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The in vitro Assessment of a Collagen/Vicryl (Polyglactin) Composite Film Together with Candidate Suture Materials for Use in Urinary Tract Surgery

I. Physical Testing

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Summary. A Vicryl (Polyglactin)/Collagen composite membrane has been developed for potential use in urinary tract surgery. The compatability of this membrane, together with its two individual components, collagen film and vicryl mesh, has been tested over a three week period in urine obtained from both healthy and from stone forming patients. The rate of degradation as indicated by changes in the mechanical strength was determined at regular intervals. In addition, absorbable suture materials such as plain catgut, chromic catgut, and vicryl were similarly tested; in the latter case, the breaking stress, and actual loss of weight of the material were compared. The whole series of experiments were then repeated in urine obtained from rabbits, the animal chosen for any future in vivo studies.

Key words: Collagen vicryl composite, Urinary tract repair, Sutures.

Introduction

Replacement of various organs in the human body has, in the past 20 years, become a commonplace occurrence. Among the most useful of such replacements are kidney transplants. Apart from the total replacement of a single organ such as the kidney or heart, there has been a considerable development of artificial materials which have been shown to be capable of repairing or substituting for normal tissues. A prime use of such materials is to be found in Orthopaedic Departments, where metals have been used to replace such major functional entities as the hip joint.

The application of artificial materials to the urinary tract is, in many respects, a more demanding excercise because of the complexity and the physical and chemical properties of urine; for this reason research into a suitable biodegradable material which can be applied to reparative work in the urinary tract has been long and frustrating.

Encouraging results in other areas of tissue augmentation have been obtained with Vicryl Mesh (Polyglactin Mesh) such as in pelvic floor reconstruction [3], in abdominal wall repair following evisceration [15, 16] and in urethroplasty [1]. It was envisaged that such an absorbable fabric, if made leakproof with a biodegradable film such as collagen, may well serve as a replacement material in urinary tract repair work.

On account of its high tensile strength, biodegradability, haemostatic properties [4], low antigenicity, [6], noncytotoxicity and ability to support cellular growth [18, 21], collagen itself has already found considerable use in the biomedical field [4, 9–11, 22, 24]; indeed recent studies [7, 20] have shown that collagen film may well have an application in urinary tract surgery.

The preparation of a collagen/vicryl composite membrane is described, and the physical properties of this material following exposure to urine are presented. The two components Vicryl Mesh and Collagen Film were assessed separately.

A comparison between the loss in breaking strength and the actual loss in weight obtained with Vicryl (Polyglactin) material following incubation in phosphate buffered saline is also described.

In addition, a number of commonly used absorbable sutures such as vicryl (polyglactin), plain and chromic catgut were also tested for their ability to withstand continued exposure to urine in vitro. Experiments were also carried out on all test materials using urine obtained from rabbits, the animal of choice for our future in vivo investigations.

Materials and Methods

Vicryl (Polyglactin) Mesh, Style 9, was obtained from Ethnor, Neuilly sur Seine Cedex, France. All suture materials were purchased from Ethicon Ltd, Edinburgh, Scotland.

Preparation of Collagen Film

Collagen film was prepared essentially by a previously described method [7]. A suspension of 4 g, lyophilised bovine hide collagen obtained from limed splits, was prepared in 1 l 0.05 M-acetic acid at 4 °C containing 0.2% v/v glycerol, and the whole homogenised for 90 s in a Waring Blender. After degassing under vaccum, the resulting slurry was poured into a plastic tray (32 x 44 cm) and the whole was allowed to dry over a stream of filtered air at room temperature forming a plasticised film which could be removed and cut to size as required. Such films were approximately 25 μ thick.

Preparation of a Collagen Coated Vicryl Mesh

A suspension of 2 g defatted lyophilised bovine hide collagen was prepared in 1 l of 0.05 M-acetic acid at 4 °C containing 0.2% v/v glycerol. The whole was homogenised for 90 s in a Waring Blender and degassed under vacuum. The resulting slurry was poured into a plastic tray (32 x 44 cm) and allowed to dry over a stream of filtered air at room temperature.

A piece of Vicryl mesh (Style 9) was placed on top of the resulting collagen film without removing it from the tray, and 1 l of a degassed collagen slurry prepared as described above was layered on top of the vicryl mesh to create a sandwich of collagen-vicryl-collagen. Any entrapped air was carefully removed, and again the whole allowed to dry over a stream of filtered air. The coated Vicryl Mesh was subsequently removed from the tray and sealed in aluminium foil.

Incubation of Materials in Human Urine

Urine was collected from both healthy and from stone forming patients (24 h specimens), and portions of collagen film vicryl mesh, and collagen/vicryl membrane (4 x 1 cm) were incubated in 150 ml aliquots for up to three weeks at 37 °C. In each case, sodium azide was added as a bacteriocide.

The tensile (breaking) strength of the prosthesis itself (collagen coated vicryl) and its two components (vicryl mesh and collagen film) were then measured at regular intervals on an Instron tensiometer test system. The gap between the jaws (effective sample length) was 1 cm, and a cross-head speed of 1 cm/min was used to break the sample.

Standard lengths of a number of absorbable suture materials were similarly incubated and tested. These included plain catgut, chromic catgut and vicryl. All sutures were 3/0 gauge.

Incubation of Materials in Rabbit Urine

A further series of experiments was then carried out in which all of the prosthetic and suture materials were incubated in 50 ml portions of pooled urine obtained from rabbits; again in all cases sodium azide was added as a bacteriocide. In one such experiment, the pH of a portion of one pooled urine was reduced to 6.0 with HCl. The materials were then incubated in both the high and low pH urine for seven days and the results compared.

Incubation of Vicryl Mesh and Suture Material in Phosphate Buffered Saline (PBS)

Approximately 10 mg portions of Vicryl Mesh (Style 9) and Vicryl coated suture material (Gauge 3/0) were dried in a dessicator, carefully weighed and incubated at 37 °C for periods of up to 35 days in 0.01 M-phosphate buffer, pH 7.0 containing 0.15 M-NaCl (PBS). Pieces of material were removed at weekly intervals, washed in distilled water, and dried in a dessicator until the weight was stabilised at a constant value. Where necessary, the pH of the incubation mixture was maintained at 7.0 by the addition of sodium hydroxide.

Simultaneously, portions of Vicryl mesh (5 x 1 cm) and standard lengths of coated Vicryl (gauge 3/0), were incubated at 37 °C in PBS and the breaking stress measured at regular intervals using an Instron tensiometer as previously described.

Results

The breaking stress of a sample of the collagen film in human urine is shown in Fig. 1 where a steady decrease in mechanical strength up to 14 days is observed followed by a more steady decline to 21 days. The urine at pH 6.5 is taken from a known stone former. Vicryl mesh (Style 9) in the same urine samples showed on average a more linear decline over the 21 day period (Fig. 2) whereas a slower loss of breaking stress at 7 and 14 days was observed in the case of the collagen/vicryl composite (Fig. 3).

Figure 4 shows the hydrolysis of coated Vicryl 3/0 suture material in three samples of urine from healthy patients. The hydrolysis obtained with a sample of rabbit urine at pH 8.2 is shown for comparison. In Fig. 5, the rate of hydrolysis of the coated vicryl 3/0 suture can be seen in three different stone forming urines, with the results obtained in rabbit urine at pH 8.2 shown on the same diagram for comparison. In both of these diagrams it can be seen that the rate of hydrolysis of the vicryl suture material is dependent upon the pH of the urine; breakdown of the polyglactin proceeding more rapidly at the higher pH values. The effect is particularly marked in the high pH rabbit urine where after 11 days, the strength of the suture has been reduced to 22% of its original value. At pH values 5.8–6.1, however the suture retains 83% of its breaking strength following incubation in human urine over the same period (Fig. 4).

To investigate further the effect of urinary pH on (i) collagen vicryl composite, (ii) vicryl mesh, and (iii) vicryl suture material, and to eliminate any difference which may arise as a result of species variation, a sample of pooled rabbit urine, pH 8.7 was divided into two equal portions and the pH of one half reduced to 6.0 with HCl. Incubations were then carried out as described under "Materials and Methods" over a seven day period. The results with these three materials are shown in Figs. 6, 7 and 8 respectively where, in all cases, a very marked effect can be observed. In the case of the composite material (Fig. 6) after 7 days at pH 8.7 only 36.9% of the original mechanical strength was retained whereas in the urine with the pH adjusted to 6.0, the strength was only reduced to 69.1%. With the vicryl mesh after 7 days (Fig. 7) the values for the breaking load were remarkably similar at pH 6.0 and 8.7 where the figures indicated 26.2% and 32.9% of the original value respectively; at the 5 day point however a marked difference (83% and 58% of the starting value) was observed. At pH 6.0 (Fig. 8), after seven days the coated Vicryl 3/0 suture material retained, as expected, most (91.5%)

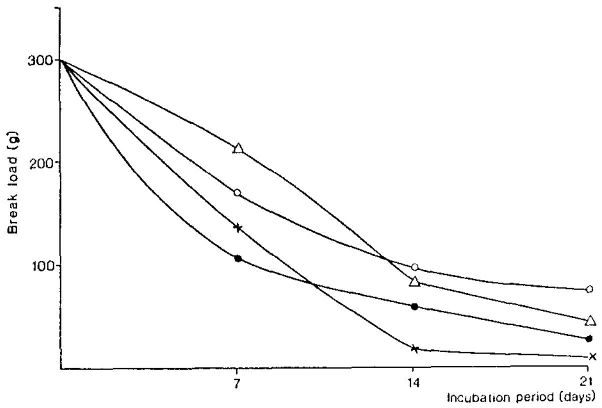

Fig. 1. Loss in breaking strength of collagen film in human urine at 37 °C. o—o pH = 5.6, △—△ pH = 5.8, ●—● pH = 5.9, x—x pH = 6.5

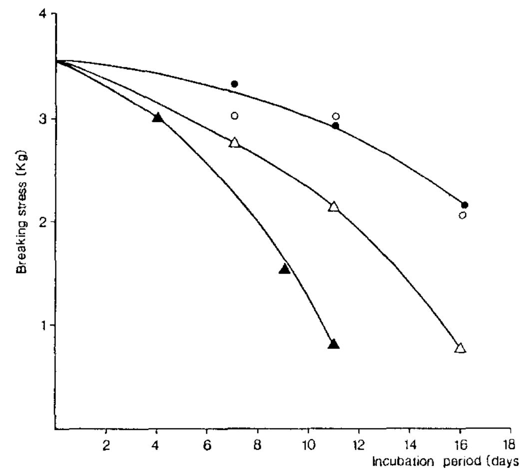

Fig. 4. Loss in breaking strength of vicryl 3/0 suture materal in normal human and rabbit urine at 37 °C. ●—● pH = 5.8, o—o pH = 6.1, △—△ pH = 7.0, ▲—▲ pH = 8.2 (rabbit urine)

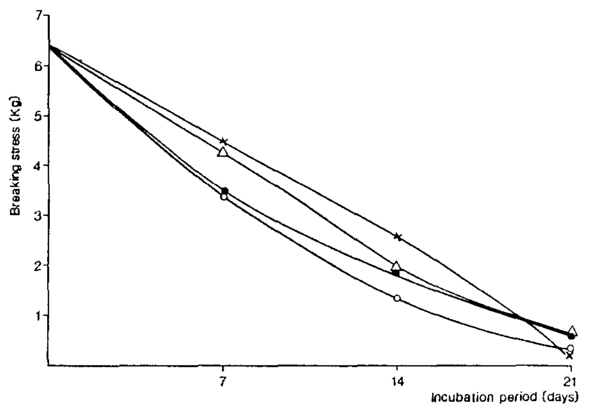

Fig. 2. Loss in breaking strength of vicryl mesh in human urine at 37 °C. o—o pH = 5.6, △—△ pH = 5.8, ●—● pH = 5.9, x—x pH = 6.5

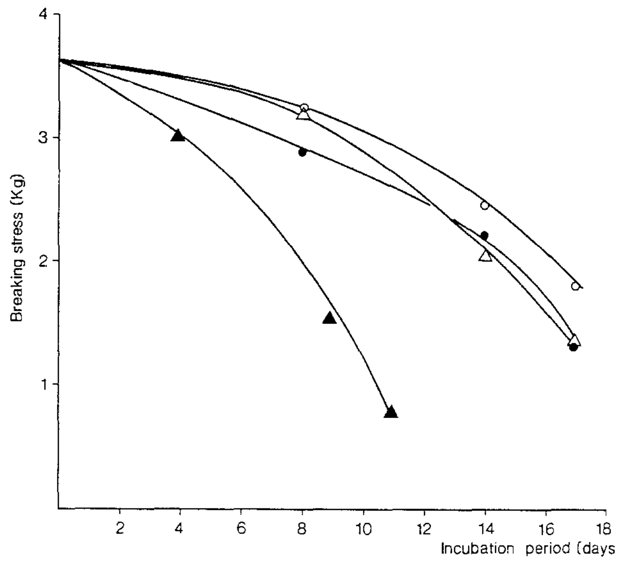

Fig. 5. Loss in breaking strength of vicryl 3/0 suture material in stone-forming human and rabbit urine at 37 °C. o—o pH = 6.2, △—△ pH = 7.0, ●—● pH = 6.2, ▲—▲ pH = 8.2 (rabbit urine)

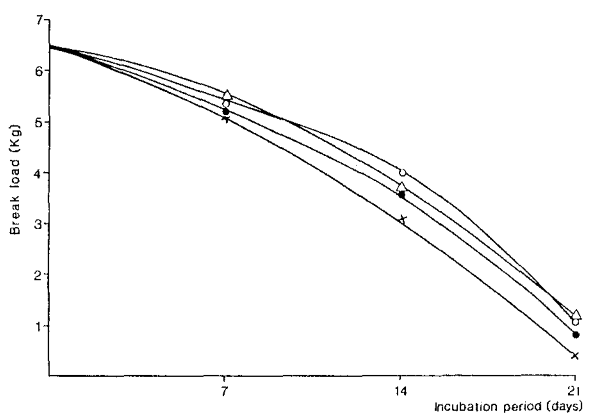

Fig. 3. Loss in breaking strength of collagen coated vicryl mesh in human urine at 37 °C. o—o pH = 5.6, △—△ pH = 5.8, ●—● pH = 5.9, x—x pH = 6.5

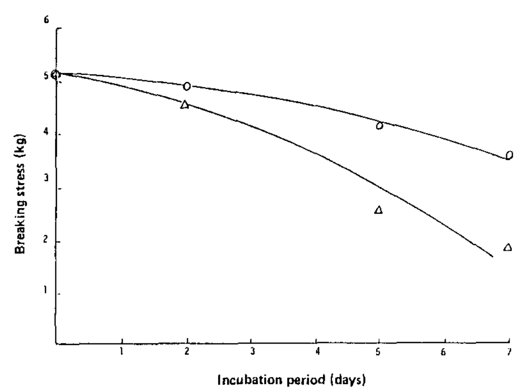

Fig. 6. Loss in breaking strength at 37 °C of collagen coated vicryl mesh in rabbit urine at two different pH Values over 7 days. o—o pH = 6.0, △—△ pH = 8.7

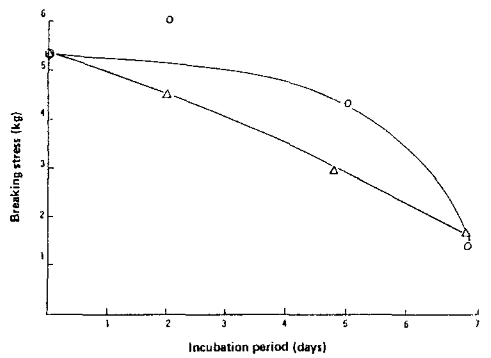

Fig. 7. Loss in breaking strength at 37 °C of vicryl mesh in rabbit urine at two different pH Values over 7 days. o—o pH = 6.0, △—△ pH = 8.7

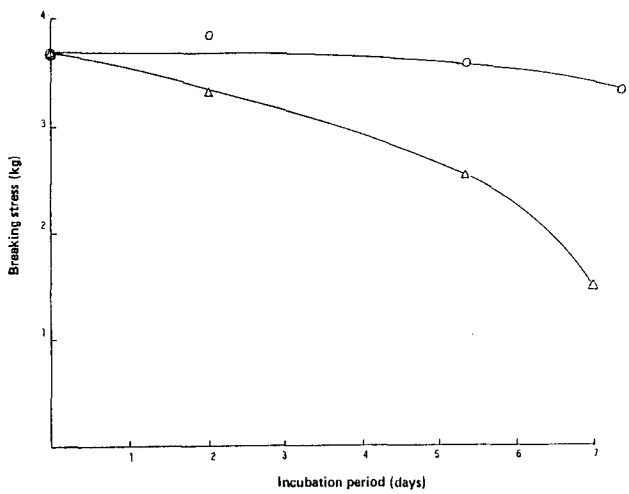

Fig. 8. Loss in breaking strength at 37 °C of vicryl 3/0 suture material in rabbit urine at two different pH Values over 7 days. o—o pH = 6.0, △—△ pH = 8.7

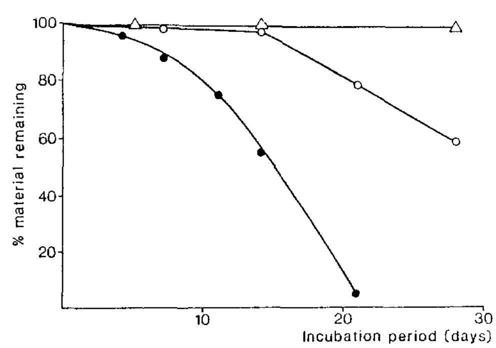

Fig. 9. Hydrolysis of vicryl 3/0 suture material showing ●—● (a) Loss of breaking strength (PBS, 37 °C), o—o (b) Loss of weight (PBS, 37 °C), △—△ (c) Published results of Craig and co-workers [5] showing % suture area remaining following intramuscular implantation. (These authors used 00 and 4/0 gauge material)

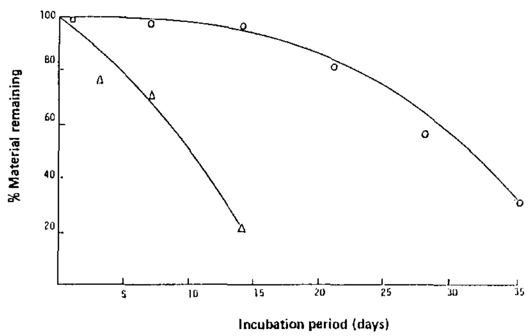

Fig. 10. Hydrolysis of vicryl mesh showing o—o Loss of breaking strength. △—△ Loss in weight

Table 1. Decrease in mechanical strength of collagen film following incubation in rabbit urine at two different pH values (Initial breaking stress of material = 553 g)

Urine pH	Breaking stress		
	2 days	5 days	7 days
6.0	198	Too low to measure	
8.7	71	Too low to measure	

of its breaking strength, but at pH 8.7 only 40.5% of the original value remained.

When the collagen film was similarly investigated it was found that, after two days the mechanical strength was reduced from 553 to 198 g at pH 6.0 (35.8% of the original value) and from 553 g to 71 g at pH 8.0 (12.8% of the original value). At five days and subsequently the values were too low to measure (Table 1).

When the percentage loss in weight of the coated vicryl suture material and vicryl mesh are compared with the decline in physical strength obtained following incubation in phosphate buffered saline, it is 14 days before any loss in weight is detectable (Figs. 9 and 10); after 28 days 60% of the original mass of both materials was retained. However, the loss in actual strength caused by hydrolysis of the vicryl proceeds much more rapidly especially in the case of the uncoated mesh. For comparative purposes (Fig. 9), the results reproduced from work published by Craig and co-workers [5] shows the rate of biodegradation of the material as measured by determining histologically the diameter of suture remaining following intramuscular implantation. Craig and co-workers following the subcutaneous implantation of vicryl sutures, produced results similar to those we have obtained in PBS at 37 °C.

Plain and chromic catgut were incubated in human and rabbit urine and the tensile strength determined at regular intervals (Tables 2a, 2b, 3a, 3b). With plain catgut, no breakdown was seen after 2 weeks incubation in human urine, but some loss in mechanical strength was observed after

Table 2a. Breaking stress of standard lengths of gauge 3/0 plain catgut following incubation in sterile human urine at 37 °C over a three-week period (Initial breaking stress of the material = 2.16 kg)

Urine no.	pH	1 week		2 weeks		3 weeks	
		Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value
1	5.81	2.46	114	2.79	129	1.57	73
2	5.70	2.30	106	2.60	120	1.85	86
3	5.57	2.11	98	2.41	112	1.93	89
4	5.78	2.45	113	2.63	122	2.06	95

Table 2b. Breaking stress of standard lengths of gauge 3/0 chromic catgut following incubation in sterile human urine at 37 °C over a three-week period (Initial breaking stress of the material = 2.22 kg)

Urine no.	pH	1 week		2 weeks		3 weeks	
		Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value
1	5.81	1.51	68	1.77	80	1.88	85
2	5.70	1.72	78	1.87	84	1.27	57
3	5.57	1.48	67	2.38	107	2.15	97
4	5.78	1.46	66	2.01	91	1.78	80

Table 3a. Breaking stress of standard lengths of gauge 3/0 plain catgut following incubation in sterile rabbit urine at 37 °C over a three-week period at two different pH values (Initial breaking stress of the material = 2.16 kg)

Urine pH	1 week		2 weeks		3 weeks	
	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value
6.0	1.74	81	1.62	75	1.60	74
8.7	1.51	70	1.78	82	1.52	70

Table 3b. Breaking stress of standard lengths of gauge 3/0 chromic catgut following incubation in sterile rabbit urine at 37 °C over a three-week period at two different pH values (Initial breaking stress of the material = 2.22 kg)

Urine pH	1 week		2 weeks		3 weeks	
	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value	Breaking stress (kg)	% Original value
6.0	1.87	84	1.53	69	1.53	69
8.7	1.85	83	1.47	66	1.76	79.3

the third week. In rabbit urine, some degradation was observed after the first week and this did not decrease significantly over the next fourteen days. Chromic catgut appeared to degrade slightly in human urine over the first week after which it remained unchanged, whereas in rabbit urine at both pH values, a decrease over the first two values was observed.

Discussion

Shrinkage of the urinary bladder can result from a variety of conditions eg. post-opertive radiotherapy. Restoration of bladder function by replacing the removed tissue with a section of excised bowel is a complex surgical procedure and can result in ionic imbalance. Clearly, it would be highly advantageous to expand the bladder by using a material which would allow the growth of tissue across its surface, together with a normal urothelium. Such a material should be biodegradable and leakproof, should not allow the replaced area to contract significantly during healing, should inhibit calculogenesis, and should be non-cytotoxic and non-antigenic.

For reasons outlined in the Introduction both collagen and vicryl mesh are materials of great potential use in the tissue augmentation field. However, when applied to the urinary tract, vicryl mesh alone will permit leakage of urine. Collagen film, although preventing leakage [7], is soft and can be difficult ot suture. When collagen film is strengthened by a suitable biodegradable mesh such as vicryl, a material is produced which merits further assessment in urinary bladder repair. Furthermore, collagen has been shown to support the growth of normal bladder epithelial cells [14, 23] and cells derived from bladder cell carcinomata [14] with excellent biocompatibility and without evidence of cytotoxicity.

The work described in this study represents the first stage of an investigation into the potential use of collagen vicryl composites for use in urinary tract surgery, and evaluates the effect of human and rabbit urine on the physical properties of this new material. Its two components, vicryl mesh and collagen film, have been assessed individually along with absorbable suture materials i.e. plain and chromic catgut and vicryl. These suture materials were studied since they are likely to be used to secure any prosthesis.

When the collagen vicryl composite material was incubated in human urine, a slightly more gradual decrease in the mechanical strength was observed over the first 14 days than with the vicryl mesh alone (Figs. 2 and 3). This effect may possibly be attributed to a protective coating formed by the collagen on the polyglactin surface which may retard its rate of hydrolysis. A simple reinforcement effect is less likely, as both mesh and collagen coated mesh had the same initial mechanical strength.

The degradation observed over the first 14 days with the collagen film (Fig. 1) may be a result of some proteolysis during this period, after which such enyzmes may tend to lose their activity. Although it is generally accepted that native undenatured collagen requires a specific collagenase enyzme [8] to effect its initial cleavage, some denaturation of the membrane either in the urine or during preparation may take place rendering the collagen more susceptible to the action of non-specific proteases. Any degradation by micro-organisms may be excluded as sodium azide was used in all incubations as a bacteriocide.

The increased loss in mechanical strength observed in rabbit urine may therefore represent an increased rate of proteolysis in this medium.

The rate of breakdown of the vicryl mesh, vicryl suture materials, and collagen vicryl composite, shows a marked dependency upon the pH of the urine, being more rapid at the higher pH values.

Interestingly, the 3/0 vicryl suture material shows in all cases studied a similar pH dependency to that of the vicryl mesh but a less rapid loss of mechanical strength. At pH values around 6, in both human and rabbit urine (Figs. 4, 5 and 8), little breakdown can be detected after 7 days, whereas at pH 8.7 over the same period in rabbit urine the mechanical strength had dropped to 41% of its original value (Fig. 8). Under similar conditions the vicryl mesh retained only 32% of its breaking stress (Fig. 7). The generally slower rate of hydrolysis of the vicryl suture may well be due to the calcium stearate coating applied by the manufacturers. Collagen probably protects vicryl in a similar fashion as shown in the present study.

When vicryl is incubated in phosphate buffered saline, the decrease in mechanical strength takes place more rapidly than the actual dissolution of the material as measured by the percentage loss in weight (Figs. 9 and 10). Vicryl (polyglactin 910) is a water soluble copolymer of glycolic and lactic acids containing 90% and 10% of each component respectively [5], and it is to be expected that some bonds in the material will hydrolyse weakening the overall strength of the material before sufficient fragmentation takes place to cause the polymer to actually dissolve. Studies [19] on another water soluble suture material, PDS (polydioxanone suture) where the decrease in average molecular weight was measured indicate that this may well be the case.

Interestingly, when the results obtained in this study are compared with those reported by Craig and co-workers [5] it can be seen that vicryl loses all of its mechanical strength and much of its weight before any decrease in the diameter of the material.

Unlike Vicryl (polyglactin 910), plain and chromic catgut are produced from natural materials which are degraded by collagenolysis and phagocytosis and, for this reason their breakdown in vivo is less predictable [2, 12, 13]. When samples of plain and chromic catgut were incubated in human and rabbit urine, some degradation was observed over the first one or two weeks, after which the suture tended to remain stable. As both of these materials consist essentially of collagen, some limited proteolysis may possibly take place for the same reason as that proposed for the collagen film.

In conclusion, we have prepared a leakproof composite membrane consisting of a collagen film reinforced with vicryl mesh. The collagen component is partially degraded by urine, and the vicryl dissolves at a rate which is dependent upon the pH. Such a prosthesis could well find a use in urinary bladder repair, provided that adequate tissue regeneration could occur over the surface of the material.

Some preliminary in vivo investigations in our laboratories [17] suggest that this is indeed the case. The choice of suture to secure the prosthesis may well be influenced by the pH of the urine. Other properties of the composite of particular importance to the urologist such as crystallisation of urinary salts on the prosthesis or any tendency of bacteria commonly associated with urinary tract infection to adhere to the surface of the membrane are currently under investigation.

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