5. DISCUSSION

5.1 Liquid Formulations

Liquid formulations represent a novel modality of TD drug delivery. There are no reports of any similar formulations in various databases scanned, although basic research in percutaneous absorption often involves application of the test compound on skin using a volatile vehicle. The formulations developed are easy to make and dispense, as well as easy to use. The excipients are commonly available commercially, and are very cheap. The laboratory-scale excipient cost of a unit dose at 1994 retail prices works out to be less than 1 paisa. The excipients are all "Generally Regarded as Safe". Stable and reproducible formulations could be prepared and uniform doses could be dispensed by ordinary droppers. There are two drawbacks associated with their use, which are as follows:

(1) There is less bioavailability of the drug from such a formulation as indicated by the studies on rats and monkeys. A smaller amount of T is released during the sustained phase of delivery in comparison to patch formulations and data reported in the literature.

(2) There is a possible environmental hazard associated with their use (Delanoe et al 1984). Contacts of patients using such formulations are exposed to a greater risk of accidental androgenization, since a part of the drug that could not be accounted for in the bioavailability studies is presumably released in the environment.

Both these problems can be minimized by providing an occlusive dressing to be placed over the in situ cast film once the vehicle has evaporated. Bioavailability of T is enhanced under occlusion (Wester and Maibach 1987), and shedding of the drug can be controlled as well.
5.2 Patches

The design strategy adopted in this case was to attempt to formulate "adhesive dispersion controlled" or Type IV TD systems, failing which, to opt for "matrix controlled" systems. Adhesive-dispersion patches could be successfully prepared by an apparatus fabricated from commonly available materials. Other methods of coating were tedious and did not permit much flexibility in controlling coating thickness. T was found to be stable in the final formulation (VAC) for a projected shelf life of 3 years if stored in the dark.

All the polymers employed in these studies were used as components of pressure sensitive adhesive formulations (Muslof 1987, Satas 1987). These belonged to three major groups: natural rubber (smoked sheet), acrylic polymers and vinylic polymers. Rubber, acrylates and EVA enjoy a high degree of popularity in use as materials in raincoats, gloves, footwear, dresses, bedsheets, towels and bandages. PVP and PVA are routinely used in the pharmaceutical industry in formulations for skin application as well as oral administration. Acrylic (Pidivyl KPS) and vinylic (Emdilith DM-45) polymer formulations used also have an impressive though anecdotal track record of safety as adhesives used in bindis, wherein there are no reports of adverse skin reactions over the past 7-12 years. The patches prepared were also demonstrated to be biocompatible by the USP XXII Direct Contact Test for biological reactivity.

5.3 Uniformity and Reproducibility

Thickness and weight determination were trivial procedures used in quality assurance of the patch formulations, but they proved to be of importance in deciding whether or not the evaluated patches were truly uniform. Considered along with the data on T content assay, these two parameters could provide assurance that patches conforming to limits had uniform content as well as dimensions. Dimensions, particularly the thickness of the release controlling adhesive layer, are very important in assuring consistent drug release profiles and thereby the bioavailability.
5.4 Physico-Chemical Properties

Polymers used in the preparation of the reported TD systems can be expected to impact on the crystallinity of T (Sekikawa et al 1978, Mackellar et al 1994a,b, Yoshioka et al 1995) and alter their own properties (Lehtola et al 1995, Lin et al 1995). These effects have been investigated using thermal analysis (Venkataram et al 1995, Yoshioka et al 1995).

The particle size of T in formulations may be expected to influence dissolution and hence permeation rates. This parameter can be evaluated by optical microscopy (Houghton and Amidon 1992). Electron microscopy and laser diffractometry may also be used for this purpose for greater accuracy and reliability. T was found to be incompatible with acrylic type adhesives. Morimoto et al (1992) have demonstrated interactions between the carboxylic functional groups of such polymers and various chemical classes of drugs such as esters, carboxyl acids, nitrates, tertiary amines and secondary amides. The hydroxyl group in T can also interact with the acrylic carboxyl according to the mechanism postulated by them.

Thermal analysis did not detect any other interaction between the various materials used for the preparation of the reported systems. However, attempts to quantify T in formulation by thermogravimetry could not achieve accuracy greater than ±10% by weight (data not shown).

Another physical property that may be expected to impact on drug release kinetics is the particle size and size-distribution of T in the formulation. This would affect the rate at which soluble T is made available for diffusion through the adhesive or film, and ultimately through the skin. Interestingly, this aspect of the pharmaceutics of TD systems is not discussed in any prominent review of literature, nor was any related report found in the survey undertaken during the present work.

Since size analysis by microscopy was hindered by the density of the particle bed, laser diffractometry was carried out. For this, it was necessary
to extract and fluidize the particles contained in the films cast from the liquid formulations. There is some possibility that the particles may have suffered some changes in shape and size during this treatment, despite all efforts to prevent such changes. However, T is practically insoluble in water, and the instrument settings were the least stressful available, so that the data obtained might be considered to be a reasonable reflection of the picture within the film.

What is most striking in these results is the appearance of a population of particles in the 2-30 μm range, that is not present in the T of the British Pharmacopoeia (1988). The latter material has particles smaller as well as larger than this. The origin of this population of particles can be accounted for on the basis of two features of the formulation: rapid evaporation of the solvent and the presence of PVP in the crystallizing bed.

The rapid evaporation of the solvent causes high rates of supersaturation to develop. Imperfect, dendritic crystals are invariably formed under such conditions (McCabe and Smith 1976). PVP has long been known to have an "anti-crystallizing" effect on various compounds (Sekikawa et al 1978, Yoshioka et al 1995). Polymer chains interact with the soluble compound and hinder the solute-solute interactions that go into making up the crystals. A crystallizing bed that is exposed to rapid supersaturation in the presence of PVP would certainly contain agglomerates that do not exhibit the crystal structure expected of well-formed crystals of the solute.

If that is so, the particles showing up in the diffractometry studies and in scanning electron microscopy represent a fraction of T that can be understood to have some properties that strongly affect their dissolution behavior (McCabe and Smith 1976). First, they would have surplus surface energy, since they are not organized into the stable crystal form. It may also be expected that this would cause such agglomerates to be metastable in view of the fact that their physical state would be in a dynamic equilibrium that tends towards crystallization on the one hand and interaction with the polymer on the other.
The foregoing arguments indicate that the diffusion of T through the LN polymer film is affected by these physical properties. There is no analogous set of experiments reported in the case of the VAC formulations, except for evidence from scanning electron microscopy. Nevertheless, the features of rapid supersaturation and presence of polymer chains are associated with the preparation of these as well, since IPA was used as a processing solvent in mixing T with the adhesive.

5.5 Testosterone Release Kinetics: In Vitro and In Vivo

A biphasic pattern was consistently encountered in both these sets of studies. The early phase of delivery from the TD systems, whether films or patches, comprised a "burst" effect. This is a common observation when formulating controlled release systems for any route of administration, and a great deal of effort has traditionally been invested in minimizing this burst to achieve "near zero-order" drug release. However, the realization that the normal T profile is itself pulsatile prompted an investigation into the possibilities of using this feature to biological advantage.

The IPA-based vehicle employed with LN formulations was initially considered as a contributing factor to the early burst in skin permeation studies. The permeation enhancing properties of this and other lower alcohols is well known. The possibility of the initial burst occurring on account of entrainment of IPA-dissolved T down a lipophilic or IPA-saturated "ramp" pathway in the skin seemed plausible. However, this possibility was negated on considering that patch formulations exhibited similar in vitro drug release kinetics, where there was no skin interposed between the patch and the receptor buffer, and only traces of IPA were left in the formulation after the coating and drying procedure.

As discussed above, certain commonalities are expected in the physical properties of T in the polymers comprising both these sets of formulations. It is tempting to conclude that the similarities in release profiles arise due to these physical properties. Such a conclusion is supported by two observations. First, the similarity resides in the early phase of release. This portion of the curve is due to a fraction of the T that diffuses rapidly. If the non-crystalline, metastable agglomerates of T actually affect the diffusion
profile, they may do so only in the early phase: it is not possible for them to
outlast stable crystals on the basis of elementary thermodynamic
considerations. Second, there is an unambiguous fall in the amount of T
released per interval after the first peak is resolved. This confirms that there
is a finite reservoir of the rapidly diffusing material.

The pharmacokinetics of T observed in bioavailability studies also
show biphasic nature. A similar observation by the Theratech group, which
has recently received FDA approval for their non-scrotal T patch bears direct
quotation here. "Both (in vitro human cadaver skin permeation and in vivo
input kinetics) curves indicate a biphasic pattern, characterized by a rapid
permeation phase during the first 8 h, followed by a slower permeation
phase during the last 12 h" (Mazer et al 1992). The time frames reported by
these authors are considerably more extended than the ones reported here,
perhaps because of formulation factors and species differences, but the
phenomenon observed is identical. This also lends support to the contention
that T exhibits biphasic diffusion behavior, skin permeation and
pharmacokinetics due to intrinsic physical properties. Similar observations
have also been reported by Katz et al (1993) in case of E2. These authors
have reported biphasic pharmacokinetics of E2 from a commercially
available TD patch.

5.6 Mathematical Modeling

Attempts to model the release kinetics on the basis of published
theory were not successful. The transient nature of the burst was the major
constraint in applying Fick's laws of diffusion. Kinetic models were found to
be inadequate to address these formulations. These invariably contain a
term to account for a fraction of the drug being released with zero-order
kinetics throughout the period of delivery.

A theoretical treatment of situations in which biphasic input kinetics of
T delivered by the TD systems interact with the prevailing oscillatory kinetics
of endogenous T was also attempted. A simple linear model was able to
illustrate the advantages of biphasic kinetics in inducing feedback inhibition
of pulsatility (Misra et al 1995c). It could be demonstrated that this effect
could be achieved with a much smaller dose of T than is used in
conventional regimens. A non-linear model of the same situation could be developed on the basis of scaled differential equations modeling the Belousov-Zhabotinskii reaction. Perturbations could also be observed in the oscillatory kinetics predicted by this model. It is unlikely that the pulse generator or Zietgeber mechanism responsible for control of endocrine pulsatility is linear in nature, since most real-world processes are not. The observation that input kinetics can perturb even non-linear oscillatory phenomena therefore assumes significance.

5.7 Physiological Relevance

Although the physiological relevance of these studies to the human system needs to be evaluated more rigorously, the studies in animal models are encouraging. However, a few caveats are in order.

The rat is not considered appropriate as a model for studying percutaneous absorption of testosterone in view of the much higher permeability of rat skin to this compound as well as many others (Guy et al 1987). Nevertheless, it is an extensively used model for studies in reproduction. The castrated rat was considered specially appropriate for evaluating pharmacokinetics of input T in absence of prevailing background levels. It could also be used for bioassay by the Hershberger Test (Brotherton 1976) or assessment of effects on accessory sex organs (Higgins and Parker 1980, Mann and Mann 1981, Ganguly et al 1992).

Monkeys immunized against GnRH (Talwar et al 1984) also represent a suitable model for studying pharmacokinetics, and are much closer to man in terms of barrier properties of the skin (Guy et al 1987). Supplementation of T in the monkeys immunized with GnRH vaccine was an important objective of this study, in view of the observation of synergistic suppression of spermatogenesis as well as maintenance of sexual behavior that is envisaged with this regimen (Bhasin et al 1988, Bagatell et al 1989, Ladd et al 1989 1994, Behre 1995).

Bioavailability profiles and bioassay results confirm that the delivery systems were capable of maintaining circulating levels of T which were adequate to maintain prostate weights in castrated rats. Serum LH estimated
by the Leydig cell assay during bioavailability studies in GnRH-immunized monkeys was suppressed, and severe oligospermia was observed 6 Wks post immunization, demonstrating the efficacy of the vaccine. Continued administration of T for 4 wks to the GnRH-immunized monkeys did not affect their oligospermic status despite the demonstration of circulating T levels close to the pretreatment range. Sperm counts after 4 weeks of treatment were monitored beyond the completion of the time period associated with one cycle of spermatogenesis, and were not observed to change to any significant degree. These results accord well with literature reports that T alone is not capable of re-initiating spermatogenesis. Ladd et al (1989) have earlier demonstrated the efficacy of T supplementation in maintaining metabolic and behavioral characteristics in the GnRH-immunized rat. It was therefore concluded that both systems were suited to the application of T supplementation therapy in androgen-deprived subjects, including subjects receiving GnRH vaccine.

Some observations in the studies on monkeys cannot be accounted for on the basis of the current knowledge of the action of the GnRH vaccine and episodic secretion of hormones. Elevated levels of T were observed in the first and/or second bleeds in placebo-treated animals. These levels were significantly higher than the random variations in the assay and showed consistent values in two assays. At the same time, analysis for bioactive LH and anti-GnRH antibodies in the same serum samples indicated that the LH was low and antibody titers at peak. Possibilities of inadvertent contamination or incorrect labeling were examined and ruled out.

The biphasic profile offers investigators a delivery system to test the hypothesis that the early-morning surge of T is relevant to male reproductive physiology and behavior. Such a profile also shows promise over the other TD systems for T delivery, in that it is one more step towards the pulsatile pattern of endogenous T release.

Inhibition of spermatogenesis by administration of androgens alone or in combination with other steroids, peptides or vaccines is an emerging application in the field of male contraception. The perturbation of theoretically modeled pulsatility by the input kinetics from the systems prepared during this study indicates an interesting possibility of application
to male contraception. It has earlier been demonstrated that pulsatile administration in the form of daily subcutaneous injections of a GnRH analog induced suppression of gonadotropins and T in men. This possibility, along with the fact that the TD route is a non-invasive and high-compliance route of drug delivery, makes a strong case for incorporating these systems in experiments relating to male contraception.