umbellata* Jacq.	0·1	Large rapid increase in rate and amplitude, followed by recovery
Ervatamia divaricata (L.) Burkh. ..	0·05	Large increase in amplitude, followed by slow recovery
Calotropis procera R.Br.	0·1	Large decrease in amplitude, followed by slow partial recovery
Cordia brownei (Friesen) Johnston ..	0·1	Transient decrease in amplitude
**Heliotropium angiospermum* Murray	0·1	Gradual large decrease in rate and amplitude, followed by slow partial recovery
Cassia emarginata L.	0·05	Transient decrease in amplitude
Cassia fistula L.	0·1	Decrease in amplitude, followed by recovery
Cucumis anguria L.	0·1	Decrease in amplitude, followed by recovery
Trichilia hirta L.	0·2	Prolonged decrease in rate and amplitude
Acacia lutea Hitch.	0·1	Transient decrease in amplitude
Albizia lebbeck Benth.	0·2	Slow decrease in amplitude, followed eventually by asystole
Bougainvillea sp.	0·05	Transient decrease in amplitude
Mirabilis jalapa L.	0·005	Sudden large increase in rate and amplitude, followed by fairly rapid recovery
Meliococca bijuga L.	0·1	Decrease in rate and amplitude, followed by asystole
**Chrysophyllum cainito* L.	0·05	Decrease in amplitude, followed by partial recovery
Capsicum frutescens L.	0·05	Decrease in amplitude, followed by recovery
Solanum verbascifolium L.	0·1	Rapid asystole followed by gradual partial recovery
**Lantana camara* L.	0·1	Rapid decrease in amplitude, followed by recovery
**Kallstroemia maxima* Torr. et Gr. ..	0·1	Large prolonged decrease in amplitude

All extracts were tested on the rabbit isolated heart, but only those extracts which had a pronounced effect are listed in this table.

TABLE 4. EFFECTS ON CAT BLOOD PRESSURE

Botanical name	Dose ml/kg	Effect
Annona squamosa L.	0·1	Decrease of 30–40% followed by slow recovery
**Echites umbellata* Jacq.	0·1	Slight increase followed by a rapid decrease of approx. 80%; slow recovery
Ervatamia divaricata (L.) Burkh. ..	0·1	Transient decrease of approx. 25%
Cassia emarginata L.	0·1	Transient decrease of approx. 30%
Terminalia catappa L...	0·1	Two successive decreases of 25–30% followed by a compensatory increase; slow recovery
Mirabilis jalapa L.	0·01	Sudden increase of 75% followed by rapid recovery
Capsicum frutescens L.	0·1	Transient decrease of 20–25%
**Lantana camara* L.	0·1	Transient increase of approx. 20%
**Kallstroemia maxima* Torr. et Gr. ..	0·1	Decrease of approx. 10% followed by a slight increase before recovery

Extacts of *Mangifera indica*, *Cordia brownei*, *Meliococca bijuga* and *Verbena bonariensis* had no effect on cat blood pressure in a dose of 0·1 ml/kg.

TABLE 5. EFFECTS ON BARBITURATE SLEEPING TIME IN MICE

Botanical name	Dose of extract ml/kg	Effect as percentage of control (= 100) and significance
Calotropis procera R.Br.	5	157, 0·01 < P < 0·02
Cassia emarginata L.	1	156, 0·01 < P < 0·02
Cassia fistula L.	10	135, 0·02 < P < 0·05
Tithonia diversifolia Gray	10	185, P < 0·001
Aristea compressa Buch.	5	155, P < 0·001
Trichilia hirta L.	10	231, P < 0·001
Polygonum chinense L.	10	130, 0·02 < P < 0·05
Capsicum frutescens L.	10	160, 0·01 < P < 0·02
Solanum verbascifolium L.	5	156, P < 0·001
**Lantana camara* L.	10	171, 0·001 < P < 0·01

For significance, P < 0·05

Extracts of *Ervatamia divaricata, Catalpa longissima, Terminalia catappa, Acacia lutea, Cissus sicyoides* and **Kallstroemia maxima* had no effect on the barbiturate sleeping time of mice in sublethal doses.

resins, a volatile oil and fats (Watt & Breyer-Brandwijk, 1962). The depressant effect of an aqueous extract of *T. hirta* on rabbit isolated heart and duodenum might have been due to the saponin component, but the cause of the clonic convulsions occurring on intravenous injection into mice and of the considerable increase in barbiturate sleeping time is not clear from our studies.

Mirabilis jalapa is probably little used medicinally in Jamaica, although in India the fresh leaf juice is taken for gonorrhoea and for uterine discharges, whilst an infusion of the leaves is said to be a diuretic and is used for dropsy. The alkaloid trigonelline, which is reputed to be non-toxic, has been isolated from the plant (Watt & Breyer-Brandwijk, 1962). In the extract tested the major constituent appeared to be a catecholamine which was shown to be present by paper chromatographic analysis. Alkaloids were not detected in the extract. The catecholamine probably caused all the effects observed in the pharmacological tests.

A number of plants in the Solanaceae contain alkaloids of pharmacological importance, e.g., atropine, (—)-hyoscine (scopolamine) and nicotine. Solanine is known to be present in *Capsicum frutescens*, and this or other alkaloids could possibly account for the pharmacological effects of this extract. The fruits of this plant are used medicinally and also as condiments (chillies and Cayenne pepper). The leaves are used in Jamaica and Africa as a dressing for wounds and sores, and one leaf boiled in a little water is thought to increase urinary excretion in babies (Asprey & Thornton, 1954). No reference has been found to the medicinal uses of *Solanum verbascifolium*, although related species are commonly used in Africa in the treatment of skin diseases, pneumonia, snake bite, colic and worm infestations. Solanine has been identified in various members also of this genus. It is possible that many of the effects observed in the tests with an extract of *S. verbascifolium* were due to the

presence of nicotine, but other alkaloids and also 5-HT may have been present as well.

Only plants with pronounced pharmacological activity have been discussed. Most of these plants are used in native medicine in their countries of origin. With the possible exception of those plants that contain tannins and are used as astringents it seems improbable that they are effective in treating the diseases for which they are administered.

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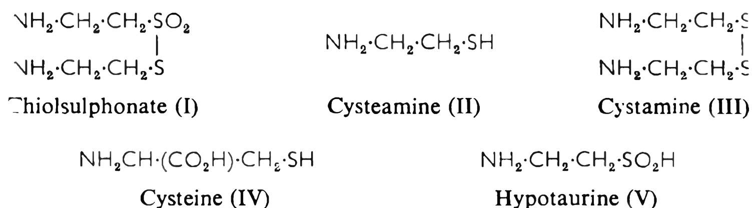
Radiation-protection of *Serratia marcescens* by a thiolsulphonate related to cysteamine

T. C. OWEN,* M. S. PARKER† AND G. M. STERN‡

A thiolsulphonate, 2-aminoethyl 2-aminoethanethiolsulphonate (also known as cystamine disulphoxide) affords significant protection to the micro-organism *Serratia marcescens* against the lethal effects of X-rays. The thiolsulphonate breaks down extensively at pH7, but the degree of protection given by it is much greater than can be accounted for by the action of decomposition products and is considered to be characteristic of the compound itself.

RECENTLY (Field, Ferretti, Crenshaw & Owen 1964), we have reported that a thiolsulphonate, 2-aminoethyl 2-aminoethanethiolsulphonate (I) related to cysteamine (II), and its *NN'*-diacetyl, *NN'*-didecyl, *NN'*-dimethyl and *NN'*-diureyl derivatives show significant radiation-protective activity in mice, comparable with the activity of cysteamine and its derivatives.

Among the many substances tested for radiation protective activity are amino-sulphur compounds closely related to cysteamine. Examples of these are cysteamine (II) itself (Bacq, Herve, Fischer, Lecomte, Blavier, Deschamps, LeBihan & Rayet, 1951), cystamine (III) (Bacq, 1956) and



cysteine (IV) (Patt, Tyree, Straube & Smith, 1949). The rationale leading to development and testing of thiolsulphonates as potential radiation protective agents derives from their ability to convert thiols into mixed disulphides (Field, Owen, Crenshaw & Bryan, 1961). The capability of certain protective agents for forming disulphide linkages involving thiol groups of tissue constituents has been suggested as an important basis for protective action (Pihl & Eldjarn, 1958). Thiolsulphonates, however, are unstable in alkaline medium. The aminothiolsulphonates of interest here are no exception, being reasonably stable in aqueous solution only as their mineral acid salts (pH *ca* 4·5) and decomposing rapidly when the pH is raised to 5·5 or above by addition of bases (Field & others, 1964).

The work here described was carried out to determine whether the radiation protective action is an intrinsic property of the thiolsulphonate,

From the Department of Chemistry and the Department of Pharmacy, College of Technology, Liverpool, 3.

*Present address: Department of Chemistry, University of South Florida, Tampa, Florida, U.S.A.

†Present address: Department of Pharmacy, University of Strathclyde.

‡Present address: Department of Biochemistry, University of Birmingham.

or whether it could be ascribed to the cystamine (a known protective agent) and hypotaurine (V) which would result from its spontaneous decomposition at body pH. The stoichiometry of decomposition approximates to the rapid formation of four molecules of hypotaurine and one of cystamine from three of the thiolsulphonate.

Testing in organisms at a pH (4·5 or below) where the thiolsulphonate would be stable is manifestly impossible in higher organisms where close maintenance of neutrality (pH *ca* 7) is essential to survival; while quantitative comparison of the protection afforded by the thiolsulphonate and by its decomposition products requires large numbers of test animals to obtain statistically significant results. Accordingly, we chose as a test organism a micro-organism, the red pigmented bacterium *Serratia marcescens*. Use of this bacterium permitted examination of the thiolsulphonate at pH 4·5 where it is stable. Although the organism does not multiply at the lowered pH it remains viable and will grow upon readjustment to pH 7·3. Furthermore a statistically significant comparison of the activity at pH 7·3 of the thiolsulphonate and of its decomposition products was possible. The results have little or no direct bearing on the behaviour of this protective agent in other organisms, but are meaningful *per se*. Several factors influenced the choice of this particular bacterium. It is relatively easily cultured and counted, its radiation sensitivity is high (Dewey, 1960) and it is currently used in these departments in a continuing study of the effect of calcium ion concentration on its radiation sensitivity. Simultaneous determination of the protection afforded by a number of other sulphur compounds was planned as corroborative information, and it was felt that this could valuably be with a micro-organism other than *Escherichia coli* which had already been most extensively studied (Kohn & Gunter, 1959).

Experimental

MICRO-ORGANISM PREPARATIONS

Bacterial suspensions were derived from 18 hr cultures of *S. marcescens* (NCTC 1377) grown at 25° on nutrient agar slopes. Suspensions for irradiation were made by adding 6 ml of air-saturated sterile buffer (pH 7·3 or 4·0 as required) to a cultured slope, agitating gently 20 times, and diluting 1 ml of supernatant suspension with 49 ml of the same sterile buffer. 10 ml portions of the resulting suspension were irradiated.

SURVIVOR COUNTS

The irradiated and control suspensions were serially diluted three times, each 1 ml to 50 ml, with sterile pH 7·3 buffer, giving a total dilution of 1 to $1{\cdot}25 \times 10^5$. Agar plates were inoculated with 0·3 ml portions of the diluted suspension. Ten plates were prepared from each sample and each control (a freshly sterilised pipette for each inoculation) making a total of either 20 or 30 plates per experiment. The inoculated plates were incubated for 24 hr at 25° and colonies then counted. As a check on the reliability of counting, and as a precaution against late development

of colonies, a number of plates were selected at random intervals and counted on three separate occasions. No discrepancy greater than 3 in an average of 60–120 colonies occurred.

IRRADIATION

Cell suspensions (10 ml) were exposed to X-rays (230 KV, 15 mA, unfiltered) at a dose rate of 1,147 rads/min. The same radiolysis bottle (25 ml capacity; stoppered) was used for each exposure, the bottle having been well "browned" by irradiation before commencement of the work. Considerable care was taken to ensure reproducible geometry of exposure. Dose rates were determined by conventional ferrous (Fricke) dosimetry using the same radiolysis bottle. The LD50 was determined by carrying out a number of irradiations at various radiation doses (Table 1). Percentage kills were converted to probits of kill by probit transformation

TABLE 1. IRRADIATION OF *S. marcescens*; LD50 DETERMINATION*

Irradiation time (sec)	Mean colony count	Standard deviation (σ)	χ^2	Probability %	Kill %	Probit of kill
0	63	2·6	0·22	>80		
30	56	2·5	0·23	>80	11·1	3·33
0	117	7·6	3·5	>80		
60	64	3·3	1·7	>99	45·3	4·37
0	149	4·0	0·44	>95		
90	74	3·6	1·1	>98	50·3	5·0
0	134	8·4	4·7	>80		
150	28	2·8	2·8	>98	79·1	5·32
0	167	3·8	0·43	>99		
180	29	3·5	2·1	>80	82·6	5·95

* Data used for construction of Fig. 1.
LD50 interpolated from probit regression line, 1,490 rads in 78 sec.

(Finney, 1962), the probits plotted against the logarithm of irradiation time and the time of irradiation for 50% kill determined from the resulting linear graph. A time of 78 sec, corresponding to an LD50 of 1490 rads was found. Direct plotting of log % survivors vs. dose indicates that an initial threshold dose is required before an exponential dose-survival relationship is established (cf. Alper, 1961 and Fig. 1).

Three 10 ml portions of bacterial suspension were used for each determination of protective activity and toxicity of each of the potentially protective substances. These were: (1) a control; (2) a toxicity check containing the protector but not irradiated; (3) a sample containing protector and exposed to an LD50 radiation dose. Ten plates were prepared from each. Duplicate experiments were made.

Oxygen consumption in air-saturated aqueous buffers exposed to this low dose is negligible, eliminating the need for aeration during irradiation and minimising any possible effects from endogenous anoxia.

MATERIALS

Cystamine (III) and the thiolsulphonate (I) were prepared by oxidation of cysteamine with hydrogen peroxide (Field & others 1961). The

resulting hydrochlorides were converted to sulphates by adding an equivalent amount of sulphuric acid to their aqueous solutions, and the water and hydrogen chloride were evaporated off under reduced pressure (Owen & Wilbraham, unpublished work). Hypotaurine (V) was prepared by a modification of Cavallini's procedure (Cavallini, de Marco & Mondovi, 1953). Cysteamine (II) was prepared by hydrolysis of thiazolidinethione. Glycerol was of B.P. quality and cysteine (IV) hydrochloride was B.D.H. laboratory chemical grade.

Buffer solutions contained (*a*) 2·0 g/litre KH_2PO_4(pH 4·0): (*b*) 1·0 g/litre KH_2PO_4 with 1·25 g/litre K_2HPO_4 (pH 7·3), and were air saturated.

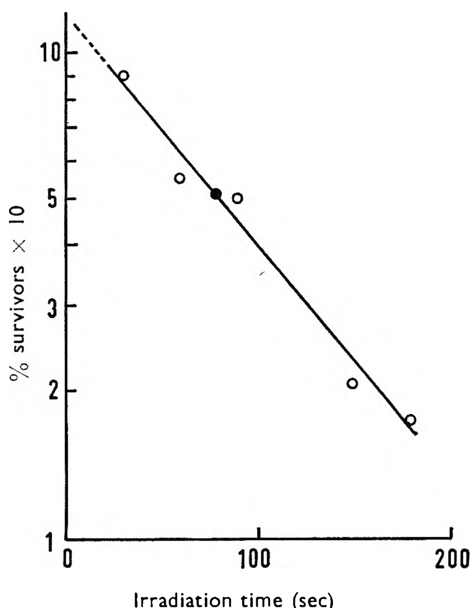


FIG. 1. Irradiation curve of *S. marcescens*.

PROTECTIVE ACTIVITIES

The protective activities and toxicities of these substances were examined by dissolving 10 mg in 10 ml suspensions of the bacteria. Preliminary work had indicated that higher concentrations were unduly toxic in certain instances and lower ones seriously impaired the significance of the protective activity observed.

Whilst the molecular weights of cystamine and the thiolsulphonate (sulphates) are roughly double those of cysteamine (hydrochloride) and hypotaurine, their equivalent weights are similar (125, 141, 113·5, 109 respectively) so that the equivalent concentrations (g equiv/litre) of the solutions used (10 mg in 10 ml) are comparable, ranging from 0·0071 for the thiolsulphonate to 0·0092 for hypotaurine. For the purposes of this

work, comparison of activities at similar equivalent concentrations is more pertinent than comparison at equimolar levels.

The protectors were added to the bacterial suspensions 5 min before irradiation, this time interval being indicated by preliminary studies. Kohn & Gunter (1959) had shown the importance of a "reactions period" (15 min) in chemical protection of *E. coli* B/r. With *S. marcescens* under our conditions no improvement of protection was observed after 5 min. The effects observed are reported in Table 2.

TABLE 2. IRRADIATION OF *S. marcescens*; PROTECTION AND TOXICITY. Radiation dose, 1,490 rads in 78 sec. Protector concentration, 10 mg/10 ml, pH 7·3*

Protective substance	Radiation dose (Rads)	Mean colony count	Standard deviation (σ)	χ^2	Probability %	Toxicity (% mortality)	Protection (a) (LD50 survival %)
None (control)	0	82	5·5	1·9	>80		
Thiolsulphonate (I)	0	45	2·9	1·3	>98	45	
sulphate (b)	1490	34	2·1	1·1	>99		76
None (control)	0	83	6·3	2·9	>80		
Cystamine (III)	0	69	5·4	3·8	>90	17	
sulphate	1490	35	2·4	0·97	>99		51
None (control)	0	114	5·5	1·6	>95		
Hypotaurine (V)	0	109	3·2	0·46	>99	4·4	
	1490	59	4·7	2·6	>90		54
None (control)	0	65	4·0	1·7	>95		
Cysteamine (II)	0	57	5·3	2·9	>80	12	
hydrochloride	1490	36	4·0	4·3	>80		63
None (control)	0	104	2·3	0·26	>99		
L-Cysteine (IV)	0	91	5·5	2·4	>90	12	
hydrochloride	1490	45	1·4	0·31	>99		50
None (control)	0	115	4·4	1·5	>99		
Thiolsulphonate (I)	0	101	7·0	2·4	>70	12	
sulphate (b) pH 4·0	1490	56	4·4	2·4	>90		55
None (control)	0	162	7·1	2·2	>90		
Dimethyl thiolsulphonate	0	9	0·79	0·56	>99	94	
hydrochloride (c)	1490	Not determinable due to high toxicity of compound					
None (control)	0	64	4·4	2·7	>95		
Glycerol	0	53	4·6	2·8	>90	17	
	1490	34	3·3	2·6	>95		64

(*a*) Number surviving irradiation in the presence of the protective substance, expressed as a percentage of the number surviving administration of the substance alone.
(*b*) 2-Aminoethyl 2-aminoethanethiolsulphonate sulphate.
(*c*) 2-Methylaminoethyl 2-methylaminoethanethiolsulphonate dihydrochloride. This compound protects mice (Field & others, 1964) but is toxic.
* Except where otherwise indicated.

STATISTICS

Since the interpretation of the results depends heavily on quantitative estimation of toxicity and protection afforded, a reliable estimate of the statistical significance of the data is essential. Appropriate values are included in Tables 1 and 2.

Discussion

While the single point method adopted is of limited utility it is adequate for the objectives of this work; that is, to compare quantitatively the protective ability of the various substances under a given, arbitrarily selected, set of conditions. We would stress that different conditions might well lead to quite different results in certain instances.

The thiolsulphonate (I) is toxic to the bacteria so that investigation at low concentrations was necessary. Even so, a considerable measure of protection was observed under the conditions of these experiments at pH 7·3 but not at pH 4·0 when the compound is more stable chemically. Of those bacteria which survived the inherent toxicity of the substance (55% of a control sample) three quarters survived an LD50 radiation dose. While this may seem only a moderate measure of protection, it is much greater than that afforded by the other substances here examined all at pH 7·3 under similar conditions. The protection afforded by the thiolsulphonate is therefore considered significant.

The data obtained for cystamine indicate moderate toxicity and no protective ability while those for hypotaurine indicate very low toxicity and very slight protection. Thus the protective activity of the thiolsulphonate cannot be ascribed to action of these substances produced by spontaneous decomposition at pH 7·3.

Even complete metabolic reduction of the thiolsulphonate to cysteamine (II), a most improbable occurrence particularly in air-saturated buffer, would not account for the level of protection observed. Cysteamine does protect *S. marcescens* under our conditions, but to a markedly lesser extent than does the thiolsulphonate. It seems, therefore, that the protective ability is an intrinsic property of this latter substance.

Several possible mechanisms of radiation protection by aminothiols have been suggested. These include (*a*) trapping of primary radiolysis radicals (H·, HO·, HO_2·) before these can interact with biochemically essential molecules (*b*) repair of damaged sites (produced by interaction of radicals with essential biochemicals) before oxygenative denaturation can occur, and (*c*) association of an essential sulphydryl group (*e.g.*, of enzyme or nucleoprotein) with the sulphydryl of the aminothiol, perhaps in a mixed disulphide linkage (see, e.g., Bacq, 1961). Quite probably all of these, and other, mechanisms may be involved.

Protection by the thiolsulphonate, seems to indicate that the mixed disulphide mechanism is at least involved here, although other mechanisms are by no means excluded. It is difficult to envisage its being a better radical trapping or repair agent than the less-oxidised cysteamine or cystamine, while its potentiality for rapid mixed-disulphide formation with thiols is well authenticated.

The observations with some of the other compounds studied merit mention. The protection afforded by glycerol, included here to give some basis for relating these studies to earlier work, was consistent with previous reports (Dewey, 1960). Surprisingly, L-cysteine showed no protective activity under our conditions; further investigation of this with different

concentrations and radiation doses is desirable. The thiolsulphonate exhibited low toxicity and negligible protection at pH 4 where it is reasonably stable, possibly because of non-absorption or metabolisation under these conditions. Finally, the rather high radiation-sensitivity of this micro-organism deserves comment. This is implicit in Dewey's observations and is confirmed by our finding of an LD50 of 1,490 rads, compared with about 3,500 rads for *E. coli* B/r (Kohn & Gunter). This makes for certain advantages (e.g. minimisation of endogenous anoxia) in studies at low doses such as those here described.

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A note on the alkaloidal content of *Datura innoxia* Miller

C. S. SHAH AND P. N. KHANNA*

Hyoscyamine, hyoscine and meteloidine have been found in the leaves, herbs and roots of *D. innoxia* Miller, but only hyoscyamine and hyoscine are present in flowers, pericarps and seeds. In addition, the roots contain 7-hydroxy-3,6-ditigloyloxytropane, (–)-3α,6β-ditigloyloxytropane, tropine and *pseudo*tropine. All the parts of the plant contain a higher percentage of hyoscyamine or hyoscyamine and meteloidine mixture than of hyoscine.

WILD *Datura innoxia* Miller is found growing widely in India. It is more abundant than *Datura metel* and *Datura metal* var *fastuosa*, which is official in the Pharmacopoeia of India (1955). The leaves of *D. innoxia* are reported to contain about 0·5% (–)-hyoscine (Trease, 1961). The total alkaloids in leaves, stems, roots, fruits and seeds of *D. innoxia* from Latin America have been reported (Gerlach, 1948) and also for the leaves, fruits and seeds from Punjab, India (Wealth of India, 1952). Evans & Partridge (1953) found hyoscine to be the main alkaloid in the leaves; hyoscyamine and meteloidine were also present. Steinegger & Gessler (1955) determined the alkaloidal pattern in leaves, stems and roots of *D. innoxia*, for the study of the relation between hyoscyamine and hyoscine at different stages of development of the plant. Evans & Wellendorff (1959) determined the percentages of individual alkaloids of roots of samples from Pakistan, from England and English grown plants from Texas seeds.

One year old plants growing on the college campus were used. They were authenticated by comparison with the description given by Santapau (1947). The individual alkaloidal content of *D. innoxia* growing in India has not previously been reported.

Methods and results

The method of Evans & Partridge (1952) was used for the extraction of total alkaloids, and the separation of individual alkaloids in the aerial parts was achieved according to the modified method of Evans & Pe Than (1962). Roots were examined using the method of Evans & Wellendorff (1959). Estimations were made according to Evans & Partridge (1952). The identities of alkaloids were confirmed by paper and thin-layer chromatography using pure substances for reference.

Hyoscine was confirmed in the ether fraction by paper chromatography (Evans & Pe Than, 1962). From the chloroform fraction, the spot on the paper chromatogram corresponding to hyoscyamine was eluted with ethanol, spotted on to thin-layer plates (8 in. × 8 in.) prepared of

From the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, 9, India.

* Senior research fellow under C.S.I.R. Scheme.

aluminium oxide G nach Stahl and developed with a mixture of chloroform:ethanol (1:1). Two spots were found, in the chloroform extracts of roots, leaves and herbs, one corresponded to hyoscyamine and the other to meteloidine. Seeds, pericarps and flowers showed only one spot corresponding to hyoscyamine.

7-Hydroxy-3,6-ditigloyloxytropane and (–)-3α6β-ditigloyloxytropane, in the light petroleum (60-80°) fraction, were confirmed on thin-layer plates (8 in. × 8 in.) prepared from aluminium oxide G nach Stahl and developed with ether.

Tropine, in the ammoniacal chloroform fraction, was confirmed by descending paper chromatography (Schwarting, 1963) on Whatman No. 1 paper, loaded with 0·5M KCl and developed with butanol:HCl (98:2) saturated with water. Tropine base, prepared according to the method of Youngken & Underhill (1962), served as a reference compound. On this paper chromatogram another spot corresponding in Rf to *pseudo*-tropine was found (Schwarting, 1963).

Spots on the paper chromatograms were developed by modified Dragendorf's reagent (Block, Durrum & Zweig, 1958), while iodine in carbon tetrachloride was used for the thin-layer chromatograms.

The results of the quantitative estimation of individual alkaloids are recorded in Table 1. This shows that all parts of the plant contain a

TABLE 1. DISTRIBUTION OF PRINCIPAL ALKALOIDS IN *Datura innoxia* MILLER

	I	II	III	IV	V
Leaf	0·564	0·031	0·270	0·276†	
Flower	0·517	0·029	0·130	0·364	
Herb	0·601	0·032	0·276	0·306†	
Seed	0·319	0·022	0·091	0·210	
Pericarp	0·146	0·028	0·033	0·037	
Root	0·610	0·106*	0·133	0·360†	0·016

I : Total alkaloids calculated as hyoscyamine.
II : Alkaloids with high Rf values calculated as hyoscyamine.
III : Hyoscine.
IV : Hyoscyamine/atropine.
V : Tropine and *pseudo*tropine. All %.

* : Mixture of (–)-3α6β-ditigloyloxytropane and 7-hydroxy-3,6-ditigloyloxytropane calculated as 7-hydroxy-3,6-ditigloyloxytropane.
† : Meteloidine present with hyoscyamine.

higher percentage of hyoscyamine or hyoscyamine and meteloidine mixture than of hyoscine. The higher percentage of hyoscyamine or the hyoscyamine–meteloidine mixture agrees with the observations of Steinegger & Gessler (1955) and the higher percentage of hyoscyamine and meteloidine mixture in the roots agrees with the findings of Evans & Wellendorff (1959).

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Letters to the Editor

Reactivity of Wistar rats to dextran

SIR,—The inflammatory anaphylactoid reaction produced in rats by the single intraperitoneal injection of dextran is mediated chiefly through a release of 5-hydroxytryptamine and histamine (Parratt & West, 1957). Recently, Harris & West (1963) found that not all rats of the Wistar strain react to this injection although the concentrations of histamine and 5-hydroxytryptamine in the skin of rats not reacting are similar to the concentrations in the skin of those reacting. Non-reactivity has since been shown to be a genetically-controlled recessive character (Harris, Kalmus & West, 1963). In the present work, we have found that rats obtained from one Wistar colony now contain the non-reactivity gene at a much higher frequency than when it was last reported. The cause of the frequency change has not so far been elucidated.

Wistar albino rats from random-mated stock of the Agricultural Research Council, Compton were injected with dextran (Intradex, Glaxo) according to the method of Harris & West (1963) and divided into two types: those which showed the anaphylactoid reaction consisting of gross oedema of the extremities (hereinafter called Reactors) and those which did not react (called Non-reactors). Although in January 1963 the percentage of non-reactors was similar to that found in the previous 4 years, by April 1963 more than half of the animals tested at each time failed to give the anaphylactoid reaction. The percentage of non-reactors reached above 80% of the total by September 1963 and has since remained at this level. These results are shown in Table 1.

TABLE 1. THE CHANGE IN PERCENTAGE OF NON-REACTOR WISTAR RATS OBTAINED FROM A.R.C., COMPTON, BETWEEN 1962–64

Year	Month	No. of rats tested	Non-reactors	
			No. found	Percentage
1962	Up to Oct.	2,600	573	22
1963	Jan.–Feb.	150	36	24
	April–June	405	206	51
	Sept.–Nov.	179	148	83
1964	Jan.–Feb.	170	136	80
	March–April	214	181	84
	May–June	252	219	87
	July–Sept.	110	90	82
	Oct.–Nov.	500	401	80
Total since September 1963		1,425	1,175	82

To maintain more than a 4 to 1 ratio in favour of non-reactivity which is a recessive character, it can be predicted that most of the reactors now being supplied are heterozygotes and carry the non-reactor gene. By selective breeding, this has proved to be so. Table 2 shows the results of various matings between reactors and non-reactors. The percentages of non-reactors found in the offspring are very close to those expected if the present reactors are mostly heterozygotes. It is hoped that these results will stimulate biologists to check their animal material for reactivity before attempting to determine the anti-inflammatory activity of a new compound by its ability to suppress the dextran anaphylactoid response.

TABLE 2. THE RESULTS OF VARIOUS MATINGS TO SHOW THAT THE REACTORS ARE PREDOMINANTLY HETEROZYGOTES

Mating	Litters	Offspring (F_1 generation)	Non-reactors		
			No. found	Percentage	% expected*
Reactor x Reactor	10	123	26	21	25
Reactor x Non-reactor	4	45	24	53	50
Non-reactor x Non-reactor ..	4	37	37	100	100

* % expected is the maximum number of non-reactors expected if the parent reactor is a heterozygote.

Department of Pharmacology,
School of Pharmacy,
University of London,
29–39, Brunswick Square,
London, W.C.1.

S. I. ANKIER
G. B. WEST

Department of Pharmacology,
School of Pharmacy,
College of Technology,
Moulescoomb,
Brighton, 7.

J. M. HARRIS
D. K. LUSCOMBE

December 1, 1964

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Sympathomimetic amines and vascular permeability

SIR,—Both dextran and egg-white increase vascular permeability when injected intradermally into rats and produce oedema when given subcutaneously into the plantar region of the foot. These reactions are prevented when large doses of adrenaline and noradrenaline are injected intravenously a short time before the dextran and egg-white (Parratt & West, 1958). A study has now been made of the relative activities of some sympathomimetic amines given *intradermally* in inhibiting these changes in vascular permeability.

Male Wistar albino rats obtained from Bengers Ltd., Holmes Chapel, were injected intravenously with azovan blue dye (7 mg/kg) and then given dextran (Intradex, Glaxo) intradermally into the ventral abdominal skin (100 μg/0·1 ml) and subcutaneously into one hind paw (6 mg/kg). In other areas of the abdominal skin, the dextran, mixed with varying amounts of the isomers of adrenaline, noradrenaline and isoprenaline, was injected in volumes of 0·1 ml whilst the other hind paw received dextran and one of the amines. (–)-Adrenaline was effective in doses of 1 μg intradermally and 5 μg subcutaneously. The relative activities of the other amines are shown in Table 1. Inhibition of the egg-white responses was also tested and found to be similar to that of dextran.

As (–)-noradrenaline is much less active than (–)-adrenaline, vasoconstriction does not appear to play an important role in these vascular permeability changes. The effect on carbohydrate metabolism is more likely since the relative activities of the amines are related to their ability to produce hyperglycaemia; in addition, exogenous glucose prevented both the dextran and egg-white responses. Bradykinin release may also be involved in these responses, and when the action of

bradykinin was tested the relative inhibitory activities of the sympathomimetic amines were similar to those recorded in Table 1. However, the doses used

TABLE 1. INHIBITION OF THE DEXTRAN RESPONSE IN RATS BY SYMPATHOMIMETIC AMINES

Route of injection of dextran	Dose of amine producing inhibition ((−)-adrenaline = 1)			
	(+)-adrenaline	(−)-noradrenaline	(+)-noradrenaline	(±)-isoprenaline
Intradermal	10	9	90	490
Subcutaneous.. ..	9	10	95	500

were much smaller; for example, 0·1 μg adrenaline was effective when given with or 10 min before the bradykinin dose (0·1 μg). In contrast, exogenous glucose exerted only a feeble inhibitory action on the bradykinin response.

Recently, Aschheim & Zweifach (1964) showed that intradermal adrenaline followed by the external application of xylene rendered rat skin resistant 24 hr later to intradermal injections of histamine liberators such as compound 48/80. They considered that the adrenaline-xylene treatment was both efficient and reliable as a method for depleting the skin of vasodilator amines. This is unlikely as we have found little release of histamine from the skin under this treatment. Moreover, when similar experiments were made with other sympathomimetic amines, the relative activities were very close to those shown in Table 1. Noradrenaline was much less active than adrenaline (minimum dose used was 0·1 μg), and isoprenaline was almost without effect. As with the bradykinin response, exogenous glucose had only a feeble action on the adrenaline-xylene treatment. It is possible that xylene inhibits the formation of bradykinin and adrenaline inhibits the action of free bradykinin, or that the adrenaline-xylene treatment fixes the tissue mast cells for a period of time.

Finally, we have evidence that adrenaline is more active than noradrenaline, and much more active than isoprenaline, in preventing the effects of thermal injury in rats (45° for 30 min). In these experiments, the sympathomimetic amines were given in divided doses to maintain suitable tissue levels and so prevent the effects of the bradykinin which is known to be released when hind paws of rats are kept at this temperature.

These experiments illustrate that adrenaline and possibly noradrenaline are likely to act as local anti-inflammatory hormones in the tissues, thereby controlling the development of inflammation resulting from injury.

Department of Pharmacology,
School of Pharmacy,
University of London,
29/39, Brunswick Square,
London, W.C.1.

R. A. Brown
G. B. West

December 17, 1964

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Degraded carrageenan and histamine-induced parietal cell hyperplasia in the guinea-pig

SIR,—Repeated administration of a depot injection of histamine acid phosphate to the guinea-pig will cause a parietal cell hyperplasia of the gastric mucosa (Cox & Barnes, 1945). Sulphated polysaccharides will reduce the volume and acidity of gastric juice secreted by guinea-pigs treated with depot histamine (Anderson, Marcus & Watt, 1962). The parietal cell is believed to be the acid secreting cell of the stomach and we report the results of an examination of the effect on the development of this histamine-induced hyperplasia in the guinea-pig of the sulphated polysaccharide degraded carrageenan (Ebimar, Evans Medical Ltd.) (Anderson, 1961) given at the same time.

Male albino guinea-pigs, 475 ± 50 g weight, maintained on a cube and cabbage diet were used in three groups: group A, control; group B received i.m. injections of histamine acid phosphate suspended in beeswax (10%)—arachis oil, 10 mg/kg thrice weekly for four weeks; group C received the same dose of histamine but had a 5% solution of degraded carrageenan *ad lib.* in place of the drinking water given to the other groups. At the end of the experiment the animals were killed and the stomachs removed.

Parietal cell counts were made by the method used by Cox & Barnes (1945) as modified by Marks (1957); staining was according to Marks & Drysdale (1957). During processing, shrinkage of the strips containing the parietal cells occurred, but this was assumed to be constant in both directions and in strips obtained from all three positions (greater curvature (posterior and anterior walls) and lesser curvature) and was, therefore, ignored. 18 to 24 counts were made and averaged for the number of parietal cells occurring from the surface to the base of the mucosa in the representative strips 0·1 mm wide and 8 μ thick. The nuclear diameter averaged 6·7 μ for all three groups. Parietal cell counts for each stomach were calculated in millions per kg total body weight. The results (Table 1) indicated that degraded carrageenan prevented the development of the histamine parietal cell hyperplasia, although there is still a significant rise in parietal cell numbers ($t = 2{\cdot}61$; $P < 0{\cdot}05$).

TABLE 1. THE REDUCTION OF HISTAMINE PARIETAL CELL HYPERPLASIA IN GUINEA-PIGS BY DEGRADED CARRAGEENAN

Group	No. of animals	Average body weight g	Parietal cell count millions per kg	
A	6	505	187	
				$t = 27{\cdot}3$; $P < 0{\cdot}001$
B	6	495	238	
				$t = 2{\cdot}50$; $P < 0{\cdot}05$
C	5	492	196	

Group A was control; group B received histamine i.m.; group C received histamine and 5% solution of degraded carrageenan in place of drinking water, consuming an average of 64 ml per animal per day.

In another control group of two animals which received only degraded carrageenan *ad lib.* (no histamine) the parietal cell count was unaltered after one month ($187{\cdot}0 \times 10^6$ parietal cells per kg; average daily intake of 5% solution of degraded carrageenan, 59 ml).

At death there was no evidence of peptic ulceration in any group. This absence of ulceration with a dose of histamine which produced peptic ulcers in fasted susceptible animals is, we believe, due to the continuous presence of food in the stomach. The stomachs in group B appeared to be larger *in situ* at death, although after fixation and preparation for photography, the stomach areas per kg body weight of the different groups were not significantly different.

Although the histamine hyperplasia is less when degraded carrageenan is administered the number of parietal cells is not reduced below the normal level in group C and the effect, therefore, appears not to be one of general parietal cell toxicity.

Evidence which suggests the possibility of a humoral action of degraded carrageenan has been found (Anderson & Soman, 1963) in guinea-pigs prepared by high duodenal ligation, where the sulphated polysaccharide, introduced distal to the ligature, diminishes histamine-stimulated gastric secretion. In the present experiments the same mechanism could operate.

Sulphated polysaccharides adhere to the stomach mucosa by combining with the protein and mucoprotein of mucin in acid conditions. During histamine stimulation the parietal cell will, in its hyperactive state, be associated with an unusually high hydrogen ion concentration which will favour such reaction in the vicinity or even on the surface of these cells, perhaps to an extent sufficient to interfere with their multiplication.

Department of Pharmacy,
University of Strathclyde,
Glasgow, C.1.
October 6, 1964

W. ANDERSON
P. D. SOMAN

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Effect of γ-aminobutyric acid upon strychnine convulsions

SIR,—It has been demonstrated that γ-aminobutyric acid (GABA) applied to the surface of the cerebral cortex of several animal species protects the animals from electrically or chemically induced seizures (Purpura & Grundfest, 1956; Purpura, Girado & Grundfest, 1957). Furthermore, several investigators have demonstrated that acute administration of GABA parenterally protects animals from electrically and chemically induced seizures (Hawkins & Sarett, 1957; McLennan, 1957; 1958).

In the course of our experiments, we found that parenterally administered GABA (3·0 g/kg) failed to afford immediate protection to rats from electrically induced seizures and also strychnine seizures. Pylkkö & Woodbury (1959) demonstrated that the CD50 of strychnine was increased in rats pretreated with GABA 72 hr before treatment with the convulsant. This observation prompted us to study further the time course of the protective properties of GABA against strychnine seizures.

Mature male albino Holtzman rats were used. γ-Aminobutyric acid and strychnine sulphate were dissolved in saline and given intraperitoneally. The

animals were pretreated with 3·0 g/kg GABA; and strychnine was administered administered 30 min, 1, 2, 3, 4, and 15 days after GABA and the CD50 values for these animals determined ($CD50_1$) according to Litchfield & Wilcoxon (1949). The CD50 values for strychnine were calculated at the same time intervals for rats not pretreated with GABA ($CD50_2$). The potency ratio (PR = $CD50_1/CD50_2$) and the fP.R. were calculated by the method of Litchfield & Wilcoxon (1949).

TABLE 1. THE EFFECT OF GABA ON CD50 OF STRYCHNINE

Weight (g)	Number of rats	$CD50_1$ with GABA mg/kg	Number of rats	$CD50_2$ without GABA mg/kg	Potency ratio $CD50_1/CD50_2$	$f_{P.R.}$
112–128	18	½ hr after GABA: 1·9 (1·7–2·1)	18	2·6 (2·3–2·9)	0·7 (0·6–0·8)	1·2
130–188	18	1 day after GABA: 2·9 (2·5–3·4)	18	2·4 (2·1–2·8)	1·2 (0·9–1·6)	1·3
124–152	18	2 days after GABA: 2·8 (2·5–3·1)	18	2·7 (2·4–3·0)	1·0 (0·8–1·2)	1·2
86–114	18	3 days after GABA: 2·5 (2·3–2·7)	18	2·0 (1·8–2·2)	1·3 (1·2–1·4)	1·1
117–134	18	4 days after GABA: 2·8 (2·5–3·2)	18	2·5 (2·3–2·7)	1·1 (0·9–1·3)	1·2
180–237	18	15 days after GABA: 3·3 (2·9–3·7)	18	2·7 (2·4–3·1)	1·2 (1·1–1·3)	1·1

The results are shown in Table 1. It is evident that 3 and 15 days after GABA administration the CD50 of strychnine was elevated significantly.

It has been established by Eccles (1956) that strychnine selectively blocks the inhibitory synaptic transmission in the central nervous system. Therefore it is of interest that the convulsant activity of strychnine is altered 3 and 15 days after a single dose of GABA.

At the present, however, it has not been established whether GABA blocks excitatory synapses or enhances inhibitory transmission in the central nervous system. Furthermore, it still remains to be determined if GABA alone or a metabolite of it is responsible for the postulated inhibitory effects of this amino-acid. If the elevation of the CD50 of strychnine 3 and 15 days after GABA is due to this amino-acid, the observations presented seems to indicate that GABA has a long onset of action as an inhibitory agent. Whether GABA alone or a metabolite of it is responsible for this phenomenon also remains to be determined.

Hazleton Laboratories, Inc.
P.O. Box 30
Falls Church, Virginia, U.S.A.
December 22, 1964

HEINZ SORER
OLAVI PYLKKÖ

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Timing of therapy in experimental poisoning with organophosphorus compounds

SIR,—In previous studies on experimental therapy of acute poisoning with organophosphorus compounds in rats using pralidoxime and atropine (Sanderson & Edson, 1959; Sanderson, 1961), some of the results suggested that intraperitoneal injection of pralidoxime and atropine together immediately after oral administration of some organophosphorus compounds gave less benefit than either drug separately. With certain of the compounds, notably morphothion, this treatment brought about a worsening and acceleration of anticholinesterase effects and mortality, during the first half hour only, which exceeded the effects produced by the organophosphorus compound alone. These effects did not occur when morphothion was given intraperitoneally, or when treatment was with atropine or pralidoxime alone.

As this observation could have important implications in therapy for organophosphorus poisoning in man, these findings have now been re-examined. In the present experiments, therapy in rats was delayed in some animals until the onset of toxic effects, thus confirming organophosphorus poisoning, otherwise the methods were unchanged (Sanderson, 1961). The results of these experiments are summarised in Table 1. As previously found, a combination of pralidoxime and atropine given immediately caused accelerated anticholinesterase effects which were usually lethal, while treatment with atropine alone, or in combination with pralidoxime, delayed till onset of effects, was beneficial.

Thus while harmful effects can arise with the combined pralidoxime-atropine therapy for poisoning if this therapy is begun before the onset of symptoms, these

TABLE 1. EFFECT OF ATROPINE AND ATROPINE/PRALIDOXIME THERAPY ON MALE RATS GIVEN ORAL MORPHOTHION

Treatment group	First injection	Deaths in test period	Time of onset	Time of deaths	Observations
I None (control)	—	5/6	30 min	8–23 hr	Normal slow anticholinesterase action
II Atropine A	Immediately after morphothion	2/6	35 min	8½–23 hr	Good control of secretions; general condition improved.
B	After onset of of poisoning	1/6	30 min	9–23 hr	Similar to previous group
III Pralidoxime + atropine A	Immediately after morphothion	5/6	5–8 min	15 min –4½ hr	Rapid development of severe anticholinesterase effects, with 4 deaths in first 20 min, then recovery apparently complete after 45 min and recurrence as treatment wore off
B	After onset of poisoning	2/6	35 min	9–23 hr	Recovery apparently complete in 60 min, then recurrence as treatment wore off, becoming marginally better than Group IIB

Doses: morphothion, 300 mg/kg orally; atropine sulphate, 17·4 mg/kg, and pralidoxime iodide, 100 mg/kg, i.p. repeated after 4 hr, then given s.c. at 8 and 24 hr.

effects are not seen when treatment with the combination is delayed until the onset of symptoms.

Medical Department, D. M. SANDERSON
Chesterford Park Research Station,
Nr. Saffron Walden, Essex.
December 11, 1964.

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Ethanolamine and anaphylactic shock

SIR,—As is well known, mepyramine and other antihistamines protect guinea-pigs against anaphylactic shock. Smith (1961) has reported that ethanolamine alone has no protective effect but potentiates the protective effect of mepyramine. In addition, he made experiments with guinea-pig ileum and suggested that ethanolamine inhibits the SRS-A liberation in anaphylaxis. Because of the fundamental interest of his observations we have repeated the protection experiments with the same method (Herxheimer, 1952), giving mepyramine and ethanolamine intramuscularly before the animals were shocked. The results were calculated according to the method of Armitage, Herxheimer & Rosa (1952) which differs somewhat from the calculation of Smith in the mathematical expression of the protection but leads to comparable conclusions. The Table shows that the combination of ethanolamine and mepyramine has no greater protective effect than mepyramine alone. It even appears that the combination of 1·0 and 0·05 mg mepyramine with 20 mg of ethanolamine protected less than mepyramine alone. The animals were fed with pellet food containing additional ascorbic acid supplemented with hay.

We therefore are unable to confirm the results reported by Smith.

TABLE 1. EFFECTS OF ETHANOLAMINE AND MEPYRAMINE IN PROTECTING GUINEA-PIGS FROM ANAPHYLATIC SHOCK

Ethanolamine + Mepyramine		Mepyramine
Ethanolamine 20 mg/kg, Mepyramine 0·01 mg/kg		Mepyramine 0·01 mg/kg
$\bar{x}$ = 30	← $P > 0{\cdot}35$ →	$\bar{x}$ = 32·5
n = 14		n = 44
s.e. = 4·9		s.e. = 4·5
Ethanolamine 20 mg/kg, Mepyramine 0·05 mg/kg		Mepyramine 0·05 mg/kg
$\bar{x}$ = 34·2	← $P < 0{\cdot}001$ →	$\bar{x}$ = 54·9
n = 42		n = 35
s.e. = 4·2		s.e. = 2·9
Ethanolamine 20 mg/kg, Mepyramine 1·0 mg/kg		Mepyramine 1·0 mg/kg
$\bar{x}$ = 68	← $P < 0{\cdot}01$ →	$\bar{x}$ = 79·5
n = 9		n = 24
s.e. = 3·9		s.e. = 2·4

$\bar{x}$ = mean antianaphylactic protection in % (ranging from 0–100%)
n = number of experiments
s.e. = standard error
P = level of significance of difference between two results

H. HERXHEIMER
E. STRESEMANN

Rudolf Virchow Krankenhaus,
Berlin 65
December 16, 1964

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Identification of norbormide, a new *Rattus* specific rodenticide

SIR,—A new rodenticide, norbormide [Shoxin, 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)norborn-5-ene-2,3-dicarboximide] has recently become commercially available. It is claimed that this substance is specific to *Rattus*, and has no harmful effects on domestic animals even when consumed in massive quantities (Roszkowski, Poos & Mohrbacher, 1964). As it seems likely to become widely used, its identification will become a matter of importance in forensic science, and it will be necessary to distinguish it from other alkaloidal compounds of more general toxicity.

Norbormide may be recovered from biological material in the alkaline-chloroform fraction of a Stas-Otto or similar extraction process. On a citrate buffered paper chromatogram (Curry & Powell, 1954) it gives a bright blue fluorescent spot at Rf 0·87, positive with iodoplatinate solution and with Dragendorff's reagent. On a thin-layer chromatogram [Silica gel-sodium hydroxide-methanol (Sunshine & Fike, 1964)] it gives a similar spot at Rf 0·84. The ultraviolet spectrum in 0·1 N hydrochloric acid shows maxima at 300 mμ and 240 mμ, a shoulder at 258 mμ and a minimum at 287 mμ. The compound gives a blue colour with ammonium molybdate/sulphuric acid (Clarke & Williams, 1955), and dense yellow rosettes, forming slowly, with platinic chloride solution (Clarke & Williams, 1955). These characteristics enable microgram quantities of norbormide to be distinguished from other basic compounds.

Department of Physiology,
Royal Veterinary College,
Royal College Street,
London, N.W.1.
December, 10, 1964

E. G. C. CLARKE

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Book Reviews

RESEARCH PROGRESS IN ORGANIC-BIOLOGICAL AND MEDICINAL CHEMISTRY. Edited by U. Gallo and L. Santamaria. Vol. 1. Pp. xvi + 583. Società Editoriale Farmaceutica, Milan, 1964. $19·00.

This volume, initially planned as a single dedicatory volume to the first centenary of the "Bollettino Chimico Farmaceutico" (1861–1961), appears as such, containing contributions only by Italian research scientists. It serves, also, as the first of what is to be a series of volumes on "Research Progress in Organic-Biological and Medicinal Chemistry", inviting contributions on an international basis. Whilst the present volume is especially to be welcomed as a centenary volume, marking the contribution of Italian scientists to pharmaceutical research and to research in allied subjects, the wisdom of launching yet

another international series of research reviews in this field is surely to be questioned. Unnecessary duplication of effort is already not unknown, and, regrettably, duplication of publication by the same authors in different series. Such repetition is wasteful of scientific effort and might well be avoided by restriction to a few strictly-controlled outlets for publication rather than their multiplication. It is to be hoped, therefore, that this new series will be marked by an insistence on originality and a firm rejection of material which has already appeared elsewhere in the same form.

The present volume contains eleven reviews covering a variety of aspects of Italian work in the field of medicinal, pharmaceutical and pharmacological chemistry. Biochemical studies are represented by a review of tryptophan metabolism in man, which includes a short and useful account of analytical methods, but which is mainly devoted to a consideration of the relationship between tryptophan metabolism and some pathological states. A useful survey of the absorption, distribution and excretion of vitamin B_{12} covers not only intestinal absorption by simple diffusion and intrinsic factor-facilitated diffusion, but also considers in some detail the nature and behaviour of vitamin B_{12} binding factors. Other reviews survey in considerable detail the isolation and chemistry of such naturally-important substances as the acid-soluble nucleotides of fungi, ethers of steroidal hormones, echinulin, and the rifamycin family of antibiotics. The review of Italian contributions to the chemistry of certain alkaloids of South American origin is disappointing; much of the work contributing to the structure of these complex compounds has been of an international character, and this no doubt accounts for the rather unfortunate scrappy appearance of an account which is very largely concerned only with Italian work in this field. In medicinal chemistry, Bovet and Rosnati's account of curarimimetics, whilst repetitive of many similar previous accounts, is both logical and acceptable in this context, and also delightful in its simplicity and clarity of exposition. Its concern in some detail, too, with the potentiation of curarimimetics by SKF 525–A, is both critical and useful in the new stimulus which it must give to those interested in the reversibility of curare-like drugs. The account is marred only by some confusion in nomenclature on pages 92 and 93, where C-curarine is occasionally referred to as curarine. A review of azetidines covers such important chemical properties as acid and heat stability and surveys a range of pharmacological actions exhibited by various compounds of this heterocyclic type. The survey of photodynamic substances, which are capable of producing damaging effect in biological systems under the influence of light, is extremely valuable, not only providing a chemical classification of substances known to produce such effects, but also selecting certain groups such as the furocoumarins, the phenothiazines and polycyclic hydrocarbons for fuller discussion. The mechanism of photodynamic actions and photodynamic sensitivity in cells and tissues are also discussed. The volume is completed by a contribution on analeptics, which is entirely pharmacological, including aspects of toxicity pneumokinetic action, cardiovascular effects and antagonism with C.N.S. depressants.

Considerable credit is due to the skill of the translators who have produced the English translation, which, apart from a few minor errors which give away its Italian origin (page 114 "schema" for "scheme"; page 158 "oxigenated" for "oxygenated"; page 59 "o.Aminohippuric acid" for "*o*-Aminohippuric acid"—both forms are used on this one page) is very well presented, interesting, and easy to read.

J. B. Stenlake

MEDICAL PHARMACOLOGY. Principles and Concepts. By Andres Goth. Second edition. Pp. 585 (including Index). Henry Kimpton, London, 1964. 88s.

The remarkable developments in the chemical and biological sciences during this century have led to the synthesis and clinical use of large numbers of potent drugs giving a measure of control of disease to an extent undreamed of even 30 years ago. So complex is the present-day picture in terms of biochemistry, biophysics and physiology, that the task of relating what he is taught of the basic biological sciences to the rational, selective, clinical use of a synthetic drug is proving increasingly—and understandably—beyond both practitioner and student.

It would be wildly optimistic to suggest that today, a course of pharmacology could be given, or a book written which would enable the student to predict the therapeutic and toxic actions of a compound he has not met before. It is, however, possible to enable similar-acting drugs, especially those closely related chemically, to be compared and the claims of manufacturers to be logically assessed. Unfortunately the task is too seldom attempted. Dr. Goth obviously has this objective in view and he meets with a measure of success. His book is, however, too short for him to be able to set out adequately what is known of the mode of action of drugs and at the same time discuss as fully as he does their clinical pharmacology and toxicology. The compass is only 547 pages which includes bibliographies, some of which are quite lengthy, and numerous well-drawn formulae and diagrams.

The layout and choice of subject-matter are disappointingly conventional, but chapters on poisons and antidotes and prescription writing, and sections on clinical pharmacology together with the general emphasis upon clinical application indicate the bias towards practical medicine. The commonly used drugs are all included and many new compounds are mentioned. The style is economical and the text clearly written.

There is a certain unevenness in the treatment. Thus adrenaline and noradrenaline receive adequate detailed discussion, histamine gets a chapter to itself, and 5-hydroxytryptamine most of another. Yet acetylcholine, a knowledge of the properties of which is the surest foundation for a study of the pharmacology of the autonomic nervous system, is dealt with only scantily, and the information is scattered about the text. Too little attention is paid to the increasingly important biochemical and physicochemical theories of anaesthesia; it is not sufficient to refer the reader to a review published some 14 years ago or even to the recent original papers. It is surprising, too, that more is not said about the mode of action of the salicylates, while structure-activity relationships are given little space. Thus although the clinical aspects of the subject matter get due consideration, the reader is too often sent to the original literature or to a review article if he shows an interest in mechanisms of action. One other criticism is, that although the diagrams are well reproduced and show evidence of careful selection they are not always adequately dealt with in the text, and the legends frequently need expansion and clarification.

Nonetheless this book has a very great deal to commend it to the reader. It contains a surprising amount of information. It is readable and interesting, while the author takes a forthright but balanced view of the use of drugs. As a text it will appeal most strongly to the student of medicine in search of a short book. American nomenclature is used throughout, but British approved and official names are included and can be found in the index. It is well worth a place on the bookshelf.

J. J. LEWIS

PERGAMON PRESS PUBLICATIONS

Pteridine Chemistry

Proceedings of the Third International Symposium held at the Institut für Organische Chemie der Technischen Hochschule, Stuttgart

Edited by W. Pfleiderer, Institut für Organische Chemie der Technischen Hochschule, Stuttgart; and E. C. Taylor, Department of Chemistry, Princeton University.

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