Tissue Response to Bioerodible, Subcutaneous Drug Implants: A Possible Determinant of Drug Absorption Kinetics

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The fibrous tissue compartments that develop in response to the subcutaneous implantation of bioerodible heat-fused rods of norethindrone and cholesterol (85 and 15%, respectively) were studied by light and electron microscopy at various intervals after implantation to determine whether the biological inflammatory response may play a role in drug absorption. Thirty-five regularly menstruating, sterilized (tubal ligation), healthy females each received four Annuelle rods. The microanatomy of seven of the largest implants (135 mg norethindrone) was studied. A dense fibrous biological compartment was found to surround each rod. By light microscopy no abnormal tissue response was revealed. Scanning and transmission electron microscopy showed that the surfaces of the rods were covered by a cellular matrix of mononuclear cells. The fibrous compartment was composed of a loose cellular bed immediately surrounding the norethindrone rod, a dense fibrous connective tissue envelope containing blood and lymphatic vessels, and an outer fatty connective tissue layer. Transmission electron microscopy confirmed that the cellular tissue immediately surrounding the rods was composed mainly of lipid laden macrophages. Norethindrone levels in tissue capsules at 3 and 10.5 months were 0.05 and 8.4% by weight, respectively. These observations suggest that the local inflammatory response plays a role in the active processing of this delivery system. This picture is qualitatively different from the general view of the fibrous capsule as a simple rate limiting membrane. The effects observed in this study suggest that a more complex, functional biological system develops in response to the subcutaneous introduction of a drug delivery device.

KEY WORDS: norethindrone; implants; bioerodible; electron microscopy; macrophages.

INTRODUCTION

Implantable, slow-release, solid dosage forms have significant advantages for delivery of medications in a number of clinical situations. Potential applications of such systems include contraception, *in situ* cancer chemotherapy, analgesia in chronic pain, and delivery of growth factors for both clinical and agricultural purposes. Systems which are bioerodible and biodegradable are, in general, preferable to bioinert delivery devices, in that they do not require surgical explantation when depleted. Historically, theoretical modeling of the release kinetics of bioerodible systems has relied on purely physicochemical concepts, such as drug concentration in the device, surface geometry, and drug solubility constants, to predict release rates (1). A heat-fused norethindrone/cholesterol rod demonstrated near-zero-order kinetics *in vivo* rather than the first-order decline predicted by a uniformly diminishing surface area (2). Therefore, we questioned whether the biological inflammatory response surrounding a bioerodible implant might play a role in determining rates of drug absorption. We sought evidence of such a role by light and electron microscopic examination of fibrous tissue capsules containing Annuelle rods removed at various intervals after implantation.

MATERIALS AND METHODS

We examined subcutaneously implanted norethindrone rods (Annuelle, Endocon, Inc., S. Walpole, MA) removed from 7 of 35 normally menstruating, sterilized females who had volunteered for a Phase I study of the pharmacokinetics of implantable contraceptives. The pharmacokinetic results of this study have been reported elsewhere (3) but Fig. 1 (Table I) shows the NET concentration against time for the "F" rods which were used for this study. Briefly, 35 regularly menstruating, sterilized (tubal ligation), healthy females each received four Annuelle rods. The rods were placed subcutaneously in the flexor surface of the forearm by means of a sterilized, disposable implanter (Harman Injector). Rods reported on in the present study were implants of norethindrone and cholesterol (85 and 15%, respectively) manufactured by a proprietary heat fusion technique (flash melting) and measuring 2.7 mm in diameter and 6 mm in length. Four rods contained approximately 135 mg norethindrone. No complaints leading to removal were related to symptoms at the sites of implantation. The rods were removed by resecting the complete connective tissue capsule through a 6-mm incision at the midpoint and perpendicular to the line of insertion. The specimens were placed in 10% formalin, labeled, and analyzed by light and electron microscopy and by mass spectrometry. Rods from the same manufacturing lot, but not implanted, were examined by the same methods.

Macroscopic features were photographed and the specimens were prepared for correlative microscopic study. The encapsulated rods were cut longitudinally and transversely without dislodging them from their connective tissue beds. In some instances tiny windows were made in the capsule in order to permit observation of the rod material without disturbing its location in the capsule. Portions of some specimens were used for the light microscopy and scanning and transmission electron microscopy using techniques previously described (4). Sections of tissue were prepared by hematoxylin and eosin, periodic acid–Schiff, periodic acid–Schiff diastase, and Gomori trichrome connective tissue stain.

In addition 1-μm plastic sections stained with toluidine blue were prepared. These specimens, unlike those for light microscopy, were prepared for study without using organic solvents, which are known to dissolve lipids (e.g., steroid

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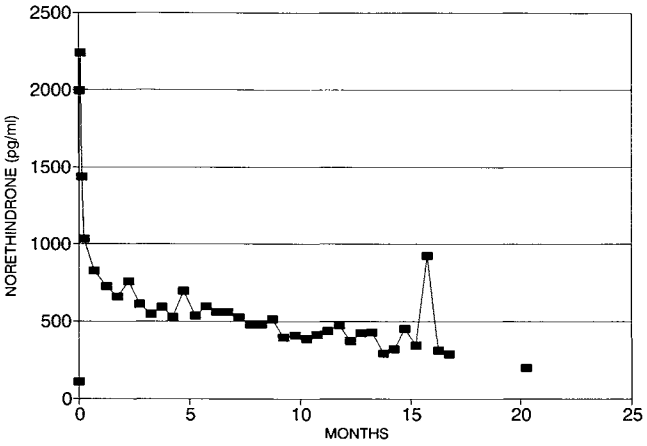

Fig. 1. Norethindrone concentration plotted against time for "F" rods (pellets).

hormones). In addition, some material from the fibrous tissue compartments of specimens taken at 3 and 10.5 months postinsertion was prepared for mass spectrometry of norethindrone content according to standard methods.

RESULTS

Macroscopic examination of excised specimens revealed a dense fibrous compartment surrounding each rod (Fig. 2) with loose connective tissue peripherally. The compartments were segmented between the individual rods (Fig. 3), thus maximizing the tissue surface area presented to the implants. As expected, the total cross-sectional area of implanted rods appeared to decrease with time postimplantation, and rods occupied less of the central areas of the connective tissue compartments.

Light microscopy revealed a highly structured compartment consisting of an inner lumen, with a lining layer of loose cellular material, a middle fibrous layer, and an outer loose connective tissue layer with some adipocytes (Fig. 4). The middle fibrous layer contained well-developed blood and lymphatic vessels, some of which had free lipid globules in their lumens (Fig. 5). The cellular component of the fibrous capsule consisted of numerous round cells and large macrophages, many of which contained lipid globules (foam cells) in palisades between layers of denser connective tissue containing fibroblasts (Fig. 6). No hyperplasia or neoplasia was seen, and granulocytes (rare) were seen only in the 3-month postimplant specimen. Relatively few foreign body giant cells were seen in any of the specimens.

Scanning electron microscopy of preimplanted rods viewed longitudinally revealed a superficial covering of loose crystals, presumably norethindrone, adherent to a true surface of apparently homogeneous crystalline aggregate of the active and excipient ingredients (Fig. 7). Scanning electron microscopy of rods after implantation showed that the surface was covered by a cellular matrix of mononuclear cells (Figs. 8A and B). The surface under this matrix was markedly irregular as compared to the surface of preimplant rods (Figs. 9A and B).

The fibrous compartments were composed of a loose cellular bed immediately surrounding the norethindrone rod, a dense fibrous connective tissue envelope containing blood and lymphatic vessels, and an outer fatty connective tissue layer (Figs. 10A and B).

Transmission electron microscopy confirmed that the cellular tissue immediately surrounding the rods was composed mainly of lipid laden macrophages, i.e., foam cells (Fig. 11). With longer durations postimplantation, the apparent number of foam cells per unit area increased in the peripheral portion of the compartment. Some foam cells appeared to be disgorging their lipid contents into the interstitial space of the capsule. In one section, a granulocyte containing what appeared to be small lipid globules was seen entering a blood vessel. The fate of the foam cells could not be ascertained in the material available for study (Figs. 12A–C).

Spectrophotometric analysis of the norethindrone content of the capsular tissue revealed that at 3 and 10.5 months postimplantation, the total norethindrone content was 0.05

Table I. Means and SE for Each Time Point of Blood Draws for Patients[a]

	[Norethindrone], pg/ml								
Time	Baseline	24 hr	48 hr	96 hr	1 wk	3 wk	5 wk	7 wk	9 wk
Mean	112	1994	2242	1438	1035	830	726	661	759
SE	0	225	569	101	62	35	53	64	98
Time	11 wk	13 wk	15 wk	17 wk	19 wk	21 wk	23 wk	25 wk	27 wk
Mean	611	547	595	526	697	536	596	562	560
SE	46	46	62	33	28	37	54	34	47
Time	29 wk	31 wk	33 wk	35 wk	37 wk	39 wk	41 wk	43 wk	45 wk
Mean	528	480	480	514	395	412	386	414	437
SE	40	52	45	114	32	39	55	51	19
Time	47 wk	49 wk	51 wk	53 wk	55 wk	57 wk	59 wk	61 wk	63 wk
Mean	478	373	425	431	293	324	455	346	928
SE	22	66	75	45	42	165	65	87	725
Time	65 wk	67 wk	69 wk	71 wk	73 wk	75 wk	77 wk	79 wk	81 wk
Mean	311	289	—	—	—	—	—	—	—
SE	—	—	—	—	—	—	—	—	—

[a] Values were assigned to the nearest 2-week period in the event they were taken off at a time point different from the "ideal" schedule.

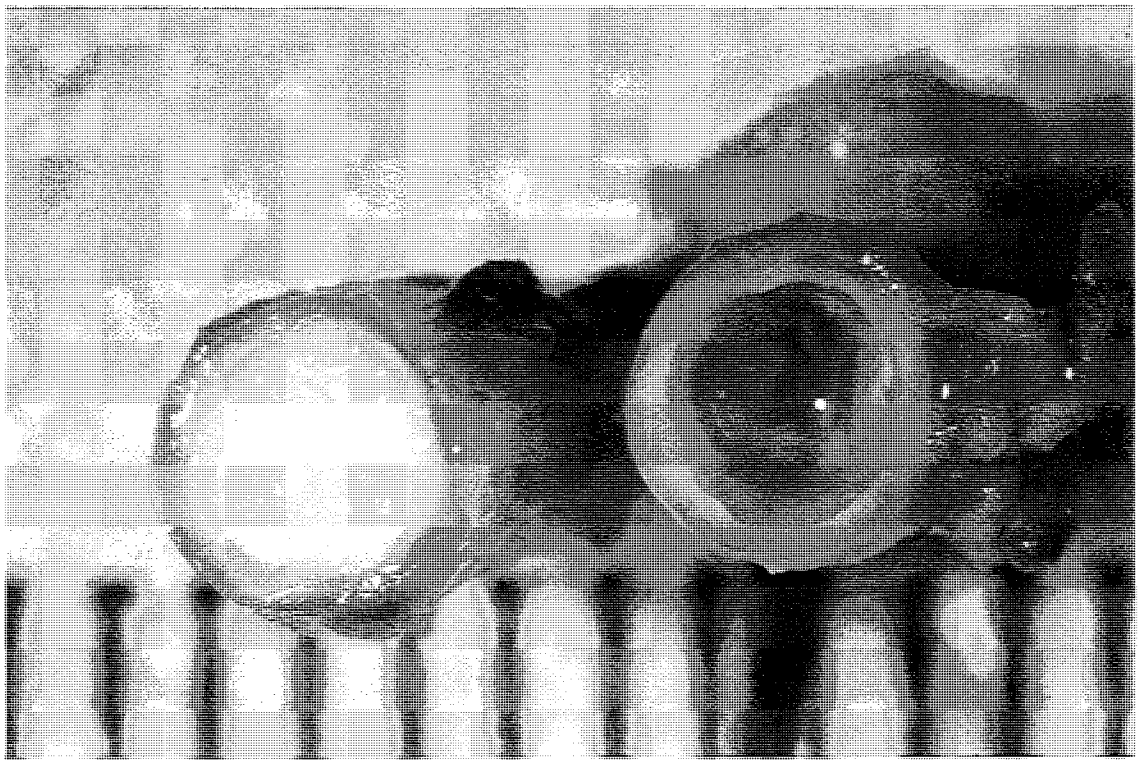

Fig. 2. Norethindrone pellet and capsule, 13 months postimplantation. Left: Norethindrone pellet inside tissue capsule. Right: Tissue capsule with pellet removed. 130×.

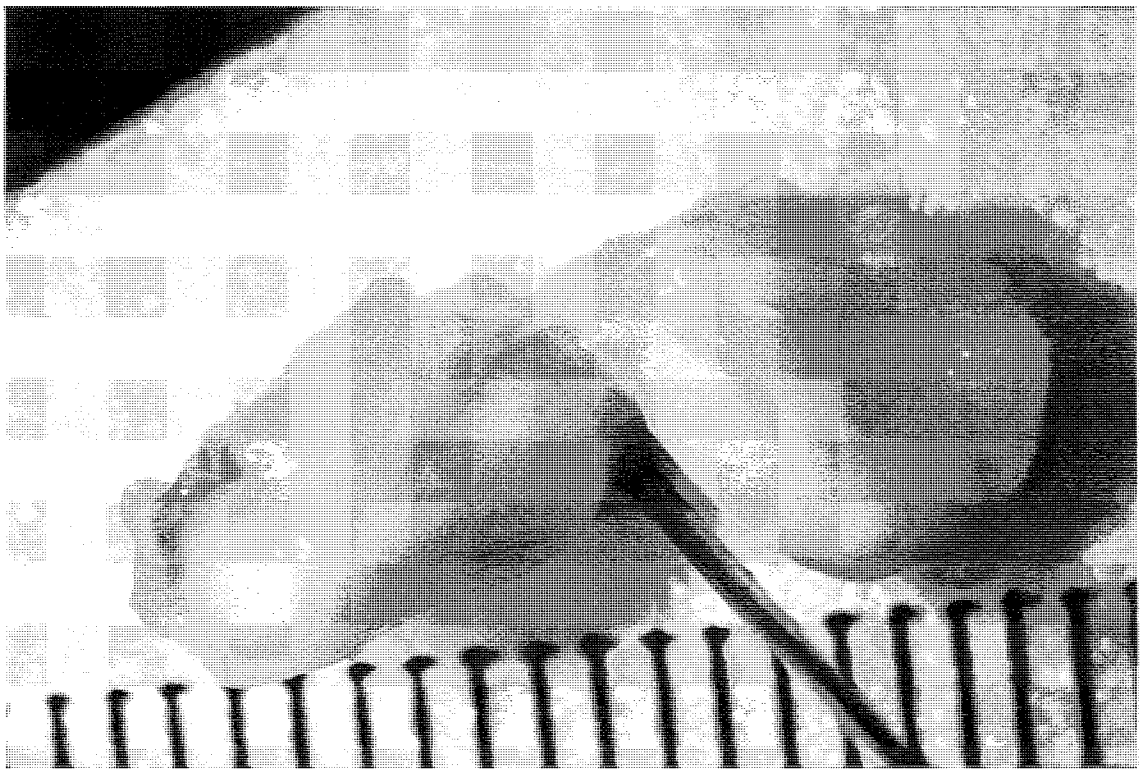

Fig. 3. Two tissue capsules, 7 months postimplantation. Each capsule surrounds a norethindrone pellet. 80×.

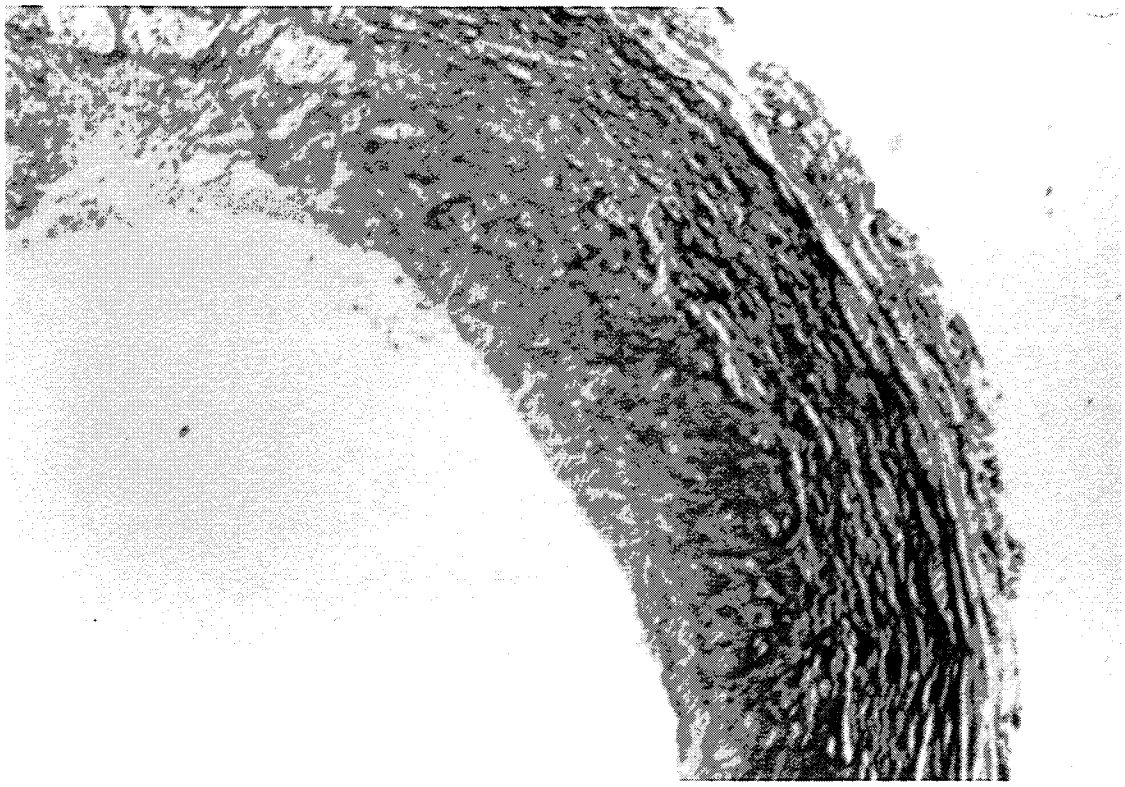

Fig. 4. Tissue capsule, 13 months postimplantation. Trichrome stain. 20×; reduced to 90% for reproduction.

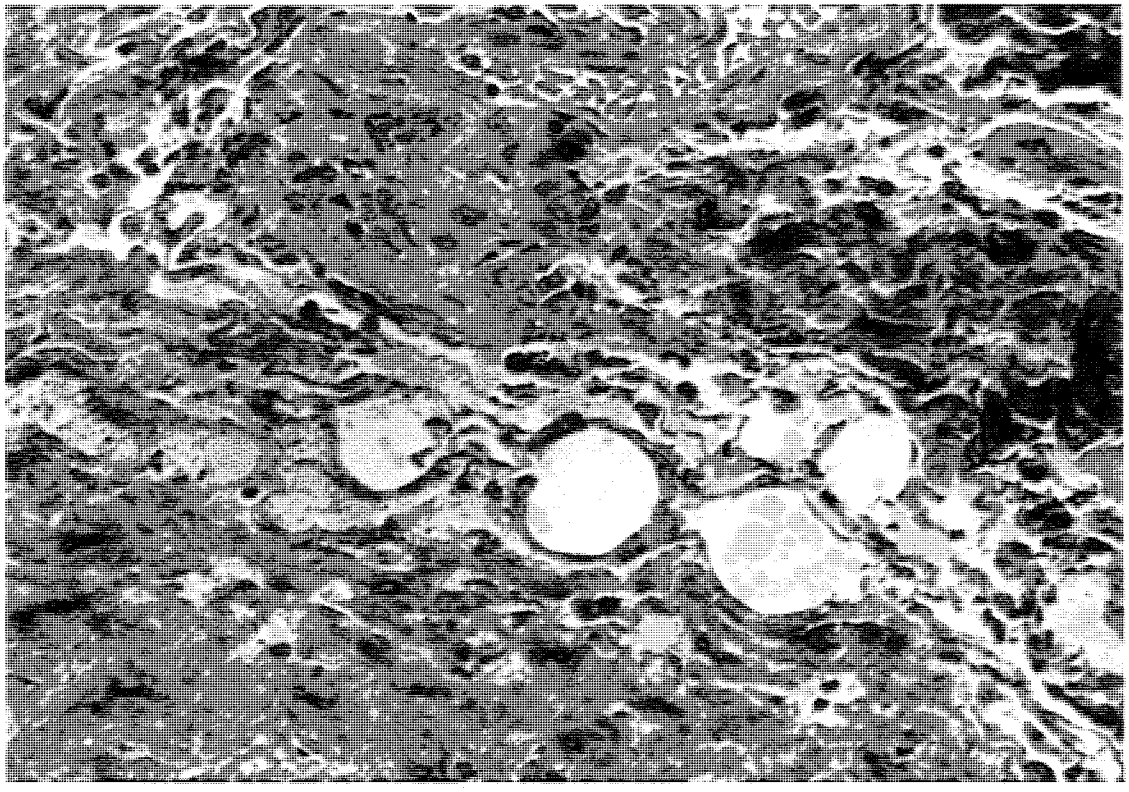

Fig. 5. Outer portions of tissue capsule, 9 months postimplantation. Toluidine blue stain, 1-μm section. Note lipid in lymph vessel. 100×; reduced to 90% for reproduction.

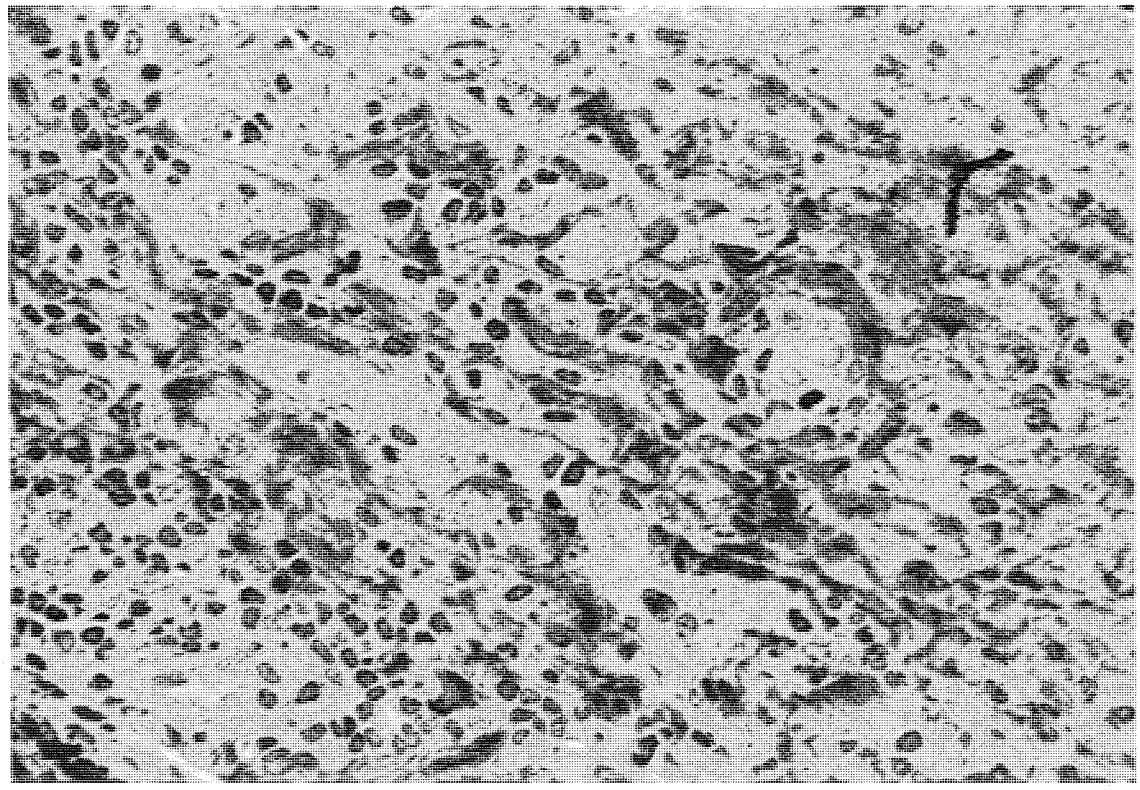

Fig. 6. Outer portion of tissue capsule, 3 months postimplantation. Toluidine blue stain, 1-μm section. Note blood vessels in capsule. 100×; reduced to 90% for reproduction.

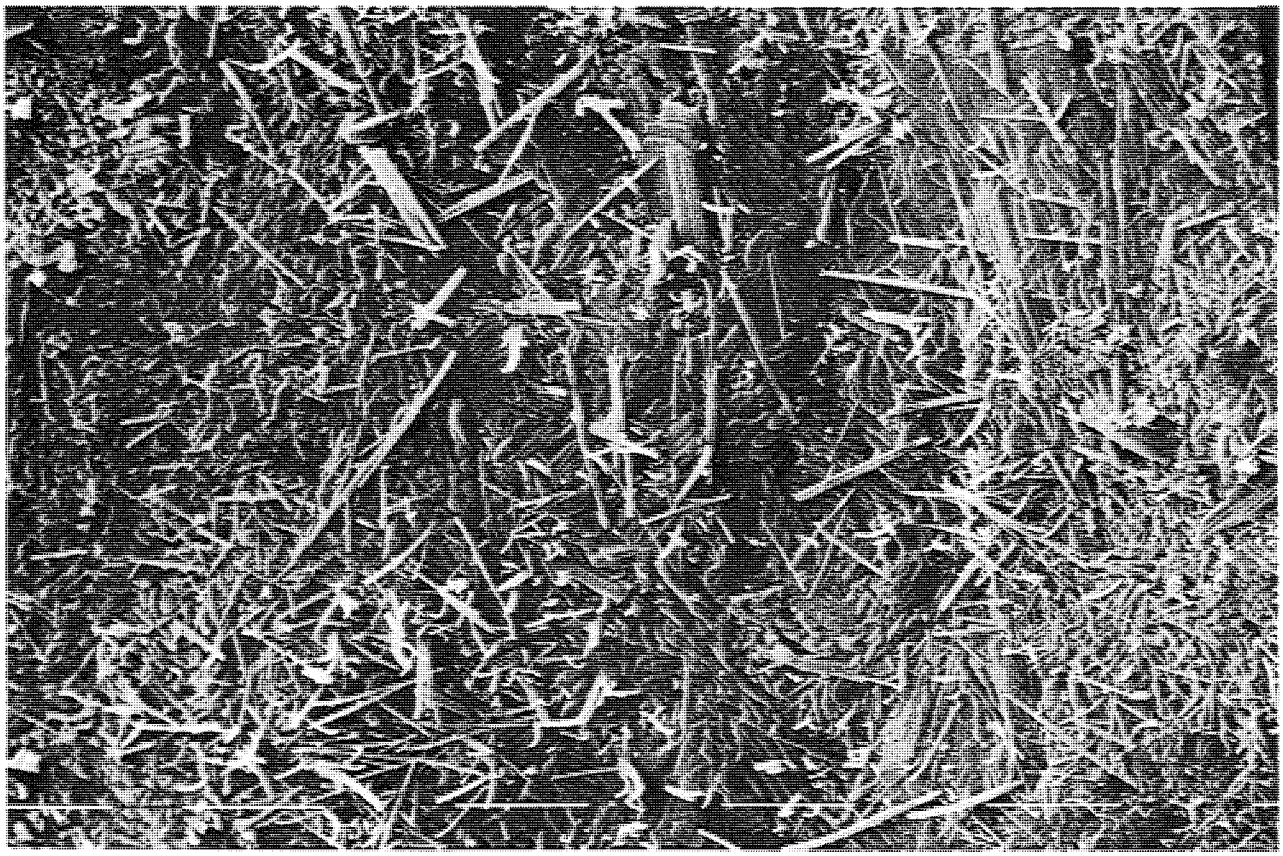

Fig. 7. Norethindrone pellet before implantation with crystals of norethindrone on surface. Bar = 10 μm.

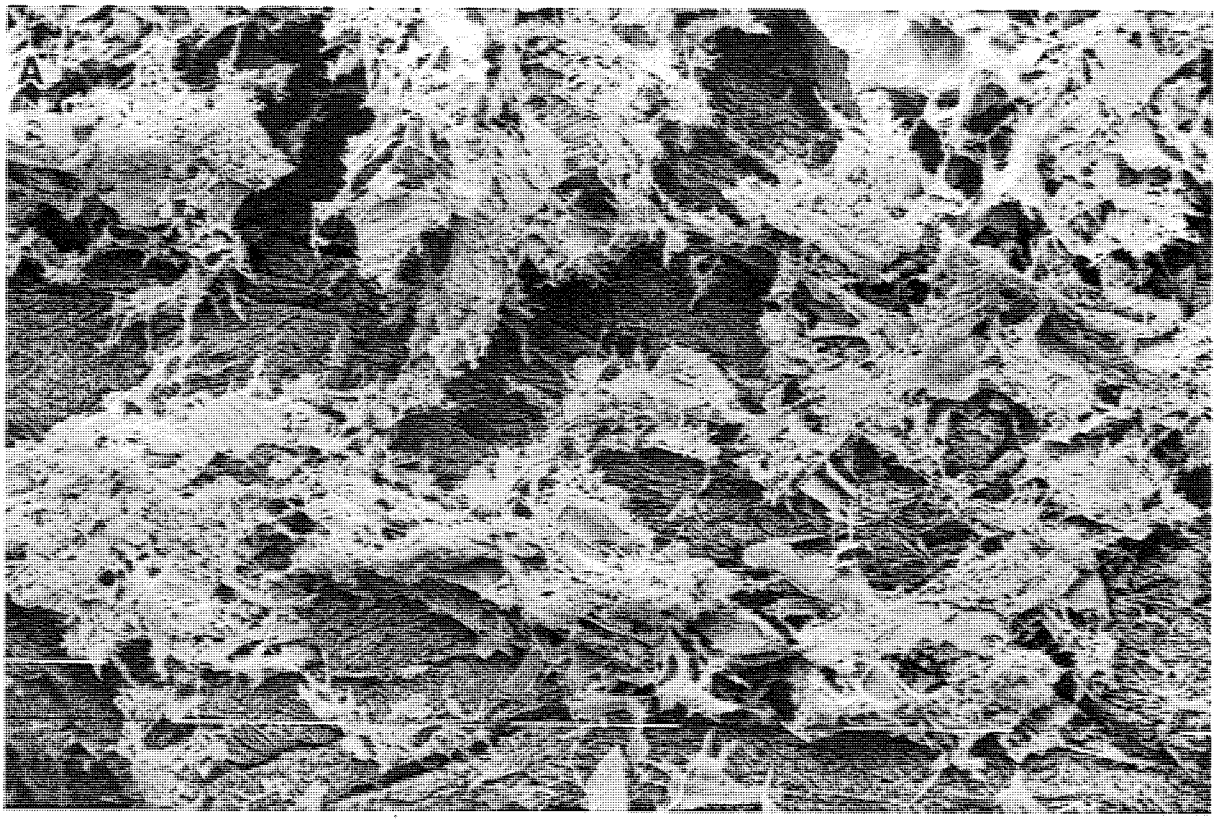

Fig. 8A. Norethindrone pellet, 7 months postimplantation. Surface of pellet is covered with amorphous, degenerating foam cells. Foam cells line capsule bed. Bar = 100 μm.

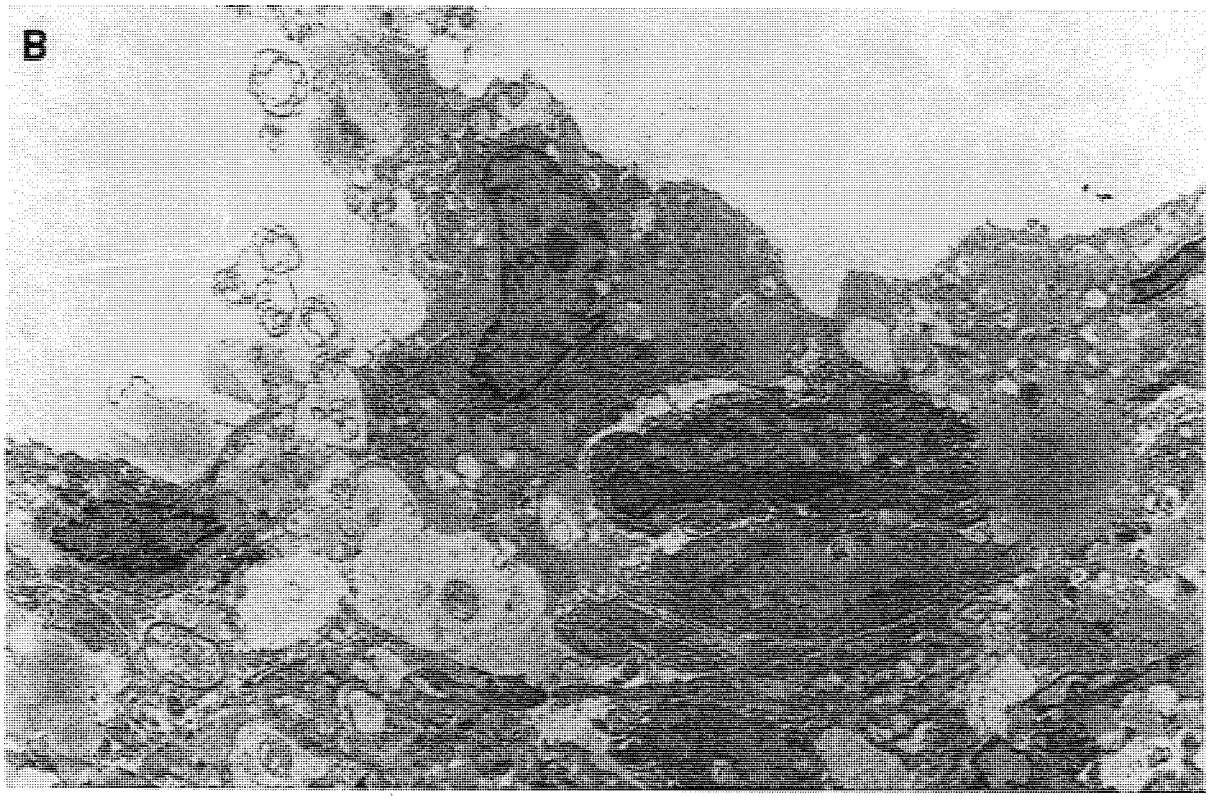

Fig. 8B. Transmission electron microscopy, depicting lipid laden foam cells of capsule bed. Foam cells form interface between norethindrone pellet tissue capsule. 4375×; reduced to 85% for reproduction.

Fig. 9A. Surface of norethindrone pellet, 9 months postimplantation, showing norethindrone crystals (right) and adherent cell debris (left). Bar = 100 μm.

Fig. 9B. Higher magnification of Fig. 8A. Note norethindrone crystals of pellet. Bar = 10 μm.

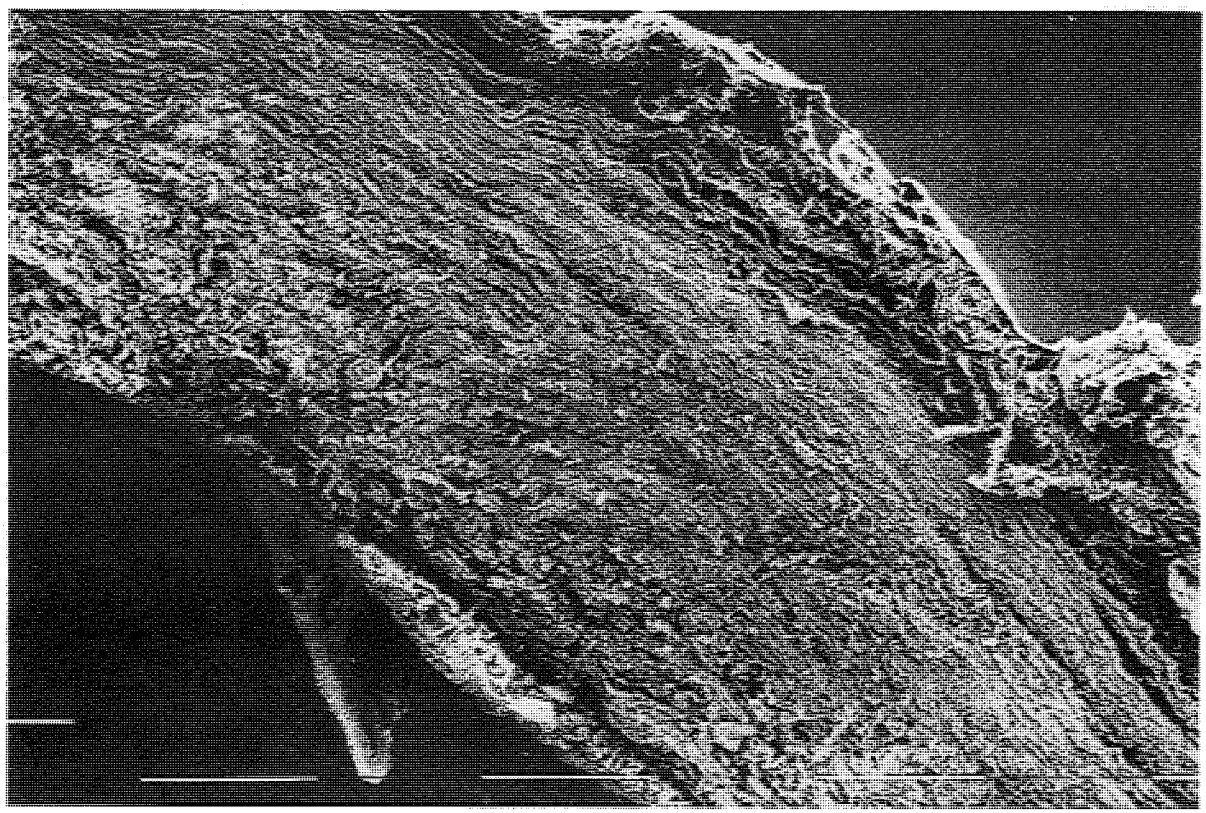

Fig. 10A. Cross section of wall of capsule in which norethindrone pellet had been implanted for 10.5 months. Note material that lines and projects into lumen of capsule (upper right). Loose, vascular connective tissue forms outer wall of capsule (upper left). Bar = 100 μm.

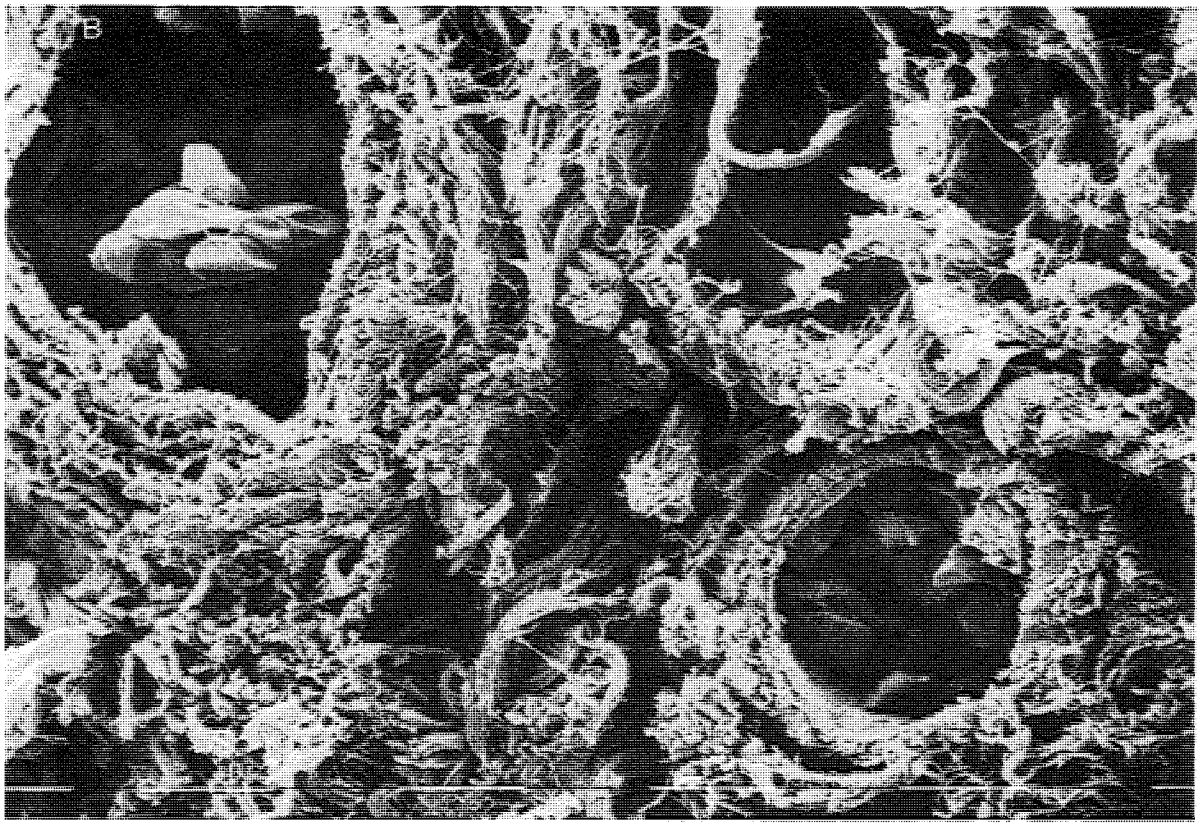

Fig. 10B. Higher magnification of out capsule wall, showing connective tissue fibers and erythrocytes (RBC) in blood vessels. Bar = 10 μm.

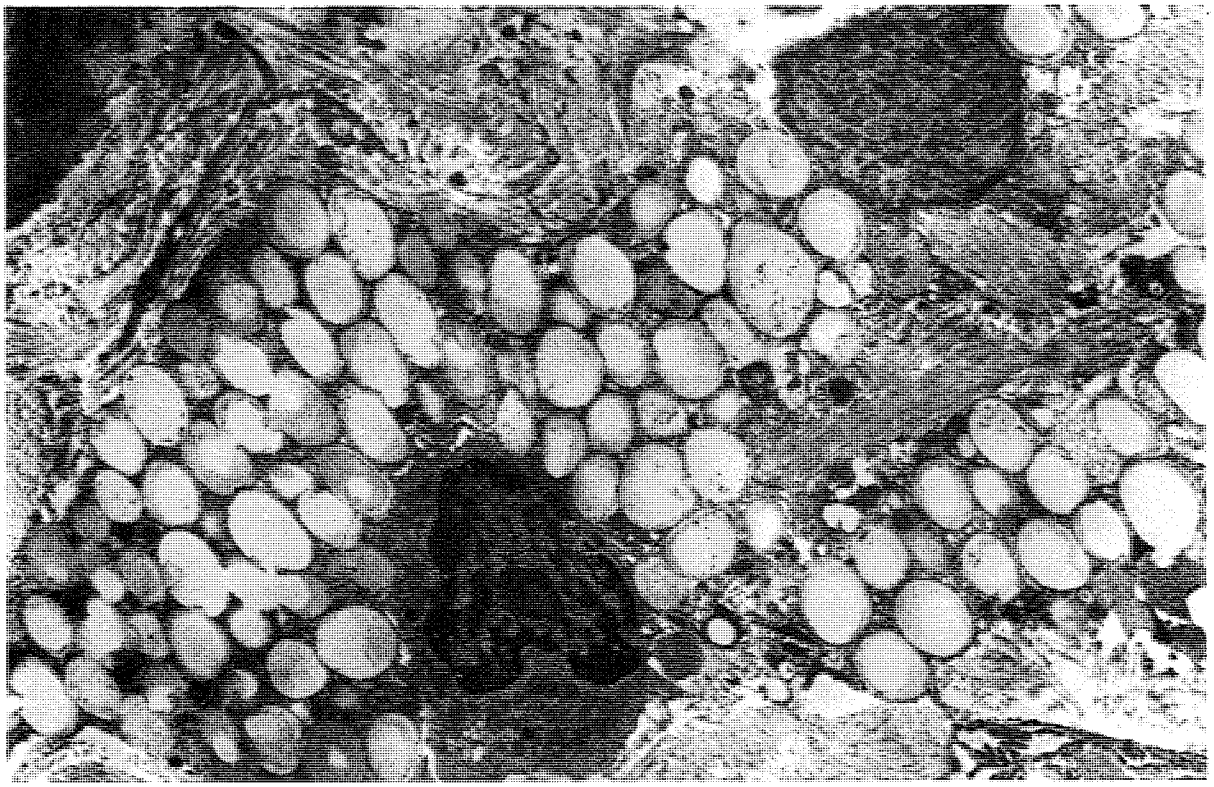

Fig. 11. Transmission electron microscopy, foam cells of inner portion of capsule near norethindrone pellet that had been implanted for 13 months. Foam cells are filled with electron lucent lipid droplets. 6000×; reduced to 85% for reproduction.

and 8.4 μg/mg of wet tissue weight, respectively. This represents an increase in norethindrone content of 168-fold and occurred during the same period in which foam cell concentration of the capsular tissue was increasing as well.

DISCUSSION

It seems logical that biodegradation kinetics might be a way to control the rate of drug delivery (5,6). This study further explores the possible functional role of the local inflammatory response as an active processing mechanism for drug absorption. The specimens studied were prepared for scanning and transmission electron microscopy without using organic solvents, which are known to dissolve lipids, and provided important new information concerning the tissue compartment that surrounds the norethindrone rod. These data could not have been obtained from specimens prepared by routine methods. Although subtle changes in cell surface morphology and intracellular organelles are not preserved in formalin fixed tissue, an interesting picture emerged, suggesting that the local tissue response plays a considerable role in the active processing of this subcutaneous delivery system. This picture is qualitatively distinct from the conventional view of the fibrous capsule as a simple rate-limiting membrane. The effects observed in this study suggest that a more complex, functional biological system develops in response to the subcutaneous introduction of a drug delivery system, as suggested by Ermini and others nearly 20 years ago (7), who studied polydimethylsiloxane implants loaded with [^{14}C]megestrol acetate. All of the capsules removed by these investigators were surrounded by a circular layer of fibrotic tissue containing granulomas and birefringent crystals. Numerous large macrophages containing dense "lysosome-like" inclusions probably of megestrol acetate were seen. This contrasts with our observation of free and macrophage ingested lipid globules and the relative absence of foreign body giant cells. NET is probably less likely to precipitate in tissue because of its solubility and its close association with the cholesterol and because it is being taken off the implant directly by the macrophages. We hypothesize that only before the "inflammatory response" has been well established does drug availability depend on simple diffusion and that the tissue response to the foreign body modulates the availability of the NET and cholesterol to the systemic circulation.

Drug absorption from an implant site is almost certainly affected by the microanatomy described above. We therefore found it valuable to compare the microanatomical picture with the clinical pharmacokinetic results.

We observed a three-phase pharmacokinetic profile. Initially, the mean serum norethindrone concentration peaked at about 2300 pg/mL and dropped rapidly to about 1000 pg/mL by 10 days after insertion. In the second phase the norethindrone concentrations declined more slowly to about 500 pg/mL. Finally, in the third phase, blood levels of norethindrone were quite stable (near-zero-order kinetics) for the next 240 days (3) (Fig. 1, Table I).

Simple hydrolysis of the loose layer of steroid crystals described above probably accounts for the initially high serum norethindrone levels. This first-stage process reflects an unregulated absorption of drug by the local vasculature.

The second stage of absorption is a transition phase

Fig. 12A. Transmission electron microscopy, foam cells between collagen fibrils in fibrous portion (middle portion) of capsule after norethindrone pellet had been implanted for 13 months. 3325×; reduced to 85% for reproduction.

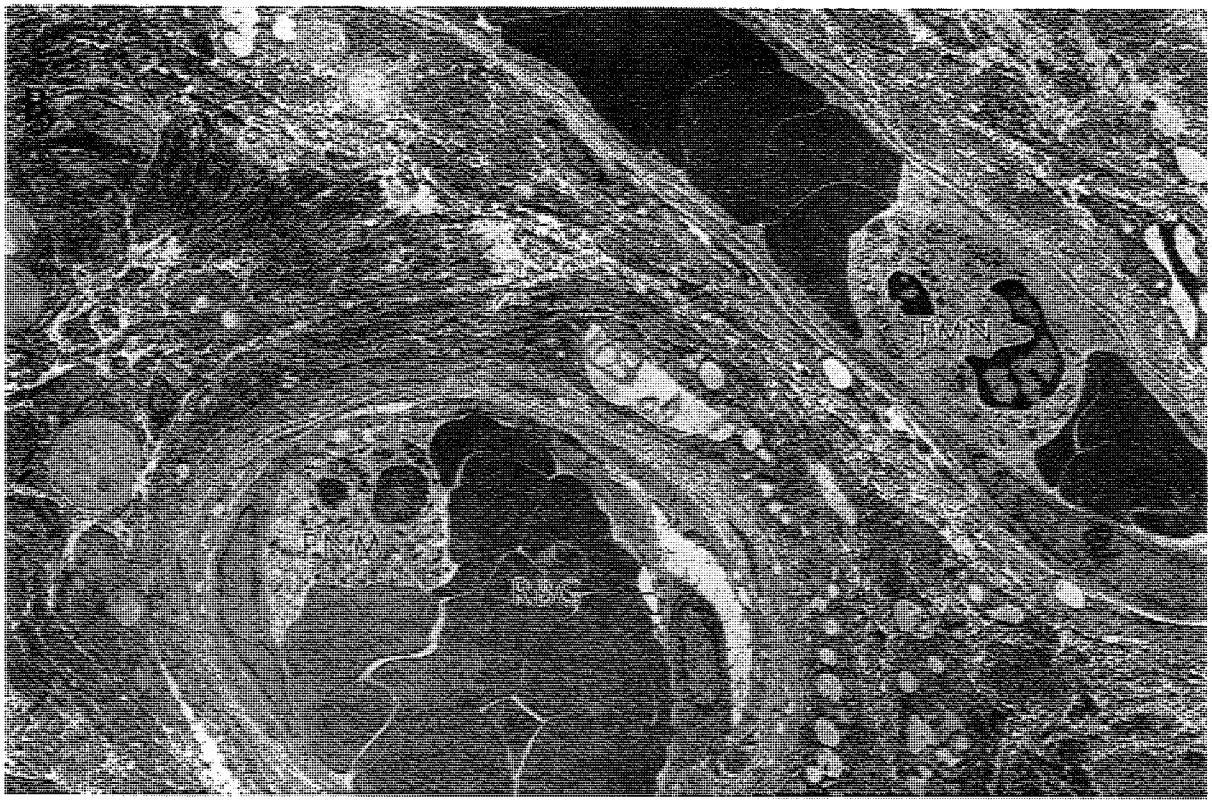

Fig. 12B. Transmission electron microscopy, depicting two blood vessels, collagen fibrils, and foam cell processes in outer portion of capsule at 6 months postimplantation. Each blood vessel contains a neutrophil (PMN) and several erythrocytes (RBC). 3325×; reduced to 85% for reproduction.

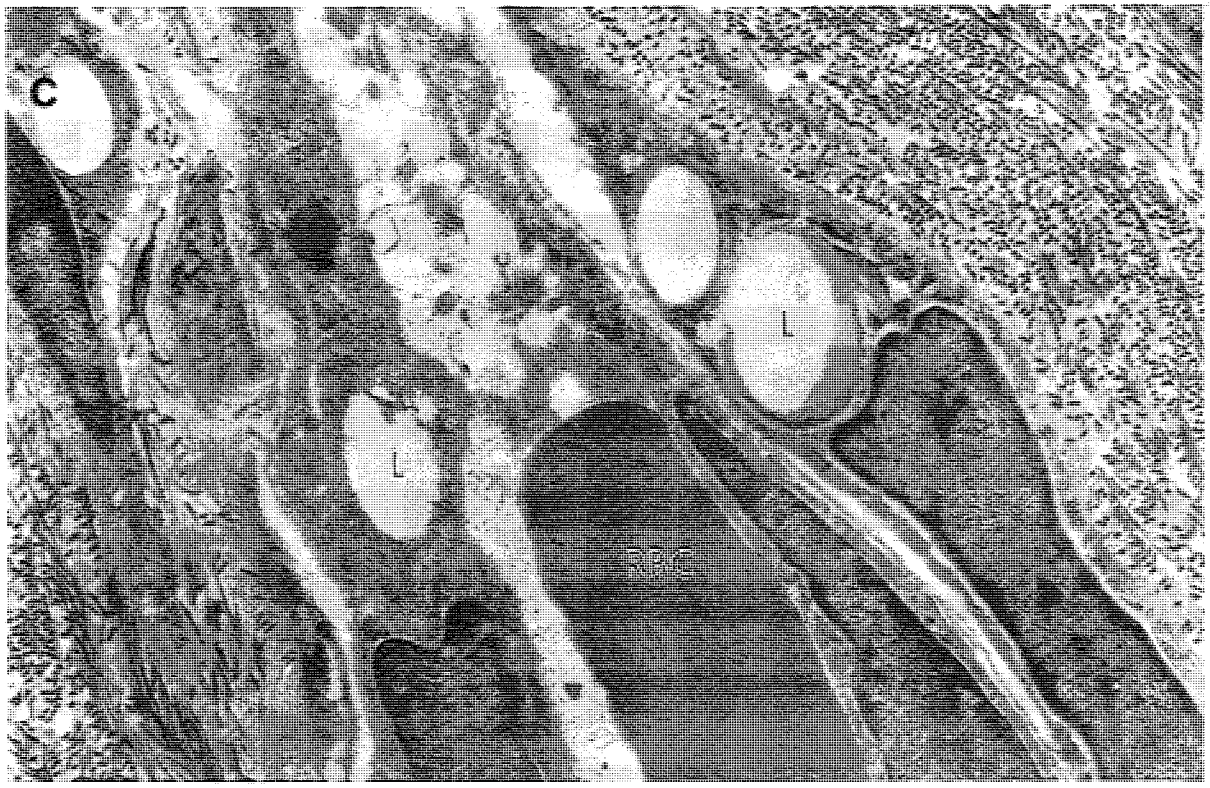

Fig. 12C. Transmission electron microscopy, capsule after norethindrone pellet had been implanted for 13 months, showing blood vessels with erythrocytes (RBC) and lipid droplets (L) in endothelial cells. 15,925×; reduced to 85% for reproduction.

during which the implant is being coated with macrophages and the fibrovascular compartment is forming. Drug absorption is in the process of becoming a function of foam cell transport to the local vasculature and lymphatics. From this study it is not possible to say what time interval is required for the implants to become coated with cells. The roughly 10-day intermediary decline in serum norethindrone concentrations could be explained by this coating phenomena as well as by the exhaustion of the supply of loose crystals on the surfaces of the implants.

It is likely that the presence of the implant and possibly lipid released during early dissolution act chemotactically to recruit monocytes from the circulatory system. Local histiocytes are probably not present in sufficient quantity to form the macrophage coat observed over the entire surface of the implants. However, local histiocytes, once activated, release cytokines, such as tumor necrosis factor, interleukin-1 and -2, and various colony stimulating factors which feed back to the bone marrow enlisting monocytes, thus augmenting the initial chemotactic stimulus (8). Other locally acting tissue growth factors derived from the macrophages stimulate angiogenesis and recruit fibroblasts and may be necessary to complete the capsule formation.

Additionally it has been demonstrated that transforming growth factor-β causes the rapid induction of fibrosis and angiogenesis *in vivo* and stimulates collagen formation *in vitro* (9). However, IL-1 and TNFα, also released by activated macrophages, oppose this effect (10). The net effect in this system, however, is the formation of a fibrous tissue capsule.

In view of these observations, we believe that a broad spectrum of potent interactive events is initiated by the slight trauma and drug release associated with the aseptically newly implanted device. The maturing of this biological compartment with its various components corresponds to the third phase of near-zero-order kinetics observed. This hypothetical model is consistent with the pharmacokinetic data as well as the microscopic evidence presented, an indication that the macrophage plays a critical role in the overall development of the functioning compartment.

As mentioned above, mass spectrometric data indicate a 168-fold increase in norethindrone in the connective tissue, which correlates with the increase in the foam cell population between 3 and 10.5 months postimplantation. Hence, it is reasonable to assume that the observed foam cells contain norethindrone. It is likely that these norethindrone-filled macrophages serve as the major source of drug absorbed during phase 3 of our model.

The perennial quest in controlled-release drug delivery has been the achievement of zero-order kinetics in an *in vitro* model. This work suggests the value of further, more quantitative analysis of the tissue response to subcutaneously implanted drug delivery devices, irrespective of their *in vitro* kinetics or intrinsic means of release, i.e., diffusion or erosion. Should future work confirm these preliminary results and even part of the attendant hypothetical models correlating these results to serum levels, the objective of achieving zero-order kinetics from controlled-release delivery devices that are placed subcutaneously warrants reevaluation. Indeed, the kinetics of such devices would amount to only a

small part of a much larger, more complex equation resulting in sustained serum concentrations of a bioactive compound. Controlled drug absorption, not merely drug release, should represent a serious objective in the rational design of implantable controlled-release drug delivery systems in the future.

ACKNOWLEDGMENTS

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