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Degradable Polymers. I. Synthesis, Characterization, and Long-Term in Vitro Degradation of a ^{14}C-Labeled Aliphatic Polyester

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Degradable Polymers. I. Synthesis, Characterization, and Long-Term in Vitro Degradation of a ^{14}C-Labeled Aliphatic Polyester

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ABSTRACT

Poly(tetramethylene adipate) has been synthesized with and without ^{14}C labeling at different molecular weights. Degradation tests of the labeled powder inoculated with microorganisms showed a decrease in molecular weight (GPC) and first an increase and later a decrease of crystallinity (DSC). These changes also occurred in the inoculated material, and the results indicate that the degradation process was abiotic hydrolysis which first takes place in the amorphous region. The radioactivity measurements showed that the microorganisms are able to degrade further the oligomers formed during the hydrolysis. The rate of degradation is related to the molecular weight. Unlabeled poly(tetramethylene adipate) was extruded and cold drawn to yield oriented fibers. These fibers retained more than 50% of the original tensile strength during 100 days of abiotic in vitro hydrolysis.

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INTRODUCTION

The first absorbable, biodegradable material used in medicine was collagen in various forms, and this material is still used today as catgut [1]. However, catgut causes inflammatory tissue response, and much research for a synthetic absorbable material has been in process.

Still, an interesting noninflammatory collagen-based material (porous, crosslinked collagen-glycosaminoglycan coprecipitate) has been designed and used as artificial skin. These membranes have protected wounds with great success from infection and fluid loss for over 25 days without rejection [2].

The first of the synthetic material to become commercially available [3] was the Dexon (Davis and Geck, Inc.) suture [4], which resulted from extensive screening of potential materials and their changes in vivo (rabbit, subcutan) and in saline after 90 days [5]. This polymer (polyglycolic acid, PGA) was soon used in copolymers, especially poly(glycolic-co-lactic acid) [6], and a suture named Vicryl (Ethicon) was produced from the 90:10 glycolic/lactic acid monomer composition [7]. This suture retains its tensile strength longer and absorbs more rapidly than Dexon [8], but its properties rely on the high levels of glycolic acid found in 50% of the chains [9]. As a result of the search for polymers with longer retention of tensile strength, several patents have emerged. Polyesters of higher aliphatic analogs of PGA like poly(ethylene succinate) [10] and their copolymers with oxalic acid [11] have been tested for absorbability in enzyme extract, and their reduced specific viscosity decreased. Poly-β-propiolactone has been prepared by electron irradiation of β-propiolactone [12], and the resulting polymer is said to lose strength in vivo but not to be absorbed over several months [8].

Para-dioxanone, which can be regarded as a modified glycolide, was polymerized to produce poly(p-dioxanone), and the resulting suture material PDS (Ethicon) [13] retained its tensile strength longer than Dexon and Vicryl [14, 15].

Recently the copolymerization of dicarboxylic acids with diols and amines to produce polyesteramides was reported [16, 17], and these materials retained their strength for up to 6 months with very slow absorption [18].

The latest development is free-radical ring-opening polymerization, which makes it possible to design slowly absorbing polyesters with long hydrocarbon chains by copolymerization of 2-methylene-1,3-dioxepane with different amounts of ethylene [19].

The reason for the search for a slowly absorbing material is that the regeneration of human tissue is slow in certain areas, particularly in tendons, ligaments, and nerves. An interesting application in this direction is the use of Vicryl mesh as a base for regeneration of arterial [20-23] and cardiac [24] tissue and also nerves [25] in pigs, whose tissue regeneration is faster than in the human system. This makes it possible to use Vicryl in this application.

The material chosen for this work is the linear aliphatic polyester poly(tetramethylene adipate) (PTMA). It is known to be biodegradable if of low molecular weight [26] but has not been tested as a high molecular weight material required for production of a long-term absorbable suture material. The degradation was followed by measurement of released ^{14}C-labeled products from the labeled polyester [27]. With this method both the biotic and abiotic degradation can be followed simultaneously.

EXPERIMENTAL

Materials

Adipic acid (reinst), tetramethylene glycol, and tetraisopropyl titanate were obtained from E. Merck, (West Germany). 1,6-^{14}C-Labeled adipic acid with 2.1 MBq/mg activity was obtained from The Radiochemical Centre, Amersham (UK).

The acid was dried in vacuum (5 torr, 50°C) for 10 h before synthesis. The glycol and the titanate were distilled under a reduced nitrogen atmosphere (1 torr), the latter being stored under nitrogen in sealed amber bottles.

Synthesis

The ^{14}C-labeled polymer was synthesized according to Billmeyer et al. [28]. The empty 5-necked reaction vessel was heated to 80°C and purged with nitrogen. The reagents, 0.42 mol (37.8 g) tetramethylene glycol and 0.40 mol (58.4 g) adipic acid (including 3.7 MBq labeled acid) were then added and the temperature slowly raised until the acid was completely dissolved.

The catalyst (tetraisopropyl titanate, 0.5% of the total weight of the reagents) was then added and the temperature was raised over 2 h to 190°C and maintained for 4-10 h. When no more water was evolved, the temperature was lowered to 140-150°C, the distilling head replaced with a stopper, and the nitrogen inlet tube replaced with a vacuum line.

The reaction mixture was held at 165°C with stirring under vacuum (1 torr) for 6-10 h or until the light brown melt became too viscous to stir.

The polyester was recovered by dissolving the contents in acetone and precipitating the polymer in cold methanol (-78°C). The slightly off-white polymer was filtered off and dried in vacuum. The yields were 80 ± 2%.

TABLE 1. Properties of Virgin Polymers

Molecular weight	$\overline{M}_n$	$\overline{M}_w$	ΔH_f, kJ/kg	Activity, GBq/g
High	14 000	52 000	95.3 ± 0.4	74
Low	4 200	15 000	104.1 ± 1.2	148
mp = 323-328 K (50-55°C)			T_g = 155 K (-118°C)	
Fiber diameter = 0.66-0.75 mm			Tensile strength = 66.0 ± 4.5 N	

Polymer Characterization

The polymer was characterized with GPC, DSC, IR, NMR, and radioactivity measurements. A Waters 6000 A pump with 6 columns (100-10^6 Å) connected to a differential refractometer was used for GPC with THF as the solvent, and the flow rate was 1 mL/min. The apparatus was run at room temperature with the solvent pressure at 6.9 MPa (1000 psi). The apparatus used for DSC was a Perkin-Elmer DSC-2 with a heating rate of 10°C/min. Sample weight was 5 mg. The IR used was a Perkin-Elmer 580B, with the sample as film on NaCl windows. The NMR used was a Bruker WP 200. The radioactivity measurements were made with a Packard Tri-Carb liquid scintillation spectrometer (Model 3375). Tensile strength was measured with an Instron 1122 equipped with pneumatic grips (No. 2714-002). The characterization of the virgin polymer is summarized in Table 1. Figures 1, 2, and 3 show the IR, ^{1}H-NMR, and ^{13}C-NMR spectra, respectively.

Equipment

The equipment is shown in Fig. 4. It consisted of five glass flasks, of which the central one served as the cultivation unit. The cultivation medium is given in Table 4. The first and the last flasks contained aqueous potassium hydroxide (2 M) to remove CO_2 from the incoming air (No. 1) and to absorb evolved volatile products (No. 5). Flasks 2 and 4 were empty safety traps. No other precautions were taken to filter the incoming air. The whole experimental assembly was placed in a dark cultivation room at a constant temperature of 25°C. Air was bubbled through the flasks at a rate of approximately 10 mL/min. With this procedure, all of the evolved $^{14}CO_2$ is presumed to be trapped [29].

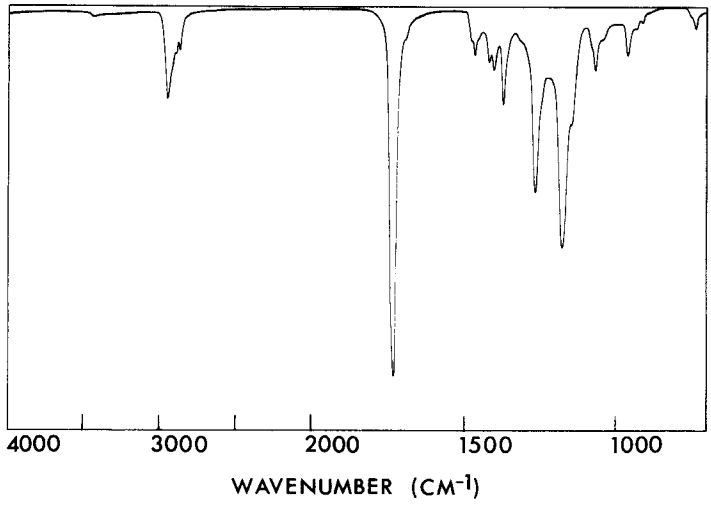


FIG. 1. IR spectrum of poly(tetramethylene adipate).

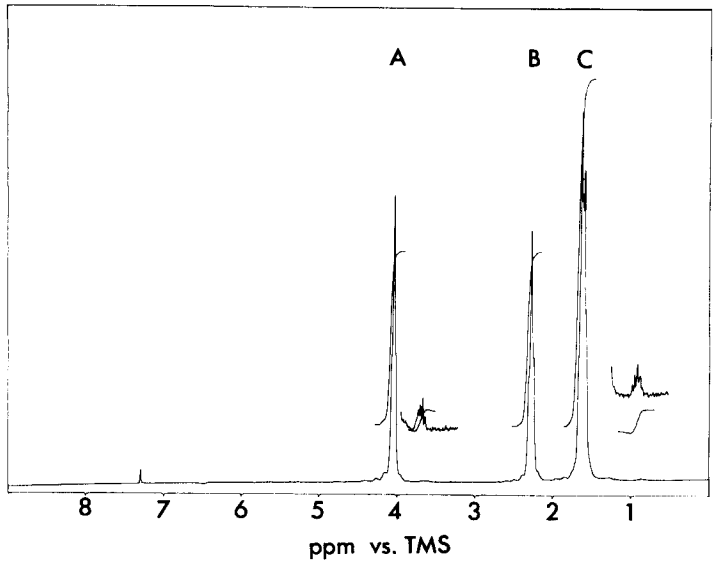
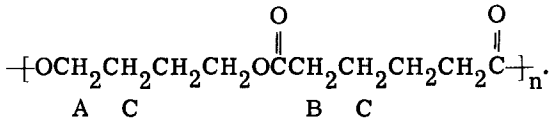


FIG. 2. ^{1}H-NMR spectrum of poly(tetramethylene adipate):



Peak assignments are shown in Table 2.

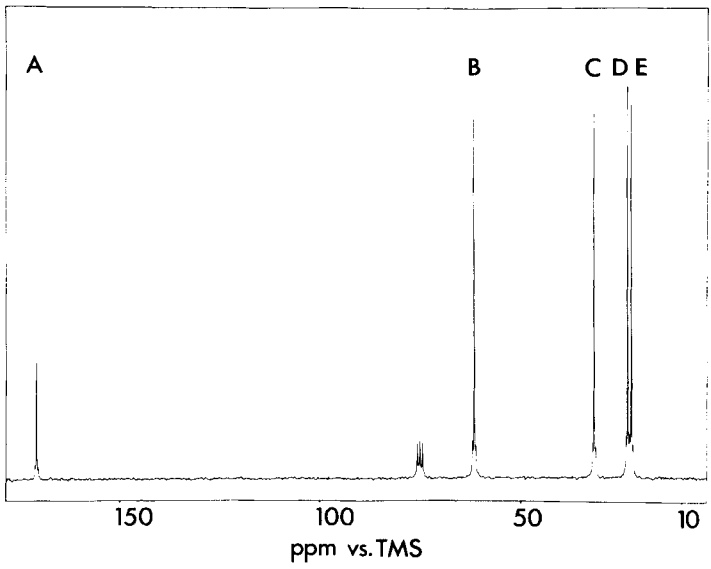
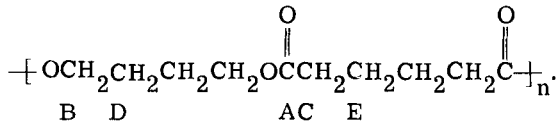
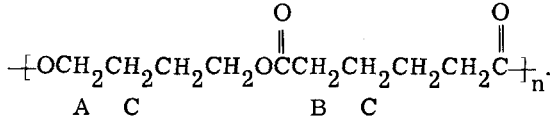


FIG. 3. ^{13}C-NMR spectrum of poly(tetramethylene adipate):



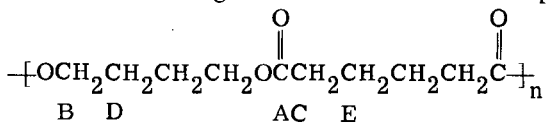
Peak assignments are shown in Table 3.

TABLE 2. Peak Assignments of the ^{1}H-NMR Spectrum



Proton type	Shift (ppm vs TMS)	
	Calculated	Observed
A	4.03	4.10
B	2.45	2.32
C	1.57	1.67

TABLE 3. Peak Assignments of the ^{13}C-NMR Spectrum



Carbon type	Shift (ppm vs TMS)	
	Calculated	Observed
A	180-170	172.5
B	64.5	63.2
C	34.4	33.3
D	28.0	24.9
E	26.0	23.9

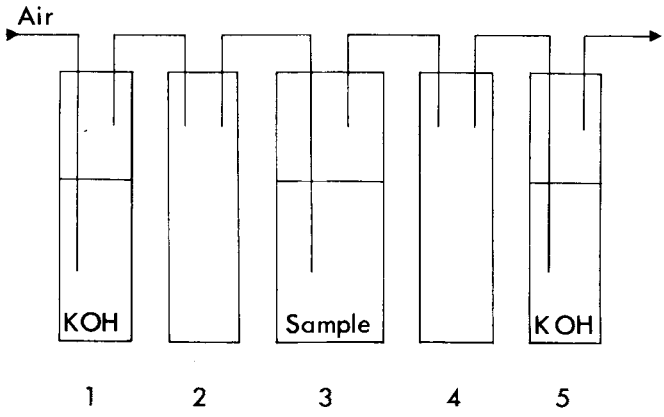


FIG. 4. Degradation apparatus.

TABLE 4. Culture Medium

Ammonium tartrate	5.0 g
Potassium dihydrophosphate	1.0 g
Magnesium sulfate (calculated as anhydrous)	0.5 g
Ferric chloride (1 wt% solution)	0.5 mL
Zinc sulfate (1 wt% solution)	4.5 mL
Distilled water to	1000 mL
pH adjusted to 5.5	

Measurements

The KOH solution in Flask 5 was exchanged each month in connection with the radioactivity measurements. The utilization of polyester was followed by measuring the activity of ^{14}C absorbed in Flask 5. The solution of potassium hydroxide was titrated with hydrochloric acid to pH 8.35 in an automatic pH-meter. After dilution to a constant ionic concentration of K^+, 1-mL aliquots were added to 10-mL scintillation solutions (Aquasol from New England Nuclear). The amount of the ^{14}C isotope was analyzed in a liquid-scintillation counter in terms of counts per minute. Finally, these values were transformed to values described as "percent degradation of polyester" after substraction of the background scintillation [29]. Similar samples without ^{14}C added were used as a measure of the background scintillation. New background samples were made each time, and the mean value was calculated.

Fibers of unlabeled polymer were extruded at 60°C in an extrusion device made in our workshop. The fibers were then necked at room temperature, and the oriented products had diameters of 0.70 ± 0.05 mm. These were placed in the buffer described in Table 5 [30], thermally stabilized at 37°C. Samples were taken after 19, 39, 89, 123, 180, and 243 days, cleaned with distilled water, and dried to constant weight.

RESULTS AND DISCUSSION

Thirty samples were inoculated with a mixture of microorganisms after 0, 30, 100, and 450 days, and to 15 of these were added $AgNO_3$ to

TABLE 5. Composition of Buffer[a]

Salt[b]	Concentration (meq/L)
NaCl	95
KCl	5
$Ca(Ac)_2.H_2O$	4
$MgSO_4.7H_2O$	2
$Na_2HPO_4.7H_2O$	2

[a]NaOH to adjust to pH 7.4.
[b]Analytical reagent grade.

stop growth and to permit measurement of the abiotic evolution of ^{14}C-labeled products. The percent degradation versus time, plotted in Figs. 5 and 6, indicates that the level of recovered volatile material rises very quickly within 50-100 days after inoculation. This is probably due to the new, fresh microorganisms being able to produce volatile materials more efficiently [31] from products of the hydrolysis, which proceeds at a constant rate. As the hydrolysis products are the only source of carbon (except the ammonium tartrate) in the cultivation medium, the population of active microorganisms decreases to an equilibrium level dependent on the rate of hydrolysis.

This behavior was also observed in the change of the molecular weight (Figs. 7 and 8). The rate of decrease during the time after inoculation was 20 times higher than in the later part, and this behavior was the same for both biotic and abiotic degradation. As shown in these figures, the high molecular weight fraction (M_w) of the polyesters rapidly decreases during degradation, whereas the low molecular weight (M_n) remains nearly constant. This indicates that the polyester is degrading by random hydrolysis and subsequent solubilization of the oligomers formed. The oligomers are then further degraded to volatile products by the microorganisms.

Generally there is a higher level of degradation for the low molecular weight material (Fig. 5) than the high molecular weight material (Fig. 6) with one exception (shown as the dotted line in Fig. 6). This sample contained a unique mixture of microorganisms, and we will continue to study the microbial degradation of polyesters with this mixture.

The change in crystallinity (measured as heat of fusion) is somewhat peculiar (Fig. 9). The crystallinity of the high molecular weight material increased for the first period to 104% of the original value

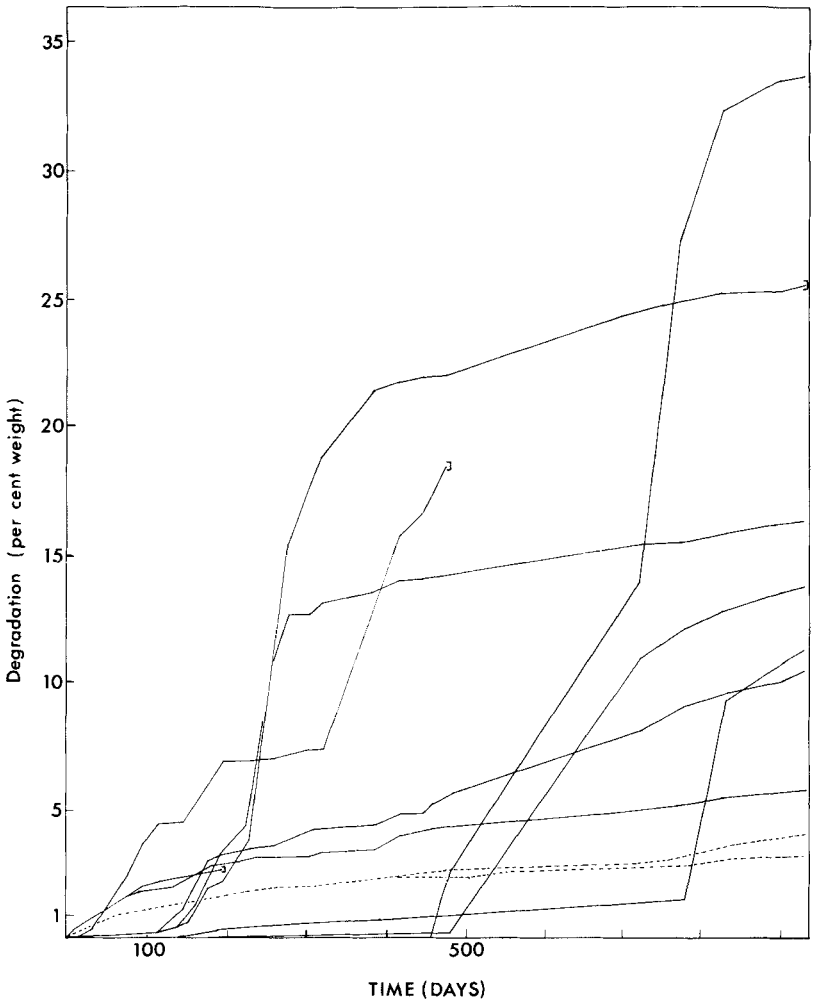


FIG. 5. Degradation of poly(tetramethylene adipate) (M_n = 4 200) expressed as percent weight recovery of ^{14}C-labeled volatile products. Broken lines represent products from samples immersed in distilled water without microorganisms. Brackets show where samples were taken for analysis.

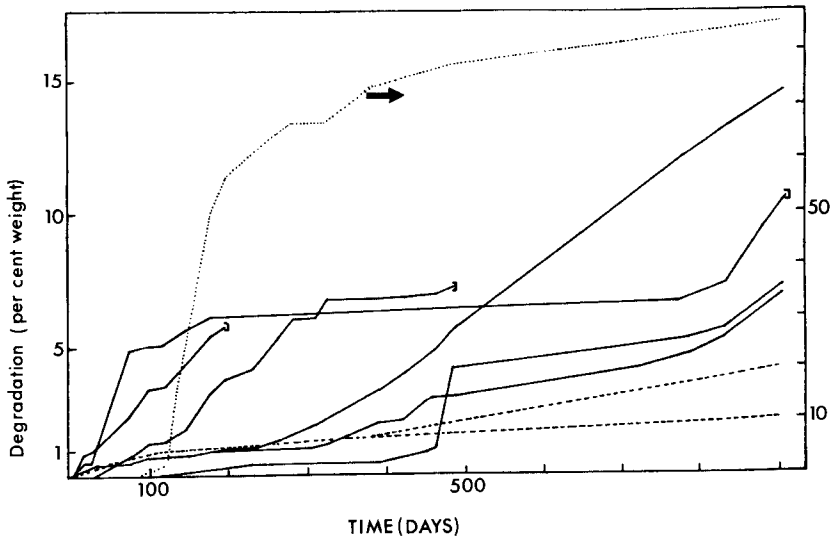


FIG. 6. Degradation of poly(tetramethylene adipate) (M_n = 14 000) expressed as percent weight recovery of ^{14}C-labeled volatile products. Broken lines represent products from samples immersed in distilled water without microorganisms. Brackets show where samples were taken for analysis.

(see Table 1) and during the second period this decreased to 78% of the original value.

However, only a decrease was observed for the low molecular weight material. A possible explanation for this behavior is that the shorter molecular chains within the crystallites are able to slip out more easily during the plasticization by the diffused water than the longer chains present in the high molecular weight material [32].

Radioactivity in the cultivation media was measured for 6 samples (Table 6) and showed that more than 90% of the biotic degradation was collected as volatile material. In the abiotic samples, however, the cultivation medium contained 90% of the degradation products up to 900 days, where the abiotic evolution of volatile material is more prominent, perhaps because of the aging of the material (oxidation) [33]. The results from the crystallinity and radioactivity measurements indicate that the rate of degradation is controlled by abiotic hydrolysis, which first takes place in the amorphous material.

The tensile strength measurements (Fig. 10) show that the polyester retains its strength much longer than, for example, Dexon. The broken line represents data taken from Chu [34].

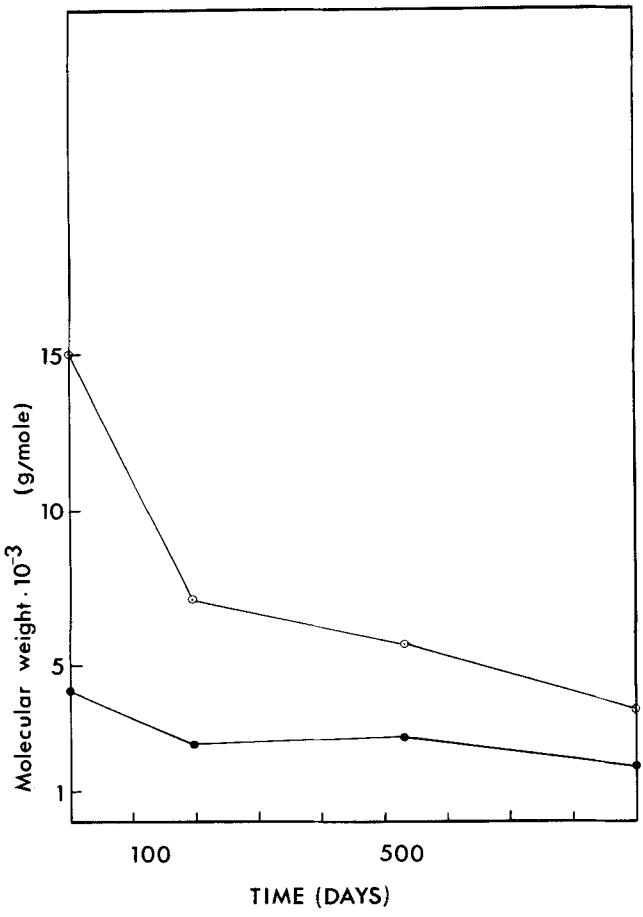


FIG. 7. Decrease of molecular weight ((○) $\overline{M}_w$, (●) $\overline{M}_n$) of poly(tetramethylene adipate) (low molecular weight material) during degradation.

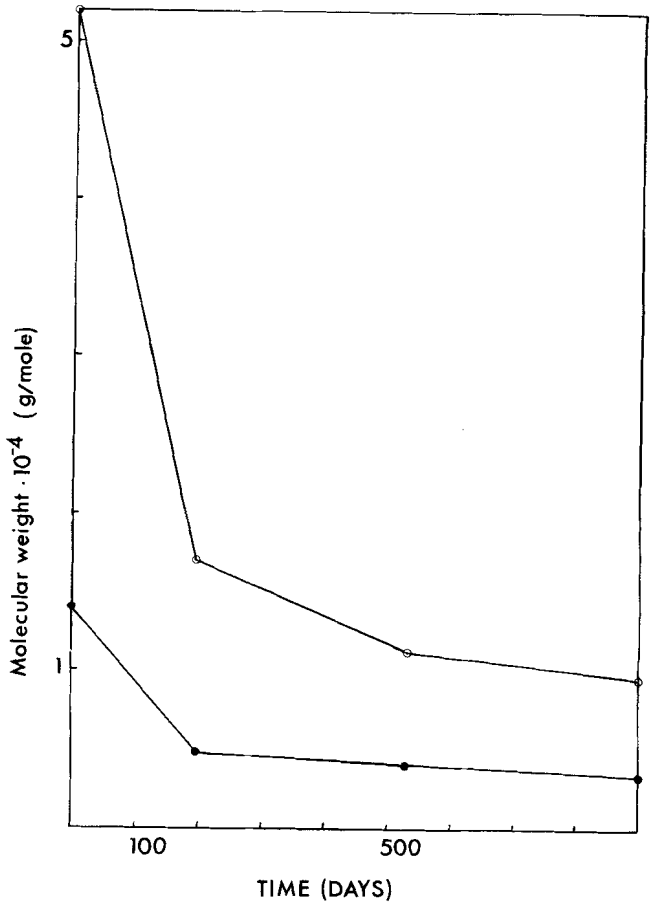


FIG. 8. Decrease of molecular weight ((○) $\overline{M}_w$, (●) $\overline{M}_n$) of poly-(tetramethylene adipate) (high molecular weight material) during degradation.

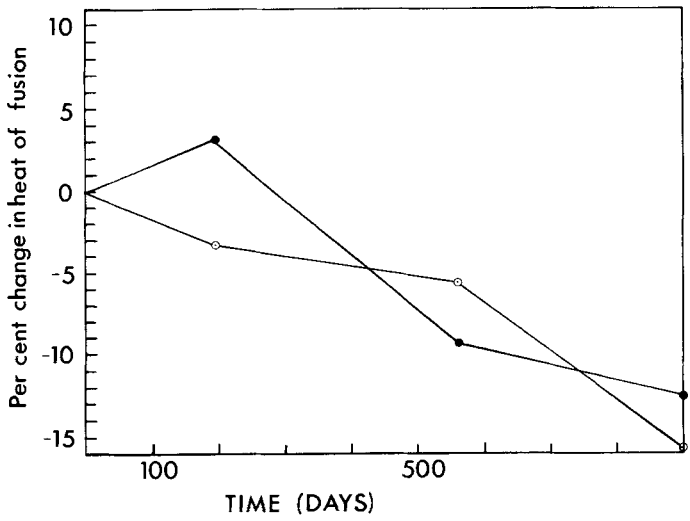


FIG. 9. Degradation induced change of crystallinity of poly(tetramethylene adipate). Low (○) and high (●) molecular weight material expressed as percent change in heat of fusion.

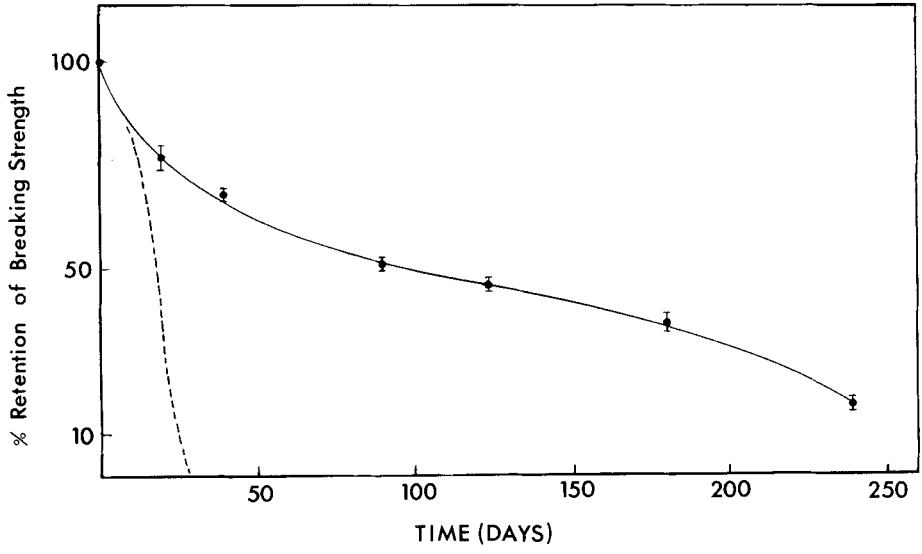


FIG. 10. The percentage retention of breaking strength of poly(tetramethylene adipate) at immersion in the buffer medium displayed in Table 5. Broken line represents the retention of breaking strength of polyglycolic acid (from Chu [34]).

TABLE 6. Percentage of Recovered ^{14}C-Labeled Degradation Products

Sample	Microorganisms	198 Days		530 Days		900 Days	
		Volatile products	Cultivation medium	Volatile products	Cultivation medium	Volatile products	Cultivation medium
Low molecular weight material	Yes	91.0	9.0	92.6	7.4	96.6	3.4
Low molecular weight material	No	1.5	98.5	6.3	93.7	68.0	32.0
High molecular weight material	Yes	94.1	5.9	91.4	8.6	94.2	5.8
High molecular weight material	No	2.8	97.2	4.5	95.5	93.9	5.8

CONCLUSIONS

We have synthesized a linear, aliphatic polyester, poly(tetramethylene adipate). It degrades in vitro by random hydrolysis, and the rate is controlled by the crystallinity and the molecular weight of the material. Poly(tetramethylene adipate) retains 50% of its original tensile strength for 100 days in vitro and, because of this, is an interesting long-term absorbable suture material for such applications as human tendon replacement and vascular grafting.

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