was local. The fact that imidazole does not inhibit but facilitates and potentiates the AFR to α-MSH further supports the suggestion that the effect of α-MSH is not directly mediated by prostaglandins, as seems to be the case with other traumatic agents to the eye[6,14]. The action of α-MSH on the disruption of the blood-aqueous barrier might join the chain of reactions after prostaglandin synthesis. It should be noticed, however, that the effects of imidazole on water permeability[16], calcium binding[16,17], pH, and osmolarity[17] might also contribute to its action on the α-MSH effect.

Pilocarpine (2%), which has some chemical structures in common with imidazole, was tested in a preliminary study (5 rabbits) for its possible effect on the AFR to α-MSH. A facilitation and potentiation of the AFR to α-MSH, similar to that of imidazole, was found[18].

Summary. MSH, like traumata to the eye, cause a permeability disturbance in rabbits, with protein leakage into the aqueous. The MSH effect was enormously increased by instillation of imidazole or pilocarpine. The MSH effect seems to engage a different mechanism than the prostaglandin-dependent action of other agents.

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[18] The synthetic α-MSH was kindly put at our disposal by Ciba Ltd., Basel, Switzerland.

The Effect of Indomethacin on Tooth Extraction Wound Healing in Rats

Inflammation is an essential feature in wound healing. Many studies indicate that anti-inflammatory agents affect the wound healing. According to some studies indomethacin retards the healing of experimental skin wounds in the rat[1,2]. However, STRUCK and HERNÁNDEZ-RICHTER[3] observed qualitative and quantitative improvement in the healing of wounds after local subcutaneous application of indomethacin. PENNERS[4] stated in his clinical report that indomethacin has a favourable effect on the healing of surgical wounds.

As there are both positive and negative views on the effect of indomethacin on wound healing, we wanted to investigate how indomethacin affects the healing of tooth extraction wounds. In this kind of investigation it is possible to determine what kind of effect indomethacin has on healing in epithelial tissue, subepithelial connective tissue and bone.

Materials and methods. A total number of 51 male Sprague-Dawley rats was studied. The age of the animals at the beginning of the study was 50 days and their average weight was 160 g. The tooth extractions were carried out under slight ether anaesthesia. The gingival tissue was first loosened very carefully from all molars on the left side with a sharp instrument. The teeth were then carefully rotated with modified forceps and then extracted with a strong vertical pull. Root fractures were uncommon and, if any were observed, the rat was excluded from the trial. The total extraction time was never more than 1 min per rat. Immediately after extractions the rats were conscious and in good condition. After extractions they were kept without food for 4 h, but they received water ad libitum.

Then 26 rats (test animals) were given indomethacin 2 mg/kg p.o. twice daily by gastric catheter in a carboxymethyl-cellulose suspension. The remaining 25 rats

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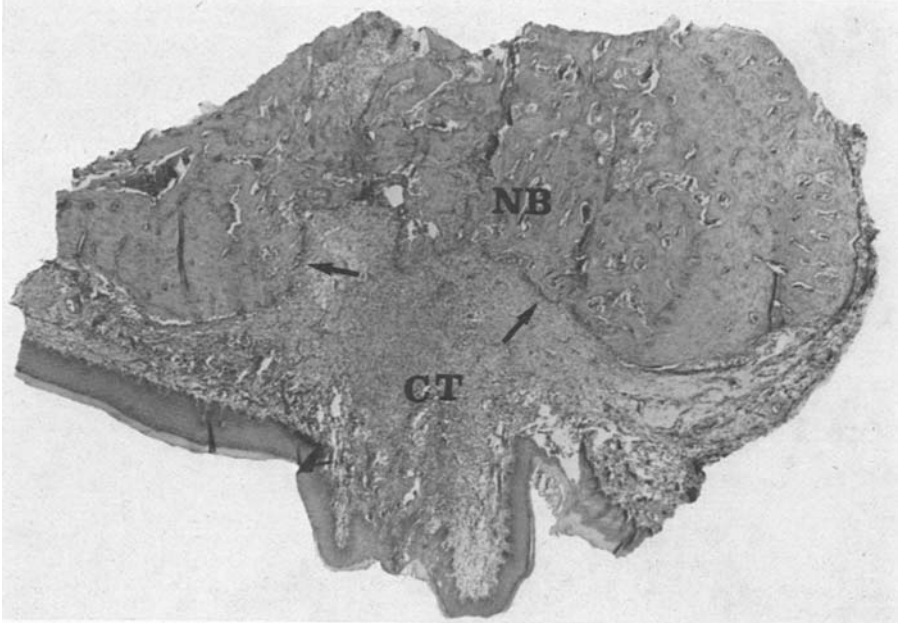

Fig. 1. 14-day-old extraction wound of the control rat. The wound is fully covered by the epithelium. New bone (NB) can be seen in the bottom half of the socket. The zone of bone forming cells (arrows) is clearly visible between new bone and connective tissue (CT). H. E., ×28.

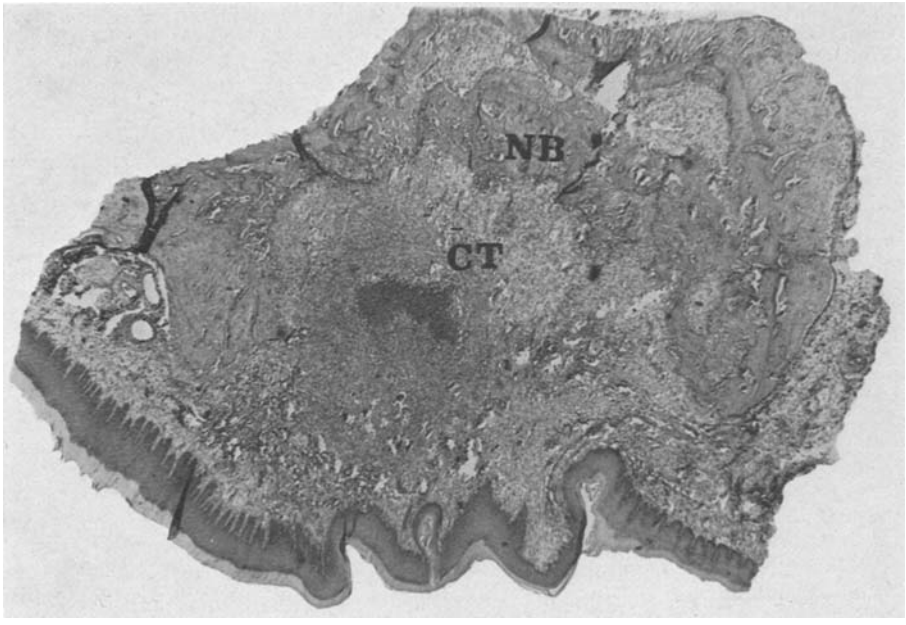

Fig. 2. 14-day-old extraction wound of the indomethacin-treated rat. There is only a narrow new-bone zone in the bottom part of the socket. H. E., ×28.

(controls) were given only carboxy-methyl-cellulose suspension. Indomethacin administration was continued for 5 days, except for 2 groups of animals from which the specimens were taken already 1 and 3 days after the tooth extractions. The first indomethacin dose was given in the morning, 3 h before tooth extractions. $1^1/_2$ h after the extractions, the oedema in the cheek was recorded.

Both the indomethacin-treated rats and the control rats were killed by cutting the aorta under ether anaesthesia in groups of 5–6 rats 1, 3, 7, 14 and 21 days after the extractions. The half of the upper jaw from which the teeth had been extracted was removed from the rats. The specimen consisted of alveolar sockets, one half of the hard palate, and the soft tissues of these areas. The specimens were fixed for 2 days in 10% neutral formalin, decalcified for 2 week in 10% EDTA and embedded in paraffin. The 7-µm sections were stained using the haematoxylin-eosin technique.

Results. $1^1/_2$ h after extraction, 77% (20/26) of the indomethacin-treated rats had visible oedema in the cheek. In the control rats, oedema was 100% (25/25). Between the figures there is a statistically significant difference ($p < 0.05$. using the χ^2-test). In general, the oedema was less severe in the indomethacin-treated rats than in the control rats. The indomethacin dose administered had no effect on the weight development of the rats.

In the control group, the healing of the extraction wounds followed the general principles which have already been established[5–7]. The indomethacin treatment had no effect on the healing of the epithelium and the subadjacent connective tissue. The epithelial proliferation at the wound edge started in both groups on the 3rd day after extractions. The whole wound was covered by epithelium in 14-day-old wounds. However, the structure of the epithelium was not fully mature. The epithelium was folded, and the keratinized layer was still thinner than the keratin layer of the adjacent mucosa. The epithelial pegs penetrating into the connective tissue were irregular. In 21-day-old wounds no difference could be observed between the wound epithelium and the epithelium of the surrounding alveolar mucosa.

A difference between the control and indomethacin groups appeared in the formation of new bone. In the indomethacin group, bone repair was retarded. The difference was clearly visible already in the 7-day-old wounds. In the 14-days-old wounds of the control animals, the bottom halves of the sockets were filled with new bone. In the indomethacin-treated rats, an equivalent amount of new bone was never observed at this stage of healing (Figures 1 and 2). The whole socket was filled with new bone in the 21-days-old wounds of the controls. At this stage, bone formation in the indomethacin group corresponded to that in control rats at 14 days after extraction.

Discussion. The indomethacin dose administered was not found to produce any toxic symptoms in the rats. WISEMAN and CHIAINI[8] report that the absorption of indomethacin after oral administration is lower in man than in the rat. Likewise, the half-life of indomethacin is shorter in man than in the rat. Therefore, it can be presumed that the indomethacin dosage (2 × 2 mg/kg p.o. for 5 days) which was used in this study is somewhat higher than the clinical dosage, though it does not produce toxic symptoms. An equivalent daily dose has been used in another study concerned with the effect of indomethacin on skin wound healing in the rat[2].

Tooth extractions cause postoperative oedema in the cheek of the rat. Indomethacin clearly decreased this oedema. This is logical since indomethacin is an anti-inflammatory agent.

The dosage of indomethacin used was not found to affect healing processes in the connective tissue or the

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epithelium. On an earlier occasion, an equivalent daily dosage of indomethacin has been observed to inhibit the healing of skin wounds in the rat[2]. The difference in results could be explained, for example, by the fact that the external surroundings of a skin wound are completely different from those of an extraction wound.

A 5-days' indomethacin treatment retarded the ossification of the alveolar socket to a certain extent. Perhaps indomethacin has some effect on the function of osteoblasts and osteoclasts. Further studies are required for resolving this problem.

Zusammenfassung. Untersuchungen an der Ratte zeigten, dass nach Zahnextraktionen die Zufuhr von Indomethazin ein postoperatives Oedem beträchtlich reduziert, dabei aber die Ossifikation der Alveolenhöhle verzögert.

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Inhibition of Adriamycin Cardiotoxicity by Acetyldaunomycin

Therapy with the anthracycline antibiotics daunomycin (D) and adriamycin (A) has frequently been associated with cardiomyopathy and congestive heart failure[1–10]. The cardiotoxic effects may be recognized early by careful monitoring of heart functions[11]. But the only practical measure to protect the patients is a strict limitation of the total dose to approximately 500 mg/m² [10].

Various approaches have been tried to reduce cardiotoxicity of anthracycline antibiotics. In acute experiments, mecamylamine, hexamethonium, guanethidine, and reserpine were shown to inhibit arrhythmia induced by i.v. infusions of D into hamsters[12]. Moreover, the chelating agents EDTA and ICRF 159 were found to counteract the increase in coronary perfusion pressure caused by D and A in the isolated dog heart[13]. A clinical application of these pharmacological observations was not tried.

Cardiotoxicity could also be reduced by administering the antibiotics in the form of DNA complexes. These are thought to be incorporated into the cells by pinocytosis, avoiding high free drug concentrations in the extracellular space[14]. Encouraging support for this concept was provided by preliminary clinical observations[15].

The present attempts to reduce cardiotoxicity of A were motivated by pharmacokinetic and toxicological observations: After i.v. injections into hamsters, a large proportion of anthracycline antibiotics were taken up by various tissues, including the heart. Within 5 min a daunomycinone-like aglucone was found in animals treated with D and A, reaching levels in the heart of 12% and 37% of the injected dose respectively. With acetyl daunomycin (AD), a drug which is N-acetylated at the sugar (daunosamine) moiety, the aglycone was found only after 30 min. Its levels never exceeded 2% of the injected dose[16]. After repeated i.p. injection in rats, the first signs of cardiotoxicity, changes of the QRS complex in the electrocardiogram (ECG), were observed with a cumulative dose of 80 mg/m² of D and 68 mg/m² of A[17]. Similar doses also caused disturbances of the metabolism of heart mitochondria[18]. AD, however, caused no significant changes of the ECG and mitochondrial metabolism at cumulative doses exceeding 800 mg/m² [17,18].

Electron microscopic studies demonstrated a direct injurious effect of D and A on mitochondria and myofibrils of the heart[19]. Since AD readily entered the heart muscle[16] but was not cardiotoxic, it was conceivable that it might compete with A and D for binding sites on structural proteins and thereby protect myocardial cells against the injurious effects of the more toxic derivatives. The experiments reported in this paper bear out this hypothesis.

Materials and methods. Groups of 6 female rats of the SIV-50 strain weighing approximately 160 g were injected i.p. 5 times weekly with A, AD, or A plus AD as indicated

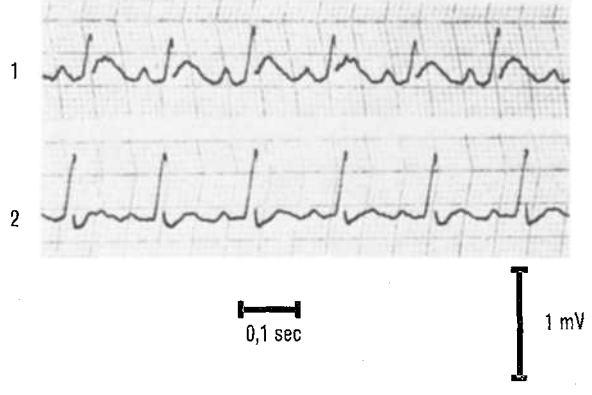

Fig. 1. 1. ECG (lead D: electrodes inserted s.c. over right scapula and sacral vertebrae) of unanesthetized, untreated rat. 2. ECG (lead D) of the same rat, after 6 injections of 4 mg/kg A. Marked widening of QRS with appearance of a distinct S-wave trough. Moderate flattening of T-wave and bradycardia.

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