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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

II. Surface Deposition of Urinary Salts

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Summary. Collagen coated vicryl mesh has been incubated with a series of urine collections from healthy and stone-forming patients. For comparison, collagen film, vicryl mesh, and a number of absorbable and non-absorbable sutures were similarly tested. Incubation in rabbit urine were also included in the study. Deposition of urinary salts, estimated qualitatively by electron microcopy, were observed on the collagen vicryl composite in approximately two thirds of the urines tested including rabbit urine. Those urines from patients with a high calcium excretion in particular caused urinary deposits on the material. Similar results were obtained with collagen film although the latter was not tested in rabbit urine. Considerably less deposits of urinary constituents were found with other absorbable materials such as vicryl mesh, vicryl sutures, and chromic catgut, whereas a higher proportion of concretions were found with the non-absorbable sutures. The results indicate that urinary salt deposition may be a problem associated with collagen based composite materials after prolonged exposure to urine. It must, however, be emphasised that great care should be exercised when extrapolating any results obtained in-vitro to the in-vivo situation.

Key words: Collage vicryl composite – Urinary tract repair – Urinary calculi

Introduction

The preparation of a biodegradable collagen vicryl prosthesis for potential use in urinary tract surgery has been described [6]. In addition to the collagen/vicryl composite, the degradative effects of urine were studied on the two individual components, vicryl mesh and collagen film. Absorbable suture materials such as vicryl and catgut, which could be used to secure such a prosthesis in-vivo were also included in the study [6].

When any artificial material is placed in direct contact with urine, for example following operative surgery on the bladder, there is always the potential complication of the deposition of urinary salts on its surface, resulting eventually in calculus formation. Any artificial material in contact with urine should therefore produce zero or minimal crystallisation before it is replaced with viable tissue. The subject under test in this study is collagen/vicryl composite.

In the current investigation, collagen coated vicryl mesh and its two components collagen film and vicryl mesh, were incubated at 37 °C in presterilised human urine for periods of up to three or four weeks after which the surfaces were examined by scanning electron microscopy (SEM) for any deposition of urinary salts or concretions. The elemental composition of any deposits was determined on the electron microscope using X-ray microanalysis. Urine from both healthy and known stone-forming patients was used in the experiments. A number of absorbable and non-absorbable sutures were also included in the study. The experiments were then repeated using pooled urine obtained from rabbits, the animal of choice for our in-vivo studies.

Materials and Methods

Collagen coated vicryl mesh and collagen film were prepared as described [6]. Vicryl Mesh (Style 9) was obtained from Ethnor, Neuilly-sur-Seine Cedex, France. All suture materials were purchased from Ethicon Ltd, Edinburgh, Scotland, and were of 3/0 gauge.

Urine samples (24 h collections) were obtained from both stone-forming and non-stone forming patients. Specimens were analysed for pH, calcium, phosphate and urate content, and were sterilised by the addition of sodium azide before use.

1 cm squares of collagen coated vicryl mesh, collagen film, and vicryl mesh were incubated in 25 ml portions of human urine at 37 °C for periods of up to three or four weeks. Following the required incubation period, samples were fixed in 4% buffered glutaraldehyde solution and processed for scanning electron microscopy. Specimens were examined in the scanning mode of a Jeol JEM 1200-Ex electron microscope, and analytical spectra obtained using

Table 1. Analysis of 24 h urine collections from healthy and stone-forming patients

Patient No.	Urine pH	Calcium		Phosphate		Urate	
		mmol/l	mmol/vol	mmol/l	mmol/vol	mmol/l	mmol/vol
1[a]	6.2	7.20	8.64	17.0	20.4	3.1	3.7
2[a]	6.1	2.80	7.10	17.5	44.6	3.4	8.7
3[a]	6.0	6.30	9.14	19.0	27.6	2.0	2.9
4[a]	6.0	0.75	2.20	3.1	8.9	1.0	2.9
5[a]	5.9	3.10	7.29	19.0	44.7	2.5	5.9
6[a]	6.7	2.55	4.08	8.0	12.8	0.9	1.4
7[a]	5.9	6.0	11.40	27.0	51.3	1.3	2.5
8[a]	6.8	0.55	0.76	11.0	14.8	0.8	1.1
9	5.64, 5.85	1.60	3.20	32.0	64.0	3.7	7.4
10	5.61	3.40	6.29	22.0	40.70	3.9	7.2
11	5.79	0.90	1.60	14.5	25.0	1.7	3.0
12	6.07, 5.69	5.05	11.60	22.0	50.6	2.7	6.2
13	5.55	1.70	3.30	23.0	44.2	2.3	4.4
14	6.21, 5.57	0.55	1.10	11.0	21.1	1.2	2.3
15	5.81	1.40	3.70	8.0	21.3	0.9	2.4
16	5.88, 5.78	1.35	3.40	14.5	36.8	2.4	6.1
17	5.58	3.40	6.70	15.0	29.7	2.4	4.6
18[a]	6.47	4.77	6.20	26.6	36.4	2.4	3.1
19[a]	6.37	5.85	5.85	18.0	18.0	2.0	2.0
20[a]	6.03	1.41	3.4	10.5	25.3	2.0	4.8
21	6.36	3.85	7.70	16.0	32.3	2.0	4.0
22[a]	6.79	1.95	5.0	11.0	28.2	1.7	4.4

Where more than one sample was obtained from the same patient, both pH values are given

[a] clinical stone former: Normal range calcium = 3.0–6.0 mmol/vol Phosphate = 13–39 mmol/vol and Urate = 2.8–4.4 mmol/vol

a Link Systems 860 Series 2 energy dispersion analyser. Analysis conditions were kept constant throughout the study.

Samples (2 cm lengths) of the following sutures were similarly incubated and processed; chromic catgut, plain catgut, vicryl (soluble braided polyester), 'Ethilon" (nylon monofilament), "Mersilene" (braided polyester) and "Mersilk" (braided silk).

Incubations in Rabbit Urine

The above materials were incubated in 25 ml portions of pooled rabbit urine (pre-sterilised with sodium azide) for up to 12 days at 37 °C. In addition, lengths of "PDS" (polydioxanone suture, soluble polyester monofilament), and "Prolene" (polypropylene monofilament) were similarly treated in this medium.

Results

The pH, calcium, phosphate, and urate contents of urine specimens used in the experiments are listed in Table 1.

Where appropriate the chemical composition of the crystalline deposits found on the test materials are shown in parentheses in the corresponding tables.

Collagen Coated Vicryl Mesh

Tables 2a–c summarise the results of the SEM examination of the composite membrane in human urine, each table listing the urines in order of calcium, phosphate and urate content respectively. From Table 2a it can be seen that in all but one of those nine urines tested in which the calcium excretion was above the normal range, calcium containing deposits were found on the membranes. Six of these urines were from known clinical stone formers. Of those eight urines where the calcium was either within or below the normal range, deposits were only found in three cases.

Table 2b illustrates the effect of phosphate excretion where from the six urines tested with an abnormally high phosphate content, deposits were found in five cases, three from known clinical stone formers. However, in the normal and below normal range, urinary deposits were found in about half of the urines tested.

The incidence of deposition of urinary salts on the membrane was evenly distributed over the whole range of urines when urate excretion is considered as a parameter (Table 2c).

A typical calcium deposit found on the composite material is shown in Fig. 1. Figure 2 shows the elemental analysis of the same crystal.

When the prosthesis was incubated in a sample of pooled rabbit urine at pH 8.2, deposits containing sodium, potassium, and phosphorus were found at 7 days, and after 17 days, the presence of calcium was detected. Also, following incubation of rabbit urine for 17 days, considerable disruption of the vicryl mesh component occurred.

Table 2a. Incubation of collagen vicryl composite collagen film and vicryl mesh in human urine. Effect of 24 h calcium excretion

Patient No.	Calcium Excretion		Collagen/vicryl composite	Collagen film	Vicryl mesh
	mmol/24 h	mmol/l			
12	11.60	5.05	+++ (Ca, P) †	–	clean
7[a]	11.40	6.00	+++ (Ca, P, K)	+ (Ca, P)	+++ (Ca, P)
3[a]	9.14	6.30	+++ (Ca, Na, P) ‡	–	–
1[a]	8.64	7.20	+++ (Ca, P)	+++ (Ca, P)	+++ (Ca, P)
21	7.70	3.85	+ (Ca, P)	–	clean
5[a]	7.29	3.10	+ (Ca, K)	clean	clean
2[a]	7.10	2.80	+ (Ca, Na, P) ‡	–	–
10 (2 experiments with same urine)	6.29	3.40	clean (both samples)	clean (both samples)	clean (both samples)
18[a]	6.20	4.77	+++ (Ca, P)	+++ (Ca, P)	clean
19[a]	5.85	5.85	clean	clean	–
6[a]	4.08	2.55	clean	–	–
15	3.70	1.40	+++ (Ca, K, P)	–	clean
20[a]	3.40	1.41	–	+ (K)	–
16 (2 samples)	3.40	1.35	both clean		clean (both samples)
9 (2 samples)	3.20	1.60	clean (1 sample) † +++ (Ca, P) (1 sample) †	+ (Ca, K, Na)	+ (Ca, FE) (1 sample) † (1 sample clean) †
4[a]	2.20	0.75	clean	clean	clean
11	1.60	0.90	clean	clean	clean
14[a]	1.10	0.55	++ (Ca, K, P)	–	–

Incubations were carried out at 37 °C for two weeks unless otherwise indicated († = 3 weeks, ‡ = 4 weeks)
+ = light deposition of urinary salts; ++ = medium deposition; +++ = heavy deposition. The elemental composition of these crystals is shown in brackets

[a] known clinical stone former: Normal range for calcium excretion = 3–6 mmol/vol (24 h)

Table 2b. Incubation of collagen vicryl composite, collagen film and vicryl mesh in human urine. Effect of 24 h phosphate excretion

Patient No.	Phosphate excretion		Collagen/vicryl composite	Collagen film	Vicryl mesh
	mmol/24 h	mmol/l			
9 (2 samples)	64	32.0	+++ (Ca, P) † clean (1 sample) †	+ (Ca, K, Na)	+ (Ca, Fe) (1 sample) † clean (1 sample) †
7[a]	51.3	27.0	+++ (Ca, P, K)	+ (Ca, P)	+++ (Ca, P)
12	50.6	22.0	+++ (Ca, P) †	–	clean
5[a]	44.7	19.0	+ (Ca, K)	clean	clean
2[a]	44.6	17.5	+ (Ca, Na, P) ‡	–	–
10 (2 experiments with same urine)	40.7	22.0	clean (both samples)	clean (both samples)	clean (both samples)
16 (2 samples)	36.8	14.5	clean (both samples)	–	clean (both samples)
18[a]	34.6	26.6	+++ (Ca, P)	+++ (Ca, P)	clean
21	32.3	16.0	+ (Ca, P)	–	clean
3[a]	27.6	19.0	+++ (Ca, Na, P) ‡	–	–
20[a]	25.3	10.5	–	+ (K)	–
11	25.0	14.5	clean	clean	clean
15	21.3	8.0	+++ (Ca, K, P)	–	–
14[a]	21.1	11.0	++ (Ca, K)	–	–
1[a]	20.4	17.0	+++ (Ca, P)	+++ (Ca, P)	+++ (Ca, P)
19[a]	18.0	18.0	clean	clean	–
6[a]	12.8	8.0	clean	–	–
4[a]	8.9	3.1	clean	clean	clean

Incubations were carried out at 37 °C for two weeks unless otherwise stated († = 3 weeks, ‡ = 4 weeks)
+ = light deposition of urinary salts; ++ = medium deposition; +++ = heavy deposition. The elemental composition of these crystals is shown in brackets

[a] known clinical stone former: Normal range for phosphate excretion = 13–39 mmol/vol (24 h)

Table 2c. Incubation of collagen vicryl composite film and vicryl mesh in human urine. Effect of 24 h urate excretion

Patient No.	Urate excretion		Collagen/vicryl composite	Collagen film	Vicryl mesh
	mmol/24 h	mmol/l			
2[a]	8.7	3.4	+ (Ca, Na, P) ‡	–	–
9 (2 samples)	7.4	3.7	+++ (Ca, P) (1 sample) † clean (1 sample) †	+ (Ca, K, Na)	+ (Ca, Fe) (1 sample) † (1 sample clean) †
10 (2 experiments with same urine)	7.2	3.9	clean (both samples)	clean (both samples)	clean (both samples)
12	6.2	2.7	+++ (Ca, P) †	–	clean
16 (2 samples)	6.1	2.4	clean (both samples)	–	clean (both samples)
5[a]	5.9	2.5	+ (Ca, K)	clean	clean
20[a]	4.8	2.0	–	+ (K)	–
21	4.0	2.0	+ (Ca, P)	–	clean
1[a]	3.7	3.1	+++ (Ca, P)	+++ (Ca, P)	+++ (Ca, P)
18[a]	3.1	2.4	+++ (Ca, P)	+++ (Ca, P)	clean
11	3.0	1.7	clean	clean	clean
3[a]	2.9	2.0	+++ (Ca, Na, P) ‡	–	–
4[a]	2.9	1.0	clean	clean	clean
7[a]	2.5	1.3	+++ (Ca, K, P)	+ (Ca, P)	+++ (Ca, P)
15	2.1	0.9	+++ (Ca, K, P)	–	clean
14[a]	2.3	1.2	++ (Ca, K)	–	–
19[a]	2.0	2.0	clean	clean	–
6[a]	1.1	0.9	clean	–	–

Incubations were carried out at 37 °C for two weeks unless otherwise stated († = 3 weeks, ‡ = 4 weeks)
+ = light deposition of urinary salts; ++ = medium deposition; +++ = heavy deposition. The elemental composition of these crystals is shown in brackets

[a] known clinical stone formers: Normal range for urate excretion = 2.8–4.4 mmol/vol (24 h)

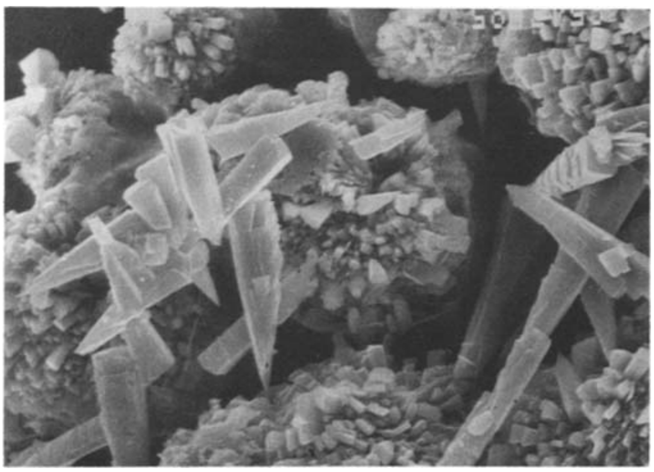

Fig. 1. Electron microscopy of typical calcium containing deposit found on surface of collagen vicryl composite after three weeks incubation at 37 °C (x 1800)

Collagen Film

The results of prolonged incubation of samples of collagen film with human urine collections are given in Tables 2a–c where it can be seen that from a total of ten urines used, deposits were found in five cases although one of these was only found to contain potassium. The same general comments can be applied to these findings as those of the collagen vicryl prosthesis described above, three quarters of the calcium containing deposits came from high calcium urines, an observation in keeping with the fact that the composite material also has a collagen surface.

No experiments were carried out with the collagen film and rabbit urine.

Vicryl Mesh

Tables 2a–c also summarise the SEM findings following the incubation of vicryl mesh in human urine. Generally, the use of simple vicryl mesh produced results similar to those obtained with the composite material in that those urines with a high calcium and phosphate content tending to form crystals on the surface. Overall, however, there appeared to be much less crystallisation on the vicryl mesh than was found either with the collagen vicryl composite or collagen film, in that only three urines out of the twelve tested produced surface deposits.

When the vicryl mesh was examined following incubation in a sample of rabbit urine at pH 8.2 for 7 and 17 days, no surface concretions could be found.

Vicryl Sutures

The results of these experiments are shown in Table 3. Of nine urines tested deposits were found in three cases, al-

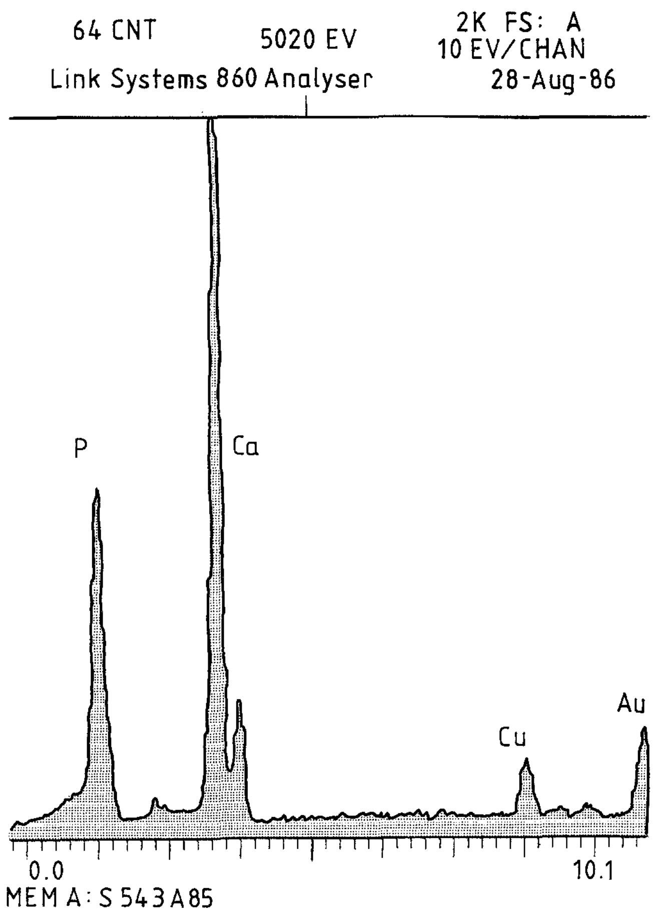

Fig. 2. Elemental analysis of the crystals shown in Fig. 1 using x-ray micro-analysis. Peaks identifying calcium and phosphorus can be clearly seen

Table 3. Incubation of absorbable sutures in human urine

Patient No.	Vicryl	Chromic gatgut	Plain gatgut
1[a]	–	clean	–
4[a]	–	clean	–
5[a]	–	clean	–
7[a]	–	clean	–
9	+ (Ca, P) ‡	–	–
(2 samples)	+++ (Ca, P) ‡		
10	clean	–	–
11	clean	–	–
12	clean	+++ (Ca, Cr) ‡	+++ (Ca, P)
13	–	clean	clean †
14[a]	–	+ (Ca, P, S) † clean ‡	clean †, + (Ca, Fe) ‡
15	clean	–	clean
16	clean	clean †	–
18[a]	++ (Ca, P)	–	–
19[a]	+ (Ca)	–	–
22[a]	clean	–	–

Incubations were carried out at 37 °C for two weeks unless otherwise indicated († = 1 week, ‡ = 3 weeks)
+ = light deposition of urinary salts; ++ = medium deposition; +++ = heavy depositon. The elemental composition of these crystals is shown in brackets

[a] known clinical stone former

though of these three only one was abnormally high in calcium.

No depositions were found following incubation in rabbit urine (pH 8.2) after 7 days.

Chromic Catgut

This material (Table 3), like vicryl, showed little tendency to act as a nidus for the crystallisation of urinary salts, even with the higher calcium urines. From eight experiments deposition was found following incubaton in two urines, only one of which had a high calcium content.

When chromic catgut was exposed to rabbit urine at pH 8.2 for seven and seventeen days, no concretions were observed. However, with another sample of rabbit urine at pH 8.5, some calcium crystallisation was found following twelve days incubation at 37 °C.

Plain Catgut

This material (Table 3) was tested solely in four samples of human urine, two of which produced surface depositions. One of these urines (No. 12) had a high calcium content. However, when incubated with rabbit urine at pH 8.2 for seven and seventeen days, or with a second sample at pH 8.5 for twelve days, no urinary depositions were detected.

Ethilon

Table 4 summarises the SEM examination of the surface of this material incubated in eight human urine samples. Of the four urines which caused calcium containing deposition of urinary salts, three had a high calcium content. No concretions were found on the Ethilon surface following incubation in the two samples of rabbit urine.

Silk

Of the three urines tested, two produced concretions (Table 4). Of these one urine was high in calcium and produced crystals in two separate experiments. No depositions were found after incubation in rabbit urine at pH 8.5 for twelve days.

Mersilene

All four urines which were tested with this material showed no urinary depositions after two weeks incubation. When, however, the experiment was prolonged to three weeks with three of the urines (Table 4), deposits were found in all cases. However, following incubation in rabbit urine at pH 8.5 for 12 days, no concretions were observed on the surface.

Table 4. Incubation of non-absorbable sutures in human urine

Patient No.	Ethilon	Silk	Mersilene
1[a]	+ (Ca, P, Na)	–	–
4[a]	clean	–	–
5[a]	clean	–	–
7[a]	+++ (Ca, P)	–	–
12	+ (Ca) ‡	++ (Ca, S, P, K, Cr) + (Ca, K, Cr) (2 samples)	clean
13	+ (No elements found – † presumably organic)	–	clean
14[a]	+++ (Ca, P)	clean †	clean (2 samples)
15	–	+++ (Ca, Cr) ‡	clean
16	clean	–	–

Incubations were carried out at 37 °C for two weeks unless otherwise indicated († = 1 week, ‡ = 3 weeks). With the Mersilene sutures, calcium containing deposits were found with urine nos. 12, 13, and 15 when incubations were extended to three weeks

+ = light depositon of urinary salts; ++ = medium deposition of urinary salts; +++ = heavy deposition of urinary salts. The elemental composition of these crystals is shown in brackets

[a] known clinical stone former

PDS and Prolene

While these materials were not tested in human urine, incubation for twelve days in rabbit urine at pH 8.5 did not produce detectable surface calculi on either of these two sutures.

Discussion

Owing to the supersaturated nature of urine, deposition of urinary salts on prosthetic materials used in urinary tract surgery is always a potential hazard, especially when one considers that such crystals can act as a nidus for the development of a urinary calculus. Indeed, this problem has been recognised for some time and over the last three decades there have been a number of relevant reports in the literature where many attempts have been made to assess an ideal suture for the urinary tract [1–5, 7–10].

In one report an insignificant occurrence of calculus formation in the bladder of guinea pigs was found after sixty and seventy days exposure to chromic catgut [1] and in a further paper these authors found no such incidences in dogs [2]. Other investigators [3] found depositions on both chromic catgut and dexon in the rabbit urinary bladder. In-vitro experiments by the same authors [3], using calcium phosphate solutions rather than urine, have shown that salt deposition could occur on silk as well as both chromic catgut and dexon. Earlier work however [4] had indicated that Dexon rather than catgut could lead to such problems following bladders closure.

Using three different sutures viz, vicryl, polyglycolic acid, and chromic catgut on experimental cystotomy wounds in dogs it was reported [5] that no significant calculus formation had taken place over thirty days with any of these materials. Other investigators [7] also found that no concretions formed after 120 days when chromic catgut or polypropylene sutures were used to close experimental wounds in the bladder of dogs. However, these authors [7], reported that concretions occurred with both silk and mersilene (a braided polyester). Using dogs a further report [8], showed that some calculus formation occurred on silk and to a lesser extent on chromic catgut, whereas polyglycolic acid showed no such tendency.

Depositions on both catgut and silk sutures were found after 1 to 2 weeks when they were employed to close experimental wounds in the bladder of rabbits [9].

An in-vitro technique was designed to assess the calculogenic potential of a number of different materials [10]. A supersaturated urine was produced by pulverising urinary stones extracted from patients and dissolving them in normal urine. Materials including silk, teflon coated dacron and cotton (non-absorbable multifilaments); nylon, polypropylene and polyethylene (non-absorbable monofilaments); plain catgut and chromic catgut (absorbable collagenous sutures), were then exposed to this urine in a circulating system. Crystalline deposits took place after 2–4 h on plain and chromic catgut and on all of the multifilament non absorbable materials. Conversely deposits were only found on the monofilament sutures following several days incubation. The results from this study, would therefore indicate that the physical structure of the suture may be of some importance.

In an in-vivo study [10] the same authors placed all of the different types of sutures into the bladders of female dogs and found concretions on all of the materials tested except for a teflon monofilament suture.

In the study presented in this report, we have attempted to assess the calculogenic potential of our collagen vicryl composite materials as well as a number of sutures which could be used to secure such a prosthesis in-vivo. Although it is not normal practice to use non-absorbable sutures in the urinary tract, a number of such materials were included for comparison. All materials were incubated in human urine over a period of up to three to four weeks. Experiments were also carried out for up to seventeen days in rabbit urine, a study which we consider relevant to our in-vivo work on these animals.

In this in-vitro evaluation we chose 24 h urine specimens both from nonstone forming and from known stone-forming patients, and urines showing a wide range of concentrations of those elements particularly likely to cause salt depositons such as calcium and phosphorus. Sutures, representing a variety of physical structures were also deliberately chosen.

While deposition of urinary salts on the test materials did not appear related to either pH or clinical stone formation, there was a tendency throughout the study for those urines high in calcium to cause concretions to form on the test materials. With the collagen coated vicryl prosthesis, approximately two thirds of the urines tested, including rabbit urine, formed deposits on the surface, a finding to be considered before commencing any in-vivo evaluation of this material. Similar results were obtained with the collagen film which may be expected as the composite has a collagen surface although the incidences of crystallisation were less. Under the conditions of our experiments much less crystallisation of urinary salts took place on both vicryl mesh and vicryl sutures (25% and 33% of cases respectively) after testing in human urine. Concretions were not found on either of these materials following incubation in rabbit urine. These findings would agree in general with those of other authors quoted above [5, 8]. Chromic catgut also appeared to cause fewer incidences of crystallisation (25%) although some deposition occurred with rabbit urine. No concretions were found with rabbit urine following incubation with plain catgut although depositions were found with human urine (2 cases out of 4 tested).

With Ethilon, a non-absorbable nylon monofilament suture calcium containing urinary deposits were observed in four of the eight human urines tested. No such crystals were found following incubation in rabbit urine. Silk, a braided suture gave similar results. With Mersilene, a braided polyester, deposits were found on three urine samples after three weeks but not after two weeks. No such crystals were found with rabbit urine. Prolene (a polypropylene monofilament) and PDS (polydioxanone suture, an absorbable monofilament) were clear of urinary salts following incubation in rabbit urine. Neither of these materials were tested in human urine.

The presence of chromium and iron found on some samples (Table 2a–c, 3 and 4) is almost certainly artefactual.

Clearly, the results presented in this study are entirely the result of an in-vitro investigation and therefore, great care should be exercised when extrapolating any of the findings to the in-vivo situation. With these limitations in mind, however, it appears that some problems with the deposition of urinary salts particularly from urines with a high calcium content could possibly occur when the collagen vicryl composite is applied to the urinary tract in-vivo and suggest that in the clinical situation it may be advisable to lower the urinary calcium before applying such a prosthesis e.g. by thiazide diuretics. The results also show that chromic catgut, vicryl sutures and vicryl mesh may be relatively "safe" materials in contact with urine. The findings with non-absorbable sutures have been included for comparison but such materials would not be used to secure the prosthesis in-vivo.

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