Effect of Nonionic Surfactants on the Transport of Testosterone across a Cellulose Acetate Membrane

P. M. SHORT*, E. T. ABBS†, and C. T. RHODES

Abstract □ Solubilization of testosterone by a series of three alkylpolyoxyethylene surfactants at 37° has been examined as a function of surfactant concentration. The effect of these surfactants has also been investigated upon the diffusion of testosterone through cellulose acetate membranes. Diffusion coefficients were calculated using a method that allows the measurements to be completed in a very short time. Possible mechanisms by which surfactants may affect drug transport are discussed. In all cases examined, the surfactants reduced the diffusion coefficient of testosterone.

Keyphrases □ Testosterone transport—cellulose membrane □ Surfactants effect—testosterone transport, cellulose membrane □ Solubilization, testosterone—surfactants □ Diffusion coefficient—testosterone

The effect of surfactants upon the biological availability and pharmacological activity of drugs has attracted the attention of a considerable number of researchers in recent years (1).

In the present work, the authors report an investigation of the solubilization of testosterone by three alkylpolyoxyethylene surfactants. The effect of these surfactants upon the diffusion of testosterone across a cellulose acetate membrane has been studied. The work was carried out in a simple closed system which may be more similar to physiological conditions than the use of a "sink." The authors utilized a method for the evaluation of membrane diffusion coefficients that is rapid and avoids the problems of back diffusion.

EXPERIMENTAL

Materials—Three *n*-alkylpolyoxyethylene surfactants of the general formula $C_{16}OE_nOH$ (A30, A45, and A60) were used.[1] The mean molecular weights of these compounds were estimated by NMR spectroscopy as previously described (2, 3). Testosterone,[2] m.p. 155.5–156.0° [lit. (4) 152–156°], glass-distilled water, spectral quality ethanol, and cellulose acetate membranes[3] were also used.

Testosterone Assay—Testosterone was assayed in 50% ethanol by UV spectrometry at 245 mμ. The molar absorptivity for testosterone at this wavelength was found to be 1.61×10^4, obeying the Beer-Lambert law. Surfactant solutions were used as blanks when required.

Solubility Determinations—An aqueous suspension of testosterone, plus the appropriate amount of surfactant, was stirred for 1 week at 37 ± 0.1° until equilibrium had been reached. Samples were filtered twice through 0.22-μm. membrane filters[4] and assayed spectrophotometrically.

Determination of Diffusion Coefficients—The apparatus used was similar to that described by Humphreys and Rhodes (3). The Perspex donor and recipient cells were separated by the membrane and stirring was effected by bar magnets activated by immersible control units. The temperature was controlled at 37 ± 0.1°. Preliminary tests showed that the surfactant did not cross the membrane, although small traces of nonsurface-active impurities did. Blank solutions, not containing drug, were therefore used as reference in the spectrophotometric assay.

In all the diffusion results, from which diffusion coefficients, D values, were calculated, the total initial concentration of steroid, $[D_w] + [D_m]$, in the donor cell was 6.93×10^{-5} M.

The thickness of the membrane, L, used in the diffusion studies was obtained from replicate determinations made using two micrometer screw gauges; the L value for the wet membrane was 6.4×10^{-5} m.

Results were calculated using an Elliott 803 digital computer.

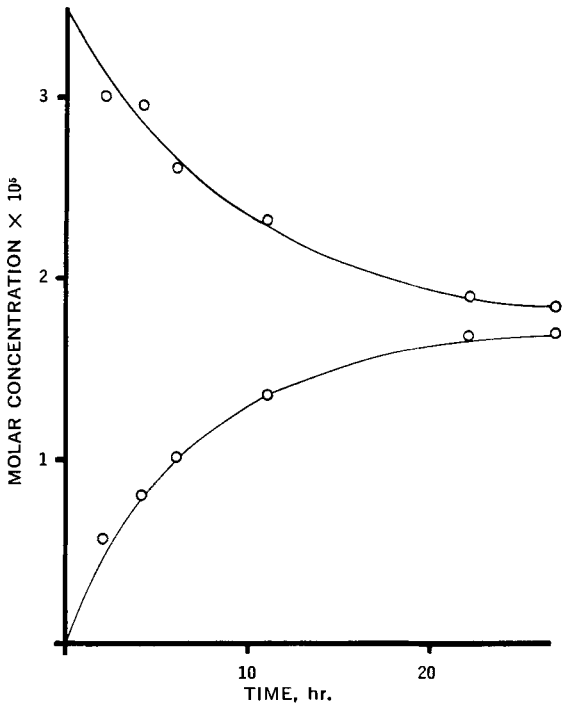

Figure 1—*Testosterone concentration in donor (upper curve) and recipient (lower curve) cells as a function of time.*

THEORY

A typical set of full-term diffusion study results showing the concentration of drug in both the donor and recipient cells as a function of time is shown in Fig. 1. It is possible, using equations based on first-order kinetic assumptions, to determine transport rates from such data. Although such determinations can be most useful, they are subject to several limitations. The transport process is often inconveniently lengthy. Also, back diffusion of drug can complicate the estimation of diffusion coefficients.

Rogers *et al.* (5) derived equations which overcome the difficulties outlined above. They investigated the diffusion of helium across glass and obtained the following equation:

$$(dp/dt) = (2A/V)SP_1\,(D/\pi t)^{0.5} \sum_{m=0}^{\infty} \exp[-(L^2/4Dt) \times (2m+1)^2] \quad \text{(Eq. 1)}$$

[1] Glover's Ltd., Leeds, England.
[2] Steraloids Ltd., Croydon, England.
[3] Visking, Scientific Instruments, Chichester, England.
[4] Millipore Ltd., Middlesex, England.

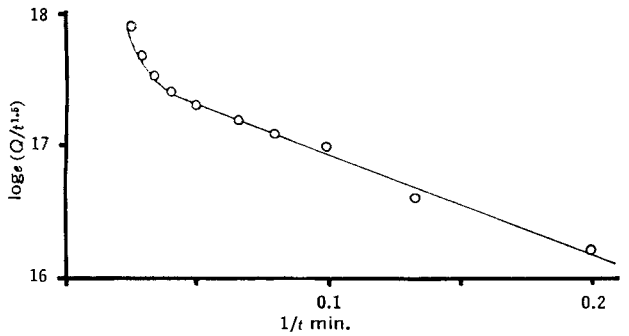

Figure 2—*Plot for the determination of diffusion coefficient.*

where P represents the pressure, t the time, A the area of the membrane, L the membrane thickness, P_1 the pressure on the donor side of the membrane, S the solubility coefficient, and D the diffusion coefficient. This equation is derived from Fick's law and includes the assumptions that D is independent of time and pressure (*i.e.*, concentration for a solution). However, measurements are made over a very short time period and the D determined is that for when $t \to 0$. Thus, these assumptions are unlikely to be of great practical significance for small concentration changes.

By integrating the original equation of Rogers *et al.*, Eq. 2 is obtained, in which the diffusion coefficient is related to the amount of drug diffused:

$$\frac{S_0 Q}{S_i C_0 L} = \frac{8}{\sqrt{\pi}}\left(\frac{Dt}{L^2}\right)^{1.5} \exp -L^2/4Dt\left[1 - \frac{6Dt}{L^2} + 60\left(\frac{Dt}{L^2}\right)^2 + \cdots - \frac{1}{9}\left\{1 - \left(\frac{6Dt}{9L^2}\right) + 60\left(\frac{Dt}{9L^2}\right) - \cdots^2\right\} \exp -2L^2/Dt\right] \quad \text{(Eq. 2)}$$

where S_0 is the solubility of drug in the solvent, S_i is the solubility of drug in the membrane, C_0 is the concentration of drug in the solvent, Q is the amount of drug diffused across the membrane, of length L, t is the time, and D is the diffusion coefficient.

Not all the terms of the integration are shown in Eq. 2 but, because of the inverted placement of t in the exponentials, this series converges most rapidly for very small values of t rather than for large values. After taking logarithms of both sides, Eq. 3 is obtained:

$$\log_e\left(\frac{Q}{t^{1.5}}\right) = \log_e\left(\frac{8C_0S_i}{\sqrt{\pi}\,L^2S_0}\right) + \frac{3}{2}\log_e D - \frac{L^2}{4D}\cdot\frac{1}{t} \quad \text{(Eq. 3)}$$

By plotting $\log_e (Q/t^{1.5})$ as a function of reciprocal time, a straight line is obtained (Fig. 2). From the slope of the line the diffusion coefficient, D, may be obtained using Eq. 4:

$$\text{slope} = -L^2/4D \quad \text{(Eq. 4)}$$

When accurate values of S_i are available, high precision values of D may be calculated by use of an iterative technique (Fig. 3). In the work reported in this paper, however, D has been calculated directly from Eq 4.

There are limitations at either end of the straight line obtained by use of Eq. 3. At the beginning of the transport process, concentrations of drug in the recipient cell are very low and errors in their analytical estimation are thus relatively high. Later when the concentration of drug in the recipient cell exceeds a critical value, back diffusion occurs and the graph starts to curve. However, results on the linear portion of the graph always returned correlation coefficients approaching unity; for example, a typical set of results of five readings gave a correlation coefficient of 0.990.

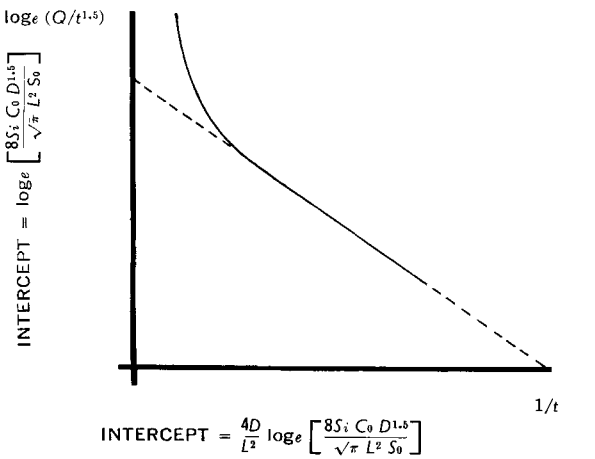

Figure 3—*Plot for the determination of high precision diffusion coefficient.*

RESULTS AND DISCUSSION

The results of the solubilization study are shown in Fig. 4. In all cases the relationship between steroid solubility and surfactant concentration was linear. This type of solubilization isotherm is indicative of micellar solubilization governed by a distribution equation (6). Distribution coefficients for testosterone in the solutions of the three surfactants have been calculated by the method of Humphreys and Rhodes (3) (Table I). When the extent of solubilization is calculated on a molar rather than a percent weight basis, the solubilization efficiency increases with chain length. This finding indicates that the solubilized steroid may be primarily located in the polyoxyethylene exterior of the micelle. Spectroscopic studies of the solubilization of several steroids have led to a similar conclusion (7).

In any aqueous isotopic surfactant solution containing a drug, the following equilibrium will exist:

$$[D_w] \rightleftharpoons [D_m] \quad \text{(Eq. 5)}$$

where D_w represents the free and D_m the bound or micellar drug. In the transport of a drug from a surfactant solution across a membrane the bound or micellar drug is not normally involved. Passive transport of drug across a membrane is a function of the concentration (or more accurately, activity) gradient of free drug across the membrane, and reduction in the value of $[D_w]$ will tend to reduce drug transport. (In those cases in which pinocytosis of micellar drug can occur the situation will, of course, be more complex.)

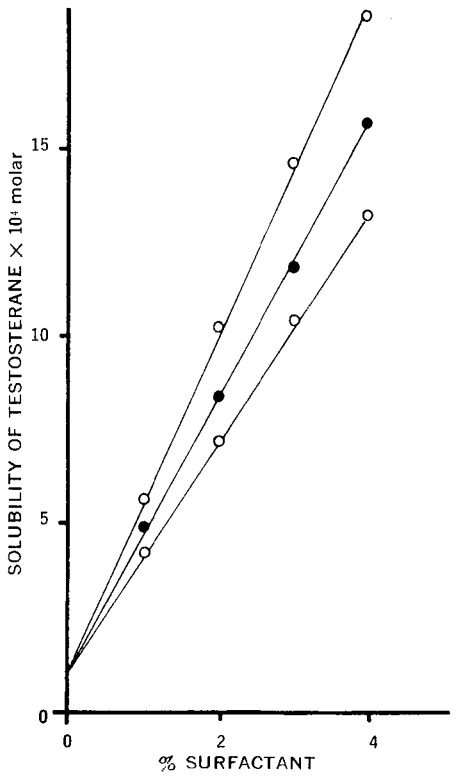

Figure 4—*Testosterone solubilization at 37° as a function of percentage n-alkyl polyoxyethylene concentration. Key: Top line, A30; middle line, A45; and bottom line, A60.*

Table I—Diffusion and Micellar Partition Coefficients for Testosterone in *n*-Alkylpolyoxyethylene Surfactant Solutions at 37°

Surfactant	No. of Ethylene Oxide Groups Estimated by NMR	K_d	Diffusion Coefficient $\times 10^7$ cm.^{2} min.$^{-1}$
—	—	—	18.80
A30	34	476	7.41
A45	62	397	10.18
A60	88	333	11.58

There are several recent reports exemplifying the reduction in the antimicrobial action of drugs caused by surfactants. However, it is apparent that other effects besides reductions in $[D_w]$ are also operative (8, 9).

The second mechanism by which surfactants can alter the transport of drugs from solution across a membrane is by modifying the aqueous diffusion coefficient of the drug. Recent work by Bloor *et al.* has shown that surfactants can increase or decrease such values (10, 11). Thus, in systems where diffusion of drug to the membrane surface is a rate-limiting factor the presence of surfactant could increase or decrease the overall transport rate. It is also possible, though rather unlikely, that monomeric surfactant might reduce the aqueous activity coefficient of the drug, the diminished activity gradient resulting in slower diffusion.

In those cases where adsorption of drug upon the membrane surface is a necessary prerequisite to membrane passage, the presence of surfactant may have further influence. Reduction of surface tension at the interface is likely to have a generalized depressant effect upon the adsorption of all species. The possibility of competition between monomeric surfactant and drug for adsorption sites also exists. However, since it is feasible that a surfactant–drug complex, such as mixed micelle, might also be adsorbed, presence of surfactant could increase or decrease the amount of drug adsorbed. The overall effect will depend upon the relative concentrations of the various species present and the values of their free energies of sorption for the membrane involved. For those substances for which active transport systems exist in a biological membrane, there is a possibility of specific interference by the surfactant with this process. There are also a number of reports which may indicate that surfactants can have direct effects on membrane permeability (12–14). Such effects could be caused by partial defatting or interaction between the surfactant and protein or phospholipid (15).

Because surfactants may affect drug transport in so many ways, there are great advantages in using initially simple *in vitro* studies which can be designed so as to allow the different mechanisms to be distinguished. Such work, of course, can not be regarded as a substitute for *in vivo* evaluation.

Diffusion coefficients of testosterone in distilled water and in aqueous 1% w/v solutions of the three surfactants, increasing in HLB value, are shown in Table I, and the effect of surfactant concentration upon the testosterone diffusion coefficient is recorded in Table II. These results show that the higher the K_d value, *i.e.*, the more the equilibrium shown in Eq. 5 is in favor of the micellar pseudophase, the greater the reduction in diffusion coefficient. Plaxco *et al.* observed similar effects with ethylene oxide chain length, and thus HLB, in investigations of drug release from suppositories (16).

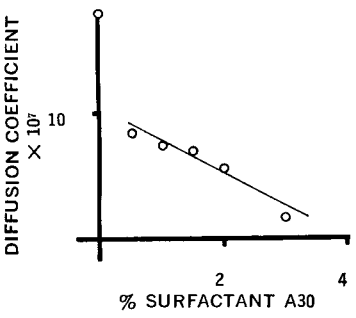

Figure 5—*Effect of surfactant (A30) concentration on testosterone diffusion coefficient.*

Table II—Diffusion Coefficients for Testosterone as a Function of Surfactant (A30) Concentration at 37°

Surfactant Concentration, % w/v	Diffusion Coefficient $\times 10^7$ cm.^{2} min.$^{-1}$
0.5	8.44
1.0	7.41
1.5	6.93
2.0	5.54
3.0	1.56

Figure 5 shows the effect of the concentration of surfactant upon diffusion coefficient. The value of the diffusion coefficient determined in the presence of surfactant decreases linearly with increase in surfactant concentration.

Extrapolation, to zero surfactant concentration, of the relationship between finite surfactant concentration and diffusion coefficient yields a value substantially different to the diffusion coefficient of testosterone determined in distilled water. It is highly improbable that this finding is due to experimental error. Since the surfactant solutions used in this investigation were stirred during the membrane diffusion measurements, this effect cannot be attributed to modification of the aqueous diffusion of the steroid. This change must therefore be attributed to some effect of the surfactant upon the membrane. It seems likely that D changes rapidly at or about the CMC (critical micelle concentration). It has been established that there is no permanent interference with the integrity of the membrane. A membrane, which had been used for the study of the effect of surfactant upon the steroid membrane transport, when thoroughly washed, behaved normally with respect to the steroid diffusion. It is suggested that this depressant effect on steroid transport is probably due either to a generalized inhibition of adsorption at the membrane–solution interface or competition between the monomeric surfactant and steroid for adsorption sites upon the membrane. Further studies of the effect of surfactants upon the transport of drugs across membranes will be published shortly.

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Hydrogenation of Substituted Isoquinolines over Nickel Catalyst II: Effects of Pressure and Temperature on the Hydrogenation of 5-Hydroxy-2-alkylisoquinolinium Salts

IAN W. MATHISON, WILEY L. FOWLER, Jr., and KATHLEEN C. FOWLER

Abstract □ A study of the effect of high pressure and high temperature on the nickel-catalyzed hydrogenation of 5-hydroxy-2-ethylisoquinolinium salt is described. The effects of these parameters on the yield and stereochemistry of the 5-hydroxy-2-ethyldecahydroisoquinolines produced are discussed. Comparisons of these data with those from analogous hydrogenations of the 5-nitro-2-methylisoquinolinium salt are included.

Keyphrases □ 5-Hydroxy-2-alkylisoquinolinium salts—hydrogenation □ Hydrogenation, isoquinolinium salts—temperature, pressure effect □ Vapor phase chromatography—analysis □ GLC—analysis □ IR spectrophotometry—structure

In a continuing study of the stereochemistry of variously substituted, fully reduced isoquinolines possessing pharmacological activity (1, 2), the authors have been recently interested in the hydrogenation of 5-substituted isoquinolines at high temperature and high pressure over Raney nickel catalyst (3). Their initial attention was directed toward the hydrogenation of a 5-nitroisoquinolinium salt in which they were able to demonstrate that increases in temperature were effective in inducing changes in the specificity of the hydrogenation while increases in pressure played little or no role in determining the stereochemistry of the desired 5-aminodecahydroisoquinolines produced. The results in regard to the effects of temperature were not totally unanticipated (4); however, an unexpected result was that increases in temperature initially resulted in increased specificity of hydrogenation up to a certain point which was then followed by a more randomized reduction. It was of significance that the *cis* ring junction decahydroisoquinoline was the heavily favored isomer produced (approximately 13:1 to 2:1, depending on conditions) (5, 6). The authors demonstrated that hydrogenolysis occurred to a significant extent at temperatures of 200° and above, while at 160° and 1500 p.s.i. an optimum yield of 71% of the desired 5-aminodecahydroisoquinolines was produced (*cis*:*trans*, 7.7:1). In view of the reported pharmacological activity of derivatives of 5-hydroxy-2-alkyldecahydroisoquinolines (2, 7) and the need for a rapid, efficient synthesis of these compounds, the authors wish to report the effects of increased pressure and temperature on the one-stage hydrogenation of 5-hydroxy-2-ethylisoquinolinium *p*-toluenesulfonate over W7 Raney nickel catalyst. The effects of these parameters on the stereochemistry of the hydrogenation and yields of the 5-hydroxy-2-ethyldecahydroisoquinolines produced will be discussed and comparisons will be drawn with the previously reported study (3).

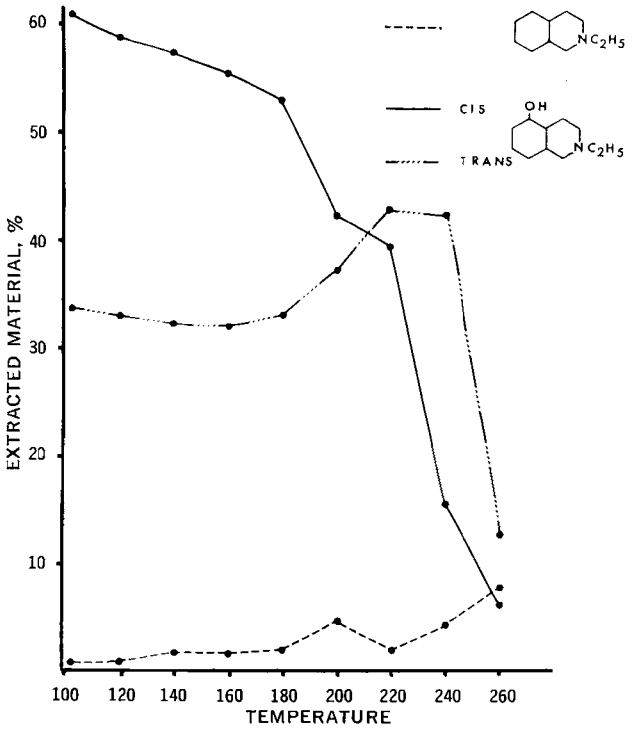

Figure 1—*Effect of temperature on reaction products at 2000 p.s.i.*

EXPERIMENTAL

The melting point is corrected. Analyses were run by Galbraith Laboratories, Knoxville, Tenn. Vapor phase chromatograms were recorded on a Varian Aerograph model 700 Autoprep chromatograph. Chromatographic peak areas were determined using a Dietzgen model D-1803-8 planimeter.