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THE ENZYMATIC SURFACE EROSION OF ALIPHATIC POLYESTERS

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The rates and mechanisms of biodegradation of a series of homo- and copolymers of ϵ-caprolactone, crosslinked to varying degrees with 2,2-bis(ϵ-caprolacton-4-yl)propane (BCP), were determined in rabbit and rat. These polymers were elastomeric, with little or no crystallinity. In contrast to the uncrosslinked crystalline homopolymer, which is subject to long induction periods prior to weight loss, poly(ϵ-caprolactone) crosslinked with 6 mole % BCP was bioabsorbed by an enzymatic surface erosion process which was detectable within two weeks in rabbit and was 80% complete after 100 weeks. The bioabsorption of crosslinked 1:1 copolymers of ϵ-caprolactone and δ-valerolactone was proportional to the crosslink density, ρ, and, for ρ < 3%, was complete within 16 weeks in rabbit and 7 weeks in rat. Concurrent with the surface erosion process, the crosslinked polymers were subject to the slower non-enzymatic hydrolysis of ester groups which is responsible for the degradation of other aliphatic polyesters such as polylactic acid in vivo *and* in vitro. *The rate of surface erosion of rods was approximately linear with time and the rate could be controlled by introducing substituents, for example via 4-t-butyl- and 6-methyl-ϵ-caprolactone, which sterically or electronically impeded the interaction of the ester group and the enzyme active site.*

INTRODUCTION

The erosion of a subdermally implanted polymer matrix, with concomitant release of a drug, represents a potentially important and frequently cited mechanism of subdermal drug delivery (for a preliminary account, see Ref. [1]). A critical review of the literature shows that, while examples of controlled chemical hydrolysis with surface dissolution are well documented [2—8], there are few unequivocal examples of the enzymatic surface erosion of polymers and no understanding of the means by which such a process may be induced. Polyglycolic acid, polylactic acid, and homologous aliphatic polyesters are commonly referred to as biodegradable polymers, but it is now known that degradation of these compounds occurs initially by non-enzymatic ester hydrolysis [9]. Phagocytosis is responsible for the bioabsorption of these and other polymers [9—11], but will only occur when the particle size is in the micron range or less. With the possible exception of nylon-6, which is reported to be subject to surface

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pitting as well as bulk chain cleavage [12, 13], the only examples of enzymatic degradation appear to be those of reconstituted natural polymers such as collagen [14], fibrin [15], chitin [16] and albumin [17], and certain polypeptides [18, 19].

Studies in our laboratory have previously shown that *in vivo* degradation of poly-(ϵ-caprolactone) (PCL) and its copolymers is non-enzymatic and substantially influenced by the crystallinity of the polymer [20, 21]. Reducing the crystallinity by random copolymerization with other lactones increased the rate of bioabsorption substantially, although at the expense of form stability. In order to retain form stability while eliminating crystallinity, crosslinked homo- and copolymers of ϵ-caprolactone and δ-valerolactone have been prepared (Fig. 1). The study of these polymers *in vivo* has provided the first unambiguous examples of the enzymatic surface erosion of synthetic polymers and serves to identify some of the structural and morphological features which determine the susceptibility of polymeric materials to heterogeneous enzymatic attack.

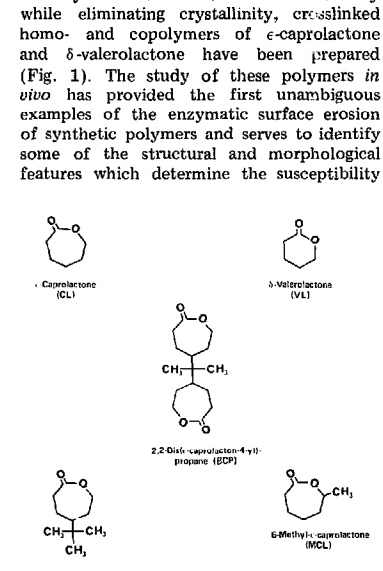

Fig. 1. Cyclic monomers used for the preparation of elastomeric polyesters.

MATERIALS AND METHODS

Monomer synthesis

ϵ-Caprolactone (Eastman Kodak, b.p. 52—54°C, 50 μm) and δ-valerolactone (Aldrich, b.p. 42—47°C, 50 μm) were twice distilled *in vacuo* using a 10 cm Vigreaux column prior to polymerization. 4-t-Butyl-ϵ-caprolactone, m.p. 57—59°C (hexane), 2,6-dimethyl-ϵ-caprolactone, *cis/trans* mixture, b.p. 53—60°C (0.5 mm), 6-methyl-ϵ-caprolactone, b.p. 69—70°C (1 mm), δ-dodecalactone, b.p. 69—71°C (20 μm), and 2,2-bis(ϵ-caprolacton-4-yl)propane, m.p. 195—211°C (ethyl acetate), *meso—dl* mixture, were prepared by Baeyer—Villiger oxidation of the corresponding ketones with *m*-chloroperbenzoic acid in CH_2Cl_2, following literature procedures [22—24]; their structures were verified by NMR, IR and mass spectrometry and their purity by TLC (Silica gel 60, EM Reagents, CH_2Cl_2 eluent) and GLC (Varian Aerograph 1400, 2% OV-17 on Supelcoport). 2,2-Bis-(4-ketocyclohexyl)propane was prepared by the method of Terada [25].

Polymer synthesis

All polymers were prepared by bulk polymerization of the purified monomers at 140°C *in vacuo* in the presence of stannous octoate (500 ppm) for 18 h. Polymerization was conducted in Teflon tubes (i.d. 1.4 mm) or between Teflon FEP films to facilitate release. Unchanged monomer(s) was removed by extraction with pentane, and the crosslink density, ρ, was calculated from the per cent conversion using eqn. (1), where n_{BCP} and n_{CL} are the mole fractions of BCP and ϵ-caprolactone in the polymer,

$$\rho = 2n_{BCP}/(2n_{BCP} + n_{CL}) \quad (1)$$

$$I = \rho P_n, \quad (2)$$

respectively. The primary chain length, P_n, was estimated by GPC analysis of the uncrosslinked polymer prepared in the absence of BCP using the same monomer batches and the same polymerization conditions; the crosslink index, I, the number of crosslinks per number-average primary chain, was derived from P_n using eqn. (2). The Young's modulus of the polymers was measured with an Instron mechanical tester, Model 1122. Chemical structure was confirmed by proton NMR spectroscopy of the uncrosslinked copolymers. The crystallinity of homo- and copolymers of ϵ-caprolactone was determined by differential scanning calorimetry (Perkin-Elmer DSC-2), measuring the heat of fusion of duplicate samples with indium as the reference standard. The crystallinity was then calculated assuming proportionality to the experimental heat of fusion utilizing the reported [26] enthalpy of fusion of 139.5 J/g for 100% crystalline poly(ϵ-caprolactone). Intrinsic viscosities were determined in toluene at 30°C at one concentration [27], using the procedure previously shown applicable to PCL. Molecular weights were determined by gel permeation chromatography (Waters Associates), using a set of five μ-Styragel columns calibrated with polystyrene standards (Waters Assoc. and Ventron Corp.) that covered a M_w range of 800—1.8 × 10^6. The polymer sample (1 mg) in THF (0.5 ml) was eluted with THF at a flow rate of 1 ml/min. The GPC traces were evaluated by the universal calibration method [28] previously applied to PCL [29].

Degradation studies

In vitro studies were conducted in distilled, deionized water and in aqueous acetic acid, both at 40°C. *In vivo* degradability was determined by subdermally implanting polymer rods (diameter 1.4 mm, length 6 cm) equidistantly about the dorsal midline of adult female New Zealand white rabbits or female Wistar rats. Polymer samples in sealed double polyethylene bags were sterilized by γ-irradiation (1.8 Mrad) prior to implantation. It was established by microbiological challenge with *B. pumilis* spore strips placed within capsules of the polymer that this dose was sufficient to achieve a 10 decade kill. No water-extractable oligomers were formed by γ-irradiation. At given time intervals, the implants were recovered and freed of adhering tissue by immersion in a 1% trypsin solution, pH 7.2, overnight. Control experiments showed this procedure did not change the properties of the polymer. Degradation was monitored by determination of the change in weight and Young's modulus. Non-crosslinked polymers were additionally characterized by GPC and viscosity measurements. The biocompatibility of the polymers was assessed by excising surrounding tissue, which was then fixed in 10% neutral buffered formalin, embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically. Selected samples were embedded in Epon and sectioned (1 μm) for further microscopic examination of cellular detail. The histopathological examination included a specific evaluation of degenerative and inflammatory response at the implant site.

RESULTS AND DISCUSSION

Polymer synthesis

A series of polymers of ϵ-caprolactone crosslinked with increasing amounts of 2,2-bis(ϵ-caprolacton-4-yl)propane (BCL) were prepared in order to define the limiting crosslink density which suppresses the formation of crystalline domains. This value was found to be about 12%, that is, an average of about eight monomer units between crosslinks. Less densely crosslinked poly(ϵ-caprolactone) crystallized slowly on standing, in some cases over a period of weeks. The Young's modulus of the completely amorphous polymer, ρ = 12%, was 4.26 ± 0.1 MPa (43.5 ± 1.0 kg/cm^2), and remained unchanged for at least one month.

In order to suppress crystallinity independently of the crosslink density, several series of copolymers of ϵ-caprolactone with other aliphatic lactones were prepared. Comonomers were chosen to retain the low glass transition temperature of PCL, but reduce the structural order by both the presence of racemic centers and by random copolymerization. The first series of polymers was prepared from equimolar amounts of ϵ-caprolactone and δ-valerolactone, with varying amounts of BCP corresponding to crosslink densities from 0.53 to 11.2%. The observed relationship between the crosslink index and the Young's modulus is shown in Fig. 2. All of these copolymers were clear stable elastomers which did not crystallize even at the lowest crosslink density. Evidently these copolymers are random, with no blocks of ϵ-caprolactone capable of crystallization.

Steric hindrance of the carbonyl group by the α-methyl substituent had an adverse effect on the polymerization of 6-methyl-ϵ-caprolactone and 2,6-dimethyl-ϵ-caprolactone. Relatively high catalyst concentrations, up to 0.7%, were necessary for homopolymerization of both monomers and only low molecular weight homopolymers (M_n < 10,000) were obtained. However, 6-methyl-ϵ-caprolactone polymerized well with ϵ-caprolactone, to give a copolymer of M_n 14,000 (η = 0.41 dl/g), and crosslinked copolymers containing 46% 6-methyl-ϵ-caprolactone, crosslink density 1.4—2.0%, were prepared with conversions > 97%.

Copolymerization of 2,6-dimethyl-ϵ-caprolactone and PCL was incomplete and the uncrosslinked copolymer obtained from equal amounts of the two monomers slowly crystallized, indicating the presence of long ϵ-caprolactone sequences and poor copolymerizability. Copolymers with ω-dodecalactone with the proportion of the latter ranging from 10 to 28 mole % were obtained. The molecular weight decreased with increasing ω-dodecalactone content but the crystallinity was 49 ± 1%, independent of the comonomer ratio. This is no different from the crystallinity of PCL with the same molecular weight [20], which suggests either cocrystallization of ϵ-caprolactone and ω-dodecalactone sequences or formation of block structures.

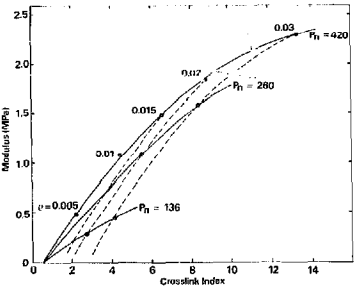

Fig. 2. Experimental relation between the Young's modulus and (a) the crosslink density (——) and (b) the primary chain length (— — —) of 1:1 copolymers of ϵ-caprolactone and δ-valerolactone crosslinked with BCP. (1 MPa = 10.2 kg/cm²).

The molecular weights of copolymers of ϵ-caprolactone with 4-t-butyl-ϵ-caprolactone were not affected by the comonomer ratio used (up to 50% of TBCL), the average M_n being 58,000 ± 6000. Even in the case of the copolymer with the lowest t-butyl content, crystallization was completely suppressed.

Polymer biodegradation

Changes in the weight, diameter, and modulus of crosslinked poly(ϵ-caprolactone) rods, ρ = 12.3%, after implantation in rabbit for times up to 107 weeks are shown in Table 1 and Fig. 3. Weight loss was detectable within 2 weeks and increased thereafter at an approximately linear rate. The weight loss was limited to surface erosion, there being a continuous decrease in the diameter of the rods with no microscopic evidence of bulk erosion. This rate of weight loss is in marked contrast to uncrosslinked

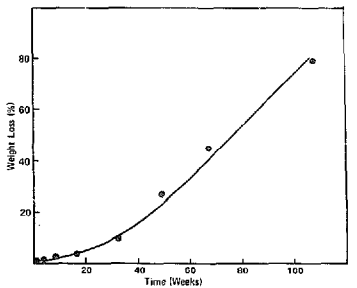

Fig. 3. Rate of *in vivo* absorption of crosslinked poly(ε-caprolactone), ε = 12.3%, after subdermal implantation in rabbit.

poly(ε-caprolactone), which, for a similar molecular weight, undergoes weight loss only after one year and then by a bulk erosion process [20]. The second property monitored, the modulus, decreased with time, indicative of the normal documented mechanism of ester degradation *in vivo*, i.e., random hydrolytic cleavage. This was further substantiated when, after 32 weeks, the recovered implants crystallized on reducing their temperature from body to room temperature, the result of a reduction in the crosslink index. Under *in vitro* conditions in water at 40°C, there was a similar decrease in modulus but no significant weight loss or surface erosion over a 7.5 week period (Table 2). In 2 *M* acetic acid, which accelerates the rate of bulk hydrolytic chain cleavage of polyesters (*vide infra*), the decrease in modulus was greater over the same time period, but again no surface erosion occurred (Table 2). These results suggested that two mechanisms contribute to the biodegradation of crosslinked poly(ε-caprolactone), an enzymatic process responsible for surface erosion as well as the normal hydrolytic process.

The relative contributions of biological and chemical mechanisms to these observations of chain cleavage and surface erosion were explored by extending the studies to the series of crosslinked 1:1 copolymers of ε-caprolactone and δ-valerolactone in which the crosslink density and modulus could be varied over a wider range with complete suppression of crystallinity. Changes in

TABLE 1

Change in Young's modulus and average diameters of rods of crosslinked poly(ε-caprolactone), ρ = 12.3%, after subdermal implantation in rabbit

Time (weeks)	Young's modulus (MPa)	Average rod diameter (mm)
0	4.69	1.33
2	4.97	1.31
	4.69	
4	4.55	1.31
	4.36	
8	4.83	1.33
	4.59	
16	4.03	1.33
	4.41	
32	3.81[a]	1.27
49	2.01[a]	1.14
67	–	1.09
107	–	0.78

[a]Sample crystallized during storage at room temperature. Moduli were determined at room temperature immediately after melting at 50°C.

TABLE 2

Degradation of crosslinked poly(ε-caprolactone), ρ = 12.3%, in water and 2 *M* acetic acid at 40°C

Time (h)	In water		In acid	
	% Weight loss	Modulus (MPa)	% Weight loss	Modulus (MPa)
0	–	4.24	–	4.24
268	0.1	4.08	0.3	3.78
431	0.2	3.98	0.3	2.66
596	0.2	3.93	0.3	2.30
781	0.2	3.79	0.3	1.72
945	0.2	3.58	0.8	1.65
1250	0.2	3.42	1.0	19.9[a]

[a]Sample crystallized.

the properties of these copolymers under *in vitro* conditions (water, 2 *M* acetic acid, 40°C) are summarized in Table 3. Weight losses in water up to 10 weeks (1658 h) were negligible and only became measurable in 2 *M* acetic acid after 7 weeks (1113 h), coincident with a loss in form stability. The modulus, *E*, decreased logarithmically with time, the rate being inversely proportional to the crosslink density, ρ. Thus, a plot of ln (E/E_0) versus (time/ρ) for all of the copolymers fell on a single straight line (Fig. 4). During the initial phase of the hydrolysis the crosslink density may be assumed constant and this decrease in modulus is due to the decrease in the primary chain length resulting from random chain cleavage of chain segments between crosslinks. The rates of bulk hydrolytic chain cleavage may then be derived from the modulus measurements of Fig. 2, where the dashed lines represent changes in modulus for a constant crosslink density but changing primary chain length. The results of such an analysis are shown graphically in Fig. 5 as a plot of reciprocal molecular weight versus time.

The kinetic expression for acid-catalyzed polyester hydrolysis is given by eqn. (3), where [COOH], [ester] and [H] are the carboxy end group, ester, and acetic acid

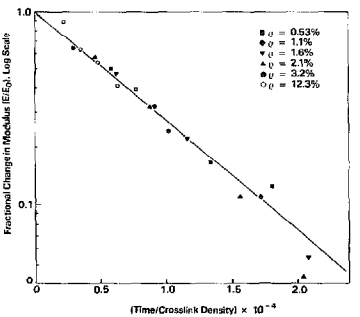

Fig. 4. Change in elastic modulus of copolymers of δ-valerolactone and ϵ-caprolactone with different crosslink densities, ρ, as a function of time/crosslink density in 2 *M* acetic acid at 40°C.

TABLE 3

Degradation of crosslinked copolymers of δ-valerolactone and ϵ-caprolactone in water and 2 *M* acetic acid at 40°C, ρ = 0.53–3.2%

Time (h)	% Weight loss					Young's modulus				
	ρ = 0.53%	ρ = 1.1%	ρ = 1.6%	ρ = 2.1%	ρ = 3.2%	ρ = 0.53%	ρ = 1.1%	ρ = 1.6%	ρ = 2.1%	ρ = 3.2%
Degradation in Water										
0	–	–	–	–	–	0.47	1.08	1.48	1.84	2.31
280	–	–	–	–	–	0.41	1.03	1.41	1.68	2.72
669	0.2	0.1	0.6	0.2	0.3	0.31	0.86	1.26	1.59	2.10
1000	0.3	0.1	0.1	0.2	0.4	0.11	0.59	0.98	1.33	1.96
1353	0.4	0.3	0.3	0.3	0.5	–	0.32	0.68	0.99	1.61
1658	–	0.3	0.4	0.4	0.6	–	0.25	0.50	0.75	1.42
Degradation in Acetic Acid										
0	–	–	–	–	–	0.47	1.08	1.48	1.84	2.31
96	–	0.1	0.1	0.1	0.2	0.06	0.35	0.69	1.06	1.50
183	–	0.6	0.4	0.4	0.5	–	0.12	0.33	0.59	1.15
324	–	0.3	0.1	0.6	0.5	–	–	0.08	0.20	0.56
424	–	–	0.5	0.9	0.8	–	–	–	0.08	0.38
1113	–	–	5.0	7.7	7.8	–	–	–	–	–

concentrations in the polymer bulk, respectively. Provided the fraction of ester groups cleaved is small, integrating

$$d[COOH]/dt = k[ester][H] \quad (3)$$

and equating the number-average molecular weight, M_n, with $1/[COOH]$, leads to eqns. (4) and (5), where M_n^0 is the initial molecular weight

$$[COOH] = [COOH]_0 + k't \quad (4)$$

$$1/M_n = 1/M_n^0 + k't \quad (5)$$

and $k' = k[ester][H]$. Figure 5 conforms to this kinetic relationship, and the rate constants k', derived from the slope of the plots for the different crosslinked copolymers, are in good agreement with the rate constant for hydrolysis of the uncrosslinked copolymer of ϵ-caprolactone and δ-valerolactone:

	mol g^{-1} h^{-1}
poly(VL–CL) (uncrosslinked)	2.01×10^{-7}
poly(VL–CL) (ρ = 1.5%)	2.07×10^{-7}
poly(VL–CL) (ρ = 2.0%)	1.75×10^{-7}
poly(VL–CL) (ρ = 3.0%)	1.30×10^{-7}

When implanted in rabbit, all of the copolymers, even those recovered after only 2 weeks, were subject to substantial tissue ingrowth; in contrast, uncrosslinked PCL controls were free of adhering tissue. Changes in the modulus and the weight as a function of time *in vivo* are shown in Table 4 and Fig. 6.

Considering first the change in modulus, which is the measure of the rate of bulk cleavage of the ester linkages, Fig. 7 compares the change in the primary chain length with the same process *in vitro* (water, 40°C). The equivalence of the rates demonstrates that the mechanism of chain scission *in vivo* and *in vitro* are identical, and must involve random hydrolytic cleavage of ester groups without any enzymatic contribution.

This parallel behavior did not extend to the surface erosion process. Under *in vivo* conditions there was weight loss from all of the copolymers, even after 2 weeks

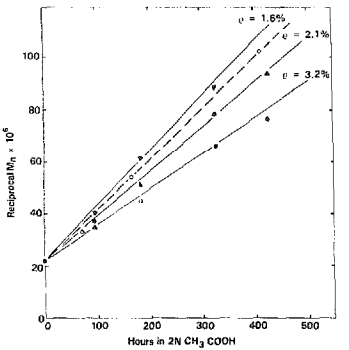

Fig. 5. Relationship between time in 2 *M* acetic acid at 40°C and the molecular weight of the primary chains of crosslinked copolymers of δ-valerolactone and ϵ-caprolactone. (— — —) Uncrosslinked copolymer. (——) Crosslinked copolymer.

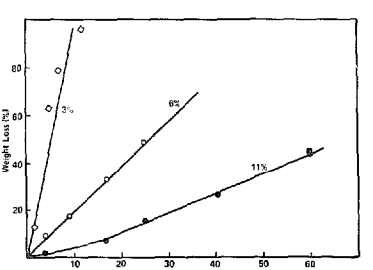

Fig. 6. Dependence of rates of *in vivo* surface bioerosion of copolymers of ϵ-caprolactone and δ-valerolactone on their crosslink density (ρ = 3, 6, and 11%, respectively).

(the earliest recovery), which increased to greater than 50% after 10 weeks and was complete by 16 weeks. As with crosslinked PCL, the weight loss occurred exclusively from the surface. *No weight loss was observed for the same polymers under in vitro*

TABLE 4

In vivo degradation of crosslinked copolymers of δ-valerolactone and ε-caprolactone in rabbit, ρ = 0.53–2.1%

Time (weeks)	% Weight loss				Young's modulus (MPa)			
	ρ = 0.53%	ρ = 1.1%	ρ = 1.6%	ρ = 2.1%	ρ = 0.53%	ρ = 1.1%	ρ = 1.6%	ρ = 2.1%
0	–	–	–	–	0.38	0.91	1.29	1.65
2	46.1	12.2	15.1	6.2	a	0.70	1.03	1.59
5	72.2	59.0	61.6	69.6	b	b	0.96	1.02
7	35.9	47.6	83.4	62.5	c	a	b	a
10	39.0	50.4	69.3	62.6	c	a	0.37	0.83
12	d	99.2	84.7	99.0	–	–	–	–
16	d	e	e	99.3	–	–	–	–

[a]Rod of very uneven cross-section.
[b]Rod broken into pieces
[c]No form stability.
[d]Sticky smear, could not be weighed.
[e]No sample found.

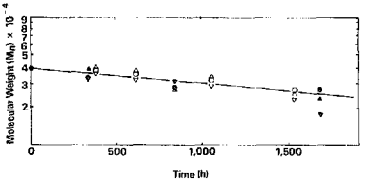

Fig. 7. Molecular weight change of primary chains of crosslinked copolymers of δ-valerolactone and ε-caprolactone during biodegradation in rabbits (solid symbols) and in water at 40°C (open symbols). (▽, ▼) ρ = 1.6%, (△, ▲) ρ = 2.1%, and (○, ●) ρ = 3.2%.

conditions (water, 40°C) during the same time period. This is considered *prima facie* evidence of an enzymatic surface mechanism.

An assessment of the species-dependence of the enzymatic process was made by implanting one of the copolymers, ρ = 1.6%, subcutaneously in rat. Duplicate samples were recovered periodically and weight loss and tissue response at the implant site were evaluated. The extent of bioabsorption was 25 ± 15% after 14 days; 40 ± 7% after 28 days and 100 ± 0% after 63 days. At 14 and 28 days, the host reaction was small, consisting of a minimal, filamentous encapsulating response, devoid of vascularity or significant inflammation. No polymer fragments were identified in the few inflammatory cells present in the vicinity of the implant. At day 63, not only had the implant been absorbed completely but also its implantation site was no longer visible, suggesting that absorption had been complete perhaps at least a week prior to 63 days. This rate of bioabsorption is more rapid than that observed in rabbit.

In order to better define the factors responsible for triggering the enzymatic process, the studies were extended to substituted polylactones. 6-Methyl-ε-caprolactone (MCL) was chosen to evaluate the effect of steric hindrance at the carbonyl site. 4-t-Butyl-ε-caprolactone (TBCL) provided an example of a hydrophobic substituent. The hydrolytic degradation of both the uncrosslinked homopolymer of MCL and its 1:1 copolymer with ε-caprolactone was measured in water and 2 *M* acetic acid, in order to determine the substituent effect on non-enzymatic hydrolysis. The rate constants, derived from the slope of the

plot of reciprocal molecular weight versus time, were found to be:

	mol g^{-1} h^{-1}
poly(MCL)	0.49×10^{-7}
poly(MCL—CL) (1:1)	1.14×10^{-7}
poly(VL—CL) (1:1)	2.01×10^{-7}

If one assumes that ester hydrolysis of poly-(CL) is confined to its amorphous region, and that totally amorphous poly(VL—CL) is a good model of this region, then introduction of the 6-methyl group reduces the rate by a factor of 4. The rate constant for hydrolysis of the copolymer of MCL and CL is, as expected, the mean of the rate constants of poly(VL—CL) and poly-(MCL—CL).

The corresponding rate constants for *in vitro* hydrolysis of uncrosslinked copolymers of ϵ-caprolactone and its 4-t-butyl derivative, with different proportions of the two monomers, were determined to be:

	mol g^{-1} h^{-1}
poly(TBCL—CL) (10:90)	1.09×10^{-7}
poly(TBCL—CL) (19:81)	1.13×10^{-7}
poly(TBCL—CL) (28:72)	1.24×10^{-7}
poly(TBCL—CL) (46:54)	1.28×10^{-7}

Comparison of these data with the rate constant for amorphous poly(VL—CL) (*vide supra*) demonstrates that the steric and electronic effect of the t-butyl group gamma to the carbonyl function is to reduce the rate of ester hydrolysis by less than a factor of 2.

The changes in the modulus and weight of the crosslinked copolymers of ϵ-caprolactone with 4-t-butyl- and 6-methyl-ϵ-caprolactone after implantation in rabbit are shown in Figs. 8—9 and Table 5. For the copolymer with MCL, surface erosion and a decrease in the modulus were both immediately apparent. The rate of surface erosion was measurably slower, decreasing by a factor of four relative to that of the analogous copolymer of CL and VL with the same crosslink density (ρ = 2.0%). Perhaps coincidently, this factor is the same observed for the rate of non-enzymatic bulk hydrolysis of ester links. The time required for total biosorption of the polymer increased from 12—16 weeks to more than 30 weeks.

The substituent effect was more pronounced in the case of the series of TBCL copolymers. The erosion rate decreased in proportion to the t-butyl content and, in each case, there was no induction period prior to surface erosion (Fig. 8). This observation, together with the absence of weight loss under *in vitro* conditions, demonstrates the enzymatic process is still operative. Furthermore, since the t-butyl group

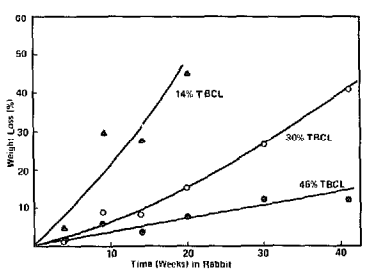

Fig. 8. Dependence of rates of *in vivo* surface erosion of copolymers of ϵ-caprolactone and 4-t-butyl-ϵ-caprolactone, crosslink density 3.6%, on the t-butyl content.

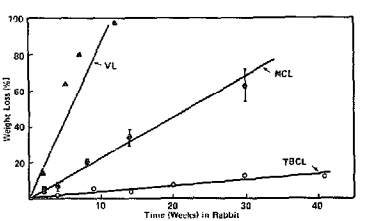

Fig. 9. Comparison of the rates of *in vivo* surface erosion of crosslinked copolymers of ϵ-caprolactone with δ-valerolactone, 6-methyl-ϵ-caprolactone, and 4-t-butyl-ϵ-caprolactone, crosslink density 2%.

TABLE 5

In vitro (water) and *in vivo* (rabbit) degradation of a crosslinked copolymer of 6-methyl-ε-caprolactone and ε-caprolactone, ρ = 2.0%

In vitro degradation			*In vivo* degradation		
Time (weeks)	% Weight loss	Young's modulus (MPa)	Time (weeks)	% Weight loss	Young's modulus (MPa)
0	–	0.49	0	–	0.57
1.1	0	0.33	2	3.1	0.64
3.7	0	0.25		4.3	0.55
6.4	0	0.18	4	3.5	0.44
11.4	0	–		8.7	0.38
16.9	0	–	8	18.3	0.25
				21.8	0.19
			14	38.7	0.05
				28.5	0.06
			24	74.8	–
			30	52.6	–
				72.0	–

TABLE 6

Change in the modulus of crosslinked copolymers of 4-t-butyl-ε-caprolactone (TBCL) and ε-caprolactone in rabbit[a]

Time (weeks)	Young's modulus (MPa)		
	14.0 mole % TBCL	29.5 mole % TBCL	46.3 mole % TBCL
0	2.28	1.61	1.15
4	1.75	1.33	0.90
9	1.12	0.87	0.62
14	0.93	0.76	0.41
20	0.44	0.35	0.23
30	b	c	c

[a]Crosslink density 3.6%.
[b]Could not be recovered.
[c]Not measurable.

had little effect on the non-enzymatic rate of ester hydrolysis, one may conclude that this substituent in some way blocks interaction with the enzyme active site, either sterically or by unfavorable hydrophobic interactions.

Interestingly, the rates of weight loss of the 4-t-butyl and 6-methyl substituted copolymers were linear, despite the fact that the surface area of the cylindrical geometry employed is expected to decrease as the radius decreases. This observation, which is relevant to the kinetics of drug release, must reflect increasing surface irregularity as the degradation proceeds. Scanning electron microscopy supported this interpretation, showing no bulk erosion but increasing surface irregularity.

CONCLUSIONS

Uncrosslinked aliphatic polyesters such as polylactic acid and poly(ε-caprolactone) undergo degradation *in vivo* by a process which begins with random chain scission by aqueous hydrolysis of ester links, and is further characterized by long induction periods before any bioerosion occurs. Crosslinked polyesters are subject to the same hydrolytic process, but undergo bioerosion by a different and new mechanism which involves immediate enzymatic attack at

the surface of the implant. The susceptibility of these polymers to enzymatic attack is believed to be related to the segmental mobility of the polymer chains which, for a low T_g, non-crystalline polymer, permits the ester group to assume the conformation necessary to interact with the active site of the esterase. The rate of attack and the subsequent erosion may be controlled by the introduction of substituents such as methyl and t-butyl, which function by steric or hydrophobic inhibition of the interaction of the enzyme active site and the ester group. Increasing the crosslink density also serves to reduce the rate of enzymatic attack, presumably by restricting the segmental motion of polymer chains and the facility with which they may assume the conformation necessary for binding to the enzyme. In quantitative terms, enzymatic attack becomes of minor importance when the Young's modulus exceeds 5 MPa.

These polymers are potentially useful for the delivery of drugs via a surface erosion mechanism, the duration of which may be tailored from several weeks to more than a year.

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