Long-Term Controlled Navel Administration of Testosterone

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Abstract □ Male fertility has reportedly been regulated by the long-term, continuous administration of testosterone. To deliver the testosterone at a controlled rate for a month or longer, a bandage-type, testosterone-releasing, disk-shaped device was developed. *In vitro* drug elution studies demonstrated that a constant release profile of testosterone was achieved. *In vivo* studies in rhesus monkeys with the medicated device directly overlaying the navel for 46 d, yielded a fairly steady plasma level and also a constant urinary excretion rate for 32 d. A greater systemic bioavailability (more than twofold) was achieved as compared with drug disposition directly onto the navel or *via* the polacebo device (*i.e.*, drug is deposited onto the prefabricated placebo device). A fairly constant cumulative urinary recovery profile was achieved for longer than 1 month, in which >90% of the dose was administered. The *in vitro* and *in vivo* relationship was analyzed and discussed.

Keyphrases □ Testosterone—long-term controlled administration, navel, rhesus monkeys, bioavailability □ Controlled administration, navel—long-term, testosterone, rhesus monkeys, bioavailability □ Bioavailability—long-term controlled administration, navel, testosterone

It is known that the spermatogenesis is an androgen-dependent process which can be maintained in hypophysectomized rats by continuous administration of testosterone (1). Also, it was reported that male fertility can be regulated by the long-term, continuous administration of testosterone or testosterone derivatives (2–4).

Recently, several medicated bandages were successfully developed and approved by the FDA for marketing to provide a continuous transdermal administration of systemically active drugs through the intact skin (5–7). Following the development of a scopolamine-releasing system for 3-d prevention of motion sickness, four nitroglycerin-releasing transdermal therapeutic systems (system A[1], system B[2], system C[3], and system D[4]) were also developed to provide 24-h continuous protection against anginal attack (6, 7).

It was recently observed in this laboratory that the transdermal absorption of testosterone in six rhesus monkeys *via* the navel area produces a substantially greater systemic bioavailability than by forearm administration (79.9 *versus* 49.9%). Bioavailability of testosterone by intravenous administration was used as the control (22). In the present investigation, we evaluated the feasibility of using the navel as the site for a long-term application of a bandage-type controlled-release drug delivery system developed to provide a continuous transdermal administration of testosterone at a controlled rate.

EXPERIMENTAL SECTION

Preparation of the Testosterone-Releasing Transdermal Bandage—To deliver the testosterone at a controlled rate for a month or longer, a disk-shaped testosterone-releasing device was fabricated, using the methodology and process outlined for the preparation of microsealed drug delivery (MDD) systems (9–11), to contain a combination of 0.041% w/w of [^{14}C]testosterone and 0.252% w/w of nonradioactive testosterone in the medicated liquid compartments. The testosterone-saturated liquid compartments were dispersed homogeneously as discrete microscopic spheres in the cross-linked polymer matrix (Fig. 1).

For the *in vivo* studies, medicated MDD disks with a surface area closely resembling that of the navel of a rhesus monkey (with an apparent surface area of 0.2 cm^2 each) were fabricated to contain 4.41 ± 0.25 μCi of [^{14}C]-testosterone. Each of the disks was then glued onto the center of a piece of commercially available plastic adhesive[5] to form a testosterone-releasing transdermal bandage.

***In Vitro* Release Studies**—The *in vitro* drug elution system, used to characterize the mechanism and rates of testosterone release from each of the MDD disks, was essentially the same as that reported previously (12), except that the medicated MDD disk was mounted in a specially designed acrylic holder (Fig. 2). The whole assembly was then rotated at a constant angular rotation speed of 81 rpm, to achieve a constant hydrodynamic condition, in a perfect sink aqueous medium (with a constant volume of 150 mL) at 37°C. The medium contains 75% v/v of polyethylene glycol 400 to enhance the aqueous solubility of testosterone and to maintain a perfect sink, which simulates the biological sink achieved by blood perfusion. The amount of testosterone released daily was determined spectrophotometrically at the λ_{max} of 241 nm.

***In Vivo* Navel Absorption Studies**—Rhesus monkeys (4.85–5.0 kg) were immobilized by the injection of 1 mg/kg of phencyclidine hydrochloride[6]

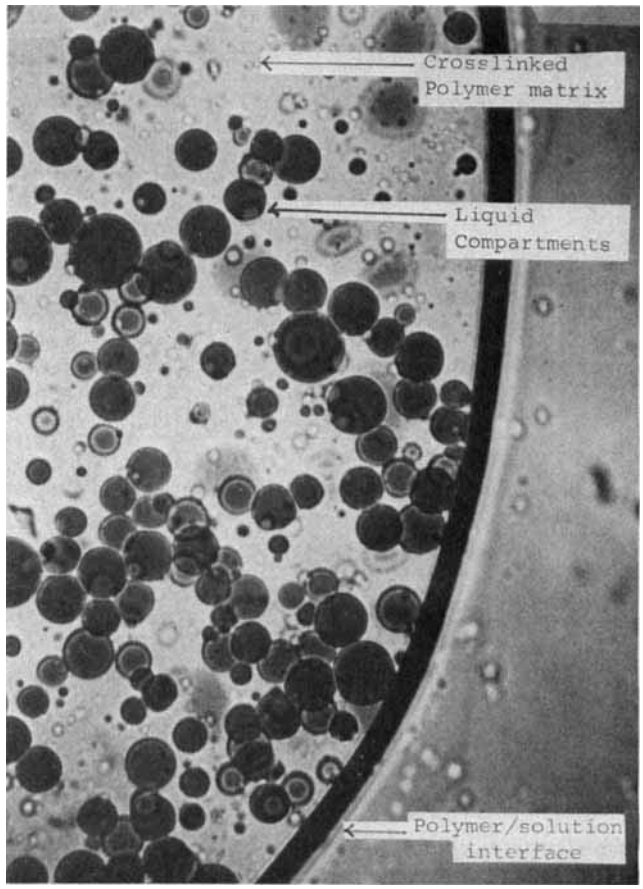

Figure 1 —*Photomicrograph (1300×) of the cross-sectional view of an MDD system. The microscopic liquid compartments (dark areas), which encapsulate drug particles, are homogenously dispersed as discrete, immobilized, unleachable spheres (with diameter <50 μm) in a cross-linked polymer matrix (light background area).*

[1] Nitrodisc; Searle Pharmaceuticals, Chicago, Ill.
[2] Nitro-Dur; Key Pharmaceuticals, Miami, Fla.
[3] Transderm-Nitro; Ciba Pharmaceutical Co., Summit, N.J.
[4] Deponit; Pharma-Schwarz GmbH.
[5] Johnson & Johnson, New Brunswick, N.J.
[6] Bio-ceutic Laboratories, St. Joseph, Mo.

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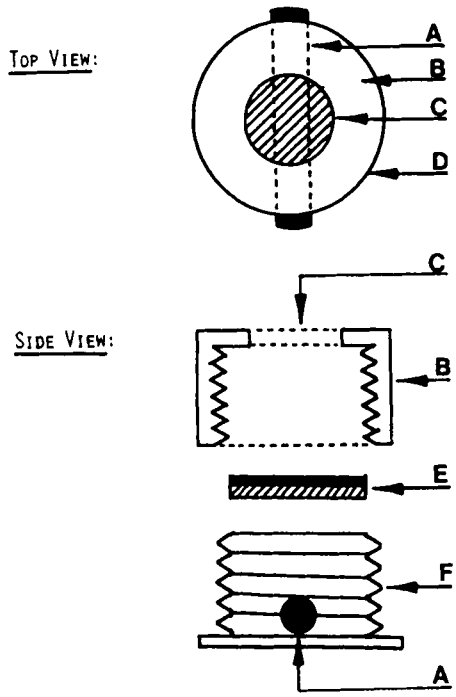

Figure 2—*Diagrammatic illustration of the acrylic holder which is specially designed for the mounting of the disk-shaped MDD system. The whole assembly can be rotated in a thermostated drug elution solution at constant rotation speed by a magnetic stirrer. Key: (A) magnetic spin bar; (B) acrylic cap; (C) opening; (D) acrylic holder; (E) MDD disk; (F) acrylic platform.*

solution. Testosterone was then administered to the navel area by the following methods.

Drug Disposition—Five microcuries (25 μg) of [^{14}C]testosterone[7] in 100 μL of acetone was applied dropwise to the navel, with the area of application controlled at 0.2 cm^2 by a template (an aluminum plate with a 0.2-cm^2 circular hole at the center). After all the drug solution was applied and quickly dried, a piece of plastic adhesive was placed over the application site and further reinforced with another plastic adhesive strip.

MDD Disk Application—One hundred microliters of drug-free acetone[8] was applied dropwise to the navel (this was done to correct for any possible effect of acetone on percutaneous absorption). After the solvent had dried, a unit of testosterone-releasing transdermal bandage was applied directly with the medicated MDD disk centered on the navel. It was then reinforced with another strip of plastic adhesive.

Placebo Disk Application—A placebo disk containing no testosterone was prepared in the same manner as described earlier for the MDD disk. After placing the placebo disk over the acetone-treated navel, 6 μCi (30 μg) of [^{14}C]testosterone in 10 μL of acetone was applied dropwise on the 0.2-cm^2 placebo disk. After drug solution was dried rapidly, a piece of plastic adhesive was placed over the placebo disk and further reinforced with another strip of plastic adhesive.

Each of the treated monkeys was placed into a jacket[9] and then secured in a metabolism chair. A 20-gauge catheter needle[10] was secured to the leg of the monkey to collect blood samples from the saphenous vein. Meanwhile, saline solution[11] was continuously infused through another catheter at a rate of 14 mL/h. Two-milliliter aliquots of blood were collected at 0.5, 1, 2, 3, 4, 5, 6, and 24 h and then every 24 h thereafter until the completion of a study. Urine was also collected on a daily basis.

At the end of each study, the plastic adhesive strips and MDD or placebo disks were removed and thoroughly extracted with methanol[8]. The area of drug application was also carefully washed to remove the residual, unabsorbed testosterone (both radioactive and nonradioactive). The extractions and washings were then combined for the analysis and determination of the fraction of dose not absorbed.

The blood samples were collected in heparinized tubes. After centrifugation, 200 μL of plasma were transferred from each sample and mixed with 5 mL of scintillation fluid[12]. Duplicate samples were prepared and their radioactivity was determined[13]. Conversion of counts per minute (cpm) to disintegrations per minute (dpm) was accomplished by the external standard channels ratio method. Data measured as dpm was recorded and converted to the corresponding total radioactivity in 100 mL of plasma (μCi/100 mL) at each sampling interval.

Samples (500 μL–1 mL) were taken in duplicate from a daily urine collection and mixed with 10 mL of scintillation fluid[12]. Total radioactivity in each sample was determined and calculated in the same manner as described earlier for plasma samples.

[7] New England Nuclear Corp., Boston, Mass.
[8] Reagent grade; MCB Manufacturing Chemists, Norwood, Ohio.
[9] Alice King Chatham, Medical Arts, Los Angeles, Calif.
[10] Longwell; Becton, Dickinson & Co., Rutherford, N.J.
[11] McGaw Laboratories, Glendale, Calif.

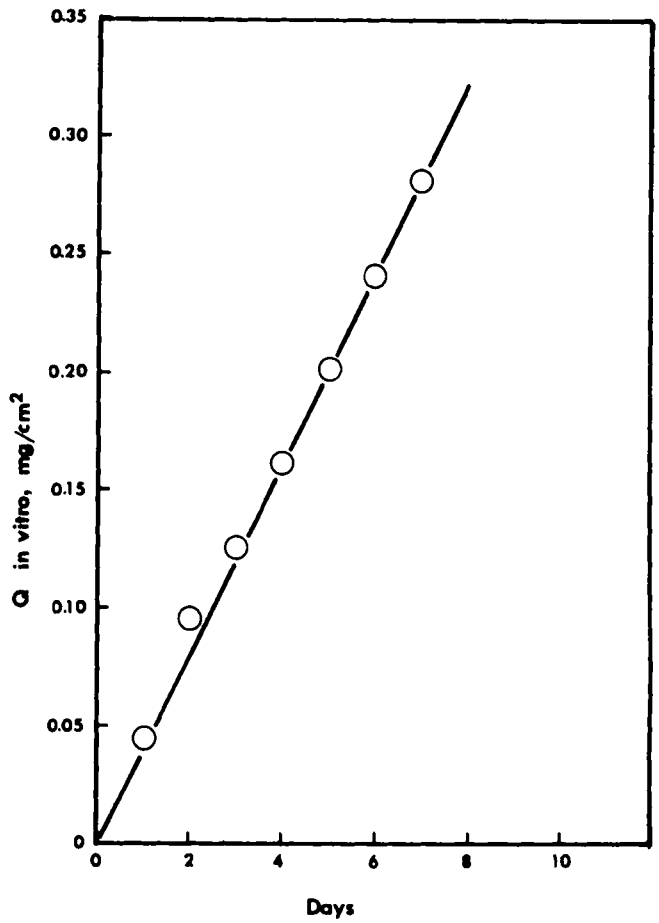

Figure 3—*Seven-day* in vitro *release of testosterone from the disk-shaped MDD system under a perfect sink condition at 37°C. The rate of release (Q/t) was determined to be 40.25 ± 0.64 μg/cm^2/d (mean ± SD).*

RESULTS AND DISCUSSION

***In Vitro* Release of Testosterone from MDD Disks**—A typical set of data on the *in vitro* release of testosterone from an MDD disk is illustrated in Fig. 3. The cumulated amount, Q, of testosterone released from a unit surface area of the MDD disk was found to be linearly proportional to the duration (in days) of elution in the elution solution. Apparently, a constant (zero-order) release profile was achieved. The observation is in good agreement with the controlled release of deoxycorticosterone acetate from the same type of MDD system (12). From the slope of the linear Q *versus* t plot, the rate of release, Q/t, of testosterone from the MDD disk was calculated to be 40.25 (±0.64) μg/cm^2/d (average of triplicate experiments with a coefficient of variation of 1.6%). The theoretical basis for the observed Q *versus* t relationship was analyzed previously (13).

Long-Term Navel Absorption of Testosterone—For the *in vivo* studies, a unit of testosterone-releasing transdermal bandage was applied to each of two rhesus monkeys with the medicated MDD disk (0.2 cm^2) directly overlaying the navel. Blood and urine samples were collected according to the protocol outlined earlier (*Experimental Section*) until all the testosterone dose incorporated was totally released (~46 d).

[12] PCS; T. M. Amersham/Searle Corp., Arlington Heights, Ill.
[13] Mark II Scintillation Counter; Searle Analytic, Inc., Des Plaines, Ill.

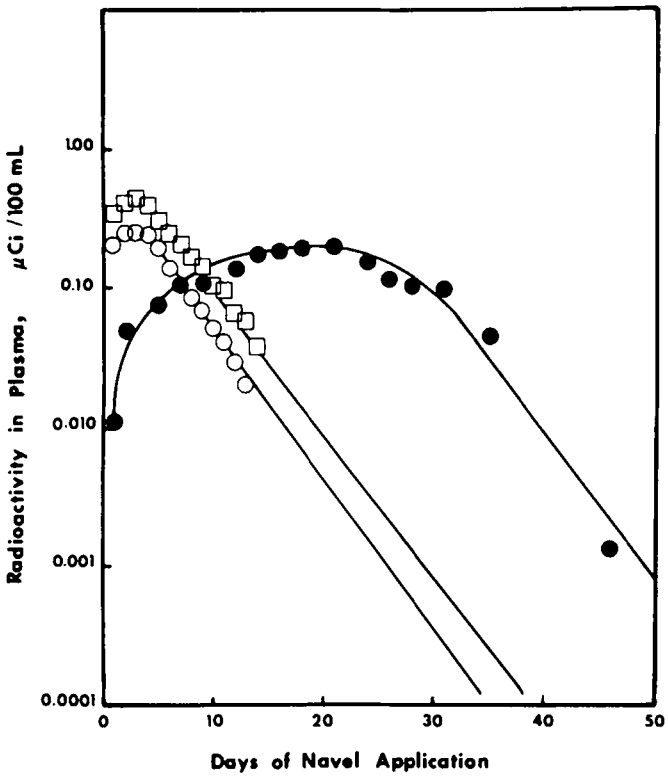

Figure 4—*Mean plasma radioactivity levels following the navel administration of [^{14}C]testosterone in two rhesus monkeys. Key: (○) drug alone (5.03 μCi); (□) disposition on a placebo disk (6.02 μCi), (●) microseal in an MDD disk (4.41 μCi). The first-order rate constants for elimination (β) were 0.0104, 0.0098, and 0.0101 h^{-1}, respectively.*

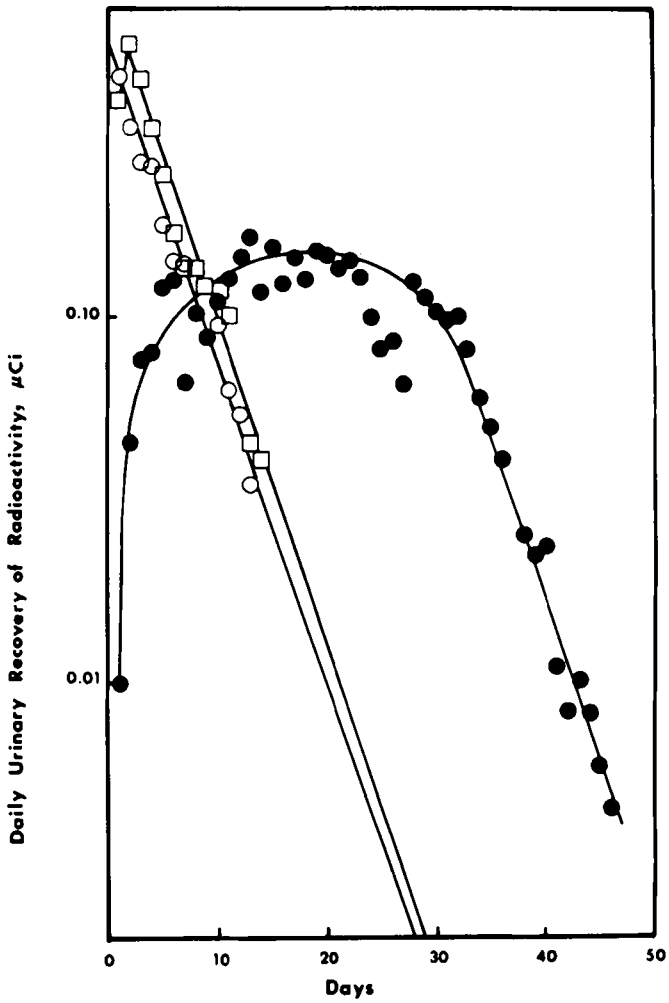

Figure 5—*Mean daily urinary recovery of radioactivity following the navel administration of [^{14}C]testosterone in two rhesus monkeys. Key: (○) drug alone (5.03 μCi); (□) disposition on a placebo disk (6.02 μCi); (●) microseal in an MDD disk (4.41 μCi). The first-order constants for urinary excretion (k_e) were 0.0085, 0.0087, and 0.0085 h^{-1}, respectively.*

Both plasma profiles (Fig. 4) and daily urinary recovery data (Fig. 5) demonstrated that the 46-d application of the MDD disk on the navel of the monkey maintained a fairly steady plasma radioactivity level at ~0.10 μCi/100 mL for a duration of slightly over 1 month and also a fairly constant urinary excretion rate of 0.10 μCi/d in the same period.

On the other hand, application of [^{14}C]testosterone directly onto the navel by the solution disposition technique or disposition of drug on a placebo MDD disk (without microsealed drug inside the device) at the time of navel application could not achieve or maintain either a steady plasma level or a constant daily urinary excretion rate of [^{14}C]testosterone (Figs. 4 and 5). The results suggested that the MDD system provides the controlled-release mechanisms to meter the release of a constant amount of [^{14}C]testosterone available for navel absorption.

The data in Figs. 4 and 5 also indicated that the rate constants for elimination (β) and for urinary excretion (k_e) of [^{14}C]testosterone were not altered by the controlled drug administration of the MDD system. These observations are expected since the β and k_e values are the intrinsic pharmacokinetic profiles of the drug and should not be modified in any way by the method of administration. Both with and without microsealed [^{14}C]testosterone in the device, the testosterone and its metabolites were eliminated and excreted basically at the same rates as those for drug alone. Testosterone and its metabolites were eliminated at a mean β value of 0.0101 ± 0.0003 h^{-1} and excreted at a mean k_e value of 0.0085 ± 0.0001 h^{-1}.

To determine the *in vivo* release rate of testosterone at the navel site, the cumulative urinary total radioactivity recovery data (expressed as percent of the administered dose) was plotted as a function of time (Fig. 6). As seen in the short-term (7-d) *in vitro* drug release studies (Fig. 3), a fairly constant urinary recovery profile was also observed in the navel absorption of testosterone when the combination of radioactive and nonradioactive testosterone was delivered by an MDD disk, for a period of ≤32 d. It was also noted that >90% of the administered dose of the radioactive testosterone was recovered in the urine during the 32-d zero-order transdermal controlled drug administration (Fig. 6).

On the other hand, it was observed that only one-half (~46%) of the administered dose was recovered from the urinary excretion when [^{14}C]testosterone was administered either by direct disposition on the navel or by depositing a placebo MDD disk (without microsealed compound in the liquid compartments) on the navel. The balance of the administered dose was recovered from the washings of the skin surface and from the extractions of the covering materials. The reasons for the low transdermal bioavailability observed are still not clear and require further study. In any case, a better systemic bioavailability of testosterone was achieved by use of the controlled-release MDD system, which released testosterone at the molecular level and controlled the amount of testosterone available for continuous transdermal administration through the navel.

As previously stated, each MDD disk contained a combination of 0.041% w/w [^{14}C]testosterone and 0.252% w/w nonradioactive testosterone. By calculation, the 32-d cumulative urinary radioactivity recovery data in Fig. 6 can be translated into the cumulative total amount (Q) of testosterone (radioactive and nonradioactive) absorbed by each of the rhesus monkeys investigated. Again, a fairly linear Q *versus* t relationship was achieved (Fig. 7), as seen in the *in vitro* drug release studies (Fig. 3). From the slope of this linearity, it was determined that the testosterone was released and absorbed from the MDD disk at an *in vivo* rate (Q/t) of 27.66 μg/cm^2/d.

***In Vitro–In Vivo* Relationships**—Comparison of this *in vivo* release rate (27.66 μg/cm^2/d) with the *in vitro* release rate (40.25 μg/cm^2/d) determined in Fig. 3 yields an *in vitro–in vivo* correlation coefficient of 0.69. The observation of a nonunity correlation coefficient, *e.g.*, 0.69, could be attributed to the existence of skin tissues in the *in vivo* situation, which act as an additional diffusion barrier for the permeation of drug molecules released from the medicated MDD disk. Drug molecules have to diffuse through this barrier before they can reach the biological sink for transport to a target organ[4]. The dissimilarity between *in vitro* and *in vivo* conditions may also contribute to the *in vitro–in vivo* correlation coefficient lower than unity. The sink condition established in the *in vitro* situation by the enhancement in saturation solubility may not simulate the interfacial partitioning of the drug from the drug delivery device toward the stratum corneum, which is a nonsink medium in nature.

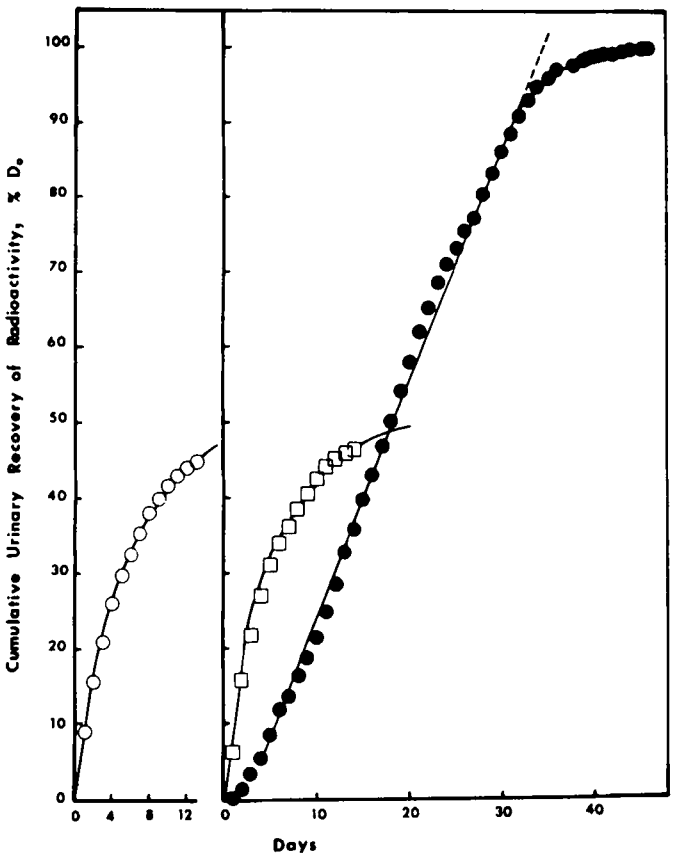

Figure 6—*Comparison of the time course for the cumulative urinary total recovery of radioactivity following the navel administration of [^{14}C]testosterone. Key: (○) drug only; (□) disposition on a placebo disk; (●) microseal in an MDD disk. The total urinary recovery is 44.8, 46.5, and 93.4%, respectively, of the administered dose (D_0).*

In a separate study with subcutaneously controlled administration of deoxycorticosterone acetate delivered by an identical MDD-type drug delivery system, an almost unity (1.09) *in vitro–in vivo* correlation coefficient was obtained (13), in which the stratum corneum does not exist between the drug delivery system and the biological sink.

However, the *in vitro–in vivo* correlation coefficient (0.69) obtained can be utilized as a working factor in the determination and projection of the long-term *in vivo* release rates from a short-term *in vitro* drug release study (13–17). This coefficient can be very useful in the development of a long-acting drug delivery system in that it can minimize the expense involved in the amount of testing required and the use of costly animals.

In conclusion, the bioavailability data generated in this investigation have clearly demonstrated that the navel can be a useful site for long-term transdermal administration of drugs, such as testosterone, *via* a bandage-type controlled-release drug delivery system [such as a microsealed drug delivery (MDD) system] to provide a prolonged plasma drug level for at least 1 month. The systemic bioavailability of the drug can also be enhanced substantially by the controlled drug-release mechanisms of the MDD system. The same conclusion was reached in the controlled subcutaneous administration of deoxycorticosterone acetate by a subdermal implant-type MDD system (13).

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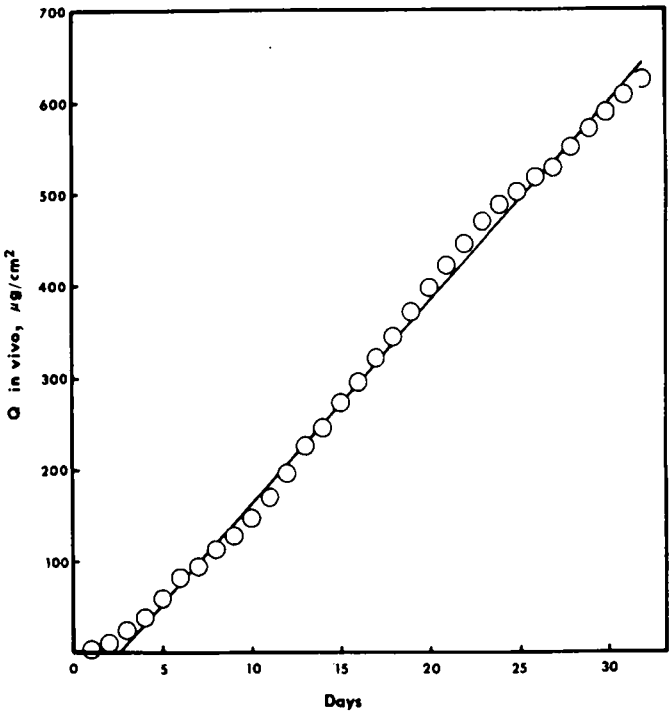

Figure 7—*Time course for the cumulative total amount of testosterone administered to the two rhesus monkeys during 32-d navel application of an MDD disk (0.2 cm^2). The data were calculated from Fig. 6 by converting the cumulative urinary radioactivity data into the total amount of testosterone administered/cm^2 of the MDD disk. The* in vivo *release rate (Q/t) was calculated to be 27.66 μg/cm^2/d.*

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