DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(9), 1405-1422 (1989)

DIADERMATIC DOSE FORMS OF TESTOSTERONE: IN-VITRO RELEASE STUDIES AND IN-VIVO ABSORPTION IN A HUMAN MALE

A. Babar, R. A. Khaleque, A. J. Cutie and F. M. Plakogiannis

Division of Pharmaceutics and Industrial Pharmacy
Arnold & Marie Schwartz College of Pharmacy and Health Sciences
Long Island University, Brooklyn, New York 11201

ABSTRACT

The study was undertaken to evaluate the in-vitro release of testosterone from various topical bases and to screen formulations with the optimum drug release for in-vivo evaluations in a human male. Several diadermatic bases containing 2% testosterone were prepared and studied for the in-vitro release of drug through the cellulose membrane using the diffusion methods. Also, additive ingredients such as, ethanol, polyethylene glycol-400, and dimethylsulfoxide (DMSO) at various concentration levels were included in these formulations to study their effects on the release rate of the drug. The general rank order of testosterone release from these bases was: water-washable base > hydrophilic base > University of California Hospital Base > gel base > cream base > water-soluble base > emulsion base. Except for the water-washable base containing 15% polyethylene glycol-400, all additives had little or no effect on the drug release. The formulations with the best in-vitro drug release were selected and evaluated for in-vivo drug release in a single male volunteer. Each formulation equal to 50mg of testosterone was applied on the fore-arm, and following each application, 24 hour urine samples were

collected and analyzed for the Urinary free testosterone and its principal metabolites, i.e. androsterone and etiocholanolone. A 50mg of oral testosterone in a plain gelatin capsule was used as the control to compare the bioavailability profiles. The ratio of urinary-free testosterone to its main metabolites was found to be (1:1) from one of the topical formulations evaluated compared to (1:20) obtained in the case of oral dose of testosterone used as the control. This suggests that more drug was able to escape the pre-systemic inactivation and resulted in the higher level of free testosterone in the body.

INTRODUCTION

Testosterone is a clinically important natural androgen which exhibits an extremely low gastrointestinal absorption profile. Accordingly, therapeutic failures have been documented with the use of oral testosterone. Gisvold (1) suggests that testosterone is inactivated by the intestinal bacteria when given orally. Estwood (2), on the other hand, indicates that testosterone, when administered orally, is readily absorbed but such absorption is completely ineffectual in as much as the hormone is altered by the passage through the liver before reaching the systemic circulation.

A clinical study comparing the efficacy of various testosterone compounds administered sublingually to hypogonadal men indicated that it needed twice as much of testosterone as methyl testosterone for the same level of therapeutic effects (3). Another study comparing various routes of administration of androgens, suggests that the unesterified testosterone, when given orally, is ineffective and does not produce any pharmaco-therapeutic effect (4).

The therapeutic efficacy of orally administered testosterone has been established by Johnsen (5). This study suggests that the clinical benefits of testosterone can be obtained when given

orally, provided the daily dose is in the range of 400mg. Recently, the improved bioavailability of testosterone has been documented by dispersing the drug in biosurfactant-lipid combinations and polyethylene glycols (6). Also, Chien (7) has shown that the systemic availability of testosterone [^{14}C] by navel absorption is relatively close to the level obtained by the IV administration of an equivalent dose, and is substantially greater than the level achieved by forearm administration (7).

Although the oral route is the most convenient and economical one, yet it is an unpredictable route of administration for some drugs and sometimes totally ineffective. Testosterone as such is not used in the clinical practice today. Instead, various ester forms such as methyl testosterone is widely used when oral androgen therapy is required in spite of its side effects. To minimize such problems, a number of important drugs, such as nitroglycerin, scopolamine, estrogens and others are successfully being used in the forms of transdermal drug delivery systems (8). There are numerous reports available, revealing the effects of vehicle composition on the release as well as on the bioavailability of relatively water insoluble drugs. Also, several additive ingredients have been found useful in enhancing the drug release from the topical bases. In light of these, the present study was initiated to study the in-vitro release of testosterone from various topical bases including the additive ingredients. The formulations with maximum drug release were then scaled up for in-vivo drug release studies in a human male. Also, one of the aims of the study was to compare the levels of free systemic testosterone from the selected topical formulations to that of an equal dose of oral drug.

EXPERIMENTAL

Materials: Testosterone[1], white petrolatum[2], Glyceromonostearate[3], Polyethylene glycol-4000[4], cetyl alcohol[5], stearyl alcohol[5],

propylene glycol[5], sodium lauryl sulfate[5], glycerin[5], Myrj 52[3], Amerchol CAB[5], Amerlate P[6], Polyethylene glycol-400[3], Alcohol USP[4], Dimethylsulfoxide (DMSO)[7], ß-glucuronidase[8], Androsterone[9], Etiocholanolone[9], Anhydrous Ether (peroxide free)[4], Benzene[4], Ethyl acetate[4], Glacial acetic acid[4], Sodium hydroxide[4], Acetate buffer (pH 4.63)[4], Anhydrous sodium sulfate[4].

Equipment: Spectrophotometer (model: Spectronic 200 UV)[10], Gas chromatograph (model: 3920B)[2], Recorder minigrator[11], pH meter (model 140)[12], Thin Layer Chromatographic Apparatus[13], etc.

Preparation of Samples: Each ingredient of the ointment formulation was accurately weighed in the percentage ratio described in each formulation in Table 1. All the aqueous phase ingredients and the oil phase ingredients were placed into separate stainless steel containers and heated to 80°C ± °C. The water phase was then slowly added to the oil phase while stirring and mixed for 20 ± 5 minutes and cooled to 50°C and testosterone was incorporated while mixing. All the additive ingredients used were also added at this stage and mixed for about 10 minutes and cooled to room temperature. The ointment samples thus prepared were then stored in tightly closed glass jars until further experimental work.

Analytical Methods:

A) In-Vitro Studies: All samples were analyzed spectrophotometrically for testosterone contents at 248 nm.

B) In-Vivo Studies: All samples were analyzed for urinary testosterone and its principal metabolites using a published procedure (9).

Content Uniformity: All ointment samples were analyzed for testosterone contents prior to their use. Only samples with drug contents 100 ± 10% were included in these studies.

Table 1

Formulations

INGREDIENT	% W/W						
	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
Testosterone, NF	2.00	2.00	2.00	2.00	2.00	2.00	2.00
White Petrolatum	25.00	14.30	20.00	-	-	-	-
Stearyl Alcohol	15.00	6.40	3.00	-	-	-	-
Cetyl Alcohol	-	5.40	-	-	4.00	-	1.00
Mineral Oil	-	21.40	-	-	-	-	5.00
Amerchol CAB	-	-	7.00	-	-	-	-
Glyceromonostearate SE	-	-	5.00	-	-	-	1.00
Myrj-52	-	-	4.00	-	-	-	-
PEG 4000	-	-	-	30.00	-	4.00	-
PEG 400	-	-	-	60.00	-	-	-
Span 40	-	-	-	1.00	-	-	-
Stearic Acid TP	-	-	-	-	18.00	-	3.00
Carbopol 940	-	-	-	-	-	0.50	-
Glucam E-20	-	-	-	-	-	6.00	-
Sodium lauryl sulfate	1.00	1.50	-	-	-	-	-
Triethanolamine	-	-	-	-	2.00	1.00	1.50
Additive(s)	-	-	-	-	-	-	-
Tween-20	-	-	-	-	-	10.00	-
Propylene glycol	12.00	-	-	-	-	-	-
Glycerin	-	-	-	-	5.00	-	4.00
Water qs to	100	100	100	100	100	100	100

*(I) - Hydrophilic Base

(II) - University of California Hospital Base

(III) - Water-washable Base

(IV) - Water-soluble base

(V) - Emulsion Base

(VI) - Gel

(VII) - Cream Base

IN-VITRO RELEASE STUDIES

A 15.0 Gm sample of each ointment was placed in a 2 ounce jar of 2" diameter with an approximate surface area of 3.4 square inches. A semi-permeable membrane with a molecular weight cut-off point of 3000 was tied around the upper lip of the jar with a silk

thread. The samples were then invertedly placed at the center of the beakers each containing 300 ml of distilled water, previously maintained at 37° ± °C in a water bath. At 15, 30, 45, 60, 90 and 120 minute time intervals, a 3 ml portion of the diffusion medium was withdrawn for the analysis of the amount of drug diffused and replaced with an equal volume of the water to keep the diffusion volume constant.

IN-VIVO RELEASE STUDIES

The formulations with the best in-vitro release of the drug were selected for in-vivo evaluation. The known amount of ointment sample equivalent to 50 mg of testosterone was applied in an area of 24.2 cm^2 of skin on the forearm of a 28 year old healthy male. Following each application sufficient water was orally consumed and urine samples were collected for a period of 24 hours. The total urine volume was recorded and frozen until analysis. Also, a 50 mg oral dose of testosterone was used as the control to compare the urinary excretion profiles. All samples were run in triplicate, and a minimum of two weeks was allowed to elapse between each experiment.

RESULTS AND DISCUSSIONS

Solubility

The solubility of testosterone was determined in the diffusion medium and was found to be 48 ug/ml in the diffusion medium.

In-Vitro Release of Testosterone

The percentage of drug release from different topical bases, over a period of two hours, are exhibited in Table 2. The general rank order of testosterone release was: water-washable base > hydrophilic base > U.C.H. base > gel > cream > polyethylene glycol base > emulsion base. The addition of the additive ingredients had little or no effect in enhancing the drug release, except for the

TABLE 2

PERCENT RELEASE OF TESTOSTERONE FROM DIFFERENT BASES

OINTMENT BASES	TIME (MINUTES)					
	15	30	45	60	90	120
HYDROPHILIC	0.114	0.176	0.190	0.240	0.280	0.300
WATER WASHABLE	0.340	0.439	0.560	0.658	0.820	0.958
U.C.H.	0.082	0.100	0.116	0.150	0.180	0.236
PEG WATER SOLUBLE	0.037	0.045	0.062	0.084	0.119	0.134
CREAM	0.042	0.076	0.082	0.086	0.122	0.156
GEL	0.037	0.051	0.075	0.090	0.129	0.164
EMULSION	0.033	0.046	0.059	0.079	0.103	0.122

NOTE: EACH READING IS THE AVERAGE OF THREE DETERMINATIONS.

polyethylene glycol at 15% level in the emulsion base which gave the higher release of the drug.

In order to interpret release data in terms of meaningful parameters, the data were treated by various kinetic principles. First, the simplified Higuchi's equation (9), which is valid when the drug release is under 30 percent.

$$\frac{q}{A} = 2C \left(\frac{Dt}{\pi}\right)^{\frac{1}{2}} \qquad \text{Equation 1}$$

where q = amount of drug released (mg), D = diffusion coefficient (cm^2/sec), t = time in seconds, A = area of the diffusion membrane, C_o = initial concentration of the drug in the ointment, and π = a constant.

The release data comply well with the requirements of the equation 1, namely, (a) only testosterone is assumed to diffuse, (b) D is constant with respect to both time and position of the ointment layer, (c) the relative large volume of the diffusion medium is assumed to provide necessary sink condition, (d) the percentage drug release is less than 30%. When the parameters D, A and C_o of the above equation are constant, then equation 1 converts to a simple form $q \alpha \sqrt{t}$. And plotting the percentage drug release versus square root of time straight lines were obtained as exhibited in Figure 1. Also, Higuchi's equation indicates a direct dependence of the release rate on the diffusion coefficient of the drug, therefore, more release of the drug is expected when there is less affinity for the base. This is manifested by the highest diffusion coefficient value of (10.6×10^{-9} cm^2/sec) as obtained in the case of the water-washable base, contrary to the lower diffusion coefficient value observed with U.C.H. base formulation. This could be attributed to the fact that more drug was soluble in the base due to the presence of a higher percentage of lipids.

Several studies have documented the in-vitro release of hydrophobic drugs using aqueous receiving media (10). Bronaugh and

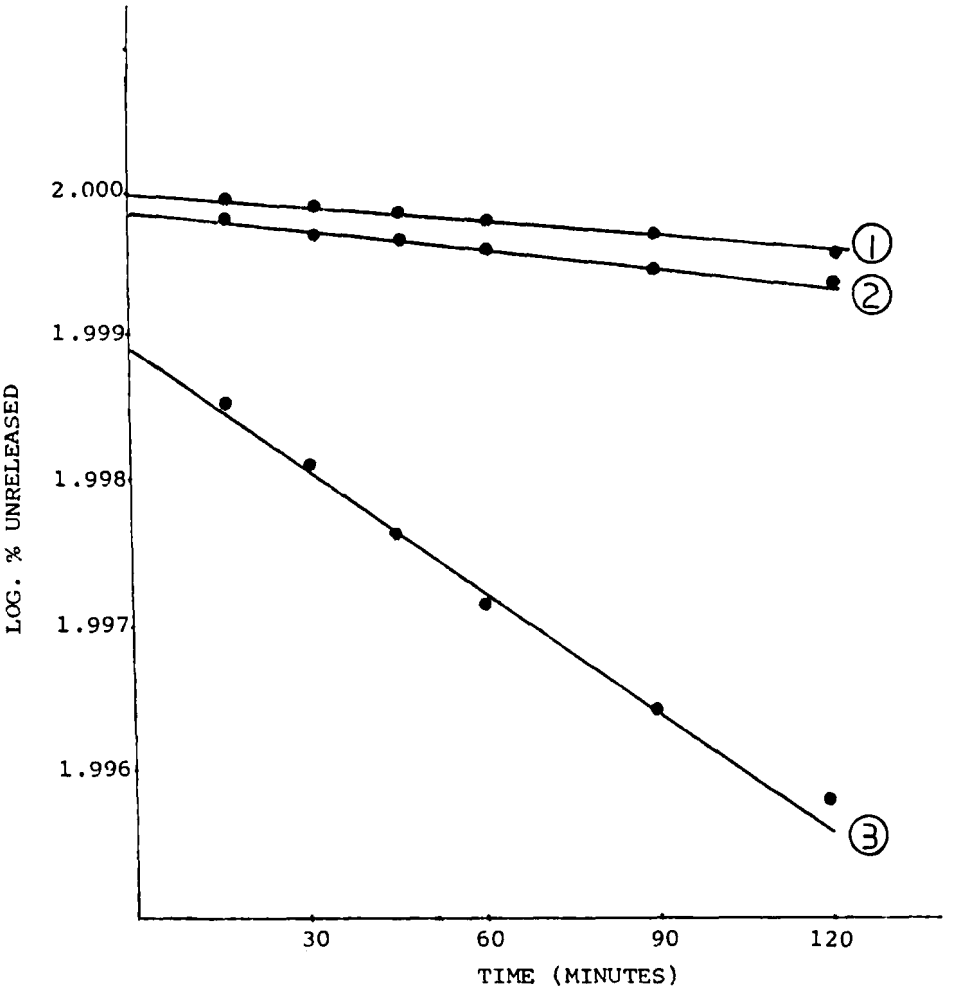

Figure 1

Release Data Plotted Versus Square Root of Time: (1) Water-Washable Base; (2) Hydrophilic Base; (3) University of California Hospital Base

Stewart (11) reported 8-96 fold increase in the permeation of hydrophobic compounds in-vivo, as compared to their in-vitro release data using aqueous receiving medium. Treating the in-vitro data in accordance with the Fick's law, the permeability coefficients of drug from different bases were calculated using the following equation:

$$q = PAC_o t \qquad \text{Equation 2}$$

where q = number of mg of drug diffused through the membrane at time t (seconds), P = permeability coefficient (cm/sec), A = area (cm^2) of diffusion membrane, and C_o = concentration (mg/litre) of drug present in the base at time zero.

Since the release of drug from the experimental formulations was very low, the drug concentration in the vehicle remained essentially unchanged. Therefore, equation 2 served well to calculate the permeability coefficients of drug from different bases and these values are exhibited in Table 3. From this one observes that the highest value (19.10×10^{-8} cm/sec) obtained from the water washable base compared to the lowest value (2.2×10^{-8} cm/sec) obtained from the emulsion base.

As the diffusivity of a substance of a similar molecular weight and shape differs only slightly (12), the partition coefficient factor has been considered as an important parameter to estimate the distribution of drug between the ointment base and the receiving medium. The relationship between the partition coefficient and the permeability coefficient can be expressed by the following equation:

$$Kp = \frac{Ph}{D} \qquad \text{Equation 3}$$

where Kp = partition coefficient, P = permeability coefficient (cm/sec), D = diffusion coefficient (cm^2/sec) aand h = thickness of the barrier (cm).

The partition coefficient values of testosterone for various formulations are also exhibited in Table 3. According to this, the lowest partition coefficient value (5.01) is obtained for the water-washable base giving the highest drug release compared to the highest partition coefficient value of (51.8) for the emulsion base giving the lowest drug release.

In order to develop an ideal kinetic model, the diffusion rate

TABLE 3

VALUES OF DIFFUSION, PERMEABILITY, AND PARTITION COEFFICIENTS

AS CALCULATED FROM THE IN VITRO DATA FOR DIFFERENT OINTMENTS

OINTMENT BASES	DIFFUSION COEFFICIENT [D] $(D \times 10^{-9})$ cm^2/sec	PERMEABILITY COEFFICIENT [P] $(P \times 10^{-8})$ cm/sec	PARTITION COEFFICIENT $[K_p]$
WATER WASHABLE	10.60	19.10	5.01
HYDROPHILIC	1.29	6.80	17.39
U.C.H.	0.50	4.20	27.70
CREAM	0.21	2.70	42.40
PEG WATER SOLUBLE	0.16	2.40	49.50
EMULSION	0.14	2.20	51.80
GEL	0.22	2.70	40.50

data were treated with first order kinetic equation. Since the rate of testosterone release remained low, the first or zero order interpretation would make no significant differences because the amount of drug remaining at time t is essentially unchanged. By plotting log of percentage drug remaining data versus the time, the straight lines were obtained for the various formulations tested as shown in Figure 2. Also, the values of the release rate constant, y-axis intercept and regression coefficient were calculated and exhibited in Table 4.

The effects of various additives (ethanol, DMSO and polyethylene glycol-400) at 5, 10 and 15% levels on the release of testosterone were studied. Except for polyethylene glycol-400 at 15% level, all other additives had adverse effects on the drug release from these bases. This could be attributed to the solvency

TABLE 4

DIFFUSION RATE DATA EXPRESSED AS THE PARAMETERS OF APPARENT FIRST ORDER KINETICS

OINTMENT BASES	10^{-5} K MIN^{-1}	Y INTERCEPT	STANDARD REGRESSION COEFFICIENT r-value
WATER WASHABLE	5.95	1.9988	0.994
HYDROPHILIC	1.72	1.9995	0.961
U.C.H.	1.46	2.0015	0.994
CREAM	1.00	1.9998	0.982
PEG WATER SOLUBLE	0.98	2.0003	0.988
GEL	1.23	2.0001	0.999
EMULSION	0.87	1.9999	0.993

K = the first order rate constant.

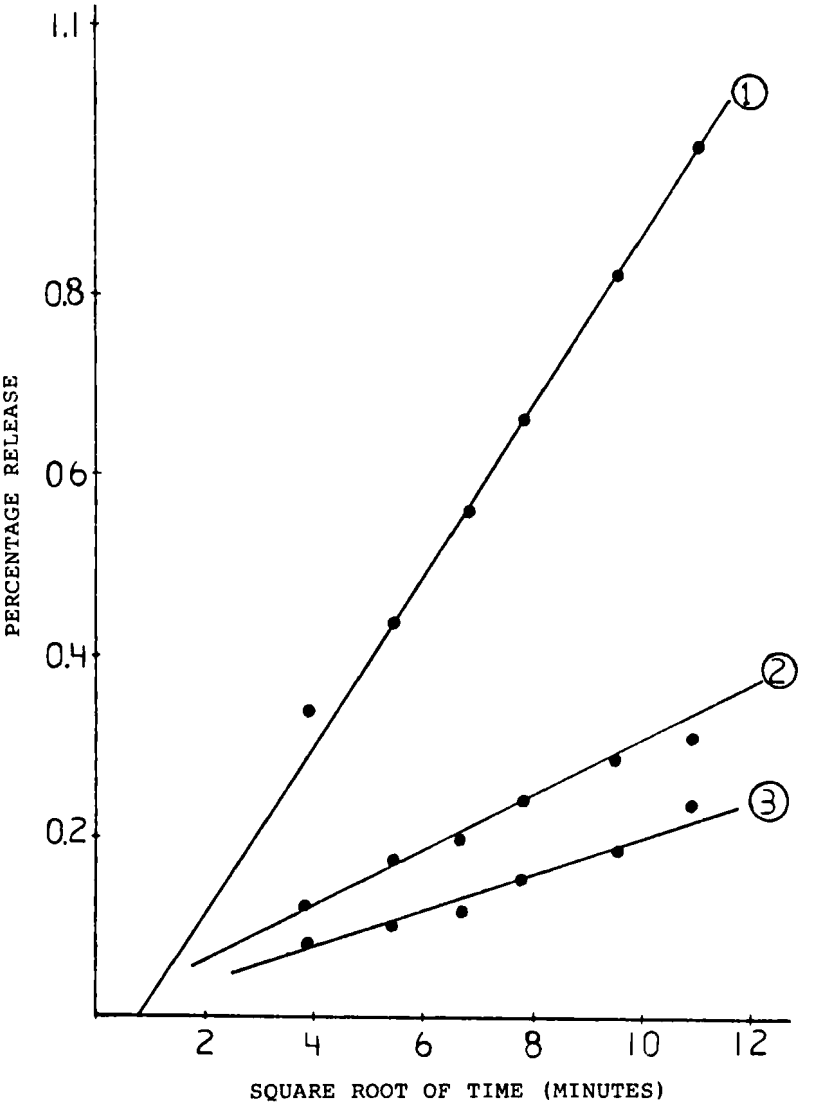

Figure 2

Release Data Of Selected Bases Expressed in First Order Kinetic Fashion: (1) University of California Hospital Base; (2) Hydrophilic Base; (3) Water-Washable Base.

effect of polyethylene glycol-400, therefore, more drug was able to diffuse into the receiving medium and caused greater release.

IN-VIVO STUDIES

On the basis of the in-vitro release data, three formulations were selected for the in-vivo studies in a single male volunteer. The in-vivo studies were run in triplicate and based on the assessment of the urinary excretion of free testosterone and its metabolites following the topical application of each of the selected formulation. The ointment samples evaluated included: 1-water washable base, 2-water washable bases with 15% polyethylene-glycol-400 and 15% DMSO respectively. A 50 mg of oral dose was also consumed and used as the control to compare the ratios of the urinary free testosterone to metabolites as obtained from the dermatological applications.

The amounts of testosterone and its principal metabolites excreted in 24 hour urine after the application of topical formulations are given in Table 5. According to these data, at the end of 24 hours, only 18.90% of the topically applied dose of 50 mg of drug was collected as (free testosterone, etiocholanolone and androsterone) in urine from the water-washable base, 8.00% from the water-washable base + 15% DMSO and 11.50% from the same base containing 15% PEG 400. And, the total urinary excretion of these compounds was approximately 25.2% from the same dose of the orally administered drug. The relatively low absorption of drug from the topically applied formulations was expected compared to the oral drug administration.

Unlike the in-vitro data, the topical absorption of testosterone was higher from the formulation consisting of the water-washable base than the same with 15% PEG-400. Table 5 shows the excretion of urinary free testosterone as the percentage of the total dose administered or applied. In general, the rank order of

TABLE 5

URINARY EXCRETIONS EXPRESSED AS

PERCENTAGE OF ADMINISTERED DOSE

SAMPLE	EXP.	METABOLITES (Androsterone & Etiocholanolone)	FREE TESTOSTERONE	% OF EXCRETED DOSE
1 - CAPSULE	1	13678	811	29.00
	2	13631	643	28.50
	3	8716	345	18.20
	Avg.	12008 (µg)	600 (µg)	Avg. 25.20
2 - WATER-WASHABLE BASE	1	8013	1169	13.36
	2	10764	1868	25.30
	3	4967	1663	13.20
	Avg.	7915 (µg)	1567 (µg)	Avg. 18.90
3 - WATER-WASHABLE BASE + 15% DMSO	1	2599	1240	7.70
	2	2424	1405	7.60
	3	2261	1341	7.20
	4	3153	1681	9.60
	Avg.	3479 (µg)	1889 (µg)	Avg. 8.00
4 - WATER-WASHABLE BASE + 15% PEG 400	1	2691	2779	11.00
	2	2594	2923	11.00
	3	2577	2668	10.50
	4	4143	2638	13.50
	Avg.	3001 (µg)	2752 (µg)	Avg. 11.50

urinary free testosterone was: water-washable base with 15% PEG-400 > water-washable base with 15% DMSO > water-washable base > oral dose.

In order to study the biological availability of testosterone in terms of meaningful parameters, the ratios of free testosterone to its principal metabolites (androsterone and etiocholanolone) were calculated and these values are exhibited in Table 6.

The results of this very limited percutaneous absorption study did not necessarily correlate with the in-vitro diffusion studies. However, it served well as a model for screening a large

TABLE 6

URINARY EXCRETIONS EXPRESSED AS THE RATIO OF METABOLITES (ANDROSTERONE AND ETIOCHOLANOLONE) TO FREE TESTOSTERONE

SAMPLE	EXP. NO.	TESTOSTERONE (μg)	METABOLITE (μg)		RATIO
1. Control					
ORAL DOSE	1	811	13678		1:17
	2	643	13631		1:21
	3	345	8716		1:25
				Avg.	1:20
2. WATER-WASHABLE	1	1169	8013		1:7
BASE	2	1868	10764		1:6
	3	1663	4967		1:3
				Avg.	1:5
3. WATER-WASHABLE	1	1240	2599		1:2.0
BASE WITH 15%	2	1405	2424		1:1.7
DMSO	3	1341	2261		1:1.7
	4	1681	3153		1:1.9
				Avg.	1:1.8
4. WATER-WASHABLE	1	2779	2691		1:1
BASE WITH 15%	2	2923	2594		1:1
PEG-400	3	2668	2577		1:1
	4	2638	4143		1:1.6
				Avg.	1:1.2

NOTE: All these values were calculated after having substracted the values of natural excretions of hormone in the urine.

group of experimental formulations. The ratio of metabolites to urinary free testosterone remained significantly different from those observed with the use of an equal dose of orally administered drug. The data suggest that more drug is able to escape presystemic inactivation and consequently produces higher therapeutic drug levels when used via the diadermatic dosage forms.

NOTES

1. Roussel Corporation, New York, N.Y.
2. Pharmaderm Inc., New York, N.Y.
3. Ruger Chemical Co., Springfield, N.J.
4. Fisher Scientific Co., Springfield, N.J.
5. Amend Drug and Chemical Co. Inc., Irvington, N.J.
6. Amerchol Corporation, N.J.
7. Eastman Kodak Co., Rochester, N.Y.
8. Cooper Biomedicals, Freehold, N.J.
9. Sigma Chemical Co., St. Louis, MO
10. Shimadzu Scientific Instruments, Columbia, MD
11. Perkin Elmer Co., N.J.
12. Fisher Scientific Co., Springfield, N.J.
13. Thomas Scientific Co., Philadelphia, PA

REFERENCES

1. Gisvold, O., The Text Book of Organic Chemistry, 6th Ed., J.B. Lippincott Co., PA, 1971, p. 796.
2. Estwood, E.B., The Text Book of Pharmacological Basis of Therapeutics, 6th Ed., by Goodman and Gilman, The McMillan Co., p. 1567.
3. Escamilla, R.F. and Gordon, G.S., J. Clin. Endocrinol and Metabol, 10, 284, 1950.
4. Foss, G.L., The Lancet, 502, March 1939.
5. Johnsen, S.G., The Lancet, 1473, Dec. 1974.
6. Babar, A. and Jarowski, C.I. J. Pharm. Sci., 72, 708, 1983.
7. Chien, Y.W., J. Pharm. Sci., 73, 285, 1984.

8. Physician's Desk Reference, 1984.

9. Higuchi, W.I., J. Pharm. Sci., 51, 802, 1962.

10. Brown, D.W.C. and Usamer, A.G., Food Cosmet. Toxicol., 13, 81, 1975.

11. Bronaugh, R.L. and Stewart, R.E., J. Pharm. Sci., 73, 1255, 1984.

12. Higuchi, T., J. Soc. Cosmet. Chem., 11, 85, 1960.