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Review Article

The flow properties of emulsions*

P. SHERMAN, M.Sc., F.R.I.C.

EMULSIFICATION is essentially a process for diluting oils so as to facilitate their use in several ways. For example, in the field of pharmacy a medicinal oil may not only be more acceptable orally when administered as an oil-in-water (o/w) emulsion but it may also be absorbed more readily. Similarly, the application of an oil to the skin in emulsion form promotes easier spreading and absorption. The performance of such products depends very much on their flow properties. Unfortunately, most emulsions, apart from very dilute ones, do not exhibit simple Newtonian flow, that is, their viscosity is not independent of the applied rate of shear, so that the interpretation of flow data is often difficult.

The flow of Newtonian liquids can best be illustrated by the following model. Imagine the space between two parallel planes separated by a distance x to be filled with liquid. If a force F is applied to the upper plane A (Fig. 1), while the lower plane B remains stationary, A will move at a constant velocity u. All the liquid between the two planes does not move at the same speed. Instead the rate varies with the distance from A, being a maximum (u) in the layer adjacent to A and zero in the layer adjacent to B. The rate of change in fluid velocity is given by du/dx. This represents the rate of shear (v) of the liquid, whilst the force per unit area applied to A represents the shearing stress (S). The viscosity of the liquid (η) is given by the ratio S/v, and since this remains constant, η can be determined by a single measurement irrespective of the magnitude of S or v.

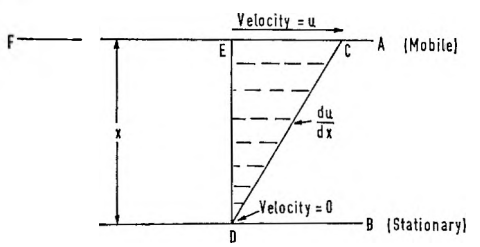

FIG. 1. Model for Newtonian flow.

Many liquids, emulsions, or suspensions which do not show this behaviour fall within the category exhibiting non-Newtonian behaviour (Fig. 2). This category includes both plastic and pseudoplastic flow. In pseudoplastic flow viscosity decreases curvilinearly with increasing rate of shear from the initial application of a shearing stress, whilst in plastic flow a minimum shearing stress (S_0) is required before flow begins.

* Based on three lectures given at the Post-Graduate School for Pharmacists, School of Pharmacy, University of London, April, 1963.

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Above S_0 viscosity decreases curvilinearly with increasing rate of shear. Ultimately, at infinitely high shear, the viscosity of either system does not alter with further increase in the shear rate.

Two other shear stress values are often quoted when reporting plastic flow data. These are the extrapolated yield value, the intercept on the shear stress axis obtained by extrapolation from the linear part of the curve, and the upper yield value, which refers to the applied shearing stress at which linear flow is first established. In dilatant flow the viscosity increases as the rate of shear increases, that is, the flow characteristics are the reverse to those encountered in pseudoplastic flow.

Since the viscosities of pseudoplastic, plastic, and dilatant emulsions vary with the rate of shear, measurements made at a single rate of shear have little significance, particularly when comparing the flow behaviour of two different emulsions. A reliable analysis involves detailed measurements over a wide range of shear rates.

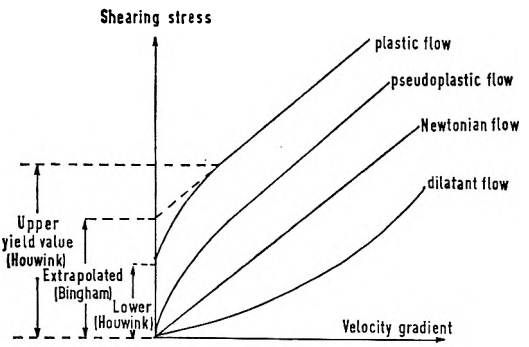


FIG. 2. Examples of flow behaviour.

Measurement of viscosity

TYPES OF VISCOMETER

Many commercial instruments are available for measuring viscosity. The principal kinds, and some typical examples of each type are enumerated in Table 1. Not all of these viscometers are designed to study the variation of viscosity with rate of shear. This applies particularly to the falling sphere and ultrasonic viscometers. Of the other three types, the cone-plate viscometer is the only one which provides uniform shearing conditions throughout the sample. In the capillary viscometer the rate of shear varies from zero at the capillary axis to a maximum at the wall surface. The measurement of viscosity in a coaxial cylinder instrument usually involves rotation of one of the cylinders, leading to a torque being transmitted through the test sample to the other cylinder. In this example the rate of shear varies from a maximum value at the rotating cylinder surface to a minimum at the surface of the other cylinder. By a suitable design, so that the thickness of the sample layer is small, the rate of shear gradient can be minimised.

TABLE 1. PRINCIPAL TYPES OF VISCOMETER FOR USE WITH EMULSIONS

	Application	
	Newtonian flow	Non-Newtonian flow
A. *Capillary*—		
U-tube; single or multi-bulb	X	X (multi-bulb only)
Variable pressure	X	X
Techne vibrating piston	X	X
B. *Coaxial Cylinder*—		
Ferranti Portable	X	X
Epprecht Rheomat	X	X
B.F.M.R.A. (Gaydon & Co.)	X	X
Rotovisko (Gebrüder Haake)	X	X
C. *Cone-plate*—		
Ferranti-Shirley	X	X
Rotovisko (Gebrüder Haake)	X	X
Weissenberg Rheogoniometer	X	X
D. *Falling sphere*—		
Höppler	X	—
E. *Ultrasonic*—		
Bendix Ultra-Viscoson	X	—

The principal practical disadvantage of capillary instruments is their unsuitability for studying the effect of time of shear on viscosity at any given rate of shear, and hysteresis effects, since the sample in the capillary is changing all the time. On the other hand, when time effects are shown, instantaneous viscosity data are obtained more easily with this instrument than with the coaxial or cone-plate instruments, unless an automatic recording device is connected up to the latter. The capillary instrument does not give rise to end effects, the Weissenberg effect, temperature fluctuation due to heat development, and to the danger of sample structure being altered due to homogenisation when sheared. All these phenomena may arise when using a coaxial instrument and a further source of error may result from incorrect alignment of the cylinders. Possibly the main advantage of a capillary instrument over a coaxial instrument is that higher rates of shear can be achieved with the former, thus extending its application to a wider range of flow behaviour.

When interpreting viscosity data obtained with a capillary viscometer the following points should be borne in mind.

(i) As the test sample moves from the wide tube in which it is initially deposited into the much smaller diameter capillary, the sample is deformed around the shoulder of the wider tube. The correction which has to be applied for this "end effect" (or more correctly, the "entrance effect") can be minimised if the capillary has suitable dimensions.

(ii) Entry of a dispersion, or an emulsion, into a capillary may be accompanied by axial migration of the disperse phase. This gives rise to concentration fluctuations across the capillary width, the principal concentration reduction occurring in the sample layers nearest the capillary wall.

(iii) Part of the applied pressure is used to impart kinetic energy to the sample when it enters the capillary. All the pressure is not used, therefore,

in shearing the sample. This "kinetic energy correction" is more important than (i).

(iv) The shearing stress (S_0) required at any point in a capillary to produce flow is given by

$$S_0 = PR/2L \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where P is the applied pressure, R is the capillary radius at that point, and L is the capillary length. Near the capillary axis R is very small, so that P would need to be infinitely large to exceed S_0. This is not possible under practical conditions. Consequently, near the axis there is always a thin layer of sample which moves through the capillary as a solid plug ("plug flow"). From this we may infer that the S—v curve for capillary viscometer data never becomes absolutely linear at high rates of shear.

Newtonian viscosity data obtained with a falling sphere viscometer are interpreted on the basis of Stokes's law for a sphere falling through a liquid at a constant speed. If the sphere is relatively large in comparison to the diameter of the tube through which it falls a more complex equation has to be used (Ladenburg, 1907; Flowers, 1914; Faxen, 1922). Recently it has been shown that using very small nylon spheres several tests can be made on a single sample (Scott-Blair & Oosthuizen, 1960). By rotating the tube between tests unsheared material becomes available for further determinations.

The ultrasonic viscometer operates very simply. It consists essentially of a probe and an electronic computer. At the end of the probe is a thin alloy steel blade, which is excited by a short electrical impulse, so producing ultrasonic shear waves in the medium around the probe. The computer translates the energy requirement for this motion into viscosity. Whilst this method lends itself to automatic control of viscosity, its range of application is very limited at present. The single available impulse permits viscosity measurement at only one rate of shear, so that it cannot be used for non-Newtonian systems.

Table 2 (after McKennell, 1956) summarises the equations required to calculate rate of shear, shear stress, yield value, and viscosity from the data obtained using the main types of viscometers discussed in this section. In capillary instruments both shearing stress and rate of shear vary from zero at the axis to a maximum at the capillary wall. When determining non-Newtonian viscosity it is customary to base the calculation on the conditions prevailing at the capillary wall.

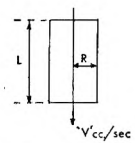
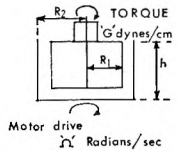
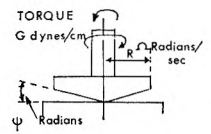
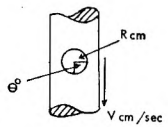
General interpretation of non-Newtonian viscosity

Many attempts have been made to define mathematically the shape of the S—v curve for plastic flow. Bingham (1922) proposed an equation for an idealised system in which the S—v relationship is linear overall provided the critical shearing stress at which flow begins (S_0) has been exceeded.

$$v = u\,(S - S_0) \quad .. \quad .. \quad .. \quad (2)$$

where u is the mobility, or $1/\eta_\infty$.

TABLE 2. EQUATIONS REQUIRED TO CALCULATE RATE OF SHEAR, SHEAR STRESS, YIELD VALUE AND VISCOSITY FROM DATA OBTAINED USING THE MAIN TYPES OF VISCOMETER DISCUSSED

	Capillary	Coaxial cylinder	Cone-plate	Falling sphere
Viscometer				
Newtonian Viscosity η	$\eta = \pi PR^4/8LV$	$\eta = \frac{G\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)}{4\pi h\Omega}$	$\eta = \frac{3G}{2\pi R^3} \Big/ \frac{\Omega}{\psi}$	$\eta = \left(\frac{2}{9}\frac{d_1 - d_2}{v}\right) gR^2$ uncorrected
Non-Newtonian viscosity η_∞ ..	$\eta_\infty = \frac{\pi R^4 g\sigma}{8Le} \frac{1}{\left(\frac{dQ}{dP} + \frac{P}{4} \cdot \frac{d^2Q}{dP^2}\right)}$	$\eta_\infty = \frac{M}{4h\Omega}\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right) - \frac{v}{\Omega} \ln \frac{R_2}{R_1}$		
Rate of shear v	$v_{\text{capillary wall}} = \frac{4Q}{\pi R^3}$ $v_{\text{axis}} = 0$	$v_{max} = \frac{2\Omega}{R_1^2\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)}$ $v_{min} = \frac{2\Omega}{R_2^2\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)}$	$v = \frac{\Omega}{\psi}$	$v_{max} = \frac{3V}{2R}$ $(\theta = 90°)$ $v_{min} = 0\ (\theta = 0°)$
Shear stress S	$\frac{PR}{2L}$	$\frac{\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)}{4\pi h}$	$\frac{3G}{2\pi R^3}$	
Yield value f	$\frac{P_0 g\sigma R}{2L}$ $P_0 = Pv_{\sim 0}$	$\frac{S\,G_2}{\ln(R_2/R_1)}$	SG_2	

G_2 = extrapolated value of torque for $\Omega = 0$. σ = density of Hg. e = constant for converting flowmeter readings into c.c/sec efflux.

Ostwald (1925), and de Waele (1925), independently modified Poisseuille's equation, and both arrived at the same result.

$$v = \frac{1}{\eta^*} S^n$$

or in more detailed form

$$V^n = \frac{\pi R^4 (P - ae^{-PR/2}}{8L\eta^*} \quad .. \quad .. \quad .. \quad (3)$$

where n is a measure of the structure developed within the test material, having a value of 1 for a Newtonian fluid, 'a' is some form of yield value, and η^* is the viscosity. Several objections have been raised against eqn 3. In some systems η^* does not have a steady value; instead it fluctuates with variation in n, which may arise from change in S. Furthermore η^* does not have the correct dimensions for viscosity.

Herschel & Bulkley (1926), and Scott (1931), developed power equations resembling eqn 3.

$$v = u (S - a)^n \quad .. \quad .. \quad .. \quad .. \quad (4)$$

In general, power equations have no theoretical significance because they will fit any viscosity data provided the constants are suitably adjusted.

Krieger & Dougherty (1959) assumed that temporary pairing of particles occurred during flow, due to localised concentration fluctuations, and that the viscosity of such a system is related to S by

$$\frac{\eta_S - \eta_\infty}{\eta - \eta_\infty} = \left[1 + \left(\frac{S}{S_c}\right)\right]^{-1} \quad .. \quad .. \quad (5)$$

where η_s, η_∞, and η are the limiting viscosities at shear stress S, at infinite shear, and at zero shear respectively. S_c depends on particle size and temperature.

Particles actually form doublets, or triplets in the absence of shear due to inter-attraction forces, and statistical considerations indicate that such aggregates take the form of long chains. To deflocculate these aggregates S and v may be related by an equation of the form (Casson, 1959):

$$S^{\frac{1}{2}} = K_0 + K_1 v^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where K_0 and K_1 are constants, their values depending on the properties of the solid and liquid phases respectively.

Williamson (1929) restricted his attention to pseudoplastic flow. He suggested a graphical procedure for calculating the total power required to achieve linear flow from the S—v curve.

$$Sv = S_p v + S_1 v \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where $S_p = PR/2L$ (plastic resistance)

$S_1 = PR/2L$ (viscous flow)

The total power (Sv) is the sum of two independent contributions. The power required to overcome plastic resistance (S_p v) is used to break

down the structure arising from particle aggregation when the system is at rest. When shear is applied the particles deflocculate; further increase in shear rate provides power (S_1 v) to overcome the viscous resistance of the deflocculated system.

Eqn 5 can be written as

$$S = \frac{S_\infty v}{\phi + v} + \eta_\infty v \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where ϕ is a measure of the curvature of the graph showing the change in S required to overcome the plastic resistance with change in v. This graph is derived from the pseudoplastic flow curve. S_∞ is S_p at infinitely high rate of shear. When $S_\infty = O$, the S—v plot will be a straight line, and Poisseuille's equation is applicable. Flow is defined by eqn 2 when $\phi = 0$.

Williamson's treatment gives satisfactory results only when the curved and linear portions of the S—v curve are well defined. Recently, (Gillespie, 1960) calculated the S—v relationship for pseudoplastic flow by accounting for link formation between particles, and for link breakage due to shear and temperature. At high shear rates his equation approximates to eqn 6.

The assumption that pseudoplasticity can be represented by the sum of two independent effects is undoubtedly an oversimplification of the true conditions. Goodeve (1939), however, developed this theme further. He believed that a Newtonian effect, where the shearing force is proportional to the rate of shear, and a thixotropic effect, where the shearing force is constant irrespective of the rate of shear, contribute to flow behaviour of concentrated suspensions and emulsions.

$$F = Rv + E$$

$$\text{or} \quad \eta_\infty = R + \frac{E}{v} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

where R and E are constants, the former representing the residual viscosity, and the latter representing a coefficient of thixotropy. The thixotropic effect is attributed to particle interaction during flow, leading to link formation. When these links are stretched and broken momentum is transferred from a moving layer to the adjacent layer.

Interaction between particles results from the prevailing forces of repulsion and attraction. In an aqueous continuous phase the former are usually electrostatic in origin, depending among other things on the electrical charge on the particles, electrolyte concentration, particle size, and the distance separating the particles (Verwey & Overbeek, 1948). In oil continuous media, the repulsion is substantially reduced (Albers & Overbeek, 1960). The attraction forces, which operate over greater distances than the repulsion forces, are unaffected by the polar nature of the continuous phase. Fig. 3 shows the characteristic shapes of the attraction (V_A), repulsion (V_R) and net potential energy of interaction ($V = V_A + V_R$) curves for an o/w emulsion. The V curve shows a peak

at a certain distance between the particles. If the particles are to come closer together this potential energy barrier (V_{max}) has to be overcome. When V_{max} has a value not greater than a few kT, where k is the Boltzmann constant and T is the absolute temperature, a certain proportion of the particles are able to get over the barrier. At this very small distance of separation in the primary minimum the particles are held together by strong forces of attraction if the layer of emulsifier around the particles prevents spontaneous coalescence. On the other hand, if V_{max} exceeds 20–25 kT the particles cannot surmount the potential energy barrier and they flocculate in the secondary minimum where the attraction forces are very weak, usually not exceeding a few kT.

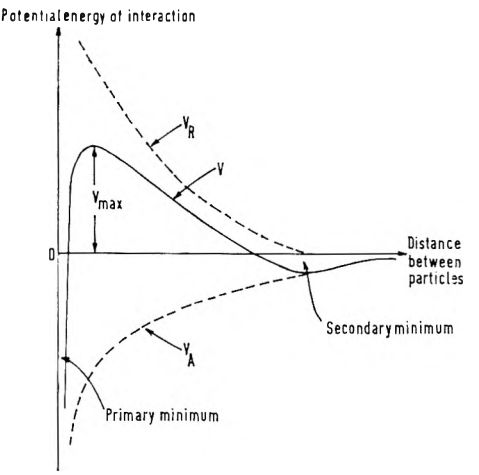


FIG. 3. Characteristic curve for the potential energy of interaction between particles in a suspension or in an emulsion.

In oil continuous media V_{max} is very small, so that the emulsifier films around the particles make contact when the particles flocculate (Albers & Overbeek, 1960). O/w emulsions stabilised by commercial grade non-ionic emulsifiers show a V_{max} which is somewhat larger than for w/o emulsions (Sherman, 1963).

Non-Newtonian flow behaviour can be interpreted on the basis of this attractive theory. The main difficulty lies in choosing the correct value for the Van der Waal's constant (A) when calculating V_A. If the value chosen is too large, V_{max} becomes too small and the secondary minimum becomes too large. In some examples the general shape of the net interaction curve may be drastically altered. For suspensions of solid particles in liquid media A can be calculated from rate of flocculation data. In emulsions, coalescence follows flocculation so that this method for deriving A cannot be used.

When particles flocculate in a suspension, or in an emulsion, part of the continuous phase is immobilised within the aggregates. Each aggregate behaves as if it had a volume greater than the sum of the volumes of the individual globules from which it is constituted (Vand, 1948; Robinson,

1948), and at very low shearing stress it rotates around its centre of mass like a single particle (Manley & Mason, 1954). In the absence of any shearing force the size of the aggregates would increase with time, and hence the viscosity at low shear rate would also increase. When shear is applied the aggregates break down along their weakest planes, and the viscosity decreases. The final stage of deflocculation involves the separation of residual particle pairs. Flow under shear not only makes them rotate, but it also sets up a tension which promotes the separation of the particles within each pair. When the tension exceeds the attraction forces between these particles they separate. The tension (v_{min}) which effects the separation of non-deformable particles in emulsions is calculated (Albers & Overbeek, 1960) from

$$v_{min} = \frac{A}{18\,\pi\eta_0\,D_m\,Z^2\sin(2\alpha)} \qquad (10)$$

where η_0 is the viscosity of the continuous phase, D_m is the mean particle size, Z is the distance between the particles and α has a value of 30°. This equation should also be applicable to small sized deformable particles.

The influence of flocculation under shear (Van den Tempel, 1963) on emulsion viscosity, and also of shear thickening (de Vries, 1963), have been recently interpreted using the Verwey–Overbeek theory. Casson (1959) considered that viscosity is controlled by the dimensions of the particle aggregates. During flow the aggregates are subjected to disruptive stresses, their magnitude depending on the size of the aggregates and the rate of shear. At any rate of shear there is an equilibrium size for the aggregates.

The viscosity of very dilute systems arises from hydrodynamic interference between particles, and their associated zones of continuous medium, during flow. In concentrated systems the interference is much greater since the particles are now closer together. Simha (1952) pointed out that particles have a finite size with respect to their distance of separation, so that the interaction between two particles on either side of a central particle will be reduced due to a "shielding effect" exerted by the latter. In the mathematical development of this theory a particle with a diameter D is enclosed within a concentric sphere of diameter M. This sphere diameter represents the maximum distance over which other particles interact with the central particle. The interaction factor λ depends on the ratio D/M.

$$\lambda = \frac{1 - D/M}{D/M} \qquad (11)$$

for D/M values greater than 0·5. Small increases in this ratio then produce larger increases in viscosity.

Ree & Eyring (1955), and Kim, Hirai, Ree & Eyring (1960), interpreted non-Newtonian flow in terms of the theory of rate processes (Glasstone, Laidler & Eyring, 1941) which assumes that before a particle can move past its neighbour it must surmount a potential energy barrier. Once

again two kinds of flow units are believed to be involved, one Newtonian and the other non-Newtonian. Several such flow units are present in every non-Newtonian system. Each unit has its own characteristic mean relaxation time β_n, and its own characteristic shear volume $a_n \times kT$. Each unit occupies a fraction x_n of the total shear surface. If all units on the same shear plane have the same rate of shear, then the viscosity (η) of the whole system is given by

$$\eta = \sum_{n-1}^{n} \frac{x_n \beta_n}{a_n} \frac{\sinh^{-1} \beta_n v}{\beta_n v} \quad .. \quad .. \quad (12)$$

The values of the parameters in the equation are calculated from given β—v relationships which are valid at the inflexion points in the η — log v curve. Unfortunately, the inflexion points are usually very difficult to identify on emulsion flow curves.

In non-Newtonian flow some form of structural change occurs as v increases. Reaction kinetics have been used to define this change (Denny & Brodkey, 1962) leading to

$$\eta_1 - \eta_\infty = \sum_{1-1}^{n} \left(x\beta/a \right)_i \quad .. \quad .. \quad (13)$$

where η_1 is the Newtonian viscosity at $v = o$. This theory makes no specific assumptions about the mechanism responsible for the structural change, and it therefore does not presuppose the presence of many flow units as in the Ree-Eyring treatment. It does assume, however, that no structural breakdown is possible at zero shear. When the theory, in its present state, is applied to emulsion viscosity data a different rate constant is derived for each volume concentration of disperse phase (ϕ). No attempt has been made to integrate the rate constants for the different values of ϕ so as to generalise the application of this theory.

Recipe ingredients and their effect on emulsion viscosity

Many factors contribute to the viscosity of an emulsion. They arise primarily from the chemical nature and properties of the materials used

TABLE 3. FACTORS WHICH INFLUENCE EMULSION VISCOSITY

1. *Internal phase*—
   - (*a*) Volume concentration (ϕ)
     inter-particle interference; flocculation; aggregation.
   - (*b*) Viscosity (η_1).
   - (*c*) Particle size, and size distribution.
     technique used to prepare the emulsion; interfacial tension; particle deformation.
   - (*d*) Chemical constitution.
2. *Continuous phase*—
   - (*a*) Viscosity (η_0).
   - (*b*) Chemical constitution, and polarity.
     effect on the potential energy of interaction between particles.
3. *Emulsifying agent*—
   - (*a*) Chemical constitution, and concentration.
   - (*b*) Solubility in continuous and internal phases; pH of liquid phases.
   - (*c*) Physical properties of film around the particles; thickness of film; particle deformation; fluid circulation within the particles; influence on the attraction forces between particles.
   - (*d*) Electroviscous effect.
     electrolyte concentration in aqueous continuous media.
4. *Additional stabilising agents*—
   Pigments, hydrocolloids, hydrous oxides, etc.

in its preparation. Table 3 lists the principal factors, and their associated phenomena, which have been reported in the literature. This form of representation is convenient but not wholly satisfactory since it suggests that each factor acts independently, whereas two, or more factors may act simultaneously. The net result is an effect which differs from the sum of their individual contributions. The following example may be used to illustrate this factor interaction. Two emulsions containing different concentrations of disperse phase are prepared using the same ingredients and the same method of pre-mixing and homogenisation. Their viscosities are then compared over a wide range of shear rates so as to determine the effect of ϕ on η. It is possible that the more concentrated emulsion will have a larger mean particle size, and broader particle size distribution than the other emulsion. Unless this is recognised, and accounted for, the wrong conclusions may be drawn from the data. Other examples of this interaction will appear in the subsequent discussion. In general, the listed factors exert a greater effect in concentrated emulsions, because the particles are packed closer together.

The interpretation of emulsion viscosity data is further aggravated by the possibility of particle deformation. This will depend, to some extent, on the physical properties of the film of emulsifier around the particles. If a particle is only slightly deformed when sheared, the deformation can be calculated (Taylor, 1934) from

$$\frac{L - B}{L + B} = \frac{vD_m \eta_o}{2\gamma}\left(\frac{\frac{19}{16}\eta_1 + \eta_o}{\eta_1 + \eta_o}\right) \quad .. \quad .. \quad (14)$$

where L and B are the dimensions of the major and minor axes, γ is the interfacial tension, and η_1 is the viscosity of the internal phase. Small particles of a few microns diameter will, therefore, undergo negligible deformation even at high shear rates. When particles in emulsions suffer little deformation under shear, conclusions regarding emulsion flow behaviour can be drawn, by analogy, from the much more detailed information which is available for suspensions of rigid spheres.

INTERNAL PHASE

Volume concentration. The viscosity of an extremely dilute ($\phi < 0{\cdot}05$) suspension of rigid spherical particles in a fluid medium is given by (Einstein, 1906; 1911),

$$\eta = \eta_o(1 + a\phi) \quad .. \quad .. \quad .. \quad .. \quad (15)$$

provided there is no interaction between the particles, and their distance of separation greatly exceeds their diameter. The constant a has a value of 2·5.

Eqn 15 can be presented in another way

$$\frac{\eta}{\eta_o} - 1 = \eta_{sp} = 2{\cdot}5\phi \quad .. \quad .. \quad .. \quad (16)$$

So that if the specific increase in viscosity (η_{sp}) is plotted against ϕ ($\phi < 0{\cdot}05$), one should obtain a straight line with a gradient of 2·5.

When the particles in dilute emulsions are deformable eqn 15 has to be modified to allow for the influence of η_1. If the emulsifier film around the particles does not prevent the transmission of tangential stress from the continuous phase, and if there is no slippage at the oil-water interface (Taylor, 1932), the relative viscosity (η_{rel}) is given by

$$\frac{\eta}{\eta_o} = \eta_{rel} = 1 + 2{\cdot}5\left(\frac{\eta_1 + \frac{2}{5}\eta_o}{\eta_1 + \eta_o}\right)\phi \quad .. \quad .. \quad (17)$$

When η_1 is large, this equation reduces to eqn 15; in all other instances η_{rel} is lower than for a corresponding dispersion of solid particles. Eqn 17 was modified by Leviton and Leighton (1936) to extend its validity to more concentrated emulsions.

$$\ln \eta_{rel} = 2{\cdot}5\left(\frac{\eta_1 + \frac{2}{5}\eta_o}{\eta_1 + \eta_o}\right)\left(\phi + \phi^{\frac{5}{3}} + \phi^{\frac{11}{3}}\right) \quad .. \quad (18)$$

At low values of ϕ eqns 17 and 18 are identical. Eqn 18 was found to agree with experimental data up to $\phi = 0{\cdot}4$. The term $\phi^{\frac{5}{3}}$ was introduced following a suggestion by Smoluchowski (1916) that it approximates to the next term in the series expansion of eqn 15; the term $\phi^{\frac{11}{3}}$ was introduced to obtain closer agreement between theoretical and experimental values of η, and it has no theoretical significance.

Eqns 17 and 18 do not recognise the effect on shear flow which an emulsifier layer around the particles may exert.

If this layer has viscoelastic properties the particles will not deform when sheared, but when the emulsifier layer is viscous, particle flow resembles that of unstabilised dispersions of fluid particles (Oldroyd, 1953; 1955). The emulsifier layer also introduces the possibility of slippage at the interface (Rajagopal, 1960).

$$\eta_{rel} = 1 + \frac{2{\cdot}5\left[\eta_1 + \frac{2}{5}\eta_o + (2V_S + 3V_B)/2{\cdot}5r\right]\phi}{\eta_1 + \eta_o + \frac{2}{5}(2V_S + 3V_B)/r} \quad .. \quad (19)$$

where r is the particle radius, and V_S and V_B are the shear viscosity of the emulsifier layer and its area viscosity—the two dimensional analogue of bulk viscosity—respectively. This equation, therefore, provides an indirect way of differentiating between plastic solid and fluid emulsifier layers. The quantity $(2V_S + 3V_B)$ has been calculated for several dilute emulsions (Nawab & Mason, 1958) and found to be within the range $0{\cdot}92 \times 10^{-4}$ to $0{\cdot}014 \times 10^{-4}$ g sec^{-1}. These values are about the same as values of V_S reported for films of surface-active agents spread at an air–water interface (Joly, 1956), although the two sets of data are not strictly comparable. Other emulsions which did not obey eqn 19 were found to follow eqn 17 provided the emulsifier layer did not inhibit fluid circulation within the particles.

As ϕ increases so does η, and when ϕ exceeds about 0·4—0·5 the emulsion becomes pseudoplastic. If it contains pigments, gums, or

hydrocolloids, the emulsion may eventually become plastic and develop a yield value. Small increases in ϕ now produce very large increases in η. When ϕ exceeds a critical value which is often, but not always, in the region of 0·74 the emulsion may invert. This will be accompanied by marked changes in η (Sherman, 1950a).

Richardson (1933) calculated the "compressibility" of an emulsion when ϕ is increased by $\delta\phi$, and from this he derived an expression for the viscosity of concentrated emulsions. At any given rate of shear

$$\ln \eta_{rel} = K\phi \quad .. \quad .. \quad .. \quad .. \quad .. \quad (20)$$

where K is a constant. This equation was later amended empirically by Broughton & Squires (1938) to obtain better agreement with their experimental data

$$\ln \eta_{rel} = K\phi + Y \quad .. \quad .. \quad .. \quad .. \quad (21)$$

where Y is also a constant. Simpson (1949) found that the modified equation held for nitrocellulose lacquer emulsions. Neither eqns 20 nor 21 fitted viscosity data for w/o emulsions satisfactorily (Sherman, 1950a). The values of the supposed constants varied with ϕ, and with the emulsifier concentration. When ϕ exceeded 0·5 the discrepancies were exceedingly large.

Hatschek (1911) proposed that η_∞ for non-Newtonian emulsions with ϕ exceeding 0·5 could be represented by

$$\frac{\eta_\infty}{\eta_0} = \frac{1}{1 - 3\sqrt{\phi}} \quad .. \quad .. \quad .. \quad .. \quad (22)$$

Sibree (1930, 1931) found that eqn 22 gave η_∞ values lower than the experimental values. The discrepancy was attributed to an increase (h) in the effective volume of the particles due to hydration of the emulsifier layer around the particles. Accordingly

$$\frac{\eta_\infty}{\eta_0} = \frac{1}{1 - 3\sqrt{h\phi}} \quad .. \quad .. \quad .. \quad .. \quad (23)$$

Most of the emulsions examined gave a value of 1·3 for h. Other workers, however, have found large variations in h (Broughton and Squires, 1938; Toms, 1941).

Another equation for concentrated emulsions has been derived by modifying eqn 15. The amended equation takes the form

$$\eta = \eta_0 (1 + a\phi + b\phi^2 + c\phi^3 - - -) \quad .. \quad .. \quad (24)$$

where b and c are constants. Table 4 summarises some of the values given in the published literature for a, b, and c. In general a retains a value of 2·5, but the values of b are widely different. Few values of c have been reported. The variation in b probably arises from differences in the particle sizes of the various systems studied. When ϕ exceeds 0·05 hydrodynamic interference takes place between the particles, the magnitude of the effect depending on particle size (Saunders, 1961).

TABLE 4. VISCOSITY OF DILUTE EMULSIONS AND DISPERSIONS OF SOLID PARTICLES

Reference	*a*	*b*	*c*
1. *Suspensions of solid spherical particles*—			
Saito (1950)	2·5	2·5	—
de Buijn (1942; 1948)	2·5	4·7	—
Eilers (1941; 1948)	2·5	4·94	8·78
Eirich, Bunzl, & Margaretha (1936)	2·5	8·0	—
Simha (1952)	2·5	12·6 (changes with ϕ)	—
Guth & Simha (1936)	2·5	14·1	—
Roscoe (1952)	2·5	—	—
Kynch (1956)	2·5	6·75–10·0 (changes with ϕ)	—
Vand (1948)	2·5	7·35	—
Higginbotham, Oliver & Ward (1958)	2·33–2·46	—	—
Mooney (1951)	2·5	—	—
Robinson (1949)	3–5	—	—
2. *Emulsions*—			
Albers (1957)	4–5	—	—
Sherman (1950)	2·3–2·8	0–9·7 (changes with ϕ)	—
Nawab & Mason (1958)	1·5–2·3	—	—
van der Waarden (1954)	2·6–5·0	—	—
Leviton & Leighton (1936)	2·44 (average)	—	—
Maron, Madow & Krieger (1951)	2·20	—	—
Saunders (1961)	2·504	6·29–7·64	26·9–36·3

Brinkman writes eqn 24 as

$$\eta_{rel} = \frac{1}{(1-\phi)^a} \qquad (25)$$

where a is 2·5. Gillespie (1963) suggests that a has this value only in systems where the particles are deflocculated. If partial aggregation occurs a will have a value greater than 2·5, particularly if liquid is held within the aggregates.

Part of the continuous phase is immobilised between particles in concentrated emulsions and dispersions. The "free volume" of this phase in which particles move past one another is then $1 - H\phi$, where H is a measure of the volume of fluid immobilised. Several viscosity equations take the general form.

$$\eta_{rel} - 1 = \eta_{sp} = \frac{a\phi}{1 - H\phi} \qquad (26)$$

For dispersions of solid particles in liquid media H usually represents the volume occupied by the particles after flocculation. The constant a has been interpreted in several ways, although it has often fitted the experimental data satisfactorily by assuming a value of 2·5 as in eqns 15 and 24.

Eilers (1941, 1943) observed that emulsions in which ϕ did not exceed 0·65 obeyed the equation:

$$\eta_{rel} = 1 + \frac{2{\cdot}5\phi}{6(1-\phi)} \qquad (27)$$

For those emulsions in which η_{rel} became infinite when $\phi = 0{\cdot}74$

$$\eta_{rel} = 1 + \frac{2{\cdot}5\phi}{2(1 - H\phi)} \qquad (28)$$

where H has a value of 1·28—1·35.

Robinson (1949, 1957) regarded the constant a in eqn 26 as a coefficient of friction, its value depending on the shape and surface roughness of the particles. According to Mooney (1951), and Maron & others (1951, 1953), H defines the crowding effect which arises when particles of more than one size are packed together. In the simple case of a dispersion with only two particle sizes present H will be a function of their size ratio.

Sweeney & Geckler (1954) found that H varied from 1·00 to 1·47, H increasing as the particle size decreased. Saunders (1961) also observed this dependence of H on particle size in monodisperse latexes with particle sizes less than 1μ. His values for H ranged between 1·118 and 1·357: the constant a was unaffected by particle size, retaining a value of 2·504 provided the thickness of the emulsifier layer was allowed for when calculating ϕ. These two publications are probably the first to point out in semi-quantitative fashion the influence of particle size on viscosity data. Since eqn 26 on expansion gives a series of the form of eqn 24 this observation is of some importance. The relevance of particle size to viscosity will be discussed in greater detail in a later section.

Viscosity. The influence of η_1 on the deformability of particles has already been discussed.

When preparing an emulsion the emulsifier is normally dissolved or dispersed in the liquid which will be the continuous phase. Sometimes, for example, when preparing an o/w emulsion with sorbitan monolaurate, the emulsifier disperses on agitation in the water phase, but it is soluble in the oil phase. When the emulsion is homogenised an appreciable part of the emulsifier migrates to the oil phase (Sherman, 1963a) so that η_1 increases and η_0 decreases. This change in the ratio η_1/η_0 affects fluid circulation within the particles, it reduces the deformation due to shear, and it also affects η_{rel}.

In concentrated emulsions any effect due to η_1 is more difficult to analyse because of superimposed particle interaction effects. Provided ϕ and particle size are kept constant, and only η_1 is altered, it should be possible to determine any change in η_{rel} due to η_1. Toms (1941) examined a range of o/w emulsions prepared with eleven different organic liquids as internal phase, and with several monovalent soaps as the emulsifier. He found no correlation between emulsion viscosity and η_1. Any influence exerted by the internal phase was attributed to its interaction with the emulsifier film around the particles. A similar conclusion was reached by Shotton & White (1960) who determined η_{rel} for a series of oil-in-acacia solution emulsions. They found that the highest η_{rel} was given by the emulsions prepared with the oil of lowest η_1.

Different values of η_1 were obtained for w/o emulsions by using aqueous solutions of glycerol, propylene glycol, sorbitol, and triethylene glycol (Sherman, 1955b). Even when η_1 was increased fifty-fold η_∞/η_0 did not alter. This was probably due to the plastic properties of the emulsifier film around the particles. When carbon black was incorporated in the oil phase η_∞/η_0 did alter, the highest values being shown by the emulsion with the lowest η_1, namely, the aqueous sorbitol solution-in-oil emulsion. The specific absorption of sorbitol solution by carbon black was greater

than the specific absorption of the other polyhydric alcohol solutions by carbon black, so that the chemical constitution of the internal phase can affect the configuration at the oil-water interface even when η_i does not influence viscosity.

Particle size, and size distribution. The bulk of published literature makes little, or no, reference to the state of dispersion of the systems examined. Nothing more precise is given than generalised statements indicating that "fine" emulsions gave higher viscosities than "coarse" emulsions of the same formulation, or that the particle size did not exceed a certain value.

Until quite recently the only observations of any value were those of Leviton & Leighton (1936) and of Richardson (1950, 1953). Unfortunately, one or other of these observations, which appear contradictory, are quoted repeatedly without appreciating that they apply only under certain limiting conditions. Leviton & Leighton (1936) found that the viscosity of dilute o/w emulsions did not change when particle diameter was reduced from $3{\cdot}0\mu$ to $0{\cdot}7\mu$. They believed that when ϕ did not exceed 0·5 any increase in the hydrodynamic volume of the particle due to adsorption of emulsifier might be counterbalanced by the increase in η_o. Richardson (1950, 1953) restricted his attention to concentrated ($\phi = 0{\cdot}75$) o/w emulsions which exhibited non-Newtonian flow. He found that η_∞ was proportional to the reciprocal of the mean particle diameter (D_m), and that η_∞ D_m remained constant provided the spread of particle sizes around D_m was narrow.

This disregard of particle size analysis is partly due to the incompleteness of viscosity equations. With only few exceptions (Oldroyd, 1953, 1955; Rajagopal, 1960), and these apply only to very dilute emulsions, no equation recognises the influence of particle size on viscosity. This problem has been studied in much greater detail for suspensions of solid particles in liquid media than for emulsions, but even here most of the equations which have been proposed (for example, Roscoe, 1952; Orr & Blocker, 1955; Mari & Otatake, 1956) do not include a specific term for particle size. Instead, alternative forms of equations are suggested depending on whether the suspension has a homogeneous or heterogeneous particle size distribution.

The importance of recognising any effect due to particle size when interpreting viscosity data, especially when particle size does not exceed a few microns, is conveniently illustrated by reference to Fig. 4. The viscosity data for a series of w/o emulsions stabilised by sorbitan sesquioleate and sorbitan trioleate, are plotted as η_{rel} against ϕ for a number of mean particle sizes. Each emulsion showed a very narrow distribution of sizes about the mean value. The shape of each curve varies with particle size, η_{rel} increasing at any given value of ϕ as D_m decreases. Thus, if these data were inserted in eqns 24 or 26 the values derived for the various constants would vary with D_m.

Pseudoplastic w/o emulsions showed a large curvilinear increase in η_∞ when the particle size fell below about 2μ (Sherman, 1960). For o/w emulsions the effect was less pronounced, and appeared only at values

of ϕ exceeding 0·5. This difference in behaviour was attributed to differences in the rheological properties of the emulsifier films around the particles. At high rates of shear the particles in a suspension are equidistant from each other. Provided they behave as rigid spheres this distance (a_m) can be calculated from

$$a_m = D_m\left(\sqrt[3]{\frac{\phi_{max}}{\phi}} - 1\right) \qquad .. \quad .. \quad (29)$$

where ϕ_{max} is the maximum volume of disperse phase which can be incorporated in the emulsion. In many cases ϕ_{max} is about 0·74, provided the particle size distribution is reasonably narrow. If $\eta\infty/\eta_0$ is plotted against a_m for emulsions of this type, an exponential relation is derived which covers viscosity data for all values of ϕ. When a_m falls below a critical value ($\sim$0·5μ), when D_m does not exceed 2–3μ, $\eta\infty/\eta_0$ increases very rapidly (Sherman, 1960). Eqn 29 indicates that with small particles the critical value of a_m is reached at lower values of ϕ than with large particles.

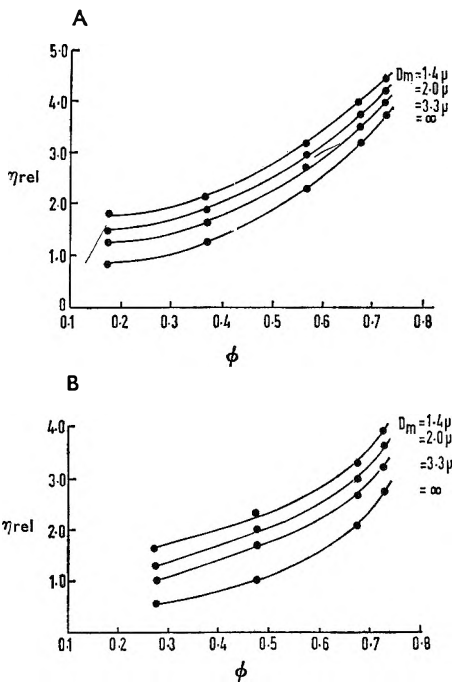


FIG. 4. Viscosity data for w/o emulsions stabilised with A, sorbitan sesquioleate and B, sorbitan trioleate.

Similar studies have been made on dilute o/w emulsions stabilised by monoglycerides and milk protein (Sherman, 1961). These studies also indicated that D_m depends on ϕ and homogenisation pressure.

Emulsions with a broad distribution of particle sizes will have a lower viscosity than comparable emulsions with a narrow distribution of

particle sizes. The depth of the secondary minimum in the potential energy curve, and the height of the potential energy barrier to flocculation in the primary minimum (Fig. 3), are both affected by D_m. This influence will be reflected in the flow behaviour at very low rates of shear.

CONTINUOUS PHASE

In spite of the lack of agreement regarding the relative effect of various factors on viscosity, all equations indicate a direct proportionality between η and η_0. It should be appreciated that η_0 represents the viscosity of the entire continuous phase, and not the viscosity of the basic fluid in which other materials may be dissolved. Thus, it is the usual practice to dissolve, or disperse, the emulsifier, finely divided pigments, and hydrocolloids in this phase, and each of these contributes to η_0.

Removal of emulsifier from the continuous phase, due to adsorption at the particle surface, will lower η_0. For emulsifiers of simple chemical structure the concentration reduction is usually too small to be of any significance.

Recent studies on thin films (Derjaguin & Samygin, 1954, 1957, 1959; Elton & Picknett, 1957; Fuks, 1958) suggest that their viscosity is very much larger than the viscosity of the same liquids in bulk, for example, a film with a thickness of 1,000 Å has a viscosity which is twice the bulk value, whilst a film of 200 Å has a viscosity which is about five times the bulk value. For aqueous films these discrepancies are attributed to electrical charge effects. Similar phenomena have now been reported for films of a non-aqueous nature. In concentrated emulsions the particles are separated by very thin films of continuous phase when they are deflocculated. If the observations on the viscosity of thin films can be applied to emulsions, it could be that the high viscosity of concentrated emulsions is partly attributable to a hitherto unrecognised unduly large value of η_0. Similarly, flocculated particles in emulsions are separated by very thin films of continuous phase. When shear is applied η decreases possibly due, in part, to a fall in η_0 as the distance between particles increases.

EMULSIFYING AGENT

Composition, and concentration. Wilson & Parkes (1936), Broughton & Squires (1938), and Sumner (1940), have all pointed out that the chemical nature of the emulsifier influences viscosity. A range of w/o emulsions with the same ϕ, but stabilised by different emulsifiers, showed quite different η_∞/η_0 values (Sherman, 1955a). The chemical structure of the emulsifier will affect the aggregation of particles when they flocculate, also the inter-particle attraction, and hence emulsion flow behaviour at low rates of shear.

Emulsifier concentration influences the value of ϕ at which an emulsion inverts, and also the optimum viscosity just before inversion (Sherman 1950a; Becher, 1958). Emulsion viscosity increases at any given ϕ with increasing emulsifier concentration. This has been attributed in some instances, for example, for protein, to increased adsorption of emulsifier at the particle surface, thus raising the value of ϕ. With many emulsifiers it

is most unlikely that the adsorbed layer is ever more than one molecule thick, so that this explanation is not universally valid. Once a monomolecular layer has been formed around the particles the excess emulsifier molecules associate to form micelles in the continuous phase. Such units immobilise fluid within themselves so that the "free" volume of continuous phase decreases, and the effective volume ratio disperse phase : continuous phase increases (Sherman, 1963a). The larger the excess of emulsifier present the greater the volume of continuous phase immobilised. Calculation indicated that for w/o emulsions stabilised by sorbitan monooleate each excess molecule of sorbitan monooleate immobilised 28×10^{-23} ml of oil.

When o/w emulsions were prepared with sorbitan monolaurate dispersed in the water phase, multiphase particles appeared, their number, size, and structural complexity, increasing as the emulsifier concentration increased. With 6·0% emulsifier the emulsion inverted at a lower ϕ than for lesser emulsifier concentrations.

Emulsifier solubility; hydrogen ion concentration. Many of the polyoxyethylene sorbitan derivatives are oil soluble and only dispersible in water. The type of emulsion obtained initially with these emulsifiers depends on the phase to which they are added, the emulsifier concentration employed, and the method used to prepare the emulsion. At some value of ϕ the emulsion inverts, and this is accompanied by a pronounced change in viscosity.

Sorbitan monolaurate behaves in a similar way. The inversion of o/w emulsions stabilised by this emulsifier, which was referred to in the previous section, is attributable to distribution of the sorbitan monolaurate between the two phases, even though it was initially dispersed in the water phase. Its rate of migration to the oil phase depends on the concentration employed (Sherman, 1963a). Similarly, if the sorbitan monolaurate is apportioned between the two phases before mixing them inversion is dependent on the emulsifier concentration (Becher, 1958).

Solubility phenomena appear to be involved also in the inversion of w/o emulsions stabilised by non-ionic emulsifiers at alkaline pH. Concentrated w/o emulsions stabilised by sorbitan sesquioleate, mannitan monooleate, and mannide monooleate, for which the water phase was a series of buffer solutions of pH ranging from 3·0 to 10·0, showed no change in η_∞ up to a pH of about 9. At a slightly higher pH the emulsions inverted to dilute o/w emulsions, and this was accompanied by a sharp drop in viscosity (Sherman, 1950b). The emulsifiers, which were insoluble in the buffer solutions at pH 7·0, became increasingly soluble as the pH approached 9·0. When $BaCl_2$ was added to the fluid o/w emulsions they inverted to more viscous w/o emulsions.

PHYSICAL PROPERTIES OF THE ADSORBED EMULSIFIER FILM

Reference has already been made to the influence of the rheological properties of the emulsifier film on the deformability of particles under shear, and on emulsion viscosity.

Experimental study of these properties has, so far, been possible only

with systems which have usually taken the form of films adsorbed at extended, flat, stationary oil-water interfaces (Criddle, 1960). It is questionable whether the results of such tests can be used to determine the rheological behaviour of emulsifier films in sheared emulsions. An attempt has been made to show theoretically how the properties of surface films are interlinked with the properties of a bulk colloidal system (Joly, 1954), but this approach has not been extended yet to the interpretation of experimental data.

ELECTROVISCOUS EFFECT

When very dilute emulsions containing electrically charged particles are sheared, the configuration of the electrical double layer around each particle is distorted. The interaction between ions in the double layer and the electrical charge on the particle surfaces is affected, leading to an extra dissipation of energy, and an increased viscosity (Conway & Dobry-Duclaux, 1960).

Smoluchowski (1916) amended eqn 15 for rigid particles to allow for this effect.

$$\eta_{sp} = 2{\cdot}5\phi\left[1 + \frac{1}{\eta_0 K r^2}\left(\frac{\epsilon\zeta}{2\pi}\right)^2\right] \quad .. \quad .. \quad (30)$$

where ϵ is the dielectric constant of the continuous phase, ζ is the electrokinetic potential of the charged particles, K is the specific conductivity of the emulsion, and r is the radius of the particles. Smoluchowski assumed that the thickness of the double layer is small compared with r. Booth (1950) developed an equation of much greater complexity in which he introduced terms for double layer thickness, ion concentration, and valency of the ions. The significant point about this equation is that it predicts a lower contribution to η by the electroviscous effect than suggested by eqn 30. This agrees with experimental data. A simpler form of Booth's equation has been developed by Street (1958).

A measurable electroviscous effect is to be expected only when r is very small, e.g. 500 Å, so that the thickness of the electrical double layer is significant with respect to r.

At higher values of ϕ than those to which eqn 30 applies the particles may be packed close enough for the double layers to repel each other. The viscosity increase which ensues is due to a second electroviscous effect. It was first observed by Harmsen, Van Schooten, & Overbeek (1953). This effect is directly proportional to ϕ^2. At constant ϕ it increases with decreasing ionic strength, because the thickness of the electrical double layer now increases, thus increasing the probability of double layer interaction.

Very little study has been made of electroviscous effects in emulsions. Van der Waarden (1954) determined the viscosity of a series of o/w emulsions stabilised by sodium naphthasulphonate for which the particle size was well below 1μ. At high emulsifier concentrations the viscosity data showed appreciable deviation from values calculated using eqn 15. The increase in viscosity was also much larger than suggested by eqn 30 or

Booth's equation, so that it was inferred that the observed electroviscous effect could not be due to distortion of the diffuse parts of the electrical double layers. The strongly ionised emulsifier adsorbed at the particle surface was believed to produce a high electric field strength (10^5—10^6 V/cm), and a layer of water molecules was strongly bound by this. Calculation indicated that the thickness of the water layer was about 30 Å irrespective of particle size.

When preparing his emulsions van der Waarden introduced the emulsifier into the oil phase. Mukerjee (1957) suggested that the observed viscosities approximated to values calculated from Booth's equation provided one allowed for passage of emulsifier into the aqueous phase during the emulsification, which involved the preparation of a w/o emulsion followed by inversion. Another complicating factor could be that when an excess of emulsifier is present, the micelles formed in the continuous phase alter the ionic concentration.

Whilst each of these emulsions was monodisperse, the particle size for different emulsions varied from 276 to 2,050 Å, and no allowance was made for the effect of this variation on the observed values of η_{rel}. If van der Waarden's viscosity data are plotted as η_{rel} against $1/a_m$ several straight lines are obtained, their gradients (G) depending on the particle size for each particular series of emulsions. When G is plotted against particle size, and compared with similar data for monodisperse latex systems of similar particle sizes (Saunders, 1961), in which no electroviscous effect was observed, it is found that the two sets of data agree fairly closely (Fig 5). With two possible exceptions, the change in η_{rel} for van der Waarden's emulsions is about that anticipated from the variation in particle size (Sherman, 1963c). It is quite possible, therefore, that no electroviscous effect was to be found in these emulsions.

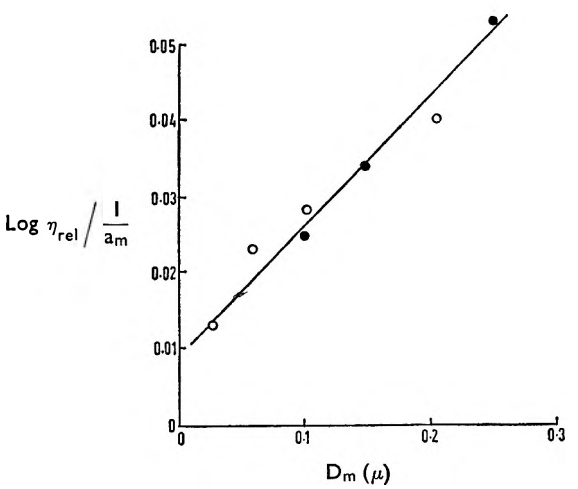


FIG. 5. Correction of van der Waal's data for particle size variation before assessing the influence of electroviscous effect.
○ Van den Waarden's data. ● Saunder's data.

Albers (1957) measured η_{sp}/ϕ for w/o emulsions containing different emulsifiers, and showing different ζ potentials. Similar values of η_{sp}/ϕ were obtained for emulsions with widely different ζ potentials. He concluded that the electroviscous effect is very small in w/o emulsions, contributing no more than 1% to the viscosity of dilute emulsions.

STABILISERS

Hydrocolloids dissolved in an aqueous continuous medium may increase η_o, thereby retarding flocculation, but may show no surface activity.

Finely divided pigments migrate to the oil-water interface and form a protective layer around the particles. Hydrous oxides, for example, the hydrated form of vanadium pentoxide, ferric oxide, or alumina, are also surface-active. Apart from any increase in the initial emulsion viscosity which may result from their use, further increases in viscosity may occur over a period of time due to progressive hydration of the oxide. Eventually a gel-like layer may form around each particle. Concentrated w/o emulsions, in which alumina was dissolved in the aqueous phase, showed this phenomenon when aged at room temperature (Sherman, 1955c). When propylene glycol was incorporated in the aqueous phase, in concentrations ranging up to 20% these changes were retarded to an extent dependent on the propylene glycol concentration. At higher concentration the formation of gel layer was completely inhibited. Other polyalcohols behaved in the same way.

VISCOSITY CHANGES IN EMULSIONS WHEN AGED

When emulsions are aged the particle size increases appreciably before the disperse phase separates in bulk. Provided the only change involved is a gradual increase in D_m, there being no appreciable change in the limits of particle size distribution, the decrease in viscosity as D_m increases should be predictable from the viscosity-D_m curves for fresh emulsions of the same formulation, calculated as described in the section on particle size. The rate of increase in D_m can be determined readily from the kinetics of globule coalescence (Lawrence & Mills, 1954; Van den Tempel, 1957), so that changes in η_{rel} on ageing for any given time can be predicted without resorting to dubious accelerated ageing techniques. Accelerated ageing by high speed centrifugation, or storage at elevated temperatures, may lead to changes in D_m which are quite different from those occurring under normal ageing conditions.

This approach has been used to predict changes in η_∞ in pseudoplastic w/o and o/w emulsions on ageing (Sherman, 1963b). The $\eta_\infty - D_m$ curves for both freshly prepared and aged emulsions were identical, thus confirming that growth in particle size, due to particle flocculation and coalescence, is the principal change during ageing. By studying the change in particle size for a few days, or in the concentration of particles per unit volume of emulsion, the rate of growth in particle size was calculated by using either of the two following equations.

$$\ln D_t = \ln D_o + Ct/3 \quad .. \quad .. \quad .. \quad (31)$$

or

$$D_t^3 = D_o^3 + \frac{8kT\phi t}{\pi\eta_o} . \exp(-E/RT) \quad .. \quad .. \quad (32)$$

where D_o and D_t are the mean particle diameters at zero time and after any ageing time t respectively, C is the rate of particle coalescence, k is the Boltzmann constant, T is the absolute temperature, and E is the energy barrier to particle coalescence. Values of η_{rel} calculated for any ageing time t agreed most satisfactorily with values determined experimentally over long ageing periods at room temperature.

Viscosity changes at very low rates of shear (η_n) are more difficult to predict, because the $\eta_n - D_m$ relationship is complicated by the superimposition of particle aggregation. At these low rates of shear an aged emulsion will exhibit a much larger degree of particle flocculation than when it is first prepared. Furthermore, the contribution of these phenomena to η_n increases with ageing time so that the $\eta_n - D_m$ curve for a fresh emulsion cannot be used to obtain information about changes in η_n on ageing without precise knowledge of the rate of flocculation and its effect on η_n.

Conclusions

It is evident from the preceding discussion that there is a great diversity of opinion regarding factors which influence emulsion viscosity, and their relative importance. In spite of this disagreement some factors are undoubtedly more important than others. For example, the electroviscous effect, and η_i when the adsorbed emulsifier film around the particles is not rigid, cannot themselves influence emulsion viscosity to any great extent. On the other hand, ϕ, D_m, particle size distribution, the chemical constitution and concentration of the emulsifier, and η_o, can be used to effect large changes in emulsion viscosity. Many of the latter series of factors involve the internal phase in some way. If η_o is to be adjusted by the use of suitable additives, then it is necessary to make an additional study of the rheological properties of the appropriate continous phases following incorporation of these additives, since they may exhibit non-Newtonian flow.

The chemical constitution and concentration of the emulsifier, and particle size distribution, usually exert a marked influence only on the viscosity of concentrated emulsions. In very dilute emulsions, viscosity is best adjusted by altering η_o.

The major difficulty in relating viscosity-rate of shear data for non-Newtonian emulsions to their practical performance is the lack of information regarding the shear stress-rate of shear conditions prevailing in practice. An attempt has been made recently (Henderson, Meer & Kostenbander, 1961) to calculate this information for some simple pharmaceutical operations, for example, spreading of an ointment on the skin, milling operations, flow of liquid through a hypodermic needle, or pouring materials out of a bottle. In milling operations, and extrusion

from a hypodermic needle, v is very high, whilst in ointment-spreading and pouring a liquid from a bottle v is comparatively low. More detailed calculations of this kind are essential if emulsion rheological data are to be used to full advantage.

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Research Papers

Dioxatrine, a potent and specific rumenal ulcer-preventing agent in rats

C. J. E. NIEMEGEERS AND P. A. J. JANSSEN

When rats are starved with free access to a 20% glucose solution, extensive rumenal ulcers are found in almost all animals at the end of the third week. The formation of these ulcers may be inhibited by dissolving adequate amounts of two anti-acetylcholine agents in the glucose solution. A minimum of about 8 mg/kg of atropine sulphate daily is required for significant activity. Submaximal mydriatic effects are observed after oral administration of this dose. Dioxatrine, a tertiary amine of novel structure, inhibited ulcer formation at dose levels devoid of significant mydriatic activity. Dioxatrine prevents experimental ulcers in rats in one twentieth the dose of atropine.

THE frequent occurrence of rumenal ulcers in starved rats was first reported by Büchner, Siebert & Molloy (1928) and was used to study the effects of drugs on ulcer formation by Grandjean (1948) by Visscher, Seay, Tazelaar, Veldkamp & Vanderbrook (1954) and by Zbinden, Pletscher & Studer (1959).

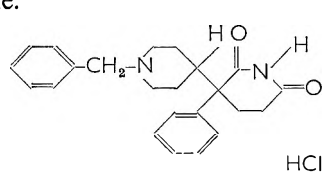
In an effort to develop an orally active drug, capable of preventing the formation of gastric ulcers at low doses without producing side-effects, we investigated many substances by a method in which rats are fasted for three weeks with free access to a 20% aqueous glucose solution containing the substance under investigation.

A few potent anti-acetylcholine drugs prevented rumenal ulcer formation in this test. All the other compounds investigated were either inactive or promoted ulcer formation, for example, reserpine.

Dioxatrine (I), an anti-acetylcholine tertiary amine of novel chemical structure recently synthetised in this laboratory, is of special interest in that it was the only compound which was capable of significantly decreasing the rate of rumenal ulcer formation without producing severe mydriasis, and of completely inhibiting the formation of ulcers in our experimental conditions.

Of the anti-ulcer compounds tested, dioxatrine was the most potent. We now present the experimental evidence of the activity of this drug compared with atropine.

(I)



± 1-Benzyl-4-(2,6-dioxo-3-phenyl)-3-piperidyl)-piperidine hydrochloride (Dioxatrine) $C_{23}H_{26}N_2O_2HCl$ = 398·92. Melting point: 300° (decomp). Solubility in H_2O: 11·7 mg/ml at 20°.

From the Research Laboratorium Dr. C. Jansen, N.V., Beerse, Belgium

Methods

THE RUMENAL ULCER TEST

Male Wistar rats of an inbred strain with an initial weight of 240 to 260 g were caged in groups of 5. Each cage (20 × 23 × 30 cm) had 12 × 12 mm mesh to prevent coprophagia. They were fasted for 21 days with free access to a 20% aqueous glucose solution in which a known amount (5, 10, 20 . . . mg/litre) of the substance under investigation was dissolved. At the end of the 21st day the animals were killed and the stomachs removed, split along the entire great curvature, rinsed with tap water and pinned on 10 × 10 cm cork plates under moderate stretching for inspection of the mucosa. A trained observer, with no knowledge of the regimen to which each mucosa had been subjected, then used the following arbitrary score system for expressing the degree of ulceration of the rumenal mucosa: score 1: no ulcers; score 2: one to ten small, or one to five big ulcers; score 3: extensive ulceration involving 25 to 75% of the total rumenal surface; score 4: widespread ulceration involving more than 75% of the surface. Two groups of five rats were used for each dose level. Ridit analysis is used for the statistical analysis of these data (Bross, 1958).

MYDRIATIC ACTIVITY

Inbred male Wistar rats with a weight of 200 to 250 g were used. Before and $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 8, 32 and 56 hr after oral administration of the substance under investigation the pupil diameter was measured with a micrometer and expressed in 1/25 mm units.

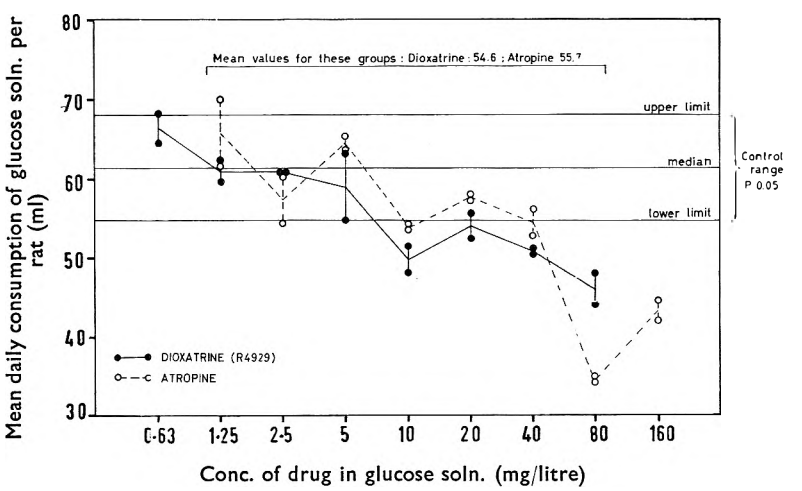


FIG. 1. The anti-acetylcholine drugs dioxatrine and atropine inhibit drinking in starved rats with free access to a 20 per cent aqueous glucose solution. Both substances are approximately equipotent in this respect. Each point represents a group of five rats.

Results and discussion

In a series of 140 control rats, fasted with free access to a 20% aqueous glucose solution, typical rumenal ulcers were found at the end of the third week in 139 animals (score 1 in one rat, score 2 in 5 rats, score 3 in 126 rats, score 4 in 8 rats). All these controls were still alive after 21 days, but their weight fell from 250 ± 10 g initially to 221 g after 7 days, 202 g after 14 days and 184 ± 10 g after a fast of 21 days. Drinking also gradually decreased from an average of 85 ml of glucose solution per rat on the first experimental day and an average of 92 ml per rat on the third day, to 68 ml on day 7, 55 ml on day 14 and 36 ml on day 20. The median control value for the entire 3 week period was 61·5 ± 6·5 ml of glucose solution per rat and per day.

Anti-acetylcholine drugs are known to block drinking by a central effect and eating by a peripheral effect (Stein, 1963). As shown in Fig. 1 dioxatrine and atropine were found to inhibit drinking of glucose solution in fasting rats. Significant inhibition was observed with both drugs in concentrations of 10 mg/litre of glucose solution or more. Both substances were approximately equipotent in this respect.

TABLE 1. FREQUENCY DISTRIBUTION OF RUMENAL ULCER SCORES IN GROUPS OF 10 RATS AFTER A FAST OF 21 DAYS WITH FREE ACCESS TO A 20 AQUEOUS GLUCOSE SOLUTION CONTAINING VARIOUS CONCENTRATIONS OF DIOXATRINE OR ATROPINE SULPHATE

	Dioxatrine-scores				Atropine-scores			
Concentration in mg/litre	1	2	3	4	1	2	3	4
0·63	0	1	9	0	—	—	—	—
1·25	0	3	7	0	1	1	8	0
2·5	2	4	4	0	0	2	8	0
5	2	3	5	0	0	3	6	1
10	2	5	3	0	0	2	7	1
20	8	1	1	0	0	1	9	0
40	9	1	0	0	4	2	3	1
80	10	0	0	0	0	6	3	0
160	—	—	—	—	4	3	1	0
Totals	33	18	29	0	9	20	45	3

No mortality, except with the two highest concentrations of atropine (1/10 and 2/10). Lowest active concentrations: 2·5 mg of dioxatrine per litre and 40 mg of atropine per litre ($P < 0·05$).

As shown in Fig. 2, the mean body weight at autopsy of rats treated with high doses of dioxatrine or atropine was significantly lower than expected from the control data. This effect is only partly due to reduced glucose consumption (Fig. 3). Eight groups of 5 treated rats, 4 with each drug, were found to have normal body weight values at autopsy in spite of a significantly reduced glucose intake, whereas only two groups, one with each drug, showed abnormally low body weights at autopsy after having consumed a normal amount of glucose solution.

The effects of both drugs on ulcer formation are shown in Table 1. Significant reduction (χ^2 test, $P \leqslant 0·05$) of ulcer formation was observed in six dioxatrine-treated groups of ten rats (2·5 to 80 mg/litre) and in three atropine-treated groups (40 to 160 mg/litre), the ratio of the lowest active concentrations being 1 to 16. With dioxatrine the stomachs of all

rats treated with the 80 mg/litre concentration were free of ulcers and the concentration protecting half of the rats was about 15 mg of dioxatrine per litre. With atropine however only 4 out of ten rats were protected with the highest and toxic (2/10 mortality) concentration of 160 mg/litre.

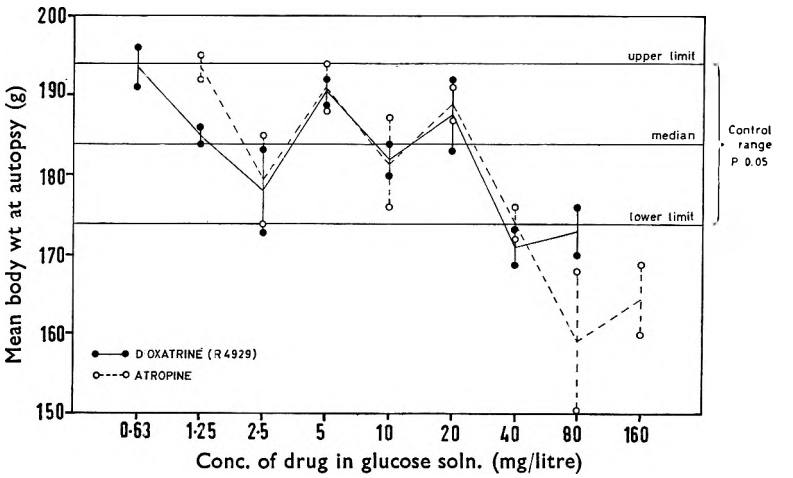


FIG. 2. The mean weight at autopsy of starved rats with free access to glucose solutions containing high concentrations of dioxatrine or atropine is significantly lower than the body weights of the control group. Each point represents a group of five rats.

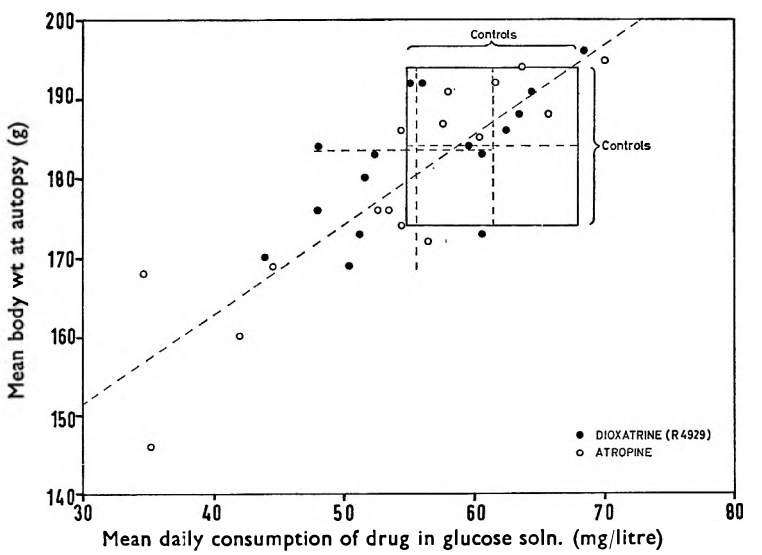


FIG. 3. Correlation of weight and glucose solution. Each point represents a group of five rats. As a whole, the weight of rats with low glucose consumption values is higher than would be expected from the control data. The square represents median values (broken lines) and confidence limits (solid lines) for the controls. Also represented are the median values for all treated rats (broken lines) as well as the calculated regression line for these animals (broken diagonal line).

Fig. 4 shows the correlation of the mean daily doses of dioxatrine and atropine absorbed by the treated animals throughout the three weeks and the degree of rumenal ulceration at autopsy, expressed in ridits. Ridit analysis (Bross, 1958) is a valuable statistical method for expressing the frequency distribution of measurements based on a nominal scale, e.g. the one to four score system used in this paper for measuring the degree of rumenal ulceration, in one meaningful symbol and for analysing its statistical significance. Using these dose-effect curves the lowest significantly active ulcer-preventing doses of dioxatrine and of atropine may be graphically estimated as respectively 0·1 and 2 mg/rat/day. Dioxatrine therefore may be said to be about 20 times more potent in this test than atropine sulphate.

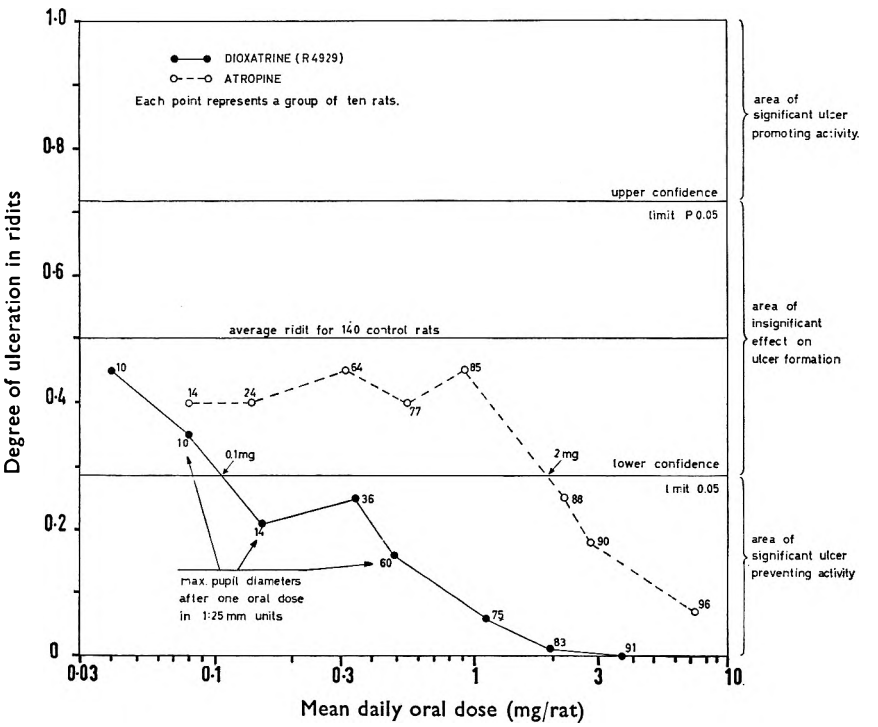


FIG. 4. Correlation of dose, degree of rumenal ulceration (in ridits) and maximal mydriatic effect. The lowest active ulcer preventing dose of dioxatrine produces almost no mydriasis and is about 20 times less than the lowest ulcer-preventing dose of atropine, which produces a pronounced mydriatic effect.

As an oral mydriatic drug, on the other hand, dioxatrine is only about half as potent (Fig. 5) as atropine, but is much slower and longer acting. With dioxatrine, mydriatic peak effects were observed about 4 hr after both oral and subcutaneous administration, as against about 3 to 4 hr with atropine.

TABLE 2. SUMMARY OF EXPERIMENTAL DATA

	Dioxatrine	Atropine	Dioxatrine / Atropine
Lowest active ulcer preventing dose level in mg./kg. orally (A)	0·4	8	1:20
Lowest active mydriatic dose level in mg./kg. orally (B)	0·5	0·3	2:1
Lowest active drinking inhibition dose level in mg./kg. orally (C)	2	2	1:1
Lowest active body weight lowering dose level in mg./kg. orally (D)	8	8	1:1
Relative values A	1	1	1:1
Relative values B/A	3/2	3/80	40:1
Relative values C/A	5	1/4	20:1
Relative values D/A	20	1	20:1

Dioxatrine is furthermore a much more specific or selective anti-ulcer agent than atropine, i.e. at dose levels producing an equivalent degree of ulcer prevention, the mydriatic effects of atropine are much more pronounced than the mydriatic effects of dioxatrine.

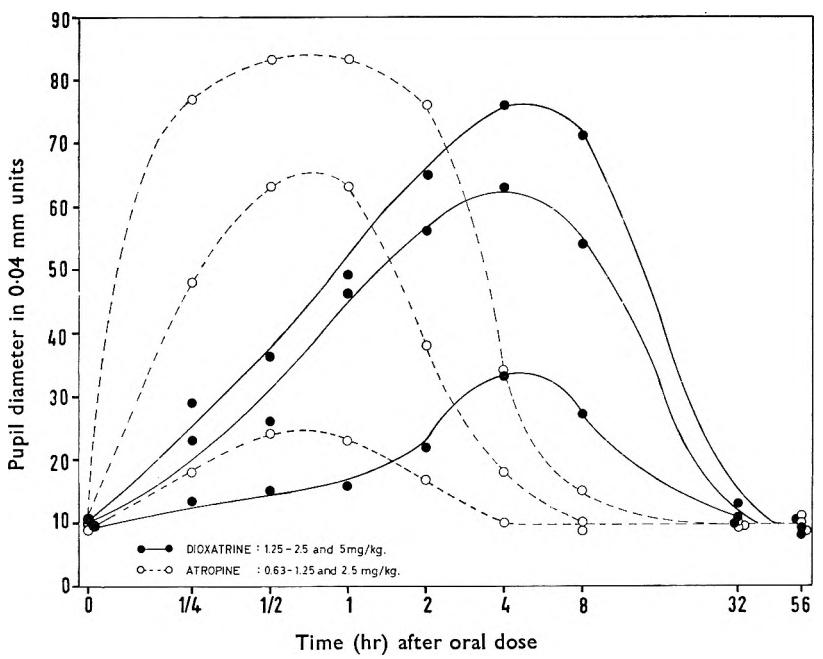


FIG. 5. As an oral mydriatic drug dioxatrine is about half as potent as atropine. Dioxatrine however has a slower onset and a much longer duration of action than atropine. Each curve represents a group of ten rats.

The lowest active oral anti-ulcer dose of dioxatrine, i.e. 0·1 mg per rat or about 0·4 mg/kg, is virtually devoid of mydriatic activity, whereas the equivalent oral anti-ulcer dose of atropine, i.e. 2 mg/rat or about 8 mg/kg produces submaximal mydriasis (Figs 4 and 5).

The most important experimental data, discussed above, is briefly summarised in Table 2.

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Some pharmacological aspects of a new water-soluble tetracycline

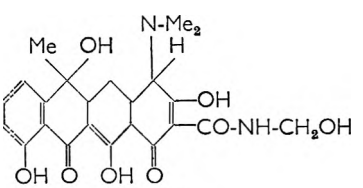
E. TUBARO, M. BARLETTA AND F. BANCI

The pharmacological properties of a new derivative of tetracycline have been described. This water-soluble compound has a low toxicity, is stable, and lacks the local side-effects common to other derivatives of tetracycline.

PARENTERAL administration of tetracycline is limited by the poor water-solubility of the base and by the acid pH of solutions of its hydrochloride. Intravenously, tetracycline, oxytetracycline and chlortetracycline cause lesions in the tissues which may lead to phlebitis, whilst gastrointestinal disturbances and changes in the intestinal flora follow their oral administration.

A new tetracycline derivative, pyrolidinomethyltetracycline (PMT), was recently obtained through a carboxamido-substitution, using the Einhorn-reaction, and was shown to lack the local reaction side-effects.

Other experiments were made with 4-(2-hydroxyethyl)-diethylenediaminemethyltetracycline (Gradnik, Pedrazzoli & Ferrero, 1960). To increase water-solubility still further, tetracyclines were then coupled with amino-acids to yield compounds of lower toxicity (De Carneri, Coppi, Lauria & Logemann, 1961; Tubaro & Raffaldoni, 1961). Using an Einhorn-type reaction (Einhorn, 1905), tetracycline and formaldehyde have now been combined to form a water-soluble tetracycline (I, TMT or methylencycline), which may be administered orally or parenterally. This paper describes pharmacological studies with TMT.



(I)

Methods and materials

A comparison was made of the properties of tetracycline hydrochloride, pyrrolidinomethyltetracycline and the new water-soluble derivative.* All drugs are considered as tetracycline base for dosage purposes.

ACUTE TOXICITY

Swiss white mice (weight 18–20 g) were fasted for 12 hr and then given the antibiotic either by injection intraperitoneally (1 ml of a 1·8–8 mg/ml solution), intravenously (0·5 ml of a 5·6–7 mg/ml solution)

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* Monosodium salt.

and orally (1 ml of a 40–80 mg/ml solution or suspension). Deaths were noted over 7 days and the LD 50 values were calculated by the method of Litchfield & Wilcoxon (1949).

CHRONIC TOXICITY

This was determined in groups of 15 mice. Test animals were given daily 250 mg/kg of the drugs intraperitoneally in 0·5 ml of solution and control animals were given 0·5 ml of physiological saline solution. The groups were weighed every 3 days for the next 60 days. Total blood counts were made when the animals were killed.

EFFECT ON BLOOD PRESSURE AND RESPIRATION

Simultaneous recordings of blood pressure and respiration were made in 6 cats anaesthetised with pentobarbitone sodium (40 mg/kg). The drugs were given in doses of 0·01 and 25 mg/kg. Dogs similarly prepared received only the higher dose of the tetracycline.

EXPERIMENTAL INFECTION

Groups of 10 Swiss white mice (weight 18–21 g) were injected intraperitoneally with 0·5 ml of *Staphylococcus aureus* culture (Smith ATCC 13709, incubated for 5 hr in brain-heart broth Difco; the infecting doses contained $15 \times 10^6 \pm 3 \times 10^6$ cells as determined by plate counts), containing 1% (w/v) of sodium glycocholate and taurocholate (Amsterdam & Schneierson, 1954). The protective action of TMT and tetracycline was then determined using both intravenous and oral routes.

BLOOD LEVELS OF TETRACYCLINES IN DOGS

The drugs were administered orally (50 mg/kg) or intravenously (10 mg/kg), and blood samples were taken from the saphenous vein. A tube-dilution method in broth was used to determine the blood levels, with *Bacillus cereus var. mycoides* as test organism.

Results

ACUTE TOXICITY

The acute toxicity of the three tetracyclines was similar for each of the three routes studied (Table 1).

TABLE 1. ACUTE TOXICITY (LD50 VALUES, mg/kg) OF THREE TETRACYCLINES IN MICE

Route of adm.	Tetracycline	PMT	TMT
Intravenous ..	160	150	170
Intraperitoneal ..	340	330	380
Oral	2,550	1,320	3,000

CHRONIC TOXCITY

The chronic intraperitoneal toxicity of the three tetracyclines is shown in Table 2.

Compound TMT was significantly less toxic than tetracycline or PMT and this was confirmed by the results of the body weight and blood counts.

TABLE 2. CHRONIC INTRAPERITONEAL TOXICITY (LD50 VALUES, mg/kg) OF THREE TETRACYCLINES IN MICE

Tetracycline	PMT	TMT
830 (750–920)	540 (460–640)	3,600 (3,270–3,960)

Mice treated daily with TMT at a dosage of 50 and 200 mg/kg intraperitoneally and 1 and 2 g/kg orally grew significantly more rapidly than controls. The blood counts showed no differences from control samples.

On the other hand, animals treated daily with tetracycline hydrochloride at a dosage of 20 mg/kg intraperitoneally grew significantly slower than controls. A dosage of 1 and 2 g/kg orally was lethal within 2–3 weeks. The blood counts did not differ from control values.

Weight curves of PTM intraperitoneally administered at the dosage of 50 and 20 mg/kg daily were similar to controls: 1 and 2 g/kg orally

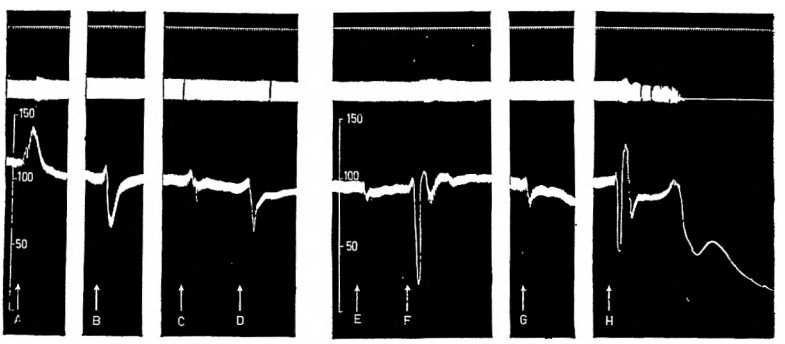

FIG. 1. Cat, female (3·2 kg) under barbiturate anaesthesia. From top to bottom: time (10 min interval), respiration, carotid blood pressure. A = adrenaline 5 μg/kg. B = histamine 5 μg/kg. C = TMT 10 and D, 25 mg/kg. E = tetracycline hydrochloride 10 and F, 25 mg/kg. G = PMT 10 and H, 25 mg/kg.

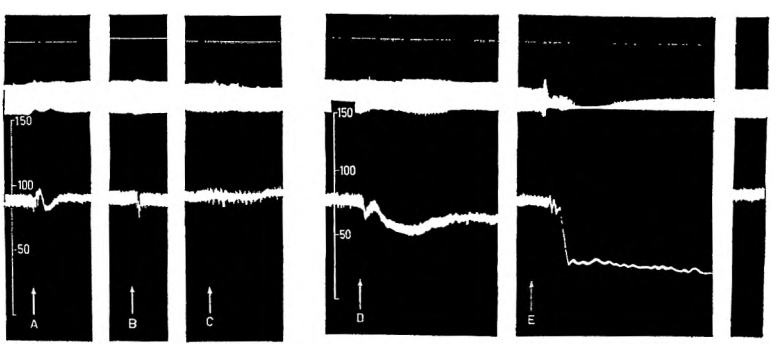

FIG. 2. Dog, female (8 4 kg) under barbiturate anaesthesia. From top to bottom: time (10 min interval), respiration, carotid blood pressure. A = adrenaline 2 μg/kg. B = histamine 2 μg/kg. C = TMT 25 mg/kg. D = tetracycline hydrochloride 25 mg/kg. E = PMT 25 mg/kg.

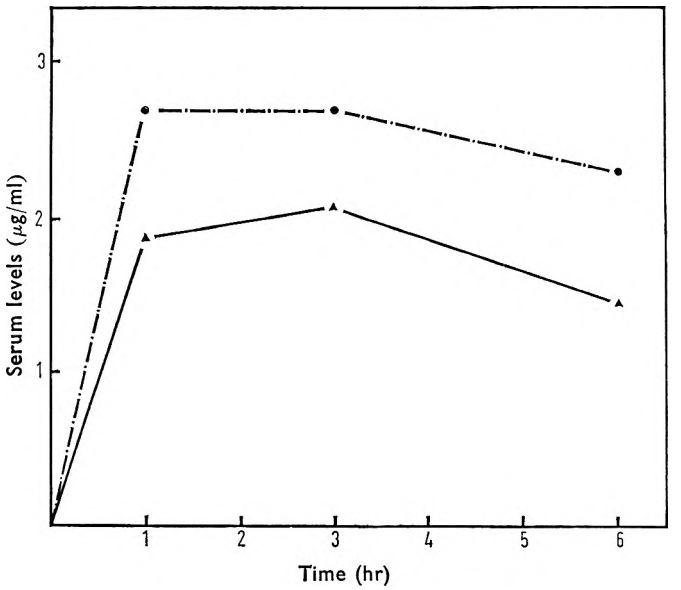

FIG. 3. Blood serum levels in dogs following one single oral dose of 50 mg/kg of tetracycline hydrochloride and TMT.

●—·—·—·—● TMT

▲————▲ Tetracycline hydrochloride

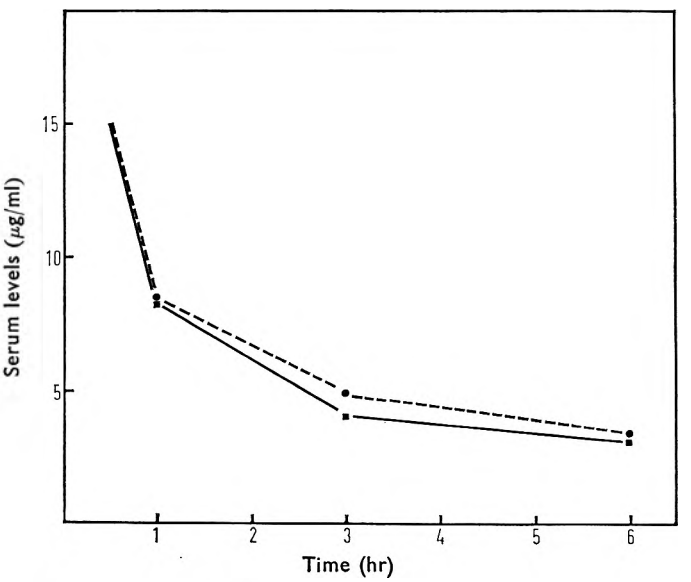

FIG. 4. Blood serum levels in dogs following one single intravenous dose of 10 mg/kg of TMT and PMT.

●— — — —● TMT

■————■ PMT

administered was lethal in 7–14 days. Again the blood counts showed no differences from control samples.

ACTION ON BLOOD PRESSURE AND RESPIRATION

Both in the dog and in the cat the action of TMT on the arterial blood pressure and respiration was less than that of tetracycline. Doses of 25 mg/kg produced only a transient fall in blood pressure in the cat (Fig 1) and no change in the dog (Fig 2) whereas corresponding doses of tetracycline were nearly lethal.

EXPERIMENTAL INFECTION

Tetracycline and TMT were equally effective in protecting mice against the *Staph. aureus* infection. The ED50 values of tetracycline, 8·8 (5·9–13·0)* mg/kg orally and 8·6 (5·9–12·5)* mg/kg intravenously did not differ significantly from those of TMT, 9·0 mg/kg (6·2–13·0)* orally and 5·6 (4·1–7·6)* mg/kg intravenously.

BLOOD LEVELS OF TETRACYCLINES IN DOGS

The blood levels of tetracycline and TMT after oral administration are shown to be similar in Fig 3.

In Fig 4, blood serum levels after a single intravenous dose of 10 mg/kg of TMT and PMT are shown also to be similar.

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* Confidence limits for a 19/20 probability.

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The urinary metabolites of 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole

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The urine of mice, rats, dogs, and men treated with 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole has been examined for metabolic products of the drug. So far, a neutral oxadiazole derivative, which has tentatively been assigned the formula of 3-phenyl-5-hydroxyethyl-1,2,4-oxadiazole, diethylamine and small amounts of unchanged drug have been isolated.

PALAZZO and Corsi (1962) showed that 10 min after the intravenous administration of the antitussive drug 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole, none was detectable in the blood while the quantity of the drug eliminated as such from urine was not more than 2% of the dose administered. Since these results indicated the drug to be almost completely metabolised, the identification of the products of its breakdown was attempted using the urine of the mouse, rat, dog and man.

Experimental methods

The experiments were made on 400 CF1 mice, of both sexes and weighing between 18 and 30 g, and on 1200 Long-Evans or CFN rats, of both sexes and weighing from 140 to 500 g. Immediately after the treatment, the animals were placed in metabolism cages and the urine collected for 24 hr in containers in which N hydrochloric acid was provided to prevent the evaporation of diethylamine. During this time food was withheld but the animals were allowed to drink. Four dogs were also used, in two of which a permanent vesical fistula had been made. In these two animals, urine was collected directly by means of a plastic bottle placed on the abdomen at the location of the vesical fistula. The other two dogs were in normal metabolism cages. The drug was also given to 60 persons and their urine was collected for 8 or 12 hr subsequent to the dose.

THE DECOMPOSITION OF THE DRUG *IN VITRO*

The base, which is an oily liquid, was suspended in water and steam distilled for 3 to 4 hr. The alkaline vapours and the oil carried over were collected in 2N hydrochloric acid where the oil was separated and the solution remaining concentrated under reduced pressure to dryness. This residue was crystallised and analysed. The oily fractions were extracted with ether, washed with diluted hydrochloric acid, purified by distillation under reduced pressure and analysed.

DETERMINATION OF DIETHYLAMINE

Sodium hydroxide was added to the urines to give a pH of 10, the solution was then distilled for 1 to 2 hr in a boiling water-bath and the distillate collected in 2N hydrochloric acid. More than 90% of added diethylamine was recovered even in concentrations as low as 10 μg/ml.

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The quantitative determination of the drug in urine was made before and after the distillation to obviate a high result for diethylamine as some of the drug is split during distillation to give the amine. Some urine samples were treated with deactivated carbon, according to Asatoor and Dalgliesh's (1956) method, to eliminate the drug. There is some loss of diethylamine, but this experimental error has been avoided by controls made each time on urine from untreated animals. The acid distillate was then treated with a 5% solution of filtered Reinecke salt. The three times crystallised precipitate from an 80% ethanol gave m.p and mixed m.p with the reineckate, separately prepared, of 259–261°. (Aycok, Eisenbraun & Schrader, 1951, give m.p 259–61°).

To determine diethylamine quantitatively, the acid distillate was evaporated to dryness, dissolved in water and transferred to a flask which was then made up to volume with glacial acetic acid. The substance was assayed according to Morgan (1958), the readings being made at 233 mμ.

The sensitivity of the method was 5 μg/ml of the final solution in acetic acid.

QUANTITATIVE DETERMINATION OF THE NEUTRAL 3-PHENYL-OXADIAZOLE DERIVATIVE

The urine was diluted when necessary with water to about 30 ml, placed in a 100 ml separatory funnel, acidified with hydrochloric acid to a pH of 2–3 and treated with ether (30 ml) the resulting emulsion being broken with ethanol. The ether layer was retained, the extraction repeated twice with ether and the extracts transferred to a 100 ml separatory funnel and washed with a 5% Na_2CO_3 solution (10 ml). The alkaline layer was separated, the ether was washed with water (10 ml) and then slowly evaporated over a water-bath to dryness. The residue was taken up with 95% ethanol (20 ml) and poured into a 100 ml flask which was made up to volume with the ethanol used for washing. The ultra-violet spectra of the urine extracts were compared first zeroing the spectrophotometer with the control set at 238 mμ. In the presence of a 3-phenyl-1,2,4-oxadiazole compound a curve is obtained with two peaks, at 276 and 284 mμ, and with λ_{max} 238 mμ. The quantitative determinations were made assuming that the neutral 3-phenyl-oxadiazole compound was 5-methyl-3-phenyl-1,2,4-oxadiazole, E(1%, 1 cm) 780. The sensitivity of the method is around 10 μg/ml of ethanol solution. 90% of added 5-methyl-3-phenyl-oxadiazole could be recovered.

SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC DETERMINATION OF THE DRUG

The urine of controls and of treated animals was acidified, extracted according to the procedure above, and then adjusted to pH 10 with 2N sodium hydroxide. This solution was shaken with ether (25 ml) in a separatory funnel. Emulsions were broken with ethanol. The ether layer was retained and the extraction repeated with ether (25 ml). The ether extract was evaporated, the residue taken up with 15 ml of 95% ethanol, transferred to a flask and the volume made up with ethanol used for washing. The ultra-violet spectra of the extracts were then run, first

zeroing the instrument with the control at 238 mμ. In the presence of the drug a curve was obtained similar to that of the neutral oxadiazole compound. Quantitative determination was made allowing for the $E(1\%, 1\text{ cm})$ of the drug citrate being 260. A complete separation from the neutral oxadiazole compound was obtained. About 90% of the product added to the urine was detected. The sensitivity was about 10 μg/ml of ethanol solution.

For the chromatographic determination of the drug, urine (1–4 ml) was made alkaline with a saturated solution of potassium carbonate one half the urine volume. The solution was extracted twice with an equal volume and twice with one half the volume of light petroleum (b.p 60–80°). The light petroleum extracts were combined, evaporated under vacuum below 40°, the residue taken up with 0·5 N citric acid (1 ml) and chromatographed using Whatman No. 1 paper, previously washed for 24 hr with the solvent (t-butanol: acetic acid: water, 60:15:25). The front travel was about 40 cm in 15 hr at 18°. The zones were localized with ultra-violet light, cut out together with the corresponding areas of the blank, and then eluted with water (8 ml). The eluate was checked with the spectrophotometer at 239 mμ, zeroing the apparatus with the blank. About 90% of added drug is detectable. The sensitivity approximates to 5 μg/ml of eluate. The chromatographic and the spectrophotometric methods furnished superimposible results, and were used interchangeably.

CHROMATOGRAPHIC DETERMINATION OF *m*- AND *p*-HYDROXY DERIVATIVES

Urine (1–4 ml) was made alkaline with 1% $NaHCO_3$ solution (0·5 ml) and then extracted with ether (5·5 ml and 3 ml). To the extracts was added 0·1% citric acid solution (1 ml) to make the product soluble in water, and the ether was removed by an air stream at 30°. The aqueous solution remaining was slowly evaporated to dryness under vacuum and the residue taken up in water (0·2–0·3 ml). The final pH should be between 4 and 5. The solution was then chromatographed on Whatman No. 1 paper previously washed for 24 hr with t-butanol: 99% formic acid: water (70:15:15). This solvent was used for development; its travel is about 40 cm in 15 hr at 18°. The zones having an R_f corresponding to that of the derivative being examined (0·82) were localised under ultra-violet light, cut out and with the corresponding blank areas were then eluted in water. The eluate was then measured at 243 mμ with the *m*-hydroxy derivative $E(1\%, 1\text{ cm})$ 168 at 243 mμ, and at 260 mμ with the *p*- hydroxy derivative $E(1\%, 1\text{ cm})$ 315 at 260 mμ. 90% of the derivative added to biological liquids can be detected with a sensitivity of about 5 μg/ml of eluate.

Assays for glycuronic, hippuric and benzoic acid were also made using established procedures.

Results

THE BREAKDOWN OF DRUG *IN VITRO*

The oily fraction extracted with ether and purified, had b.p 85° at 0·2 mm, an ultra-violet absorption curve corresponding to that of 3-

phenyl-1,2,4-oxadiazoles with λ_{max} at 232 mμ. Found: C, 69·8; H, 4·8; N, 16·3. Calc. for $C_{10}H_8N_2O$: C, 70·0; H, 4·7; N, 16·3. The quantity of product recovered accounts for all the drug treated. A direct comparison with 3-phenyl-5-vinyl-1,2,4-oxadiazole has furnished the proof of the identity of the two substances.

The basic fraction from distillation has been identified as diethylamine. The quantities obtained tally with the theoretical ones.

IN VIVO BREAKDOWN

In experiments on rats treated with 1000 mg/kg of drug orally the following fractions were isolated from urines:

(i) unchanged drug in amounts from 1 to 5% of the administered dose;

(ii) diethylamine, in amount corresponding to the metabolism of about 10% of the administered drug;

(iii) a neutral residue, with ultra-violet spectrum corresponding to a 3-phenyl-1,2,4-oxadiazole, in amounts corresponding to the metabolism of 1 to 5% of the administered drug;

(iv) hippuric acid, in amounts corresponding to the theoretical metabolism of 5 to 15% of the administered drug;

(v) a glycuronide in quantities corresponding to about 150 mg for every kg of animal weight.

The neutral 3-phenyl-oxadiazole compound is detectable in urines in larger amount after acid hydrolysis, which suggests that it may be eliminated, acetylated or conjugated with glycuronic acid. On the basis of the results obtained from the *in vitro* decomposition of the drug, the neutral oxadiazole derivative might be 5-hydroxyethyl-3-phenyl-oxadiazole, from the addition of H_2O to 3-phenyl-5-vinyl-oxadiazole. In the fractions containing the glycuronide there are large amounts of benzoic acid and this metabolite may be identified as a benzoylglycuronide. No *m* or *p* hydroxy derivatives were found in urines. The urines of a second group of rats treated orally with 500 mg/kg of the drug citrate yielded the same products as those in the preceding experiment in quantities proportional to the administered dose. A third group treated orally with 50 mg/kg furnished similar results again except that diethylamine was absent or present only in slight traces, while the glycuronide was present in quantities corresponding to about 50 mg/kg of animal weight.

In mice given an oral dose of 500 mg/kg of the drug, hippuric acid, a glycuronide, and traces of diethylamine were found in the urine. But neither drug nor the neutral 3-phenyl-oxadiazole residue was found. In the dog given 100 or 200 mg/kg of drug orally, diethylamine appeared in the urine in amounts of about 5 to 12% of theoretical. Elimination was in the first 6 hr after administration. Hippuric acid, conjugated glycuronic acid, and a neutral 3-phenyl-oxadiazole derivative were also observed.

In man treated with single doses of 100 to 300 mg of the drug citrate, 8 and 12 hr urines contained hippuric acid and conjugated glycuronic

acid. Hippuric acid was nearly all eliminated within 4 hr while glycuronic acid was excreted in greater amounts in the subsequent 4 hr. The drug was present in quantities up to 1–2% of the doses. Diethylamine was absent. The amounts of secondary amines found after treatment did not differ from control values.

Discussion

The 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole molecule can be broken down *in vitro* with the formation of diethylamine and 3-phenyl-5-vinyl-1,2,4-oxadiazole. In the urine of treated animals the fractions so far isolated are diethylamine, a neutral 3-phenyl-1,2,4-oxadiazole derivative and small quantitites of unchanged drug.

The presence of diethylamine on one hand and the other fractions on the other suggest that the metabolism of the drug *in vivo* initially resembles that observed *in vitro*, i.e. the rupture of the lateral alkylamino-ethyl chain. In its turn, the 3-phenyl-oxadiazole nucleus seems to be mainly transformed to benzoic acid, which is excreted in the form of hippuric acid or glycuronide.

The amounts of metabolites, allowing for the experimental loss, account for much of the drug administered, so far as the phenyl-oxadiazole moiety is concerned. Diethylamine was found in about 10% of theoretical quantity for high doses and was either found only in traces or not at all, for lower doses.

It is possible that diethylamine can be eliminated in other ways. Few examples exist of studies on the formation of diethylamine during the metabolism of drugs but we believe this is due more to a lack of specific investigations, rather than to a peculiarity of the metabolism of the drug.

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The hydrolysis of propyl benzoate in aqueous solutions of surface-active agents

A. G. MITCHELL

The effect of cetrimide and sodium lauryl sulphate on the alkaline hydrolysis of emulsions and solutions of n-propyl benzoate has been investigated and compared with results obtained previously in cetomacrogol solutions. Evidence is presented to show that the rate of reaction depends on the degree of saturation of the dispersion expressed as a "Saturation Ratio", which is the ratio of ester concentration to its solubility in a given concentration of surface-active agent. The hydrolysis rate of solubilised ester decreases with increase in surface-active agent concentration while the effect of such an increase in concentration on the hydrolysis rate of emulsified ester depends on the nature of the surface-active agent. The initial rate in sodium lauryl sulphate and cetomacrogol is independent of concentration, but in cetrimide the rate increases with cetrimide concentration until sufficient is present to solubilise the ester.

STUDIES on the hydrolysis of n-propyl benzoate in the non-ionic surface-active agent cetomacrogol (Mitchell, 1963) and the oxidation of aldehydes in various non-ionic surface-active agents (Carless & Mitchell, 1962) have shown that rates of reaction in aqueous solutions of non-ionic surface-active agents depend on the degree of saturation of the dispersion. The degree of saturation can be expressed as a saturation ratio R, in which

$$R = c/c_s \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where c is the concentration of reactant and c_s its solubility in the solution of surface-active agent. For a given saturation ratio the rate of reaction divided by the concentration of reactant is a constant, i.e.

$$\text{rate} = kc \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where from (1) $c = Rc_s$.

The relation between saturation ratio and reaction rate is valid for reactions in aqueous solutions of non-ionic surface-active agents, but does not appear to hold for the oxidation of aldehydes in cationic and anionic surface-active agents (Mitchell, 1960) nor for dispersions in the ampholytic betaines (Carless & Swarbrick, 1962). The present paper reports an investigation into the relation between saturation ratio and the alkaline hydrolysis of n-propyl benzoate in aqueous solutions of cetrimide, a cationic surface-active agent, and sodium lauryl sulphate, an anionic surface-active agent. The results are compared with those obtained earlier using cetomacrogol (Mitchell, 1963).

Experimental

MATERIALS

Cetrimide B.P. 1958 containing 96·8% alkytrimethyl ammonium bromides calculated as $C_{14}H_{29}(Me)_3N,Br$, sodium lauryl sulphate B.P. containing the equivalent of 59·8% of total alchols (B.P. assay), n-propyl benzoate fractionally distilled under reduced pressure, b.p 231°, $[n]_{1\cdot499}^{22°}$.

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DETERMINATION OF SOLUBILITY AND HYDROLYSIS

The experimental procedures have been described previously (Mitchell, 1962, 1963).

Results

The solubilities of n-propyl benzoate in cetrimide and sodium lauryl sulphate solutions at 35° are shown in Fig. 1. The influence of concentration of surface-active agent on the alkaline hydrolysis of a fixed amount

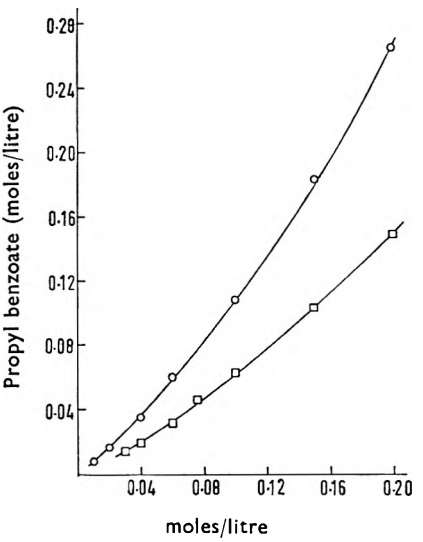

FIG. 1. Solubility of propyl benzoate in cetrimide and sodium lauryl sulphate solutions at 35°.

○ cetrimide. □ sodium lauryl sulphate

of the ester is shown in Figs 2 and 3 and Tables 1 and 2. Hydrolysis in cetrimide solutions followed the pattern reported for emulsions and solutions of ethyl benzoate and diethyl phthalate in cetrimide (Mitchell, 1962).

In the initial stage of the reaction the hydrolysis rate of emulsified ester increased with cetrimide concentration reaching a maximum when sufficient cetrimide was present for complete solubilisation (Fig. 2, curve 3). Addition of cetrimide in excess of that needed for solubilisation caused a fall in the initial rate. As hydrolysis takes place, emulsions become solutions and solutions become progressively less saturated with ester and in the final stages of the reaction the rate decreased with increase in cetrimide concentration.

Ester dispersed in sodium lauryl sulphate solutions was hydrolysed in a manner similar to that found in cetomacrogol (Mitchell, 1963). The initial hydrolysis rate of emulsions was independent of sodium lauryl sulphate concentration and was the same as suspensions of ester in water. In the solubilised state the initial rate of reaction decreased with increase

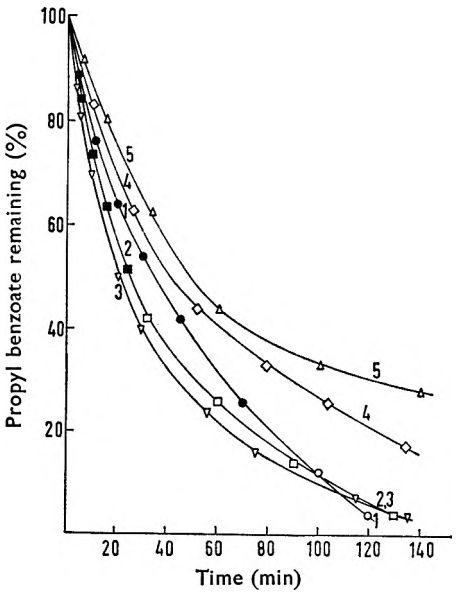

FIG. 2. Influence of cetrimide concentration on the alkaline hydrolysis of propyl benzoate (0·05 moles/litre) at 35°.

Cetrimide concentration (moles/litre): 1, 0·01; 2, 0·03; 3, 0·054; 4, 0·25; 5, 0·80. Closed symbols, emulsions. Open symbols, solutions.

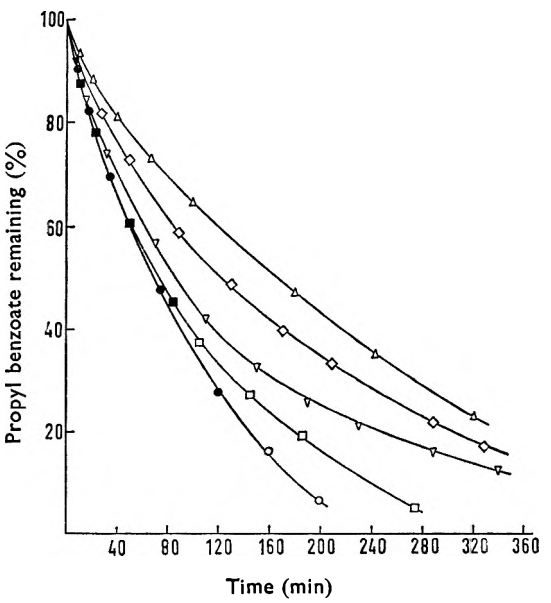

FIG. 3. Influence of sodium lauryl sulphate concentration on the alkaline hydrolysis of propyl benzoate (0·05 moles/litre) at 35°. Sodium lauryl sulphate concentration (moles/litre): ○ 0·02; □ 0·04; ▽ 0·08; ◇ 0·15; △ 0·25. Closed symbols, emulsions. Open symbols, solutions.

in the amount of surface-active agent. In the final stages the rate of hydrolysis decreased progressively with increase in sodium lauryl sulphate concentration as in cetrimide.

Unlike reactions in cetomacrogol the data could not be fitted to first order rate plots. Hence comparisons were made on the basis of half

TABLE 1. DEPENDENCE OF ALKALINE HYDOLYSIS OF PROPYL BENZOATE IN CETRIMIDE SOLUTIONS ON THE SATURATION RATIO

Saturation ratio R	Propyl benzoate moles/litre		Cetrimide moles/litre	Initial rate moles/ litre/ min × 10^4	K $\text{min}^{-1} \times 10^3$ $\left(\frac{\text{Initial rate}}{c}\right)$	$t_{\frac{1}{2}}$ min
	c	c_s				
0·1	0·125	0·125	0·116	1·26	10	65
0·1	0·025	0·25	0·193	2·75	11	67
0·25	0·025	0·10	0·096	6·28	25	28
0·25	0·050	0·25	0·164	13·2	26	27
0·5	0·0125	0·025	0·029	3·74	30	22
0·5	0·0250	0·050	0·054	7·60	30	21
0·5	0·0375	0·075	0·077	11·7	31	22
0·5	0·050	0·100	0·096	18·5	37	19
1·0	0·025	0·025	0·029	10·4	42	25
1·0	0·050	0·050	0·054	19·2	38	24
1·0	0·075	0·075	0·077	31·2	42	22
1·0	0·100	0·100	0·096	40·0	40	25
2·0	0·05	0·025	0·029	13·2	26	30
2·0	0·10	0·050	0·054	22·7	23	31
2·0	0·15	0·075	0·077	34·1	23	34
2·0	0·20	0·100	0·096	50·0	25	34
4·0	0·05	0·0125	0·014	9·33	19	43
4·0	0·10	0·025	0·029	18·2	18	44
4·0	0·20	0·050	0·054	39·2	20	43

$R = c/c_s$
where c = ester concentration
c_s = solubility of ester in cetrimide.

TABLE 2. DEPENDENCE OF ALKALINE HYDROLYSIS OF PROPYL BENZOATE IN SODIUM LAURYL SULPHATE SOLUTIONS ON THE SATURATION RATIO

Saturation ratio R	Propyl benzoate moles/litre		Sodium-lauryl sulphate moles/litre	Initial rate moles/ litre/ min × 10^4	Initial rate constant K $\text{min}^{-1} \times 10^3$ $\left(\frac{\text{Initial rate}}{c}\right)$	$t_{\frac{1}{2}}$ min
	c	c_s				
0·25	0·025	0·1	0·148	1·55	6·2	172
0·25	0·050	0·2	0·250	2·69	5·4	168
0·5	0·0125	0·025	0·049	1·03	8·3	120
0·5	0·0250	0·050	0·086	1·79	7·2	131
0·5	0·0375	0·075	0·120	2·50	6·7	120
0·5	0·050	0·10	0·148	3·82	7·6	124
1·0	0·025	0·025	0·049	2·43	9·7	86
1·0	0·050	0·050	0·086	4·67	9·4	85
1·0	0·075	0·075	0·120	6·75	9·0	88
1·0	0·10	0·10	0·148	9·90	9·9	88
2·0	0·05	0·025	0·049	4·76	9·5	72
2·0	0·10	0·050	0·086	9·90	9·9	68
4·0	0·05	0·0125	0·028	5·11	10·2	63
4·0	0·10	0·0250	0·049	9·90	9·9	67

$R = c/c_s$
where c = ester concentration
c_s = solubility of ester in sodium lauryl sulphate.

lives $t_{\frac{1}{2}}$, and initial rate constants K, calculated by dividing the initial rate by the ester concentration. The results at various saturation ratios are given in Tables 1 and 2.

Discussion

From the initial rate constants, K and half lives, $t_{\frac{1}{2}}$, given in Tables 1 and 2, it would appear that the hydrolysis of propyl benzoate in aqueous solutions of cetrimide and sodium lauryl sulphate depends on the saturation ratio as defined in equation (1). A similar relation between hydrolysis rate and saturation ratio has been shown previously for the hydrolysis of propyl benzoate in cetomacrogol (Mitchell, 1963). This dependence of reaction rate on saturation ratio provides a basis for comparing the effects of different surface-active agents on rates of hydrolysis.

When $R > 1{\cdot}0$, both the true aqueous "phase" and the micelles are fully saturated with ester. Ester in excess of its solubility is present as emulsion droplets stabilised by an adsorbed film of surface-active agent. The effect of an increase in the amount of surface-active agent, and thereby the number of micelles, is to transfer ester from the emulsion droplets to the micelles. The enlargement of the interfacial area of dispersed ester may be expected to facilitate both hydroxyl ion attack and diffusion of ester from the micelles into the true aqueous "phase". In sodium lauryl sulphate and cetomacrogol however the initial rate constant for emulsions is independent of the nature and amount of surface-active agent and therefore the rate of reaction is not controlled by the interfacial area of dispersed ester (Fig. 3). On the other hand, in cetrimide the initial hydrolysis rate of emulsified ester increases with cetrimide concentration, reaching a maximum when sufficient is present to solubilise the ester, i.e. when $R = 1{\cdot}0$ (Fig. 2, curve 3). The increase in rate constant is probably due to attraction of hydroxyl ions to the enlarged interface presented by the positively charged cationic micelles.

Addition of each surface-active agent in excess of that needed for solubilisation so that $R < 1{\cdot}0$, is accompanied by a decrease in rate of hydrolysis. It has been suggested previously (Mitchell, 1962, 1963) that the additional surface-active agent reduces the amount of ester in the true aqueous "phase" relative to that in the micelles. Ester in the micelles is less accessible to hydrolytic attack and the rate of reaction falls.

Rates of hydrolysis as indicated by the half life, depend on the nature of the surface-active agent. At the same saturation ratio the rates of reaction are in the order cetrimide > cetomacrogol > sodium lauryl sulphate. As suggested above, it is likely that the rate of reaction is controlled partly by the charge on the micelle. Thus hydroxyl ions will be attracted to cationic micelles, repelled by anionic micelles and should be unaffected by non-ionic micelles. Half lives in cetomacrogol therefore should be intermediate between those in cetrimide and sodium lauryl sulphate. These suggestions are supported by the results in Tables 1 and 2 and previous work (Mitchell, 1963).

The present paper and others in this series have shown that the hydrolysis of esters and the oxidation of aldehydes dispersed in aqueous solutions of surface-active agents depends, with the exception of oxidation of aldehydes in charged surface-active agents, on the saturation ratio. The saturation ratio can be altered by varying either the concentration of surface-active agent or the concentration of reactant. In the solubilised state a decrease in saturation ratio is accompanied by a decrease in the rate of hydrolysis and oxidation. From a consideration of the nature of these reactions it is probable that the explanation is different in each case. In the case of hydrolysis it has been considered reasonable to assume that ester dispersed in the true aqueous "phase" will be more readily hydrolysed than ester within the micelles. In contrast, oxidation of aldehydes proceeds by a chain reaction (Bäckström, 1927; Cooper & Melville, 1951) and is therefore more likely to be favoured by the local concentrations of aldehyde associated with the micelles. In both cases, however, the addition of surface-active agent in excess of that needed for solubilisation leads to a decrease in reaction rate. Solubilisation can be regarded as a distribution phenomenon in which the water-insoluble material is distributed between the micellar "phase" and the true aqueous "phase" (McBain & Hutchinson, 1955). Since the solute is preferentially soluble in the micelles an increase in the concentration of surface-active agent will increase the amount of solute in the micellar "phase" at the expense of that in true solution. With ester it has been suggested that this is responsible for the observed decrease in the rate of hydrolysis. At the same time, however, an increase in the number of micelles will lead to a reduction in the number of reactant molecules per micelle. This will not affect the rate of a hydrolytic reaction but in the oxidation of aldehydes where a chain mechanism is operating, a decrease in reaction rate is not unexpected.

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The adsorption of iodine from solution by micro-organisms and by serum

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The uptake of iodine by baker's yeast, serum, *E. coli* and *Staph. aureus* from aqueous dilutions of an iodine non-ionic surface-active agent complex was compared with that from aqueous dilutions of an ethanol and potassium iodide solution. The form of adsorption isotherms depended upon the iodine system and the substrate. A characteristic of all isotherms was the high affinity at low iodine concentrations. Except where high ethanol concentrations remained, uptake was greater from the ethanol: potassium iodide dilutions. The pH of the iodine system also influenced the uptake, the effect varying with the iodine system and the substrate.

EVANS & Fishburn (1943) have suggested that the first stage of disinfection by water soluble bactericides was an adsorption to the surface of the bacteria, followed by a chemical reaction between the adsorbed bactericide and the active proteins of the bacteria. Knaysi & Gordon (1930) were able to show that iodine was adsorbed by yeast from an aqueous iodine and iodide solution according to the Freundlich isotherm, whilst Habs (1932) undertook an investigation of the binding of iodine by bacterial cultures, from which he postulated the existence of an adsorption process involving an irreversible and a loose binding by the bacteria. Aqueous iodine and cetomacrogol systems do not produce the characteristic blue colour with starch, itself an adsorption phenomenon, and do not usually stain fabrics, in contrast to solutions of iodine and potassium iodide in ethanol. It would appear, therefore, that there is a fundamental difference between the release of iodine from the two systems. Accordingly the uptake of iodine from these two systems by yeast, serum, *Escherichia coli* and *Staphylococcus aureus* was investigated.

Experimental

MATERIALS

An iodine cetomacrogol complex and solution of iodine and potassium iodide in ethanol (Hugo & Newton, 1963); fresh baker's yeast; sterile horse serum containing no chemical preservative (Burroughs Wellcome & Co.); *Escherichia coli* Type I, formerly NCTC 5934, and *Staphylococcus aureus* NCTC 6571; the chemicals were analytical reagent grade.

METHODS

The yeast and serum were suspended in distilled water so that the dry weight of the suspensions were approximately equal to those of the bacterial suspensions, the dry weight being determined by drying to constant weight at 105°. The bacterial suspensions were prepared by washing 24 hr cultures from the surface of agar slopes, centrifuging at 3500 revs/min for 2 min to remove agar and large clumps, shaken

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for 5 min with glass beads to break up clumps, followed by further centrifuging for 5 min to remove the remaining clumps.

Since the effect of temperature on adsorption occurring from solution has been found to be small (Freundlich, 1926), the reactions were carried out at room temperature with the two iodine preparations simultaneously. The suspensions were mixed with an equal volume of the required concentration of iodine in stoppered containers and agitated gently. At the required time interval a sample was removed and the solid matter separated by centrifuging at 4,000 revs/min for 15 min in closed centrifuge tubes. The clear supernantant liquid was sampled and the iodine content estimated by titration with sodium thiosulphate and an amperometric endpoint. For the uptake by serum, separation could not be achieved by the above process. It was found, however, that by adding an equal volume of a 10% solution of trichloroacetic acid, the serum could be precipitated without affecting the iodine content of either system. This precipitate was removed by centrifuging as before. Buffer solutions were prepared from sodium acetate: hydrochloric acid and disodium hydrogen phosphate: citric acid systems (Vogel, 1951), the pH being measured by a Cambridge pH meter with glass and calomel electrodes.

Results and discussion

The uptake of iodine from the two systems by yeast, serum, and bacteria had an initial rapid stage, followed by a slower stage extending over a period of 4 to 6 hr (Fig. 1). To cover both these stages, the uptake

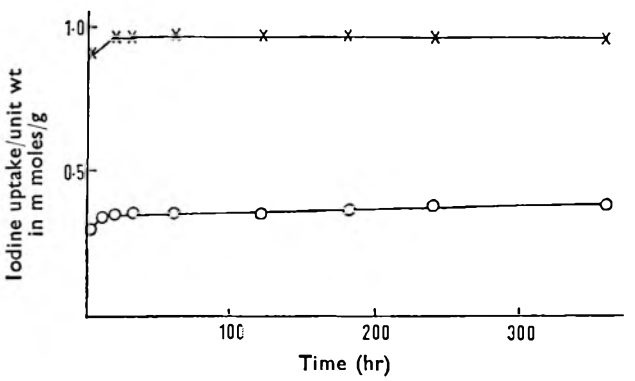

FIG. 1. The rate of uptake of iodine by *Staph. aureus* from iodine formulations. The behaviour of *E. coli*, yeast and serum was similar and over the same range. Original iodine concentration, 1,000 μg/ml. Dry weight of suspension, 1,740 μg/ml. × Iodine solution. O Iodine: cetomacrogol complex.

after 2 min and 5 hr was measured. Whether the mechanism by which iodine is removed from solution involves chemical, or physical mechanisms or a combination of both, cannot be stated from the work carried out. If, however, the uptake of iodine from the two systems is plotted in the form of adsorption isotherms, they show curves the forms of which

can be considered in the light of the classification of isotherms presented by Giles, MacEwan, Wakhwa & Smith (1960) (Figs 2 and 3). In this classification, the isotherms for the adsorption of organic solutes are

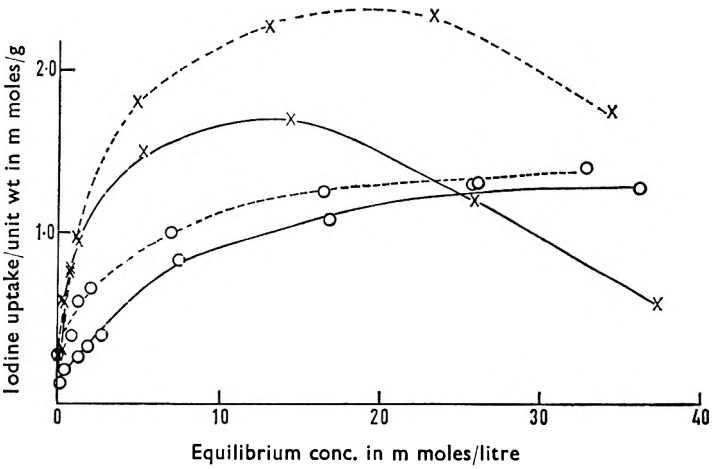

FIG. 2. Adsorption isotherms for the uptake of iodine by yeast (—) and serum - - -) iodine formulations, after 5 hr. Yeast, dry weight of suspension, 2,910 μg/ml for iodine solution and 2,890 μg/ml for the iodine: cetomacrogol complex. Serum, dry weight of suspension, 2,656 μg/ml. × Iodine solution. ○ Iodine: cetomacrogol complex.

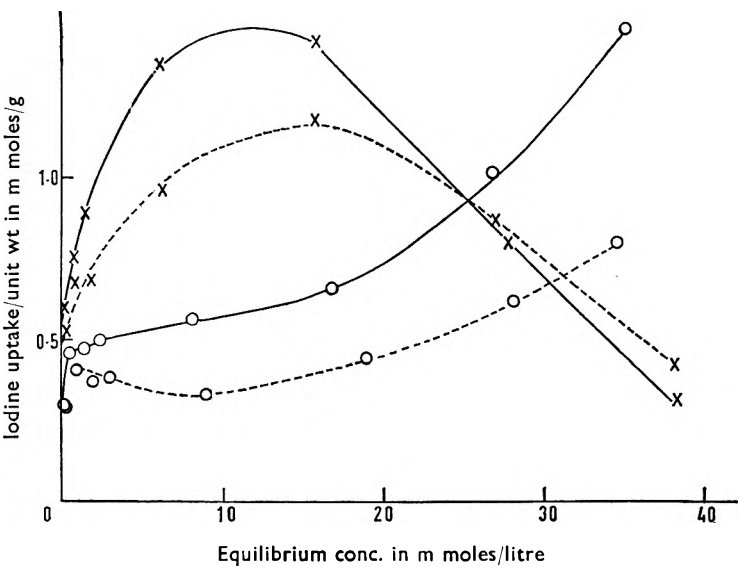

FIG. 3. Adsorption isotherms for the uptake of iodine by *E. coli* (—) and *Staph. aureus* (- - -) from iodine formulations, after 5 hr. *E. coli*, dry weight 2,690 μg/ml for iodine solution and 3,190 μg/ml for the iodine: cetomacrogol complex. *Staph. aureus*, dry weight 3,430 μg/ml for iodine solution and 3,060 μg/ml for the iodine cetomacrogol complex. × Iodine solution. ○ Iodine: cetomacrogol complex.

divided into four main classes, S, L, H and C according to the nature of the initial portion of the curve. Thus for the isotherms of the S type, adsorption is facilitated as the solute is adsorbed, the curve being convex to the x axis. The L type isotherm is of the familiar Langmuir pattern, where adsorption is hindered as the solute is adsorbed, the curve being concave to the x axis. The H type isotherm is similar to the L type, but the affinity of the solute for the adsorbent is so high that the curve commences at a positive value on the y axis. The C type isotherm (constant partition) shows a linear relation between the amount adsorbed and the equilibrium concentration. Each of these four classes are then further divided into five sub-groups, 1, 2, 3, 4, and mx, according to the subsequent shape of the isotherm. The significance of these different isotherms in the diagnosis of adsorption mechanisms is discussed by Giles & others (1960).

In each experiment (Figs 2 and 3) the initial stages of the isotherms follow the H type curve which suggests a high affinity of the iodine for the substrates, such that in dilute solution the amount of iodine remaining in solution could not be measured. After the initial stage, however, the shape of the isotherms varied, depending upon the iodine system and the substrate. There was no difference in the shape of the 2 min and the 5 hr curves, only a displacement to show greater uptake after the longer time.

The whole range of the isotherms investigated for iodine solution takes the same general form for all substrates. From the classification of Giles & others (1960), this shape would be included in the sub-group mx, which is characterised by the occurrence of a maximum, considered to be due to association of the solute. Thus with an increase in concentration the solute:solute attraction begins to increase more rapidly than the solute: substrate attraction. The maximum could also be due to

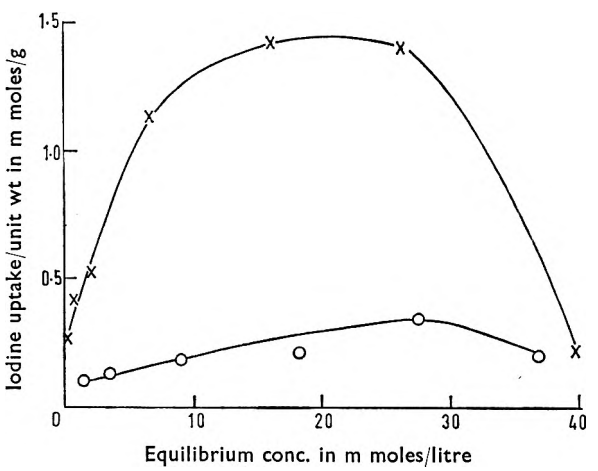

FIG. 4. Adsorption isotherms for the uptake of iodine by yeast from iodine solution, (×) diluted with water and iodine solution (○) diluted with 78% ethanol and 2% potassium iodide, after 2 min. Dry weight of suspension, 3,100 μg/ml.

the change in the solvent which occurs when the iodine solution is diluted with water. To investigate this point, the iodine solution was diluted with the original solvent, that is ethanol: potassium iodide. Fig 4 compares the 2 min uptake by yeast and illustrates the effect of changing the solvent on dilution. Considering the isotherms up to the maxima, they may be classified as H2. This represents the state where as more sites on the substrate become filled, it becomes increasingly difficult for the iodine to find vacant sites, until eventually all the sites are filled with either iodine or solvent and there is a high energy barrier to further adsorption. As these curves are modified Langmuir isotherms, they follow the Freundlich isotherm over a range of iodine concentrations (Fig. 5).

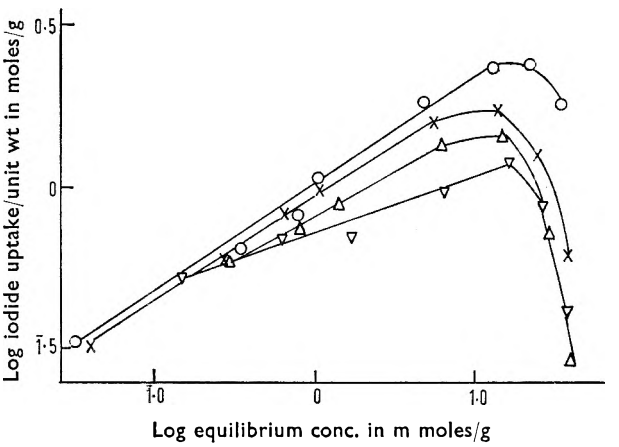

FIG. 5. Freundlich adsorption isotherms for the uptake of iodine by serum (○); yeast (×); *E. coli* (△) and *Staph. aureus* (▽) from iodine solution, after 5 hr. Dry weight of suspension, serum, 2,652 μg/ml; yeast, 2,910 μg/ml; *E. coli*, 2,690 μg/ml and *Staph. aureus*, 3,430 μg/ml.

The type H sub-group 2 isotherm was obtained for the uptake of iodine by yeast and serum from the iodine: cetamacrogol complex (Fig. 2) and thus the same kinds of mechanism are presumably involved. The uptake by the bacteria, however, takes a different form (Fig. 3). Initially there was the same high affinity, but soon a maximum was reached, with a rise as the iodine concentration increased. Thus again there appears to be evidence of the sub-group mx isotherms, with adsorption related to the changes in attraction with concentration. Salton (1951) found that the adsorption of cetyltrimethylammonium bromide by six species of bacteria (including *E. coli* and *Staph. aureus*) was of the H2 type, but irregular isotherms have been noted for the adsorption of cationic surface-active agents by cellulose (Sexsmith & White, 1959) and anionic surface-active agents by cotton (Meader & Fries, 1952). Thus the irregular isotherms for the adsorption of iodine from the iodine: cetomacrogol complex may be related to the surface-active properties of the complex.

In all cases it will be noted that, as anticipated, there was a greater uptake of iodine from the iodine solution, except where the solvent effect dominated the uptake. This suggests that the iodine may be adsorbed from the cetomacrogol system in the form of a complex, or that there is a greater affinity of the iodine for the cetomacrogol than the ethanol: potassium iodide solution. There is also the factor of interfacial tension, Freundlich (1926) considering that adsorption is greatest where the interfacial tension between solvent and substrate is high. The cetomacrogol

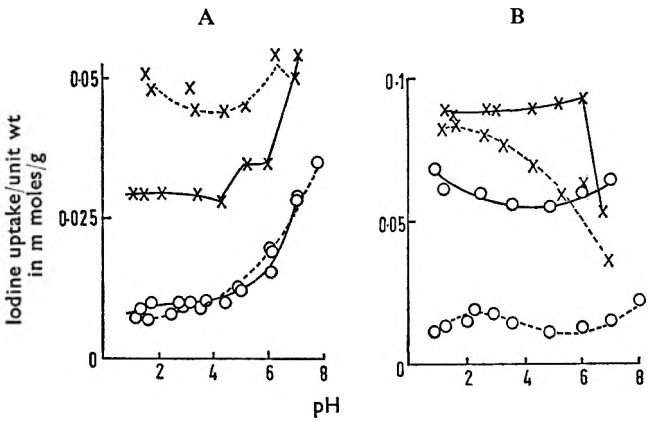

FIG. 6. The effect of pH on the uptake of iodine by (A) *E. coli* (- - -) and *Staph. aureus* (—) and (B) yeast (- - -) and serum (——) from iodine formulations, after 2 min. Original iodine concentration, 1,000 μg/ml.

		Dry weight of suspension μg/ml			
		E. coli	*Staph. aureus*	Yeast	Serum
×	Iodine solution	3,180	3,260	3,100	2,652
O	Iodine: cetomacrogol complex	2,985	3,040	3,200	2·652

can be expected to lower interfacial tension and this may be involved in the smaller uptake. All these factors plus others, however, may be involved in the process. Beckett, Patki & Robinson (1959a,b) found that cetomacrogol reduced the uptake of hexylresorcinol by *E. coli*, and not only prevented the changes in turbidity which took place in aqueous solutions, but considerably reduced the bactericidal activity. The effect of cetomacrogol on the bactericidal activity of iodine will be described by Hugo & Newton (1964).

The influence of pH on the uptake of iodine was dependent on the iodine system and the substrate (Fig 6). The uptake of iodine by yeast from the iodine: cetomacrogol complex changed little with increase in pH whereas a decrease occurred from iodine solution. In connection with this, it was observed that in unbuffered systems there was a fall in the pH after contact with yeast, whilst the converse occurred after contact with bacteria, which show an increased uptake with increase in pH. The difference in the uptake of iodine by yeast is probably related to the difference in the two iodine systems, whilst that between the uptake by yeast and bacteria, to the differences in the nature of the surface of the

adsorbing particles. The increased iodine uptake by bacteria which occurs with increase in the pH is in contrast to the reported decrease in the bactericidal and sporicidal activity which occurs as the pH rises (Gershenfeld & Fox, 1948; Gershenfeld & Witlin, 1949; Chambers, Kalber, Malaney & Bryant, 1952; Hugo & Newton, 1964 and Wyss & Strandskov, 1946).

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Chronic toxicity of brilliant blue FCF, blue VRS, and green S in rats*

W. A. MANNELL AND H. C. GRICE

Groups of twenty rats were given weekly subcutaneous injections of one of the food colours Brilliant Blue FCF, Blue VRS, and Green S. The dose, 20 mg in isotonic saline, was given for 45 weeks after which the rats were observed for an additional 26 weeks. There was ulceration and abscess formation at the site of injection in rats given Blue VRS. The other two colours produced no noticeable local or systemic effects. No subcutaneous fibrosarcomas were seen in any of the rats. Two rats given Blue VRS developed rhabdomyosarcomas in the area of the injection site. This finding is being investigated.

BRILLIANT Blue FCF (C.I. 1924, No. 671), Light Green SF Yellowish (C.I 1924, No. 670) and Fast Green FCF (C.I 1956, No. 42053), three food colours permitted for use in Canada, have been reported to produce fibrosarcomas in rats when given by subcutaneous injection (Nelson & Hagan, 1953). Two colours that have been suggested as alternatives to the ones mentioned are Blue VRS (C.I 1924, No. 672) and Green S (C.I 1924, No. 737). These are on the permitted list of food colours in Great Britain and some other countries. These two colours, as well as Brilliant Blue FCF, were studied using the procedures described by Nelson & Hagan (1953).

Methods

Eighty rats, originally of the Wistar strain, were divided into four groups when they were 5 weeks of age. There were ten males and ten females in each group. The animals were housed in colony cages, ten rats to a cage and given regular laboratory chow and water *ad libitum*.

The rats were given weekly subcutaneous injections of 0·5 ml of one of the following: (1) Isotonic saline (control group). (2) 4% Brilliant Blue in isotonic saline. (3) 4% Green S† in isotonic saline. (4) 4% Blue VRS‡ in isotonic saline.

Thus each rat in groups 2, 3 and 4 received 20 mg of colour per week. The injection site, on the back, was clipped regularly. The rats were examined weekly when injected and were weighed every other week. Forty-five injections were given for a total dose of 900 mg of colour. The experiment was terminated at 71 weeks, i.e. 26 weeks after the last injection. Rats that died during the test were examined to determine cause of death. At the end of the experiment the survivors were killed and gross examination was made of the tissues and organs. Portions of skin, fascia and muscle from the area of injection and other grossly

From the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada

* The sixth of a series on the Toxicity of Food Colours.

† Manufactured by Imperial Chemical Industries Ltd., England.

‡ Manufactured by L. J. Pointing & Son, Ltd., England.

abnormal tissues were embedded in Paraplast* after formalin fixation, and stained by haematoxylin, phloxine and saffron, and by Mallory's phosphotungstic acid haematoxylin technique.

Results

MORTALITY

The % mortality at intervals during the test is given in Table 1. Although there appeared to be differences among the groups, pathological examination indicated that almost all the deaths were due to respiratory infections. None of the deaths were attributed directly to the effects of the injection of food colour.

TABLE 1. PER CENT MORTALITY IN RATS GIVEN SUBCUTANEOUS INJECTIONS OF FOOD COLOURS

Colour	No. of weeks on test					
	12	24	33	44	59	71
None (Control)	0	5	5	15	45	60
Brilliant Blue FCF	0	10	15	15	45	55
Green S	5	30	45	55	85	90
Blue VRS	0	5	10	45	80	85

PATHOLOGY

Gross examination of the skin was made throughout the study and material was available for histologic examination from those animals dying of pneumonia before termination of the test. The skin reaction was most marked in male rats injected with Blue VRS. There was ulceration and abscess formation within 3 weeks of the beginning of treatment in some animals. Focal alopecia and dermatitis developed in other rats on this colour, followed by abscess formation and ulceration. There were irregular exacerbations and remissions of these skin conditions during the experiment. Ultimately there was pachydermia as a result of the chronic sclerodermatitis and focal cicatrisation. There were no similar skin lesions in any of the other three groups.

Tumours were observed in two female rats at the site of injection of Blue VRS. The tumours were similar in size and appearance. One measured 3·5 cm by 2 cm and the other 3 cm by 2 cm. They were roughly round and flattened. The skin overlying the tumours was ulcerated and there was crater formation in both. The skin was adherent near the rim of ulceration but peripherally it could be separated by blunt dissection. In both animals attachment to the underlying fascia and muscles was firm and there appeared to be invasion of these structures by the tumour. One mass was soft, smooth and friable in some areas and dense and firm in others. It was pink to white in colour. The other tumour was firm or rubbery except near the area of ulceration and was pink to grey-white in colour.

Histologically the skin lesions progressed from the areas of normal

* Manufactured by Biological Research Inc.

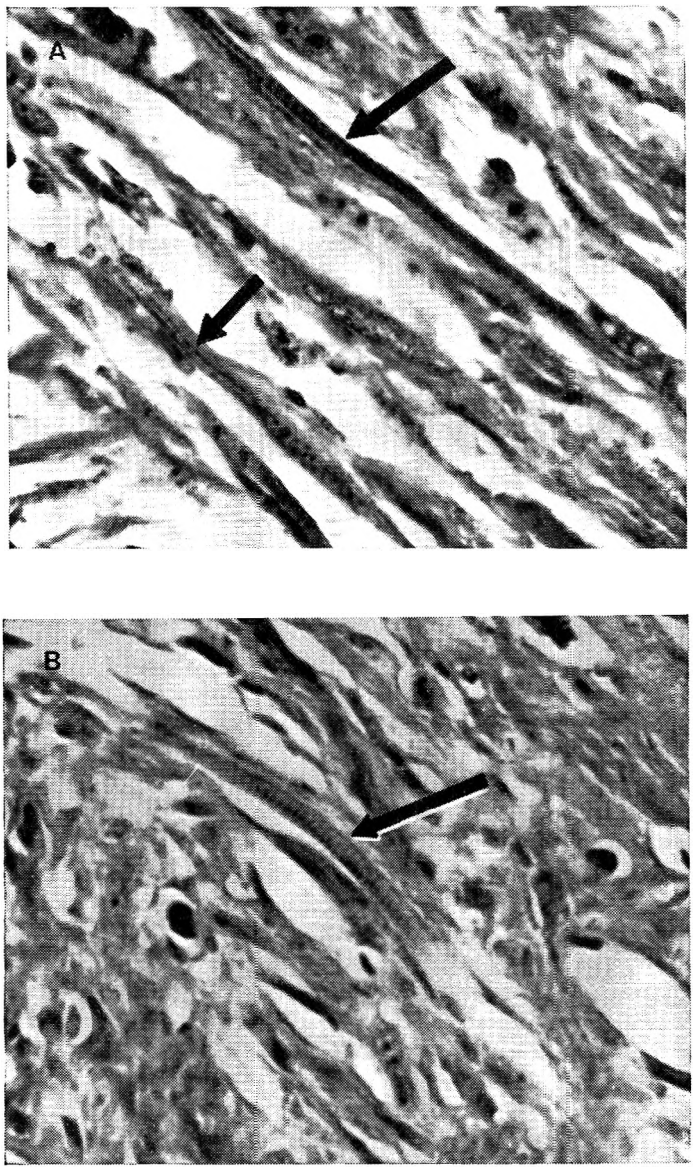

FIG. 1. Sections taken from tumours found at injection site in two rats treated with Blue VRS.

A. Arrows point to rhabdomyoblasts. Cross striations are plainly visible. One elongated cell runs diagonally through centre of field. Mallory's phosphotungstic acid haematoxylin. × 320.

B. Cross striations are visible in one cell (Arrow). Cellular pleomorphism is marked. Mallory's phosphotungstic acid haematoxylin. × 160.

epidermis to pachydermic areas that were characterised by a fibro-granulomatous reaction surrounding areas of ulceration or abscess formation. The latter were located principally in the dermis and were composed of a dense granular necrotic core surrounded by large numbers of leucocytes, predominantly polymorphonuclear neutrophiles, and macrophages and plasma cells. Dense collagen bands that encapsulated the abscesses blended with collagen bundles of surrounding dermis.

The histologic pattern of the two tumours was similar (Fig 1A and B). There was a haphazard arrangement of bands and bundles of cells. These varied greatly in shape and size from round to elliptical to strap shaped. Some cells were short and stubby while the cytoplasm of others extended across the high power field. There was great variation in shape and size of nuclei but oval shapes predominated and many had indentations. Nuclear chromatin was stippled and roughly granular. Most cells contained several nucleoli. Mitotic figures and bizarre giant cells were numerous. Cross striations were clearly exhibited in both tumours (Fig 1). These features led to the diagnosis of rhabdomyosarcoma.

The only other tumours found were a benign uterine polyp and a haemangioendothelioma of the epididymus. Both were in control rats.

Discussion

The object of this experiment was to confirm the work of Nelson & Hagan (1953) with Brilliant Blue and at the same time to see if similar results could be obtained with Blue VRS and Green S, two other triphenyl methane colours. The rats given Blue VRS tolerated the injections poorly and for this reason the treatment was discontinued after 45 weeks. We were thus not able to duplicate the conditions of the experiment of Nelson & Hagan who gave injections of Brilliant Blue for as long as 94–99 weeks. They reported, however, that tumours appeared after 40–45 weeks treatment and it was thought that by observing our animals throughout their life-span some tumours might be found.

This did not occur with rats given Brilliant Blue. It was necessary to end our experiment after 71 weeks because of high mortality from respiratory disease and poor condition of the survivors. Up to this time, however, no indication of fibrosarcoma was seen in any of the rats of any group.

The presence of rhabdomyosarcoma at the injection site, observed in two rats given Blue VRS, has not, to our knowledge, been reported in any of the numerous studies on subcutaneous injection of dyes. Investigation of this finding is planned.

Acknowledgements. Thanks are due to Miss Isabelle Dupuis and Mrs. Anita Hatch for capable technical assistance.

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Letters to the Editor

Potentiation of chlorpromazine-induced behavioural changes by anticholinesterase agents

SIR,—A recent report indicated that phenothiazine derivatives may exacerbate the symptoms of poisoning induced by phosphate insecticides (Arterberry, Bonifaci, Nash & Quinby, 1962). Because of current interest in the possible interaction of therapeutic agents with pesticides, an investigation of the effects of chlorpromazine with several anticholinesterase agents has been undertaken in conditioned animals.

A group of 10 male albino rats (375–450 g), trained for discrete avoidance responding to a level of 95% or better, was used (Goldberg, Johnson, Knaak & Smyth, 1963). Ten control sessions of 120 avoidance trials/hr revealed an average of 4·7 ± 0·6 (s.e.) shocks/animal during the first hr of a 2 hr session. Drugs were injected intraperitoneally twice weekly, with control sessions at a similar time of day three times a week. Chlorpromazine was given at a dose of 1·23 mg/kg. The three reversible cholinesterase inhibitors and the doses used were 1-naphthyl *N*-methyl carbamate [Sevin, 1·25 mg/kg (low dose) and 5·00 mg/kg (high dose)], 3-isopropylphenyl *N*-methyl carbamate (Compound 10854, 0·50 mg/kg) and physostigmine (eserine, 0·16 mg/kg). When given in combination experiments, chlorpromazine and the cholinesterase inhibitor were administered as separate injections. A theoretical additive value of any anticholinesterase agent when given in combination with chlorpromazine is equal to the sum of the individual effects minus the average control values for the animals.

The results, as summarized in Table 1, clearly indicate that potentiation of chlorpromazine-induced behavioural alteration is accomplished with the concomitant administration of any of the cholinesterase inhibitors studied regardless

TABLE 1. ACTION OF CHLORPROMAZINE AND ANTICHOLINESTERASE AGENTS ON DISCRETE AVOIDANCE BEHAVIOUR

Treatment	Shocks/animal ± s.e. (first hr after drug)	Degree of significance from controls	Theoretical additive shock values
Controls	4·7 ± 0·6	—	—
Chlorpromazine	24·5 ± 5·1	$P < 0.01$	—
Sevin (low dose)	4·3 ± 2·2	$P > 0.05$	—
Sevin (high dose)	18·3 ± 6·6	$P < 0.05$	—
Compound 10854	10·5 ± 4·9	$P > 0.05$	—
Eserine	9·4 ± 3·0	$P > 0.05$	—
Chlorpromazine + Sevin (low dose)	43·5 ± 12·8	$P < 0.01$	24·1
Chlorpromazine + Sevin (high dose)	70·4 ± 10·3	$P < 0.001$	38·1
Chlorpromazine + Compound 10854	62·1 ± 13·2	$P < 0.001$	30·3
Chlorpromazine + Eserine	59·0 ± 14·2	$P < 0.001$	29·2

TABLE 2. ACTION OF CHLORPROMAZINE AND SEVIN IN 15 MIN INCREMENTS ON DISCRETE AVOIDANCE BEHAVIOUR

Treatment	Shocks/animal (min after injection)							
	0–15	15–30	30–45	45–60	60–75	75–90	90–105	105–120
Control	1·3	1·1	1·2	1·1	1·0	1·1	1·2	1·1
Chlorpromazine	6·8	6·4	6·3	5·0	5·5	5·2	4·4	3·9
Sevin (high dose)	8·2	5·7	2·2	2·2	2·6	2·7	2·0	1·1
Chlorpromazine + Sevin (high dose)	14·7	16·4	20·4	18·9	17·8	18·6	18·2	18·4

of whether significant behavioural alteration was accomplished by the enzyme inhibitor when given alone. In addition to an exaggerated response which occurred during the first hour, a prolongation of behavioural disruption was apparent. This is revealed in Table 2 in which the average number of shocks during each 15 min period after treatment is given for one of the cholinesterase inhibitors.

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Toxicity of a nucleotoxic agent, mustine hydrochloride, and its enhancement by 5-hydroxytryptamine pretreatment

SIR,—It has been shown by numerous workers that 5-hydroxytryptamine (5-HT) possesses a marked radioprotective activity. The effects of nucleotoxic drugs show certain similarities with the effects produced by ionizing radiation. Many radioprotective agents also provide a protection against the nucleotoxic substances (Scarborough & Thomas, 1962). We have now examined the influence of 5-HT upon the toxicity of mustine hydrochloride (nitrogen mustard), a typical representative of radiomimetic poisons. For testing the specificity of the phenomenon to be described, another toxic agent, chloral hydrate, was used.

Three groups of 20 albino rats were injected intravenously with mustine hydrochloride. The first group received saline, the second 5-HT creatinine sulphate and the third chloral hydrate intraperitoneally 30 min before being given the agent. The survival was observed every 12 hr during 30 days. The results are summarised in Table 1.

TABLE 1. ENHANCEMENT OF MUSTINE HYDROCHLORIDE TOXICITY BY 5-HYDROXYTRYPTAMINE PRETREATMENT

Treatment	Pretreatment	Mortality rate after 30 days (percentage)	Mean survival time in days ± s.e.m.	"t" test (survival time)
Mustine HCl 1 mg/kg i.v.	Saline i.p.	15	26·3 ± 2·0	—
"	5-HT creatinine sulphate 21·2 mg/kg i.p.	55	17·3 ± 2·7	$P < 0.02$
"	Chloral hydrate 270 mg/kg i.p.	20	25·0 ± 2·3	$P > 0.05$

A significant decrease in mean survival time in the group pretreated with 5-HT was noted. The animals pretreated with chloral hydrate showed no significant alteration in survival after mustine hydrochloride. The doses of 5-HT

and chloral hydrate represent the same percentage of their LD50 and given alone had no lethal effect in preliminary tests.

The enhancement of the toxicity of mustine hydrochloride by 5-HT does not seem to be due to simple addition of toxicities since chloral hydrate (in equitoxic dose) did not alter the mean survival time. It is likely, therefore, that the described effect is a specific one.

The present finding is consistent with the experimental data of Field, Mireles & Dolendo (1962) who found that KB 95 (benzpiperylon, 4-benzyl-2-(1-methyl-piperid-4-yl)-5-phenyl-3-pyrazolone) an antagonist of 5-HT, provides a marked protection against mustine hydrochloride intoxication in mice.

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Antagonism of some spasmolytic drugs by calcium on guinea-pig isolated ileum

SIR,—We have recently pointed out that different mechanisms of action may be involved in the spasmolytic activity of the main papaverine derivatives (Santi, Ferrari & Contessa, 1963). This conclusion appears to be supported by our recent findings that changes in ionic environment may affect the *in vitro* activity of some spasmolytic drugs. The most striking effects have been observed by increasing the calcium concentration in the bath fluid.

The ileum of the guinea-pig was suspended in a 30 ml bath containing Tyrode medium at 37°; air was bubbled through the bath fluid, and the spasmolytic drugs [papaverine hydrochloride, eupaverin sulphate (1-benzyl-3-ethyl-6,7-dimethoxyisoquinoline) isoxsuprine hydrochloride] were added at concentrations, ranging from 1 to 8 μg/ml and allowed to act for 2 min before the addition of acetylcholine or histamine. Some experiments were made in anoxia, by replacing air bubbling through the bath fluid with 95% nitrogen and 5% CO_2.

Under these experimental conditions it was observed that $CaCl_2$ (300–400 μg/ml) strongly counteracted the spasmolytic activity of the drugs tested. When added after the failure of acetylcholine to stimulate the isolated gut pretreated with spasmolytic agents, $CaCl_2$ was able to restore a prolonged tonic contraction (which is abolished by atropine). This effect occurred after both isoxsuprine and eupaverin in concentrations ranging from 2 to 8 μg/ml. It was also detectable after 1–2 μg/ml papaverine but readily disappeared after increasing the papaverine concentration. Thus, $CaCl_2$ was less active against papaverine than against eupaverin and isoxsuprine. Furthermore, $CaCl_2$ was unable to remove the inhibition of the tonic phase of the acetylcholine or histamine-induced contraction caused by 2,4-dinitrophenol and by oxygen lack.

The data so far obtained indicate a clear antagonism between the excess calcium and the activity of some spasmolytic agents. Since calcium ions are believed to play a key role in muscular contraction as an excitation-contraction

coupling factor (Bianchi, 1961), it is tempting to assume that an interference with calcium activity may take a part in the mechanism of action of some spasmolytic drugs. On the other hand, the myolytic effect of 2,4-dinitrophenol, ascribed to impaired synthesis of high energy phosphate bonds by West, Hadden & Farah (1951), was not relieved by $CaCl_2$. This fact may account for the lower activity (compared with eupaverin and isoxsuprine) that calcium exercises against papaverine which strongly inhibits oxidative phosphorylation (Santi, Contessa & Ferrari, 1963). Thus, it seems reasonable to investigate whether papaverine may have a dual mechanism of action involving both inhibition of oxidative phosphorylation and an interference with the role of calcium, which is presumably the predominating factor in other spasmolytic agents. Finally the results with isoxsuprine, which is an isoprenaline congener, appear to be in agreement with other findings suggesting that isoprenaline may prevent the entry of calcium into the cell (Schild, 1963).

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Teratogenic activity of drugs

SIR,—Many chemical substances have the power, when administered to pregnant animals, of producing congenital malformations in the young. Methods by which new drugs possessing this action may be readily distinguished are therefore of major importance. By administering drugs initially daily throughout pregnancy and later only during the first trimester, an estimate may be made of the drugs which are most likely to exhibit teratogenic activity in man.

In the course of testing over 40 compounds by this procedure, three types of drug have emerged. Firstly, drugs which kill the mother before any effect is observed on the foetuses; secondly, drugs which kill most of the foetuses before any effect is observed on the mother; and thirdly, drugs which do not kill the mother but which produce changes within the foetuses. As previously suggested (West, 1962), an indication of a teratogenic risk may be obtained by relating foetal resorptions to the doses administered to the mother. When this relationship is made for reserpine, guanethidine and thalidomide (three drugs at one time widely used in human pregnancy), straight line graphs of quite different slopes are obtained. These are shown in Fig. 1. For this work, daily intraperitoneal doses of the drugs were given to groups of 4 rats throughout pregnancy and then to groups during the first trimester. Animals were killed on the 20th day of gestation, and foetal mortality was calculated from the number of live and dead foetuses found. For guanethidine, a horizontal line was obtained since this drug killed the mother before it was lethal to the foetuses. For reserpine, a very steep line was obtained since this drug killed all the foetuses before it was lethal

to the mother. For thalidomide, the slope was very gentle since this drug did not kill the mother and was lethal to only a small proportion of the foetuses.

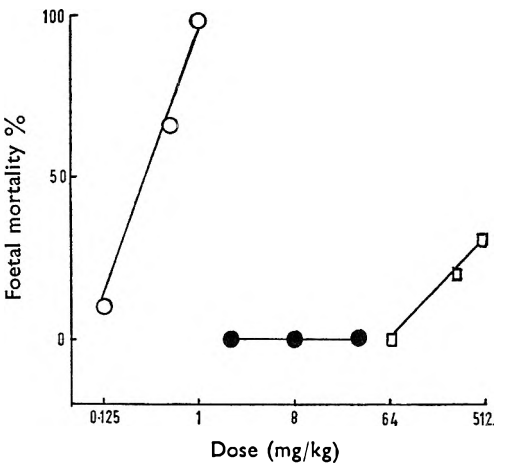

FIG. 1. The relation between foetal mortality and log dose of reserpine (○), guanethidine (●) and thalidomide (□) in rats. The highest doses of guanethidine killed the pregnant rats but did not kill the foetuses.

This last type of effect appears to be more likely to produce congenital malformations in the young since a 10-fold increase in dose produces only a slight increase in lethal action and there is more opportunity to modify the differentiation of tissues in those foetuses which live.

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Research Papers

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