Solubility of Cholesterol and Hormone Drugs in Water

DEVENDRA K. MADAN* and DONALD E. CADWALLADER▲

Abstract □ The aqueous solubilities of cholesterol, testosterone, progesterone, and diethylstilbestrol at 30° were determined using a radioactive assay procedure.

Keyphrases □ Cholesterol—radioactive determination of water solubility □ Testosterone—radioactive determination of water solubility □ Progesterone—radioactive determination of water solubility □ Diethylstilbestrol—radioactive determination of water solubility □ Solubility, water—cholesterol, testosterone, progesterone, diethylstilbestrol, radioactive assay procedure

A search of the literature indicated that solubility values for hormone drugs in water varied considerably and in some cases were not available (1–11). Scattered data reported in the literature for the aqueous solubilities of steroid hormones are presented in Table I. No values were found for diethylstilbestrol. As part of a study on the effects of macromolecules on the solubility of cholesterol and some steroid hormones (12), it was necessary to consider the solution behavior of these compounds.

EXPERIMENTAL

Materials—Progesterone[1], diethylstilbestrol[2], and testosterone[3] were recrystallized from alcohol or diluted alcohol, and their melting points were in good agreement with published values. Cholesterol[4] was purified by recrystallizing from acetic acid. The crystals were dried at 90° in an oven. The sample was then recrystallized from a 70% alcohol–water mixture and dried in a vacuum desiccator. The melting point was found to be 148.5°, and the IR spectrum was in good agreement with reported spectra.

Solubility Experiments—A stock solution of cholesterol in benzene was prepared containing 1.0 mg. of untagged cholesterol and 10 μc. of cholesterol-26-^{14}C[5]. Stock solutions of testosterone and progesterone were prepared to contain 200 mg. of untagged compound and 5 μc. of the labeled compound (testosterone-4-^{14}C and progesterone-4-^{14}C)[5] in 100 ml. of benzene, and a similar stock solution of diethylstilbestrol [using diethylstilbestrol (monoethyl-1-^{14}C)][6] was prepared in methanol. A known amount of a particular stock solution was transferred into a 125-ml. iodine flask, and the solvent was evaporated under a mild stream of nitrogen with constant shaking. A 50-ml. volume of dissolution medium (distilled water or 0.9% saline) kept at 30° was added to the flask. Dissolution studies were carried out at 30 ± 0.5° using a controlled environment unit[6] to maintain the temperature. The solution was stirred at approximately 250–300 r.p.m. using a magnetic stirrer and a 2.7 × 1-cm. Teflon-coated spin bar.

For each dissolution study, the amount of compound used was approximately 10 times in excess of its determined aqueous solubility. At predetermined intervals, 5 ml. of sample was withdrawn from the system and filtered immediately through a filtration assembly[7] containing a 0.45-μ filter. Additional samples containing cholesterol were also filtered through 0.22-μ filters.

[1] Sigma Chemical Co.
[2] Matheson, Coleman and Bell.
[3] Shering Corp.
[4] Fisher Scientific.
[5] Nuclear Chicago.
[6] Hot Pack model 1278-8.
[7] Millipore.

Table I—Solubility of Steroids in Water

Compound	Solubility in Water, mcg./ml. This Work (30°)	Literature
Cholesterol	0.09[a]	2600 (20°) (1), 52 (25°) (2)
	0.041[b]	0.025 (30°) (3), 2 (25°) (8), 0.06 (30°) (4)
Progesterone	11.0[c]	9 (25°) (5), 6.6 (25°) (6), 16.8 (37°) (1)
Diethylstilbestrol	25.0[d]	No values found
Testosterone	30.0[e]	24 (25°) (5), 27 (25°) (6), 36 (37°) (6), 29 (25°) (7), 25 (25°) (9–11), 28 (37°) (14), 48 (25°) (2), 125 (37°) (3)

[a] Mean of eight experiments (range 0.07–0.12) using 0.45-μ filter. [b] Mean of eight experiments (range 0.022–0.050) using 0.22-μ filter. [c] Mean of eight experiments (range 8–12). [d] Mean of six experiments (range 21–28). [e] Mean of eight experiments (range 26–33).

Dissolution experiments were carried out in water and 0.9% NaCl solution for cholesterol, testosterone, progesterone, and diethylstilbestrol. A 100-ml. volume of dissolution medium in a 250-ml. iodine flask was used for testosterone studies.

Radioactive Assay Procedure—A 0.20-ml. volume of the particle-free filtrate was pipeted into a liquid scintillation vial. To this was transferred 15 ml. of dioxane–naphthalene scintillation cocktail (13). The scintillation vial was tightly capped and shaken for about 30 sec. to ensure thorough mixing of the experimental solution and the phosphore. At the same time, a blank was prepared in the same manner except that the compound was omitted from the solution. The standard was prepared by pipeting 0.10 ml. of the standard stock solution of the experimental compound into a scintillation vial. After the solvent (benzene or methanol) was evaporated, 0.20 ml. of water and 15 ml. of dioxane–naphthalene scintillation cocktail were added and the vial was shaken. The samples were counted directly using a liquid scintillation system[8]. Sufficient time was allowed for the samples to cool to 4.0° to minimize quenching. The samples were counted for 10 min. and the counts per minute (c.p.m.) were obtained by dividing the 10-min. counts by 10. The solubility values were obtained from the ratio of the counts per minute of the internal standard and the sample.

RESULTS AND DISCUSSION

By using a radioactive assay procedure, the aqueous solubilities of cholesterol, testosterone, progesterone, and diethylstilbestrol were determined at 30° (Table I). Solubilities were also determined in 0.9% NaCl (Table II). Solubilities of hormone compounds were determined as they approached equilibrium and after equilibrium had been established. The solubility values reported in Tables I and II are the solubility values at equilibrium, and the complete solution behavior of these steroids is presented in Figs. 1 and 2.

Variations in water solubility of cholesterol, progesterone, and testosterone were in general accord with the principle that solubility increases with the number of polar groups. As expected, cholesterol

[8] Unilux II, Nuclear Chicago.

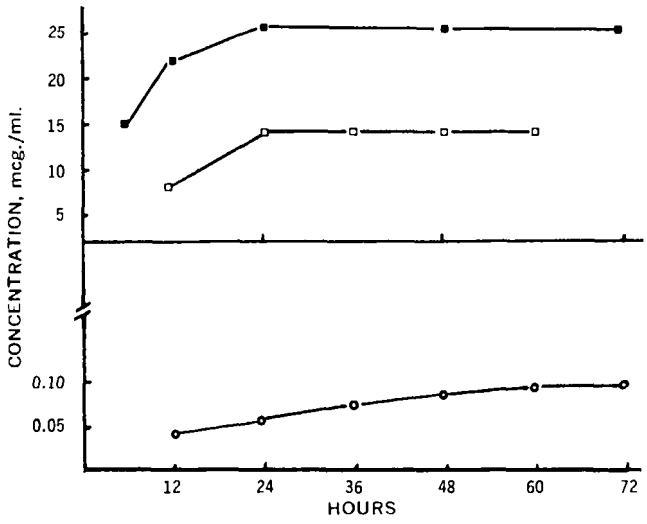

Figure 1—*Aqueous solubility of cholesterol* (○), *progesterone* (□), *and diethylstilbestrol* (■) *at 30°.*

was the least water soluble since it was the most nonpolar and bulkiest of the compounds studied. Testosterone, with one α,β-unsaturated ketone group and a free hydroxyl group, had the highest water solubility of the hormones studied.

Although diethylstilbestrol has a lower molecular weight (268) than testosterone (288), its aqueous solubility was lower; however, its melting point is considerably higher (170°) than testosterone (154°). Therefore, it is quite conceivable that due to its symmetric nature, it may form crystals of high lattice energy as compared to the relatively less symmetrical molecule of testosterone.

Using ^{14}C-labeled cholesterol, Gemant (8) studied the solubilization of cholesterol using a partition chromatographic method. He assumed that, in a solution that solubilized cholesterol, the cholesterol would travel with the moving phase; the stronger the solubilizing action, the farther it would travel. Determination of cholesterol distribution along the paper was accomplished by a radioactive tracer method. ^{14}C-Labeled cholesterol was applied to the paper and, after a suitable time of development, the strip was cut into sections and the activity of each section was measured. He found the solubility of cholesterol to be 0.2 mg./100 ml. of water (= 2 mcg./ml.), more than 20 times the solubility found in the current study.

In more recent work, Saad and Higuchi (3) found the solubility of cholesterol to be 0.025 mcg./ml. in both water and 0.9% NaCl. They equilibrated the cholesterol-water or cholesterol–0.9% NaCl for 30 days and used 0.10- and 0.22-μ filters to clarify the solutions before assay. When 0.45-μ filters were used, significantly higher results (1.5 and 2 times greater) were obtained. The results obtained

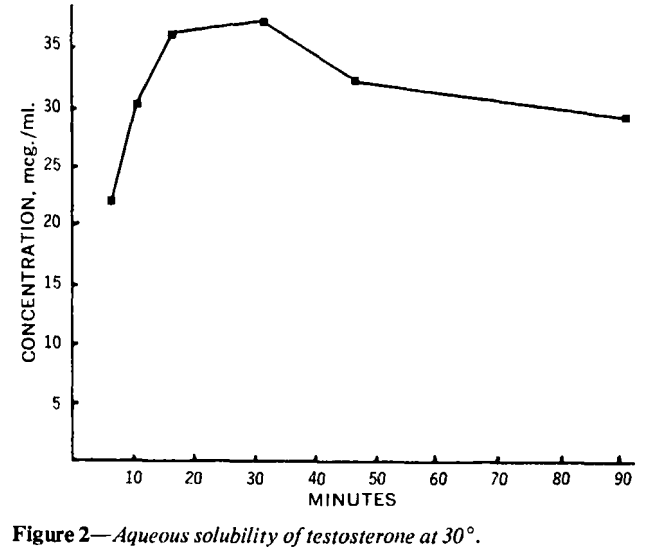

Figure 2—*Aqueous solubility of testosterone at 30°.*

Table II—Solubility of Steroids in 0.9% NaCl

Steroid	Solubility, mcg./ml.[a] This Work (30°)	Literature
Cholesterol	0.017	0.025 (30°) (3)
Progesterone	10.0	6.6 (25°) (6)
Diethylstilbestrol	24.5	No values found
Testosterone	29.5	36 (37°) (6), 27 (25°) (6)

[a] Average of two experiments.

in the present study using 0.22-μ filters (0.041 mcg./ml. water and 0.017 mcg./ml. 0.9% NaCl) are in good agreement with the findings (0.025 mcg./ml.) of Saad and Higuchi (3) considering the extremely low solubility levels of cholesterol. When 0.45-μ filters were used in the present study, the aqueous solubility was more than 2 times higher than the solubility data for 0.22-μ filters.

Several experiments were carried out in which sodium chloride was added to saturated solutions of cholesterol (0.09 mcg./ml.) to give a 0.9% salt concentration. The cholesterol solubility values for these solutions decreased to 0.017 mcg./ml. The marked decrease in solubility indicated the possibility that cholesterol crystal nuclei were salted out of solution by the addition of sodium chloride, leaving a true solution of cholesterol. These experiments appear to substantiate that the Coulter counter method used by Saad and Higuchi (3) was able to ascertain the true solubility of cholesterol by detecting crystal nuclei formation.

The solubility values for progesterone, testosterone, and diethylstilbestrol were essentially the same in 0.9% sodium chloride as in water (Tables I and II). There was no significant decrease in hormone concentration when 0.9% sodium chloride was added to saturated solutions of these compounds.

The solution behavior of testosterone is presented in Fig. 2. The equilibrium was approached from supersaturation and not from undersaturation. The decrease in high initial solubility and its subsequent leveling off suggest the conversion of a metastable crystalline form possessing a higher solubility to a form of lower solubility. Thakkar and Hall (9–11) examined the aqueous solution behavior of anhydrous and hydrous testosterone and observed an initial supersaturation with a subsequent leveling for the anhydrous form. The anhydrous form dissolved faster than the hydrous form. They also compared the X-ray diffraction patterns of the original anhydrous form and the residue remaining after the equilibration of the anhydrous form and found that an alteration in the crystalline structure did take place.

In 1954, Bischoff and Stauffer (6) studied the solution behavior of testosterone and found that short equilibration periods gave rise to supersaturated solutions. They also observed that if the solvent was changed after 5 hr. of equilibration, the residue did not produce supersaturated solutions. Heating this residue to slightly below the melting point restored it to the form that produced supersaturated solutions. They hypothesized that this difference in the solution behavior could be due to a difference in the particle sizes of the two samples of testosterone, *i.e.*, one that gave rise to supersaturated solution and one that did not. Actual microscopic examination revealed, however, that there was no difference in the particle size of the two samples. These workers postulated that an explanation of the anomolous solution behavior of testosterone may lie in the existence of polymorphic forms.

Recently, Thakkar and Hall (9–11) attempted to explain the observation of Bischoff and Stauffer (6) by stating that the testosterone sample which produced a supersaturated solution was clearly an anhydrous form whereas the water equilibrated residue which did not was a hydrated form. As might be expected, this hydrate lost its water of hydration and reverted to the anhydrous form when heated to slightly below the melting point. That such a conversion does really occur was shown by the similarity of the X-ray diffraction pattern of the heat-dehydrated hydrate sample to that of the anhydrous form.

Other workers (15, 16) also observed similar solution behavior differences in various solvents for a number of organic compounds including steroids, cholesterol, and fluorohydrocortisone acetate. The decrease in solubility with time once the peak solubility is attained has been explained as being due to nucleation and crystallization of the more stable hydrate.

The peak solubility value displayed during the dissolution of the anhydrous form of testosterone (Fig. 2) may correspond to a short-term steady-state situation 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. However, the solubilities of both forms of testosterone appear to approach the same value with time (11).

REFERENCES

(1) A. Siedell, "Solubilities of Organic Compounds," 3rd ed., Van Nostrand, New York, N. Y., 1940, p. 823.

(2) W. E. Lange and M. E. Amundsen, *J. Pharm. Sci.*, **51**, 1102(1962).

(3) H. Y. Saad and W. I. Higuchi, *ibid.*, **54**, 1205(1965).

(4) P. Ekwal and L. Mendell, *Acta Chem. Scand.*, **15**, 1404 (1961).

(5) P. Kabasakalian, E. Britt, and M. D. Yudis, *J. Pharm. Sci.*, **55**, 642(1966).

(6) F. Bischoff and R. D. Stauffer, *J. Amer. Chem. Soc.*, **76**, 1962(1954).

(7) D. Abelson, C. Depatie, and V. Cradock, *Arch. Biochem. Biophys.*, **91**, 71(1960).

(8) A. Gemant, *Life Sci.*, **6**, 233(1962).

(9) A. L. Thakkar and N. A. Hall, *J. Pharm. Sci.*, **56**, 1121 (1967).

(10) *Ibid.*, **57**, 1394(1968).

(11) *Ibid.*, **58**, 68(1969).

(12) D. K. Madan and D. E. Cadwallader, *J. Pharm. Sci.*, **59**, 1362(1970).

(13) G. A. Bray, *Anal. Biochem.*, **1**, 279(1960).

(14) L. Sjoblom, in "Surface Chemistry," P. Ekwal, K. Groth, and V. Reio, Eds., Academic, New York, N. Y., 1965.

(15) D. E. Wurster and P. W. Taylor, *J. Pharm. Sci.*, **54**, 670 (1965).

(16) E. Shefter and T. Higuchi, *ibid.*, **52**, 781(1963).

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* Present address: Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

▲ To whom inquiries should be directed.

Central Cholinomimetic Actions of 3,3-Dimethyl-1-butanol Carbamate

LYNN WECKER* and C. Y. CHIOU▲

Abstract □ The action of 3,3-dimethyl-1-butanol carbamate on the CNS was studied to determine the mechanism of its convulsant activity. Dimethylbutanol carbamate penetrated into the brain with maximum levels at 15 min. after intraperitoneal injection. The LD_{50} of dimethylbutanol carbamate in mice was 21 mg./kg., and atropine pretreatment protected animals against the toxic effects of dimethylbutanol carbamate, whereas physostigmine enhanced dimethylbutanol carbamate toxicity. Dimethylbutanol carbamate potentiated nicotine-induced convulsions in mice and had no effect on brain cholinesterase activity. Therefore, it appears that dimethylbutanol carbamate is a centrally active agent that produces convulsions *via* a mechanism similar to that postulated for nicotinic agents, although it produces antinicotinic actions peripherally.

Keyphrases □ 3,3-Dimethyl-1-butanol carbamate—mechanism of convulsant activity, central cholinomimetic actions, mice □ Cholinomimetic actions—3,3-dimethyl-1-butanol carbamate, mice □ Convulsant activity—3,3-dimethyl-1-butanol carbamate, mice

3,3-Dimethyl-1-butanol carbamate, a synthetic carbachol analog containing a carbon in place of the quaternary nitrogen atom, is an unusually interesting compound with a potent peripheral antinicotinic effect (1, 2) as well as a central convulsant action in mice, producing tonic-clonic convulsions (3, 4). The effects of this agent on cholinergic-induced drinking behavior in rats have been studied, and it was reported (5) that dimethylbutanol carbamate, when implanted into the lateral hypothalamic region of the rat brain, initiated a response similar to those of acetylcholine and carbachol. These results suggested a central cholinomimetic action of dimethylbutanol carbamate. It has been hypothesized that alterations in brain levels of acetylcholine may be correlated with behavioral changes and that there is a decrease in the acetylcholine content of the brain during convulsions (6–8). When an increase in cortical activity is evident, there is a release of acetylcholine from its storage sites in nerve terminals, resulting in an overall decrease in brain acetylcholine content (6–8).

Dimethylbutanol carbamate was found to cause a significant increase in the release of acetylcholine from the minced guinea pig cerebral cortex (1). Therefore, the present study investigated the possibility that the CNS actions of dimethylbutanol carbamate are due to a direct-releasing action of acetylcholine from storage sites inside nerve endings, a mechanism similar to that reported for the action of nicotinic agents (9). The unusual character of this compound could be used as a tool to elucidate cholinergic mechanisms.

EXPERIMENTAL

ICR Charles River mice, weighing 20–35 g., were used for all studies. To determine the extent of brain penetration of dimethylbutanol carbamate, mice were injected intraperitoneally with 20 mg./kg. ^{14}C-dimethylbutanol carbamate (0.7 μc./mg.). Dimethylbutanol carbamate was synthesized in this laboratory according to the method described previously (1). The animals were sacrificed by cervical dislocation at various times, and their brains were removed, weighed, homogenized (1 ml. water/1 g. tissue), and solubilized[1] at 50° for 12–15 hr. The samples were allowed to cool, and

[1] NCS solubilizer, Amersham Searle, Arlington Heights, Ill.