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Evaluation of the tissue reactions in the skin and body wall of koi (*Cyprinus carpio*) to five suture materials

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Five different suture materials (silk, monofilament nylon, polyglyconate, polyglactin 910, and chromic gut) were placed in the skin and body wall of 10 Doitsu (scaleless) koi (*Cyprinus carpio*). After seven days the sutures were retrieved from five of the fish in 5 mm and 6 mm punch biopsies, and after 14 days they were retrieved in the same way from the other five. The tissue reactions were evaluated by gross visual inspection and by histological examination. The total inflammatory reaction was graded on a scale from 0 (no inflammation) to 5 (severe inflammation). The synthetic suture materials generally induced a moderate inflammatory reaction that decreased after seven days. After 14 days the superficial reaction to monofilament nylon was substantial, and the tissue reactions to the organic suture materials were slightly greater than the reactions to the synthetics. The inflammatory response to silk was greater after 14 days than after seven, and chromic gut induced a moderately severe inflammatory response after seven days; the chromic gut sutures fell out before the biopsies were taken after 14 days. The organic materials induced intense inflammatory reactions which did not subside if the suture remained in the tissue.

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INTEREST in fish medicine is growing rapidly, and there has been an increasing demand for veterinary expertise in treating ornamental fish but, until recently, surgery has not been applied routinely to fish in clinical practice (Hart and Summerfelt 1975, Petering and Johnson 1991, Gilliland 1994, Lewbart and others 1995, 1998, Harms and Lewbart 2000, Lewbart 2001).

As a result of the growing interest in surgical techniques for fish, the authors investigated five suture materials for use in koi. An ideal suture would provide high tensile strength and knot security, resist infection and contamination, and react minimally with the tissue in which it was embedded (Bellenger 1982, Fossum 1997). Tissues respond to sutures as they would to any foreign material. If the body's response to the suture is intense, the suture itself may be broken down, or the tissue surrounding the suture may be destroyed. The possible consequences of a severe tissue reaction include oedematous and friable tissue that has less holding capacity for the sutures, infection that enhances the inflammatory response, and dehiscence that results from the loss of integrity of the incision (Stashak and Yturraspe 1978, Varma and others 1981, Sanz and others 1988). It would therefore be ideal to utilise a suture material that would induce minimal tissue responses. The 'perfect' suture material may never become available, and surgeons will have to examine each surgical problem and select the appropriate materials accordingly (Wood and others 1984, Greenwald and others 1994, Bennett and others 1997, Fossum 1997).

This paper describes the evaluation of the tissue reactions to five suture materials placed in the body wall of Doitsu (scaleless) koi (*Cyprinus carpio*), and is, to the authors' knowledge, the first to be carried out in this species, and a first step towards understanding the reactions to sutures in fish. The following suture materials were investigated: silk (Ethicon), monofilament nylon (Dermalon; Davis and Geck, American Cyanamid), polyglyconate (Maxon; Davis and Geck), polyglactin 910 (Vicryl; Ethicon) and chromic gut (Ethicon). Similar studies have been made in other species, including dogs, cats, birds, rats and human beings (Postlethwait 1970, Postlethwait and others 1975, Wood and others 1984, Freeman and others 1987, Sanz and others 1988, Greenwald and others 1994, DeNardo and others 1996, Bennett and others 1997), and some of the results suggest that some materials are tolerated equally by different species; however, these results may not apply to fish owing to the significant environmental and anatomical differences. Achieving a sterile field is a formidable task in fish surgery, and after surgery the fish is immersed in a sea of potential pathogens that can readily invade if the protective epidermal cuticle is compromised (St Louis-Cormier and others 1984, Stoskopf 1993, Noga 2000, Lewbart 2001). In spite of these differences, some research has suggested that the processes involved in the repair of fish tissues and their inflammatory responses are similar to those observed in homeothermic vertebrates, even though they may take longer to occur (Finn and Nielson 1971, Mawdesley-Thomas and Bucke 1973).

MATERIALS AND METHODS

Ten Doitsu koi (mean weight 94 g) were obtained from a koi farm in North Carolina. They were kept in a single 300 litre

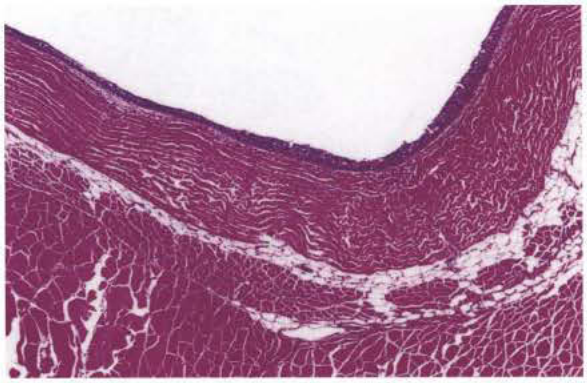

FIG 1: Control koi, day 7, showing the relatively uniform epidermal layer (top) only a few cell layers thick, the lack of scales, and the subjacent muscle layers that constitute the body wall. The layer of multiloculated clear spaces in between the muscle layers is normal adipose tissue, whereas the clear spaces between individual muscle bundles are due to artifactual separation during the manipulation of the surgical biopsy and immersion fixation. Haematoxylin and eosin. x 13·2

circular tank in dechlorinated city water maintained at 21±1°C, fed a maintenance ration of commercial koi feed (Hikari Gold; Kyorin Food), and kept on a 16-hour light, eight-hour dark cycle. They were allowed to acclimatise for one month before the study began.

All the surgical procedures were performed in one day by the same surgeon (D. C. B.). Anaesthesia was induced by immersing each fish in a 200 mg/litre solution of tricaine methanesulphonate (MS-222) (Finquel; Argent Chemical Laboratories) buffered with sodium bicarbonate, and a surgical plane of anaesthesia was maintained with a 100 mg/litre solution of buffered MS-222 (Harms 1999, Lewbart and Harms 1999). The anaesthetic agent was constantly delivered by an anaesthetic delivery system, which was constructed according to methods outlined by Lewbart and Harms (1999). After preparing each site by gently wiping with a sterile gauze sponge, three approximately 5 mm long by approximately 2 mm deep stab incisions were made in the left lateral body wall of the koi with sterile scalpel blades. Polyglyconate, polyglactin 910 and silk sutures were placed to appose the skin edges of the incisions made in the body wall. Two similar stab incisions were made in the right lateral body wall of the koi, and monofilament nylon and chromic gut sutures were placed to appose the skin edges of these incisions. One simple interrupted tie was used to close each incision, and each of the five types of suture was used in all the fish. Each fish served as its own control (Fig 1). After the surgery, each fish was given 0·1 mg/kg butorphanol tartrate (Butorphanol; Ben Venue Laboratories) intramuscularly for analgesia, and then placed in a 20 litre tank of fresh water. When it had recovered, it was replaced in the original 300 litre circular tank (Harms and Lewbart 2000).

Seven days after the surgery, five fish were randomly selected and anaesthetised. A single 5 mm diameter by approximately 3 mm deep punch biopsy of each incision site was taken, with the sutures in situ. A sixth punch biopsy was taken from the right lateral body wall as a control. This procedure was repeated on the remaining five fish 14 days after the surgery. After the biopsies, 0·1 mg/kg butorphanol were administered intramuscularly and povidone iodine ointment was applied to each biopsy site to help check bleeding and prevent infection. The biopsy sites were left to heal by second intention and no additional sutures were used to close the biopsy wounds. All 10 fish survived the study.

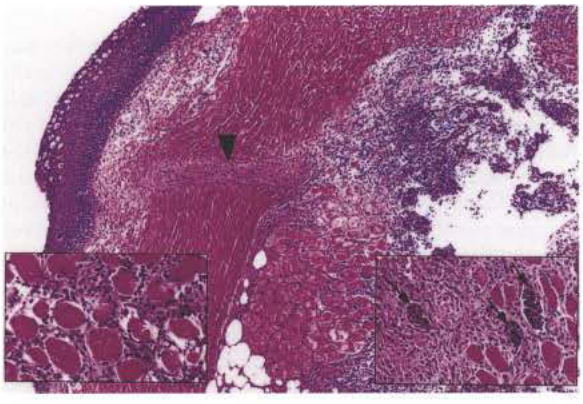

FIG 2: Silk, day 7. Example of a grade 3 inflammatory score, showing the pale area of fibrosis, perpendicular to the epidermal surface, which is the tract left by the silk suture material (arrowhead). Deep to this is a focally intense infiltration of mixed inflammatory cells, with necrosis and loss of musculature (clear space). Such an intense reaction can compromise overlying tissues and interfere with healing. Haematoxylin and eosin. x 16. Left inset Silk, day 14. Skeletal muscles bundles are separated and surrounded by inflammatory cells, and several are pale, swollen and beginning to show loss of striations. Haematoxylin and eosin. x 80. Right inset Silk, day 14. Several small blood vessels are occluded by fibrinocellular thrombi (arrows), with necrosis and loss of adjacent muscle bundles. Haematoxylin and eosin. x 80

TABLE 1: Ranges and median inflammatory scores associated with five suture materials in the body wall of scaleless koi, seven and 14 days after surgery

Suture material	Seven days Range	Seven days Median	14 days Range	14 days Median
Silk	3-3	3·0	3-5	3·5
Monofilament nylon	3-4	3·0	3-4	3·0
Polyglyconate	2-4	3·0	2-4	3·0
Polyglactin 910	3-4	4·0	2-4	3·0
Chromic gut	3-4	4·0	NA	NA

NA Not applicable

The tissue samples were immediately fixed in 10 per cent neutral-buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin for examination by light microscopy. The tissue sections were randomised and evaluated blindly by a single pathologist (J. M. L.). A six-point grading scale was used for each section as follows: grade 0 No apparent changes from normal skin and underlying muscle/subcutis; grade 1 Very mild changes, with minimal inflammatory cells and oedema, the suture tract having minimal fibrosis and well-organised granulation tissue; grade 2 Mild changes; grade 3 Moderate changes, these sections had moderate mixed inflammatory infiltrates, including a significant complement of macrophages and scattered foreign body giant cells which expanded and replaced areas of the dermis and subcutis, there was also moderate oedema and scattered necrosis of individual muscle bundles; grade 4 Moderately severe changes; and grade 5 Severe changes. Grade 5 sections had marked inflammation, oedema and larger areas of coagulative necrosis of muscle bundles; the suture tract area was poorly organised, with more exuberant granulation tissue, and there were superficial clear spaces representing separation/clefting at the dermal-epidermal junction, probably due to the action of lytic enzymes produced by infiltrating leucocytes.

The data were analysed by the Friedman two-way analysis of variance. When significant differences were found, multiple comparison procedures were used to determine which sutures were different. The non-parametric Wilcoxon matched-pairs signed-rank test was applied to the data. $P<0.05$ was regarded as statistically significant.

RESULTS

Friedman's two-way analysis of variance showed that there was a significant difference between the treatments after seven and 14 days ($P<0.001$). However, the Wilcoxon matched-pairs signed-rank test showed no significant difference between the sutures on each day. Since all the control samples were assigned an inflammatory score of 0, they did not have to be subtracted from the data.

Table 1 shows the ranges of the inflammatory scores and the median scores associated with the suture materials after seven and 14 days. All the biopsies were associated with varying degrees of tissue oedema, necrosis of muscle bundles, granulation and fibrosis of the suture tracts. After seven days, all the sutures remained in place and the amount of inflammation observed in the tissues around each type of suture was quite similar; acute or a mixture of acute and chronic inflam-

matory cells were observed in all of them. After 14 days, the inflammatory response to silk had increased whereas the tissue reactions to polyglyconate and polyglactin 910 had decreased slightly (Fig 2), although foreign body-type giant cells were identified engulfing the residual polyglyconate and polyglactin 910 suture materials (Fig 3). The tissue response to monofilament nylon after 14 days was similar to that observed after seven days, but there had been a significant superficial erythematous reaction where the nylon suture tags contacted the skin. All the chromic gut sutures had been lost in less than 14 days. None of the sutures induced an inflammatory response containing large numbers of eosinophils.

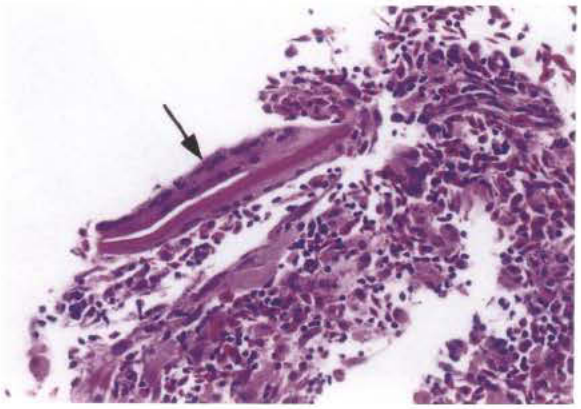

FIG 3: Polyglyconate, day 14. Within the dermis, there is a focal, moderate inflammatory reaction consisting of macrophages, lymphocytes and plasma cells. A multinucleate giant cell can be seen (arrow) with an engulfed fragment of suture material. Haematoxylin and eosin. x 100

DISCUSSION

The passage of a needle through tissue can cause an inflammatory reaction, and this reaction may be difficult to distinguish from the reaction to the suture material itself, at least initially (Bennett and others 1997). By examining the incisions seven and 14 days after surgery, it was hoped that it would be possible to make such a distinction.

Silk

Manufactured from the material produced by the silkworm, silk sutures are multifilament, organic and non-absorbable. Of the 10 suture materials evaluated by Greenwald and others (1994), silk was shown to have the least strength and toughness in vivo. Silk has been shown to wick bacteria into a wound, and significant tissue reactions to silk have been observed in many species (Knowles 1976, Bellenger 1982, Wood and others 1984, DeNardo and others 1996). Gammaglobulin has been shown to bind to silk and result in the activation of complement and the initiation of an acute inflammatory reaction. Polymorphonuclear chemotaxis and degranulation results in the release of an arsenal of hydrolytic and cytotoxic enzymes which result in tissue necrosis (Bart and Dunham 1990, Kennedy-Stoskopf 1993). Of the biomaterials evaluated by Wood and others (1984), silk caused a severe inflammatory response in the linea alba of dogs that increased with time; in contrast, in people and rabbits, silk tended to cause a less severe reaction that decreased with time. In the koi, the silk sutures induced a moderate inflammatory response in all the fish biopsied after seven days (Fig 2) which was similar to the responses induced by the nylon and polyglyconate sutures. Chromic gut and polyglactin 910 induced higher median inflammatory scores with a wider range than silk. After 14 days, silk induced the highest average histological grade, the inflammatory response having intensified grossly and histologically. The results are consistent with those of Varma and others (1981) who reported that, in dogs, silk induced a mild neutrophilic tissue reaction in infected wounds after six and 10 days, but a more severe reaction characterised by activated macrophages and fibroblasts after 20 and 40 days. Silk sutures were easy to manipulate and handle, but its use in fish surgery may be limited by the severity of the tissue reaction observed and by the likelihood of its wicking bacteria into surrounding tissues after the surgery.

Monofilament nylon

Nylon is a synthetic, non-absorbable, amide-containing thermoplastic that has been successfully used to close wounds in fish (Stoskopf 1993). Degraded by slow hydrolysis, biologically inert nylon sutures are relatively permanent and induce minimal tissue reactions (Bellenger 1982, Bennett and others 1997). DeNardo and others (1996) used monofilament nylon sutures to close wounds in the oral cavity of cats; they observed a moderate tissue reaction and showed that the nylon sutures were the most resistant to bacterial adherence. The tissue response in rock doves (*Columba livia*) was mild and consisted of a mixed inflammatory infiltrate of macrophages, lymphocytes, plasma cells and occasional granulocytes (Bennett and others 1997). In the koi, the inflammatory reaction to nylon was graded 3 to 4 after seven and 14 days, and was thus slightly more reactive than the other two synthetic sutures (polyglyconate and polyglactin 910). After seven days, it was histologically slightly more reactive than silk and slightly less reactive than chromic gut, but after 14 days silk was more reactive than nylon. Grossly, the superficial skin reaction to nylon was severe with slight oedema and significant erythema. These results differ to some extent from the results of other studies in which milder tissue reactions have been observed to monofilament nylon.

Nylon may be an adequate choice for sutures in fish, but the significant superficial reaction to the material in fish skin cannot be overlooked. The superior performance of the absorbable synthetics (polyglactin 910 and polyglyconate) make these a better option for sutures in the body wall of koi.

Polyglyconate

Polyglyconate is a synthetic, monofilament, absorbable copolymer of glycolic acid and trimethylene carbonate that induces a mild tissue reaction in most species. It has great strength and toughness and maintains good knot security (Greenwald and others 1994). In the koi, the polyglyconate sutures induced lower average histological grades after seven and 14 days than any of the other natural or synthetic materials. These results are consistent with those reported by Sanz and others (1988), who showed that 16 days after surgery polyglyconate had induced a milder inflammatory reaction in the facial tissue of rats than chromic gut or polyglactin 910. The population of cells that characterised the inflammatory reaction in koi was consistent with the findings of Freeman and others (1987), Sanz and others (1988) and Bennett and others (1997). After seven days there was a mixture of acute and chronic inflammatory cells and, after 14 days, there was an infiltrate of fibroblasts, macrophages and multinucleate giant cells. One polyglyconate suture fell out before the biopsies were taken after 14 days.

These results suggest that monofilament polyglyconate may be the most appropriate suture material to use in the body wall muscle and skin of koi. This synthetic, absorbable product is slowly degraded by hydrolysis and induced the mildest tissue reactions after seven and 14 days. Given the high risk of bacterial contamination of the surgical site during fish surgery, a monofilament material is desirable to decrease the risk that bacteria can be wicked into the tissue surrounding the suture tract.

Polyglactin 910

Polyglactin 910 is a synthetic, multifilament, absorbable copolymer of glycolide and lactide. It is slowly degraded by hydrolysis and is more susceptible to breakdown and absorp-

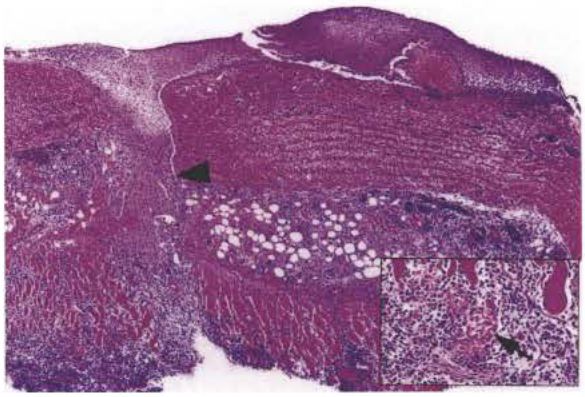

FIG 4: Chromic gut, day 7. Example of a grade 4 inflammatory score. The suture tract (arrowhead) extends from the epidermal layer to the deeper layers of the dermis. Wicking of pathogens by this suture material from the moist surgical field induced a marked inflammatory response that resulted in panniculitis and steatitis that can undermine overlying tissues. Haematoxylin and eosin. x 10. Inset Chromic gut, day 7. Detail of inflammatory reaction showing an individualised muscle bundle (arrow) undergoing necrosis which is pale, swollen, and fragmented, with loss of striations. Haematoxylin and eosin. x 80

tion in alkaline environments (DeNardo and others 1996). Generally, polyglactin 910 is considered a strong material that gives good knot security and induces a mild tissue reaction in many species (Bellenger 1982, Fossum 1997). DeNardo and others (1996) reported that the tissue reaction to polyglactin 910 sutures placed in the oral cavity of cats was moderate to marked and that bacteria were consistently observed in the interstices of the braided suture. The tissue reaction in the linea alba of cats developed from a mildly purulent reaction after one day to a severely fibromononuclear response after 14 days (Freeman and others 1987). Bennett and others (1997) showed that, in the body wall of rock doves, the reaction to polyglactin 910 was characterised by fibroblasts, macrophages and multinucleate giant cells, and was the most severe induced by the suture materials they evaluated. Gilliland (1994) showed that polyglactin 910 sutures had a mean absorption time of seven weeks in largemouth bass (*Micropterus salmoides*) maintained at 18°C, suggesting that they may be suitable when an incision required extended support and intact sutures will not be an irritant during recovery; he also reported that the grossly observable tissue response to polyglactin 910 was greater than that to chromic gut and polydioxanone. In the koi, the median inflammatory score of the tissue reactions to polyglactin 910 was moderately severe after seven days and moderate after 14 days. The population of cells that characterised the inflammatory reaction was consistent with the findings of Freeman and others (1987), Sanz and others (1988) and Bennett and others (1997). After seven days the inflammatory response was similar to the responses observed with the natural suture materials silk and chromic gut and, after 14 days, fibroblasts, macrophages and multinucleate giant cells were present. Compared with the other synthetic products (nylon and polyglyconate), it was the most intense after seven days; this finding is consistent with the results of Sanz and others (1988) who evaluated inflammatory reactions to sutures in the facial tissue of rats. After 14 days, the inflammatory reaction had decreased rather than intensified as it had with the silk sutures. Polyglactin 910 did not induce a very severe reaction that intensified over time, like that described by Bennett and others (1997). The polyglactin 910 suture is braided, but was easy to manipulate and handle. However, its use in fish surgery may be limited because of the inherent risk of contamination of the surgical site by the wicking of bacteria into the tissues.

Chromic gut

Chromic gut is an organic, monofilament, absorbable suture material that is commonly used in mammals and birds (Stashak and Yturraspe 1978, Bennett and others 1997). Catgut, which is made from bovine or ovine intestine, is treated with chromium salts to increase its tensile strength, delay its absorption, and decrease its tissue reactivity (Bellenger 1982). It is degraded by the activity of proteolytic enzymes, macrophages and giant cells. In many species, the tissue reaction is locally severe because, except in cattle and sheep, it acts as a xenograft (Knowles 1976, Varma and others 1981, Sanz and others 1988, DeNardo and others 1996, Fossum 1997). In the koi, the chromic gut sutures, which were acting as transclass grafts, generally induced a moderate to moderately severe inflammatory response after seven days (Fig 4), a finding consistent with the results of other studies. Of the 10 suture materials evaluated in rats by Greenwald and others (1994), chromic gut induced the most severe inflammation, with extensive cellular infiltrates and wide 'reaction zones'. In the linea alba of cats the sutures induced an inflammatory reaction that progressed from moderately purulent, to severely fibromononuclear, to fibrous in 14 days (Freeman and others 1987). In rock doves, Bennett and others (1997) observed a similar transclass graft reaction to that observed in the koi; the tissue reaction was marked, and the suture material induced a prominent granulocytic infiltrate that gradually resolved after 30 days (1997). Gilliland (1994) showed that, in largemouth bass maintained at 18°C, chromic gut had a mean absorption time of five weeks, suggesting that sutures of the material may be suitable when healing is expected to be rapid and rapid absorption of the sutures is desirable.

There were no chromic gut sutures left in the fish biopsies 14 days after surgery, and no suture remnants were observed histologically in the suture tract; it is therefore more likely that the sutures were lost rather than absorbed. In light of this finding and the moderate to severe reactions observed after seven days, chromic gut sutures may not be appropriate for use in koi skin, especially if the sutures are required to remain in place for longer than seven days.

CONCLUSION

The results of this study suggest that monofilament polyglyconate is the most appropriate suture material to use in the body wall muscle and skin of koi. This synthetic, absorbable product induced the mildest tissue reactions after seven and 14 days and, given the high risk of bacterial contamination of the surgical site during fish surgery, a monofilament material is desirable to reduce the risk of bacteria being wicked into the tissue surrounding the suture tract. For this reason, multifilament polyglactin 910 is likely to be less suitable. Monofilament nylon may be an adequate choice for sutures in fish, but there was a significant superficial reaction to the nylon in the skin of the koi. The synthetic products were generally better than the organic products, silk and chromic gut; silk, a multifilament material, was the most reactive material, and the chromic gut sutures were reactive after seven days and then lost. The superior performance of the monofilament, absorbable, synthetic polyglyconate renders it the best of the materials evaluated for the placement of sutures in the body wall of koi.

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