Micellar Solubilization of Testosterone III: Dissolution Behavior of Testosterone in Aqueous Solutions of Selected Surfactants

ARVIND L. THAKKAR and NATHAN A. HALL

Abstract □ The solution behavior of testosterone was studied with a view toward explaining an anomalous initial supersaturation observed in solubilized systems containing testosterone. This anomalous behavior was related to the conversion of an anhydrous crystal form of testosterone to a hydrate crystal form. The solution behavior of the anhydrous form was studied in several concentrations of polysorbate 20, dodecyltrimethylammonium bromide, and potassium laurate. In low concentrations of polysorbate 20 and potassium laurate, the time required to attain the solubility peak was less than that in distilled water. An increase in surfactant concentration resulted in a decrease in the solubility peak time. At higher concentrations, dodecyltrimethylammonium bromide exhibited a pattern similar to that of polysorbate 20 and potassium laurate; at a low concentration, however, a prolongation of the peak solubility time was noted.

Keyphrases □ Micellar solubilization—testosterone □ Testosterone dissolution—aqueous solution □ Surfactant effect—testosterone dissolution □ Anhydrous, hydrated forms—testosterone □ X-ray diffraction—identity □ IR spectrophotometry—identity

In earlier reports (1, 2) the authors discussed the micellar solubilization of testosterone in aqueous solutions of nonionic and ionic surfactants. It was noted that the approach to equilibrium in the solubilized systems was not from undersaturation as might be expected. These systems displayed an anomalous initial supersaturation with respect to the equilibrium solubility of testosterone.

Although the solubilizing properties of surfactants have been extensively investigated and reviewed (3–6), there appears to be no report of an initial supersaturation in approaching equilibrium in these colloidal systems. Mulley (4) has pointed out that high molecular weight solids of low water solubility may give rise to equilibration difficulties; however, no anomalous behavior has been reported for solubilized systems containing steroids. Sjöblom (7) has examined the approach to equilibrium solubility of estrone in aqueous solutions of polysorbate 20, sodium lauryl sulfate, and sodium cholate. He found that equilibrium solubility was attained from undersaturation, and the equilibration time depended upon the nature and the concentration of the surfactant. In general, the equilibration time increased with increasing concentration of the surfactant. In the published reports (8–11) dealing with the solubilization of testosterone, apparently no attempt was made to study in detail the approach to the solubility equilibrium.

This report deals with the dissolution behavior of testosterone in aqueous solutions of polysorbate 20, dodecyltrimethylammonium bromide, and potassium laurate. These surfactants, which have comparable lipophilic portions in their molecules, were chosen as representative of the nonionic, cationic, and anionic types.

EXPERIMENTAL

Materials—The methods of preparation and/or purification of polysorbate 20, dodecyltrimethylammonium bromide (DTAB), and potassium laurate (KL) have been described previously (1, 2). Solutions of the surfactants were prepared using water redistilled in the presence of alkaline permanganate from an all-Pyrex glass apparatus. Three concentrations each of polysorbate 20, DTAB, and KL were used. These three concentrations of the surfactants related to the critical micelle concentration (CMC) as follows: (*a*) the lowest concentration was below the CMC (with the exception of polysorbate 20 where it was at the CMC); (*b*) the intermediate one just above the CMC; (*c*) the highest one well above the CMC.

Testosterone NF,[1] obtained as micronized crystalline powder, was used as received. Since this steroid is reported to exhibit polymorphism (12, 13), the sample of testosterone used in this and earlier (1, 2) studies was characterized definitively. The X-ray diffraction pattern (Debye-Scherrer) and the IR spectrum (mineral oil) of the testosterone sample matched those of Form A prepared by the method of Mesley and Johnson (12). This crystalline form of testosterone, which conformed to the NF specifications and contained no moisture, is designated as the anhydrous form to distinguish it from a hydrate form which will be referred to later.

Dissolution Studies—Preliminary experiments revealed that the initial supersaturation in solubilized systems containing testosterone occurred within the first 0.5 hr. Since it was not practical to withdraw sample vials and analyze their contents at time intervals less than 0.5 hr., using the water-bath shaker that was employed for determining equilibrium solubility (1), it was necessary to use a different apparatus and experimental procedure, described below.

All the dissolution studies were carried out at 25 ± 0.1° in an apparatus designed after Shefter and Higuchi (14). It consisted of a 300-ml. conical flask placed in a Plexiglas jacket through which water at constant temperature was circulated from a water bath equipped with a thermostat and circulating pump.[2] A magnetic stirrer[3] was placed beneath the Plexiglas jacket. The solution in the thermostated flask was agitated by a Teflon-covered magnetic stirring bar 2.7 × 1 cm. ($1^1/_{16} \times {}^3/_8$ in.) rotating at the maximum speed.

For each dissolution study with a particular surfactant solution, the same weight of testosterone was used. This amount of testosterone was approximately eightfold in excess of that needed to saturate the surfactant solution. The weighed sample of testosterone was added rapidly to 200 ml. of the surfactant solution being stirred at 25°. At predetermined time intervals, 4-ml. samples were withdrawn from the system with a syringe and filtered immediately through a Swinnex[4] filter adapter containing a 0.45-μ

[1] Generously supplied by Schering Corp., Bloomfield, N. J.
[2] Haake circulator, series ED, Brinkmann Instruments, Inc., Westbury, L. I., N. Y.
[3] Thomas model 15, Arthur H. Thomas Co., Philadelphia, Pa.
[4] Millipore Corp., Bedford, Mass.

Millipore filter disk.[4] The particle-free filtrates were rapidly diluted to avoid the possibility of precipitation. The appropriately diluted solutions were analyzed for their testosterone content as described previously (1). All the dissolution behavior studies were conducted in duplicate and the results recorded represented the averages of the two determinations.

RESULTS AND DISCUSSION

Since initial supersaturation was observed in aqueous solutions of both the ionic and nonionic type of surfactants containing testosterone, it was felt that this could be attributed to the steroid rather than the particular surfactants or micellar systems in general. The decrease in the high initial solubility and its subsequent leveling would point to the conversion of a metastable crystalline form possessing a higher solubility to a stable form of lower solubility. If such were the case, a similar solution behavior would be expected even when the dissolution medium contained no surfactant. An examination of the solution behavior of the NF grade, anhydrous testosterone does indeed show (Fig. 1) an initial supersaturation with a subsequent leveling in testosterone solubility. A qualitatively similar dissolution behavior was displayed also by a nonmicronized sample which was prepared by recrystallization from chloroform. This sample had an X-ray diffraction pattern and an IR absorption spectrum identical to those of the micronized sample. The possibility that the anomalous initial supersaturation could be due to the testosterone sample being micronized was thus ruled out. The occurrence of this initial supersaturation and a consequent failure to establish a true equilibrium may well explain the conflicting reported values (15–19) of the aqueous solubility of testosterone.

The residue remaining after equilibration (4 days at room temperature) of excess anhydrous sample of testosterone with water was collected and dried on a filter paper under ambient conditions. A comparison of an X-ray diffraction pattern of this sample with that of the original anhydrous sample indicated that an alteration in the crystalline form had occurred. In the discussion that follows, this crystalline form is referred to as the hydrate form. Preliminary work on the crystalline modification using various techniques such as thermogravimetric analysis, differential thermal analysis, and Karl Fischer titration has shown that it is indeed a hydrate. Further work on the characterization and methods of preparation of this crystalline modification of testosterone is under progress.

In 1954, Bischoff and Stauffer (15) made some observations about the solution behavior of testosterone in water. They found that short equilibration periods gave rise to supersaturated solutions. They also observed that if the water was changed after 5 hr. of equilibration, the residue did not again produce a supersaturated solution. Heating this residue to slightly below the melting point restored it to the form that produced supersaturated solutions. Bischoff and Stauffer hypothesized that this difference in solution behavior could be due to a difference in the particle sizes of the two samples of testosterone, *viz.*, one that gave rise to supersaturated solution and one that did not. However, microscopic examination of the two samples revealed that there was no difference in the particle size of the two samples. These workers postulated that the anomalous solution behavior of testosterone might be due to the existence of polymorphic forms.

The observations of Bischoff and Stauffer are readily accounted for in the light of tbe present study. The testosterone sample which

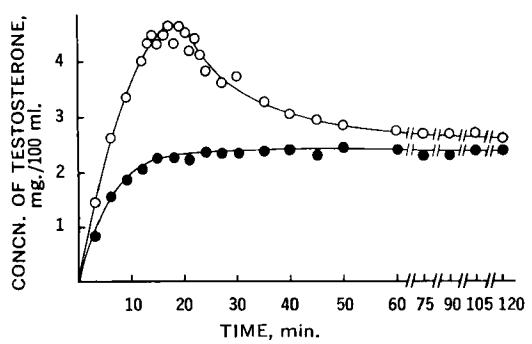

Figure 1—*Solution behavior of testosterone in water. Key:* ○, *anhydrous form;* ●, *hydrate form.*

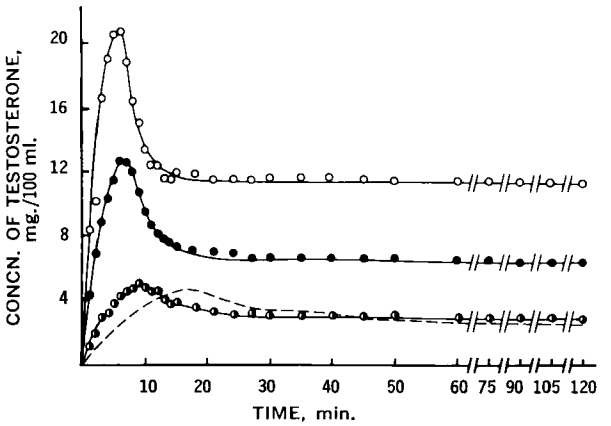

Figure 2—*Solution behavior of testosterone in solutions of polysorbate 20 (w/v). Key:* ◑, *0.01%;* ●, *0.50%;* ○, *1.00%;* - - -, *in water.*

produced supersaturated solutions was clearly the anhydrous form, whereas the water-equilibrated residue, which did not, was a hydrated form. As expected, this hydrate lost its water of hydration and reverted to the anhydrous form when heated to slightly below the melting point. This crystal conversion was confirmed by the similarity of the X-ray diffraction powder patterns of the heat-dehydrated-hydrate sample and the original anhydrous form.

The dissolution behavior in water of both the anhydrous and the hydrate forms was examined. The results are plotted in Fig. 1. It can be seen that the anhydrous form dissolves faster than the hydrate sample and produces concentrations supersaturated with respect to the equilibrium solubility. Similar differences in solution behavior of two anhydrous forms and a hydrate of prednisolone have been observed by Wurster and Taylor (20). Shefter and Higuchi (14) have also reported similar solution behavior differences for a number of organic compounds including the steroids, cholesterol and fluorohydrocortisone acetate. Guttman *et al.* (21) have investigated the solubilization of prednisolone, methylprednisolone, and fluorometholone in aqueous solutions of the nonionic surfactant, ethoxylated tertiary octylphenol formaldehyde polymer.[5] Two of the steroids that they investigated were shown to undergo crystalline modifications (20, 22) in aqueous environment which would be expected to give rise to anomalous solubility values during the approach to equilibrium in solubilized systems. Although Guttman *et al.* did not report any anomalous behavior, they utilized long equilibration times and two different methods to insure equilibrium in systems containing prednisolone. One of these methods involved dissolving an excess of prednisolone at an elevated temperature and then readjusting the system to the experimental temperature. The other method equilibrated the system directly at experimental temperature. It is possible that the different methods were used to overcome an unusual solubility pattern arising from the interconversion of crystalline forms of prednisolone.

The decrease in solubility with time after the peak solubility has been explained for other steroids as being due to nucleation and crystallization of the more stable hydrate (20). The peak solubility value, displayed during the dissolution of the anhydrous form (Fig. 1), may correspond to a short-term steady state involving equal rates of dissolution of the anhydrous form and crystallization of the stable hydrate. On the other hand, the peak solubility could also correspond to the solubility of the anhydrous form (14). As might be expected, the solubilities of both the forms appear to approach the same value with time.

The solution behavior of the anhydrous form alone was studied in the presence of various concentrations of the three surfactants. Figure 2 shows the solution behavior of testosterone in 0.01, 0.5, and 1% (w/v) polysorbate 20. In order to facilitate a comparison of the solution behavior in the presence and absence of the surfactant, the figure also includes the dissolution curve in distilled water. It may be seen that the time required for attaining peak solubility is appreciably less in 0.01% (w/v) polysorbate 20 than in distilled

[5] Triton WR-1339, Rohm and Haas, Philadelphia, Pa.

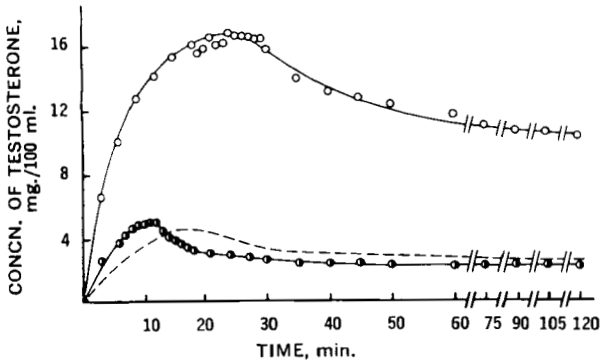

Figure 3—*Solution behavior of testosterone. Key:* ○, *in 0.01* M *dodecyltrimethylammonium bromide* ◑, *in 0.01* M *potassium laurate;* - - -, *in water.*

water. In 0.5% (w/v) polysorbate 20 there is a further reduction in this time but no further reduction is observed when the polysorbate concentration is raised to 1% (w/v).

Figure 3 shows the solution behavior of testosterone in 0.01 *M* solutions of DTAB and KL and in distilled water. It may be seen that the time required for attaining peak solubility in 0.01 *M* KL is shorter than in water, whereas in 0.01 *M* DTAB it is longer. Figures 4 and 5 illustrate the situation in higher concentrations of DTAB and KL, respectively.

The reduction in the time required for attaining the peak solubility in the presence of 0.01% (w/v) polysorbate 20 and 0.01 *M* KL may be explained by the ability of the surfactants to lower the interfacial tension between the solid testosterone and water. The consequent improved wetting of the solid testosterone would enhance the dissolution rate. There is a further reduction in the time required for attaining the peak solubility at polysorbate 20 or KL concentrations higher than the CMC (Figs. 2 and 5), but, as may be expected, the effect becomes practically constant.

A different situation, however, exists in case of DTAB. As may be seen in Fig. 3, the time required for peak solubility in 0.01 *M* DTAB is actually longer than that required in distilled water. Clearly, another factor which dominates the interfacial tension lowering effect must be present. The portion of the solution behavior curve which depicts the decrease in the solubility with time is indicative of the rate at which the less soluble hydrate is crystallized out of the solution. If there is an appreciable reduction in the rate at which the hydrate form crystallizes, then the time required for establishment of a steady state between this rate and the dissolution rate, *i.e.*, the time required for peak solubility, will be prolonged. The presence of the cationic surfactants, dodecyltrimethylammonium chloride and hexadecyltrimethylammonium bromide, have been shown to reduce the crystallization rate of adipic acid (23). This effect has been attributed to the formation of a film at the crystal-solution interface. Other reports that surface-active agents may reduce the crystallization rate also have been made (24, 25). At higher concentrations of DTAB, however, the time required for attaining the peak solubility is reduced (Fig. 4), indicating a surfactant concentration-dependent effect.

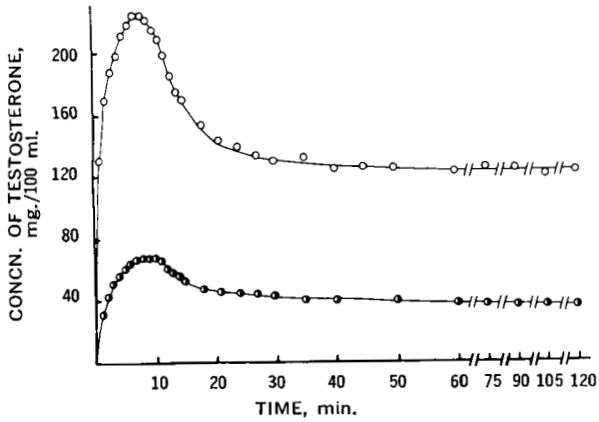

Figure 4—*Solution behavior of testosterone. Key:* ◑, *in 0.02* M *dodecyltrimethylammonium bromide;* ○, *in 0.05* M *dodecyltrimethylammonium bromide.*

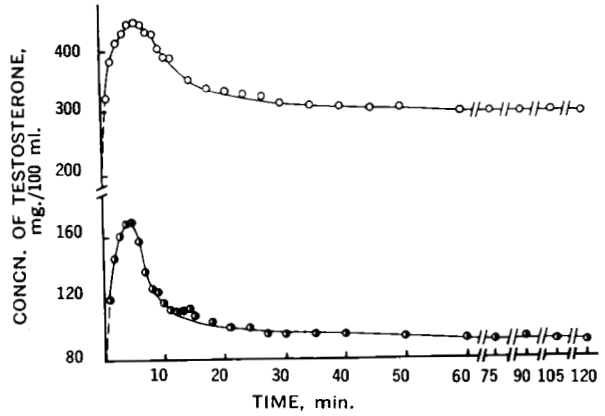

Figure 5—*Solution behavior of testosterone. Key:* ◑, *in 0.04* M *potassium laurate;* ○, *0.08* M *potassium laurate.*

Another effect of the various concentrations of surfactants that is evident from Figs. 2–5 is the extent of solubilization. This phenomenon has been discussed in the previous papers of this series (1, 2). An interesting observation noted from these solution behavior plots is that with an increase in the surfactant concentration, the time required for the testosterone solubility to fall from its peak to a plateau is reduced. This observation may be explained as being due to a greater degree of supersaturation of testosterone in the higher surfactant concentrations, which would be expected to facilitate nucleation of the hydrated crystalline form. The increased time for attaining peak solubility and a subsequent delay in attaining an apparent solubility plateau in case of 0.01 *M* DTAB solution suggests further that this surfactant provides an effective film at the interface which impedes crystal growth. The interfacial film effect is probably obscured by the solubilization effect at higher DTAB concentrations.

In view of the relatively short periods (120 min.) over which these studies were made, it is realized that the solubility values may not be equilibrium values even when successive values constitute an apparent plateau. Also, the agitation conditions provided by the magnetic stirrer apparatus are different from those of the water-bath shaker employed in the previous studies (1, 2); therefore, it would not be realistic to expect more than qualitative similarities in solution rates under the two different conditions. In view of the fact that the particle size and surface area were not rigidly controlled, it is realized that the data of this study are not amenable to mathematical treatment. However, the data presented explain adequately the seemingly anomalous initial supersaturation in solubilized systems containing testosterone. One should expect to encounter similar behavior when working with other solubilizates which exhibit polymorphism or solvate formation.

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Influence of the Route of Administration on the Area Under the Plasma Concentration-Time Curve

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Abstract □ The so-called law of corresponding areas has been used to assess the percent of absorption by comparison of the relative areas under the plasma concentration-time curves after oral and intravenous administration. These analyses are based on the presumption that the resultant areas are independent of the route of administration. After oral administration, every absorbed molecule must pass through the gut wall and when absorbed *via* the hepatic portal system must traverse through the liver, before reaching the central compartment, from which samples are obtained for analysis. However, after the usual i.v. administration, less than 30% of the molecules traverse through the liver in the first circulatory pass. Therefore, if a significant degree of metabolism takes place in the gut or in the liver, the resultant areas will not be identical. This has been verified in dogs by comparison of areas after infusion of acetylsalicylic acid by the vena cava, by the hepatic portal vein, and after oral administration. The data obtained with acetylsalicylic acid indicate metabolism occurs in both the gut wall and in the liver, thereby causing a large reduction in the areas obtained after oral dosing or hepatic portal vein infusion when compared to the area obtained after infusion by the vena cava.

Keyphrases □ Plasma concentration-time curve area—administration route □ Acetylsalicylic acid—i.v., oral administration □ Areas, concentration-time curves—i.v., oral administration □ GLC—analysis □ Fluorometry—analysis

Dost (1) and Gladtke (2, 3) have shown that the area under the plasma concentration-time (PCT) curve is proportional to the dose of drug administered. They utilized *p*-aminohippuric acid and some sulfonamides in their experiments and administered these compounds intravenously and orally. The proportionality between dose and the area under the PCT curve has been referred to as "the law of corresponding areas" and is based on the presumption that distribution, metabolism, and excretion may be expressed in terms of first-order kinetics within the dose ranges studied.

The model in Fig. 1 for the disposition of a drug in the body depicts the manner in which the rate of metabolism and elimination may be proportional to the plasma concentration. This system is an open two-compartmental model in which *Cp*, the plasma concentration, represents the concentration in Compartment 1 and *Ct* represents the concentration in Compartment 2. Metabolite(s) and excreted unchanged drug are represented by *ME* and *kel* represents the first-order rate constant of metabolism and excretion. This model has been shown to describe the disposition in animals of many exogenous compounds. Among such compounds are *p*-aminohippuric acid (4), creatinine (5), aldosterone (6), and acetylsalicylic acid (7). The proportionality between dose and the area under the PCT curve in the model shown in Fig. 1 is based on the following differential equation,

$$\frac{dM}{dt} + \frac{dE}{dt} = \frac{dME}{dt} = kel\ VpCp \qquad \text{(Eq. 1)}$$

where $kel = ke + km$, the first-order rate constants of excretion and metabolism, respectively. The total amount of the metabolized and excreted compound is represented by *ME*, the plasma concentration by *Cp*, and the volume of Compartment 1 by *Vp*. Since it is