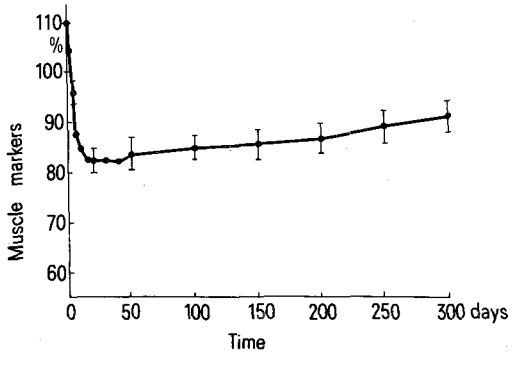
Rabbit digastric muscle sarcomere lengths (μm)

	Control (mean ± SD)	Experimental (mean ± SD)	Exp./Cont. (%)
Immediate post-shortening	2.75[a] (4) ± 0.06	3.06 (4) ± 0.09	111
2–3 weeks post-shortening	2.67[b] (12) ± 0.13	2.52 (12) ± 0.17	94
12 months post-shortening	2.62 (7) ± 0.13	2.55 (7) ± 0.12	97

[a] $p < 0.01$. [b] $p < 0.02$. Number of muscles measured in parentheses.

animals fixed by arterial perfusion of 10% formalin. The jaws of the rabbit were held in the closed position during fixation. Sarcomere lengths were measured directly from the slides with a micrometer eyepiece and an oil immersion objective. Sarcomeres were measured in rows of 10. At least 10 different fibres in each specimen were measured.

Results. The changes in muscle and tendon length following tendon shortening may conveniently be considered as short-term and long-term. These changes were determined by measuring the distance between the implanted metal markers in serial radiographs. Tendon shortening produced a significant short term increase in the length of the muscle belly (Figure). Within a day, the distance between the tendon markers began to increase, suggesting that the tendon was being pulled out. The tendon continued to lengthen until it had reached a length much greater than just prior to tendon shortening. The lengthening of the tendon is reflected in the shortening of the muscle belly in the Figure. Thus, the short term stretch placed on the muscle belly by tendon shortening is soon converted into a marked shortening of the muscle belly. Subsequently, the muscle belly tended to return toward its length prior to tendon shortening. Measurement of muscle sarcomere lengths revealed that tendon shortening produced a significant short term increase in muscle sarcomere length when compared to sarcomere lengths in the unshortened control muscle of the pair (Table). As the muscle shortened, the measured sarcomere lengths also reflected this change (Table). In the long-term, however, muscle sarcomere lengths were not significantly different from the controls, even though the muscle itself had not re-established its original length. This discrepancy between muscle length and muscle sarcomere length suggests that a mechanism exists to restore muscle sarcomere length to a normal or ideal functional length. Such a mechanism would have to add or remove serial sarcomeres as required to adjust total sarcomere number to total muscle length.



Change in muscle marker distance expressed as a percent. Vertical bars are SEM, $n = 7$ for each point on thg graph.

Summary. Tendon shortening in the digastric muscle of the rabbit resulted in a short term increase in gross muscle length and sarcomere length. Subsequently, muscle and sarcomere lengths decreased to less than control values. Long-term measurements suggested that a return to control sarcomere length may have been achieved by a reduction in sarcomere number.

Z. F. MUHL and A. F. GRIMM

Departments of Orthodontics and Histology, University of Illinois at the Medical Center Chicago, P.O. Box 6998, Chicago (Illinois 60680, USA),
18 April 1975.

Facilitation by Imidazole of the Aqueous Flare Response to α-Melanocyte Stimulating Hormone

The eye responds monotonously to both chemical and mechanical local traumata with a break-down of the blood aqueous barrier[1]. The rabbit eye is especially liable to show impressive permeability disturbances. Even small traumata disturb temporarily the blood aqueous barrier and the increased protein content in the aqueous gives a flare which can be measured photoelectrically without touching the eye[2].

Local application of arachidonic acid (AA)[3] and prostaglandin (PG)[4] gives a similar trauma reaction. Aspirin-like drugs and indomethacin, which inhibit the conversion of AA to PG[3,5] are capable of blocking the effect of local traumatic agents[6]. PG is therefore suspected of being the common mediator of the inflammatory reaction to different traumata[7].

Apart from locally applied agents, it has also been shown that the melanocyte stimulating hormone (α-MSH) given in microgram doses subcutanously is capable of producing effects which are indistinguishable from those of a local trauma, even histologically[8,9]. In an unselected material of pigmented rabbits, the percentage of positive aqueous flare responses (AFR) to α-MSH is about 40%[10]. The ultimate reason for this variability in reaction is unknown but is to some extent dependent on the age of the animals.

The effect of α-MSH on the eye appears to be in accor-

dance with the theory that its activity might fundamentally consist of an activation of membrane-bound phospholipase. This releases unsaturated free fatty acids which are rapidly transformed into PG[11]. Another suggested effect of α-MSH is its inhibition of cAMP phosphodiesterase[12]. As cAMP has been suggested as the ultimate effector of the permeability disturbance[13], α-MSH might in this way influence the blood aqueous barrier.

Whichever the important mechanism may be, it should be of interest to investigate the influence of substances which block the PG effect or that of its precursor, arachidonic acid, on the MSH action.

Recent experiments have shown that the α-MSH effect is not blocked by pretreatment with indomethacin, like the trauma reaction to other agents; in fact there was a slight increase of the α-MSH response in the indomethacin-treated eyes[14].

The present report deals with the ability of imidazole to affect the aqueous flare response (AFR) to α-MSH in rabbits. Imidazole increases the phosphodiesterase activity in vitro[15] and is supposed to antagonize the effect of AA[16] and PG[17] to the eye by its lowering effect on the intraocular concentration of cAMP. The course of the AFR was followed by quantitative measurements of the aqueous flare in the intact eye by means of a photoelectrical instrument[14]. The AF was measured in arbitrary units and the results are given as

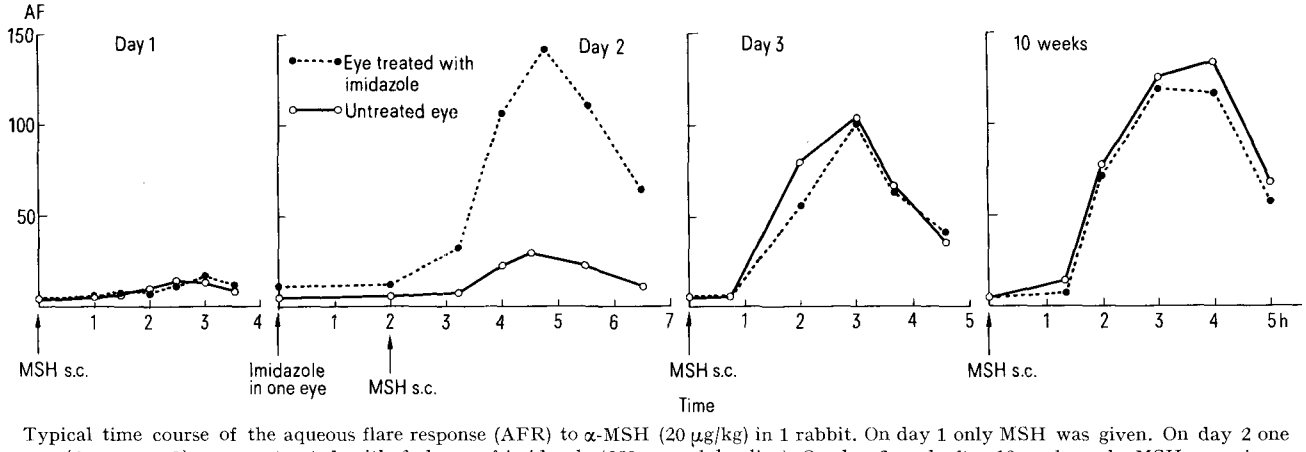
$$Q_{max} = \frac{\text{maximum flare density after treatment}}{\text{flare density before treatment}}.$$

Pigmented rabbits, 3-4.5 kg, were given 20 μg/kg α-MSH s.c. on the 1st day of the experiment, after which the AF was measured every half-hour until the flare maximum was covered. There was a significant elevation ($Q_{max} \geq 1.5$) of the aqueous flare in at least 1 eye in about 40% of the rabbits. In those cases where a flare response was elicited (8 rabbits), the Q_{max}-values varied between 1.5–5.0 (arithmetic mean 2.8, standard error of the mean 0.23). On the 2nd day 1 eye was treated topically with 2 drops (50 μl) of imidazole (250 mg ml^{-1} saline) and 2 h later 20 μg/kg α-MSH was injected s.c. In the eyes pretreated with imidazole (9 eyes), there was a modest to dense flare in 100% and the Q_{max}-values varied between 4–32 (mean 12.8, SE 2.7). In the contralateral eye, there was a positive AFR in 7 out of 9 eyes and the Q_{max}-values varied between 1.0–30.0 (mean 6.0, SE 3.0). On the 3rd day, 20 μg/kg α-MSH was given s.c. without any pretreatment with imidazole. A positive AFR was produced in both eyes in 100% (9 rabbits). The Q_{max}-values of the eyes that were given imidazole the day before varied between 3.5–26.7 (mean 14.6, SE 2.5), and in the contralateral eyes the Q_{max}-values varied between 4.0–32.0 (mean 14.8, SE 2.8). 10 weeks after these experiments, the AFR to α-MSH was again tested without repeating the application of imidazole in the meanwhile. The Q_{max}-values of the eyes (4 rabbits) that had got imidazole 10 weeks earlier varied between 8.7–61.5 (mean 33.7, SE 10.9) and in the contralateral eyes the Q_{max}-values varied between 7.0–50.0 (mean 31.2, SE 8.9). In all experiments, the flare maximum occurred in both eyes 2–3 h after stimulation with α-MSH and the aqueous flare returned to physiological values in 5–6 h. (A typical series of experiments is shown in the Figure).

In a control series (6 rabbits), only imidazole was given as in the series mentioned. There was no significant elevation of the AF in either eye, though there was a moderate external irritation with vasodilation and chemosis of the conjunctiva in the eyes treated with imidazole.

It is remarkable that strong AFR to α-MSH was elicited in all cases pretreated with imidazole, even in those rabbits which did not give a significant response to α-MSH only. The effect of imidazole seems to be long-lasting and general, though the mode of administration

[1] K. Wessely, Arch. J. Augenheilk. *60*, 97 (1908).
[2] K. Dyster-Aas and C. E. T. Krakau, Invest. Ophthal. *3*, 127 (1964).
[3] S. M. Podos, B. Becker anf M. A. Kass, Prostaglandins *3*, 7 (1973).
[4] M. B. Waitzman and C. D. King, Am. J. Physiol. *212*, 329 (1967).
[5] J. R. Vane, Nature New Biol. *231*, 232 (1971).
[6] A. H. Neufeld, L. M. Jampol and M. L. Sears, Nature, Lond. *238*, 158 (1972).
[7] B. R. Beitch and K. E. Eakins, Br. J. Pharmac. *37*, 158 (1969).
[8] H. K. Dyster-Aas, Acta ophthal., Copenh. *43*, 30 (1965).
[9] H. K. Dyster-Aas and C. E. T. Krakau, Acta Univ. lund. Sectio II *20* (1965).
[10] K. Dyster-Aas and C. E. T. Krakau, Acta endocr. *49*, 166 (1965).
[11] F. C. G. van de Veerdonk and E. Brouwer, Biochem. biophys. Res. Commun. *52*, 130 (1973).
[12] M. Goldman and M. E. Hadley, Br. J. Pharmac. *39*, 160 (1970).
[13] M. B. Waitzman and W. D. Woods, Expl. Eye Res. *12*, 99 (1971).
[14] E. Bengtsson Invest. Opthal., *14*, 306 (1975).
[15] R. W. Butcher and E. N. Sutherland, J. biol. Chem. *237*, 1244 (1962).
[16] P. Puig-Parellada, J. M. Planas and P. Puig-Muset, Ophthal. Res. *7*, 189 (1975).
[17] H. A. Zink, S. M. Podos and B. Becker, Nature New Biol. *245*, 21 (1973).



Typical time course of the aqueous flare response (AFR) to α-MSH (20 μg/kg) in 1 rabbit. On day 1 only MSH was given. On day 2 one eye (● . . . . . ●) was pretreated with 2 drops of imidazole (250 mg ml^{-1} saline). On day 3, and after 10 weeks, only MSH was given. Ordinate: AF in arbitrary units. Abscissa: time in hours.

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.

ELISABETH BENGTSSON and C. E. T. KRAKAU

Department of Experimental Ophthalmology, University Eye Clinic, S-221 85 Lund (Sweden), 4 March 1975.

[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

[1] J. J. P. MORTON and M. H. MALONE, Archs int. Pharmacodyn. Thér. *196*, 117 (1972).
[2] K. H. LEE and T. G. TONG, J. Pharm. Sci. *59*, 1036 (1970).
[3] H. STRUCK and H. J. HERNÁNDEZ-RICHTER, Arzneimitt.-Forsch. (Drug Res.) *21*, 1840 (1971).
[4] R. PENNERS, Arzneimitt.-Forsch. (Drug Res.) *21*, 1842 (1971).

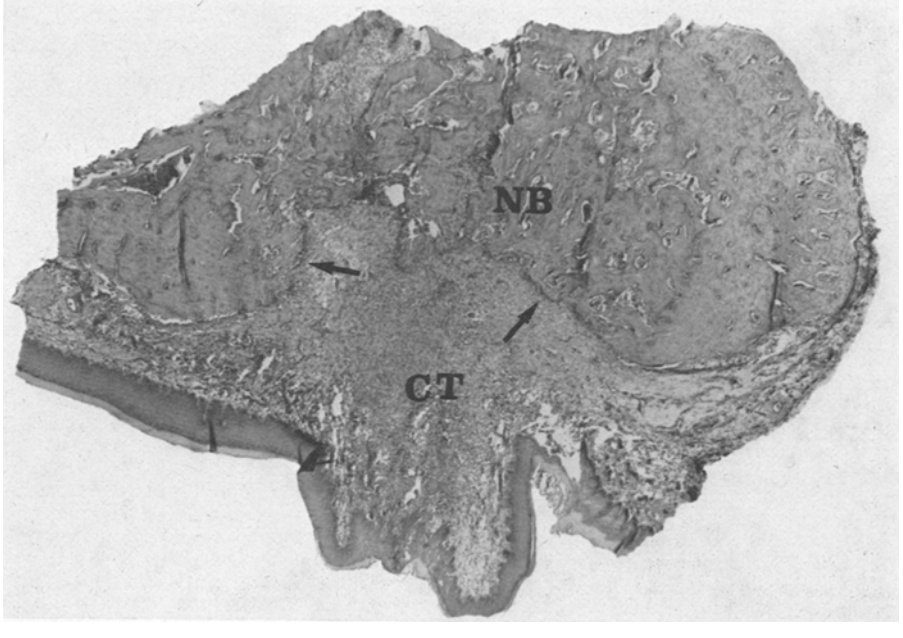


Fig. 1. 14-day-old extraction wound of the control rat. The wound is fully covered by the epithelium. New bone (NB) can be seen in the bottom half of the socket. The zone of bone forming cells (arrows) is clearly visible between new bone and connective tissue (CT). H. E., ×28.