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Comparison of ear tissue regeneration in mammals

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INTRODUCTION

Rabbits have the capacity to regenerate new tissue for repairing holes in their ears. This phenomenon was discovered by Markelova in 1953, as cited by Vorontsova & Liosner (1960). The growing numbers of investigations have been reviewed regularly since then (Joseph & Dyson, 1966*a*; Goss & Grimes, 1972; Carlson, 1978*a*; Williams-Boyce & Daniel, 1980). As ear tissue regeneration is one of the few good examples of epimorphic regeneration in mammals, it is important to know what other species besides the rabbit have this capacity.

Goss & Grimes (1975) claimed that the lost ear tissue of sheep and dogs will not regenerate, and that rabbits are unique as the only mammals capable of regenerating ear tissues. These authors attributed this failure of regeneration in sheep and dogs to the lack of development of epidermal downgrowths, which they believe form as a result of a chondroepithelial interaction. Goss (1980) suggests that, in the absence of epidermal downgrowths, scar tissue forms and thereby interferes with blastema cell accumulation, so that ear tissue also does not regenerate in deer, armadillo, opossum, chinchilla, guinea-pig, hamster, gerbil, cavy, rat and mouse. Goss demonstrated ear tissue regeneration in hares, pika and cats. In the ears of fruit bats holes were not closed, but in two species of insectivorous echolocating bats the ear holes were filled but the regenerated areas had no cartilage. Carlson (1978*a*) also implies that rabbits are unique in this capacity to regenerate lost ear tissues, although no specific attempts in other animals are cited.

In the present study, to contribute to the expanding list of mammals tested and to confirm or refute the existing reports, fifteen genera of animals, normally maintained in laboratory, zoo or agricultural facilities, were ear punched to determine what other species might possess the capability to regenerate lost ear tissues.

MATERIALS AND METHODS

Examples from four orders of mammals were compared with the rabbit of the order *Lagomorpha*. Representatives of four families of the order *Rodentia* were selected, namely, mice, rats, chinchillas and the springhare. The latter animal occupies a similar ecological niche in Africa to that occupied by the rabbit in North America. Three families from the order *Primates* were selected (1) to compare Old and New World monkeys and the marmoset, and (2) as potential clinical models. Three families from the order *Artiodactyla* were used; cows, pigs and sheep as agricultural models and the dik-dik as an exotic model. From the order *Carnivora*, the dog was selected as a potential clinical veterinary model. When available, both males and females

had their ears punched to determine whether the same variation existed between sexes in success of regeneration as observed in the authors' initial studies of the rabbit (Williams-Boyce & Daniel, 1980).

Anaesthesia was used to prevent discomfort to the animals and to aid in restraining them. Rabbits, chinchilla and springhare were tranquillised with intramuscular injections of Innovar-Vet, 0·3 ml/kg body weight. Mice and rats were anaesthetised with Avertin, 0·1 ml/5 g body weight. Dogs were anaesthetised with Tiopental. Primates were anaesthetised with ketamine hydrochloride, 10 mg/kg. Livestock species were tranquillised with acepromazine administered intravenously.

The animals were restrained, the ears shaved and cleaned with alcohol and holes punched in them with gasket punches ranging in diameter from 2 to 10 mm, dependent on the size of the animal's ear. Holes were made in areas of both ears between the medial ear artery and the marginal ear veins, where there are few major vessels. Any excess bleeding was stopped with slight pressure from a gauze pad. The rate of ingrowth of the ear tissue was recorded as a percentage of the original wound diameter.

For histological preparations, tissues from the original punched area and from punches made after wound closure were fixed in Bouin's fixative, dehydrated in alcohol, cleared in xylene, embedded in paraffin, sectioned at a thickness of 10–15 μm, stained with haematoxylin and eosin and photographed.

Since the regeneration of the cartilage layer was used in the original work as a sign of successful regeneration in the rabbit, the histology of the cartilage was also used here to evaluate ear tissue regeneration success. Comparisons were made between distinctive histological characteristics of the rabbit ear tissue regenerates and those of the other mammals.

RESULTS

Success of regeneration

Table 1 lists the animals tested, the success of closure of the ear wound, with or without cartilage regeneration, and the duration of the observation, and/or time needed to close and regenerate.

It can readily be seen from the Table that wound closure was not necessarily synonymous with regeneration, since there were animals that closed their wounds without replacement of the cartilage layer (i.e. non-regenerating). In the rabbit, closure was accompanied by continued growth of the tissues until morphogenesis was complete, but some individuals of the other mammals tested closed their wounds with fibrous scar tissue or epidermal proliferation but showed no sign of cartilaginous regeneration. In some of those other animals whose wounds closed, thickened regions composed of immature cartilage cells extending distally over the wound were seen histologically.

Histological observations on the regeneration of the cartilage layer

The normal ear contains a middle layer of elastic cartilage with its thin layer of perichondrium. On each side of the cartilage there is dense connective tissue covered by dorsal and ventral skin. The primary ear punches of all the animals tested were composed of these same tissues and differed very little histologically.

In histological sections taken from regenerated tissues, two different types of cartilage replacement were seen. In one type cartilage regrowth appeared to have

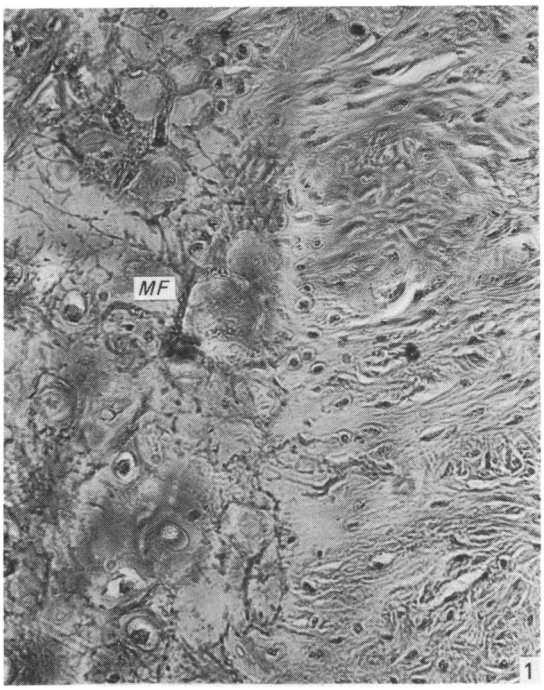
Table 1. *Ear tissue regeneration in mammals*

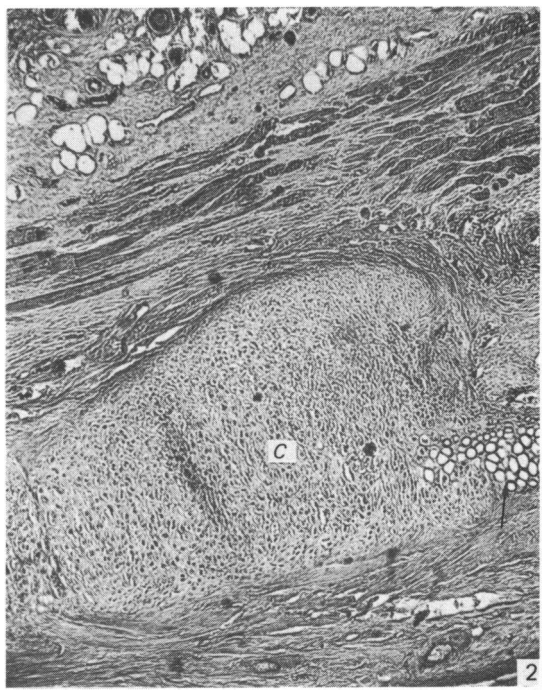
Animal*	Number and sex	Closure†					Closure time‡
				Complete regeneration			
		None	Partial	Non-Reg.	Reg.	% Reg.	
Rabbit	10 male	0	6	0	14/20	70%	5–6 weeks
Oryctolagus cuniculus	9 female	0	12	0	6/18	33%	5–6 weeks
Chinchilla	5 male	0	2	2	6/10	60%	4–11 months
Chinchilla laniger	5 female	0	7	1	2/10	20%	6–10 months
Cow	3 female	0	0	2	4/6	67%	1·5–2·0 months
Bovis bovis							
Pig	3 male	0	1	1	4/6	67%	2–2·5 months
Sus scrofa	3 female	0	1	3	2/6	33%	2–2·5 months
Rat	5 male	0	0	8	2/10	20%	5 days
Rattus rattus							
Mouse	5 male	1	7	1	1/10	10%	3 weeks
Mus musculus	4 female	1	0	6	0/7	0	1·5–2 weeks
Marmoset	5 male	0	9	0	1/10	10%	7 months
Callithrix jacchus							
Stumptail monkey	2 male	0	3	0	1/4	25%	5 months
Macaca arctoides	1 female	0	2	0	0/2	0	5 months
African green monkey	3 female	0	6	0	0/6	0	5 months
Cercopithecus aethiops							
Rhesus monkey	3 male	0	6	0	0/6	0	5 months
Macaca mulatta							
Squirrel monkey	2 male	0	4	0	0/4	0	5 months
Saimiri sciureus	1 female	0	2	0	0/2	0	5 months
Springhare	2 male	0	0	1	1/2	10%	4 months
Pedetes capensis	1 female	0	1	0	0/1	0	6 months
Sheep	2 female	0	0	2	0/2	0	2 weeks
Ovis aries							
Dog	1 male	0	0	2	0/2	0	11–15 days
Canis familiaris	1 female	0	0	2	0/2	0	11–15 days
Kirk's dik-dik	2 male	2	0	0	0/2	0	6 months
Madoqua kirkiz							

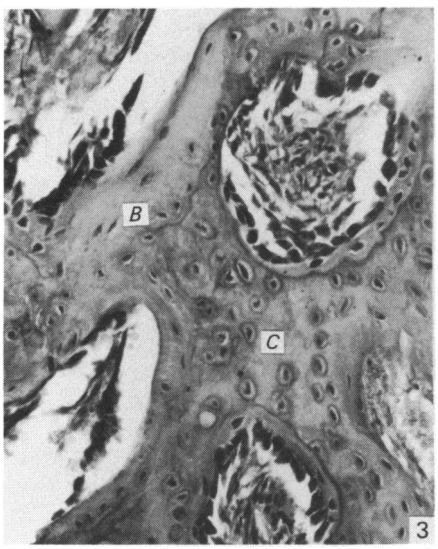
* Arranged in decreasing order of success.
† Tabulated as number of ears.
‡ Duration of observation.
Non-Reg, no visible cartilage regeneration; Reg, visible cartilage regeneration.

occurred from the perichondrium, and in the other type from 'undifferentiated' mesenchymal or blastema cells.

Figure 1 shows ear regeneration as seen in the cow. The original cut edge of the mature cartilage was easily visible in the elastic matrix. Within thick collagenous strands at the wound centre there were rounded cells with a mesenchyme-like appearance. These 'blastema' cells were seen to have aggregated along the edge of the mature cartilage. In some locations cells with an intermediate appearance, more like that of immature cartilage, were seen at higher magnifications. Even though the ear punch wounds in the cow were closed by two months, regeneration of the cartilage layer was never complete in that time.







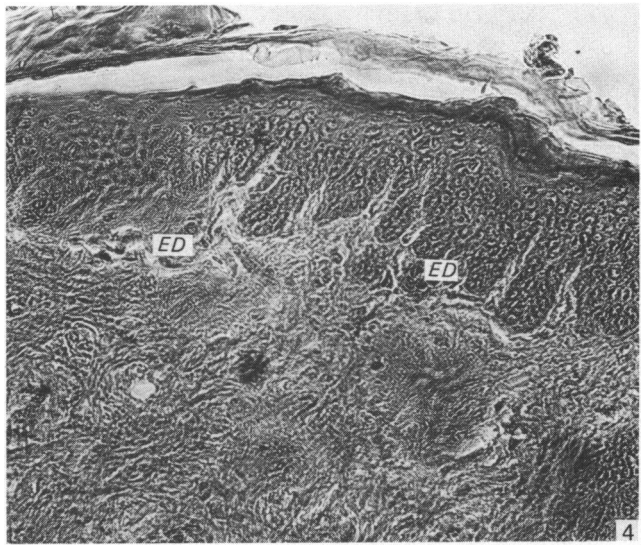


Fig. 1. Section from the regenerating tissues of a cow's ear, showing the line of biopsy with ear punching. Tissue to the left is mature cartilage with obvious matrix fibres (*MF*); to the right, newly formed tissue containing mesenchyme-like or blastema cells, collagenous fibres and scattered fibroblasts. × 65.

Fig. 2. The cartilage 'cone' region (*C*) of the regenerating ear tissue of the chinchilla 10 months after punching. Arrow points to the old cartilage sheet with its obvious lacunae which is seen at the right of the section. × 26.

Fig. 3. Section from the regenerating ear tissue of a rabbit 256 days after punch. Bone (B) surrounds the marrow cavities and cartilage (*C*) is found in the region between. The marrow cavities are lined with chondroblasts. × 113.

Fig. 4. Section from the leading edge of the epithelium in the regenerating ear tissues of a stumptail monkey. Epidermal downgrowths (*ED*) are seen around the periphery of the wound. × 65.

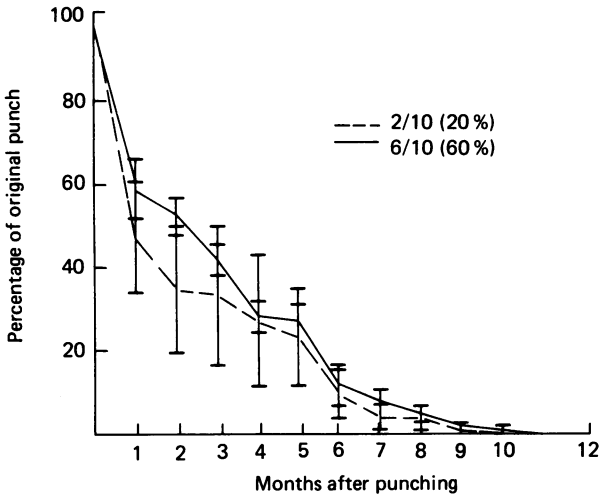


Fig. 5. Duration of closure after ear punching in adult chinchillas. Broken line, females; solid line, males. Ratios are number of ears closing/number of ears tested.

Sections of the cartilage proliferation region in the chinchilla and rabbit ear are shown in Figure 2. This region was distinguished by a 'cone' of aggregating immature cartilage cells. The cone appeared to have arisen from a proliferation of the perichondrial region and was made up of chondroblasts which would form the chondrocytes of the new cartilage. At the periphery of the cartilage cone there was a layer very similar to the perichondrium found in the normal ear cartilage, and it was continuous with the perichondrium of the mature cartilage. Chondrogenesis occurred at the cut edge of the old sheet of cartilage, with collagen organised into linear bundles (identified by trichrome staining). In the chinchilla, a perichondrial cone was never seen in sections where regeneration failed to occur.

This same observation, of cone proliferation from the perichondrium and of collagen appearing in linear bundles at the site of regeneration, was also seen in both rabbit and cow. In the cow, the bundles were short, numerous and more randomly arranged (Fig. 1).

One unique event in the regenerating cartilage layer was seen in a rabbit ear, biopsied at 265 days after punching. Not only was the original layer of cartilage replaced, but in the thickened layer of perichondrium which surrounded it a network of trabecular bone was found (Fig. 3). This resembled sections of immature bone as seen in the developing mammalian embryo. The bone was surrounded by cartilage which on staining with haematoxylin and eosin was basophilic, whereas the bone was acidophilic. In this sample, the perichondrial layer was greatly thickened surrounding the regenerated cartilage and it was within this thickened layer that the bone developed. The bone trabeculae enclosed spaces which were similar to both Haversian canals and marrow cavities. Osteoblasts with their darkly staining cytoplasm were arranged along the surface of the trabeculae. In some regions, the bone was similar to compact bone with a more regular matrix and smaller Haversian canals. No evidence of bone was seen in the regenerated tissue of any other species.

In addition to the distinctive characteristics of the regenerating cartilage layer, epidermal downgrowths were seen in the regenerating ear tissues of a stumptail

monkey. In Figure 4, the downgrowths can be seen at the dermal–epidermal interface. These downgrowths were not as large as those seen in the rabbit but these animals were also not as successful at regeneration. The tissue under the epidermis contained large quantities of randomly arranged collagenous fibres.

Location of the punch

To test the effect of location of punch on success of regeneration, adult chinchillas of both sexes were punched on the distal and proximal portions of the ear. The animals were unsuccessful in replacing tissues lost in punches placed near the outer margin of the distal portion of the ear, but when the punches were placed proximally regeneration was successful. Closure of a 0·5 cm hole took an average of 10 months. There was also variation in success of regeneration in different sexes. Males were 75 %, 60 % and 67 % successful, for the rabbit, chinchilla and pig respectively, while females of the same species were 33 %, 20 % and 33 % successful. Chinchilla data are shown graphically in Figure 5.

DISCUSSION

Ear tissue regeneration cannot be considered a phenomenon limited to rabbits. The rabbit remains the most successful of all mammals tested, but the chinchilla, cow, pig, rat, mouse, marmoset, stumptail monkey and springhare also show limited success by the methods used in this study. These results differ from those of Goss (1980) in regard to chinchilla, rat and mouse but confirm the earlier report for dogs and sheep. Some of these differences may reflect the length of the period of observation, the sex of the animal or the interpretation of 'regeneration' or 'wound closure'.

One factor especially in the reported lack of success with other animals may have been the location of the punch. This phenomenon of 'location effect' was demonstrated by punching three different sites simultaneously on the rabbit ear (Williams-Boyce & Daniel, 1980). Proximal punches closed faster and more frequently than medial or distal punches. No experimental evidence has been obtained to explain this phenomenon but it may be related to differences in vascularisation and/or the thickness of the cartilage layer (Williams-Boyce & Daniel, 1980; Goss & Grimes, 1972).

The sex of the subject animal may also contribute to differences. The same variation in success of regeneration in the different sexes, previously observed in the rabbit (Joseph & Dyson, 1965; Williams-Boyce & Daniel, 1980) was also seen in the pig and chinchilla. Anabolic androgens have been implicated in the higher regeneration rate of males (Dyson & Joseph, 1968; Joseph & Dyson, 1966*b*).

In the normal processes of appositional growth and repair, the perichondrium serves as the source of new cartilage cells (Junqueira, Carneiro & Contopoulos, 1977). It is reasonable to expect that the perichondrium may serve a similar function in regeneration. The cells of the perichondrium are capable of proliferation, migration and differentiation, all requirements of cells needed to replace the lost cartilage and perhaps serve as the precursors of blastema cells. Cartilage cells, both *in vitro* and *in vivo*, can lose their distinctive chondrocyte structure, become more fibroblastic, and will even stop producing cartilage-type collagen in favour of the fibroblastic type (Hay, 1959; Steen, 1968; Benya, Padella & Nimni, 1977; Uitto, 1979).

The chinchilla and rabbit show an active region of chondrogenesis at the tip of the old cartilage sheet, but none of the other animals shows such a dramatic proliferation. From evidence noted in other investigations and from the present histological

observations, it is hypothesised that (1) the perichondrium may serve as an active source of blastema cells; (2) cartilage regeneration in the mammalian ear may be both from a blastema and tissue proliferation of the perichondrium, as noted by other authors (Joseph & Dyson, 1966*a*; Vorontosova & Liosner, 1960; Hay, 1959). Rabbit ears, whose cartilage is surgically removed while leaving the perichondrium intact through the excised region, will regenerate cartilage from proliferation of the perichondrial fibroblasts and their subsequent differentiation into chondroblasts (Skoog, Ohlsen & Sohn, 1972; Skoog & Johansson, 1976; Wasteson & Ohlsen, 1977). Those studies also revealed that cartilage devoid of perichondrium will not regenerate, and biochemically, the newly formed perichondrial chondrocytes will form the extracellular matrix component, chondroitin sulphate, by a metabolic pathway similar to that seen in embryonic development.

The finding of bone in one of the older regenerated rabbit ear tissue samples reflects the earlier report by Goss & Grimes (1972), who described it as being deposited in the ear by the process of endochondral ossification. Goss (1983) notes that ossification in regenerating rabbit ear tissue tends to occur circumferentially "just within the original edges of the wound".

Collectively, these studies substantiate the idea that cells of the adult organism are not only capable of redifferentiating the specific lost tissues of the ear but are also capable of using part of the genome they have never expressed by forming a tissue that was never there originally.

Other examples of 'spontaneous' bone formation exist. Under pathological conditions in postnatal life, tiny pieces of bone may develop in the scars of major wounds, as in the tonsils or kidneys (Ham & Leeson, 1961) or other severely traumatised tissue. Bone nodules are occasionally found in muscles or in Achilles tendons subjected to trauma or ischaemia (Carlson, 1978*b*); complete bones are found in regenerated newt limbs from which the bones were removed prior to amputation (Weiss, 1925; Thornton, 1938), and mixtures of highly differentiated tissues, including bone, are often found in teratocarcinomas and teratomas (Carlson, 1981). Transplants to the anterior chamber of the eye of slices of articular cartilage from young rats can induce bone formation (Urist & Adams, 1968), and bones were stimulated to develop in the abdominal muscles of guinea-pigs by a bone morphogenetic protein (BMP) extracted from guinea-pig diaphyses (Urist, Iwata & Strates, 1972). Recent reviews by Urist (1983) and Urist, Delange & Finerman (1983) present evidence that bone morphogenetic protein, from several sources, acts on a variety of tissues by inducing activity of chondrogenic DNA which is closely associated with osteogenetic DNA functions.

Regeneration in the ear is more complex than that of simple wound healing. Foreign tissue formation never occurs in simple wounds (Peacock & Van Winkle, 1976), but it is found infrequently in severe wounds, tumours and regenerated tissues.

SUMMARY

These studies, on a variety of laboratory, zoo and agricultural animals, show that the phenomenon of replacement of tissues lost in an ear punch is common to some other mammals as well as the rabbit, although the rabbit appears to be the most proficient in this process. It is suggested that the cartilage may be replaced in two ways, namely through the formation of a blastema as originally hypothesised by

Markelova (Vorontsova & Liosner, 1960), and also from the perichondrium. The finding of bone tissue is supportive evidence enabling the suggestion that dedifferentiation, followed by deviant redifferentiation, does occur in the process of regeneration.

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