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EFFECT OF INDOMETHACIN ON FRACTURE HEALING IN RATS

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The healing of closed, non-immobilized femoral fractures in rats was seriously impaired by indomethacin given orally at a dose of 2 mg/kg daily. The fracture haematomas were larger and disappeared later in the animals receiving indomethacin. Mechanical strength testing of fracture healing showed that maximal tensile strength, elastic stiffness and maximal bending moment between fragments were significantly diminished in the indomethacin-treated animals. Radiological examination showed a smaller amount of mineralized callus and a more pronounced angulation between the fragments in these animals than in the placebo-treated ones. Histological examination showed bridging between the fragments by callus tissue 24 days after fracture in placebo-treated animals, whereas indomethacin treatment was followed by histological findings resembling those seen in early pseudarthrosis development.

Key words: anti-inflammatory agents; bone; callus; fractures; fractures, non-union; indomethacin; pseudarthrosis; rats

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Earlier reports dealing with the effect of potent nonsteroidal anti-inflammatory drugs on fracture healing have mainly been concerned with short-term therapy used to alleviate the immediate post-traumatic or postoperative reaction (Connell et al. 1961, Allgöwer et al. 1963, Penners 1968, Eschberger 1973). No adverse effects on fracture repair have, to the authors' knowledge, been reported. It has recently been shown, however, that indomethacin inhibits the healing of mechanical lesions in heterotopic bone in rabbit ear chambers. Furthermore, it has been postulated that these findings are of relevance also for the healing of fractures in orthotopic bone (Sudmann 1975).

The present study was undertaken to assess the effect of indomethacin on the healing of non-immobilized fractures of rat femur.

MATERIAL AND METHODS

A total of 129 male adolescent Wistar-Møllegaard albino rats, divided into two weight-matched groups, were given indomethacin ($n = 64$) and placebo ($n = 65$), respectively.

* Dr. Rø died suddenly in April 1976 after the completion of this paper.

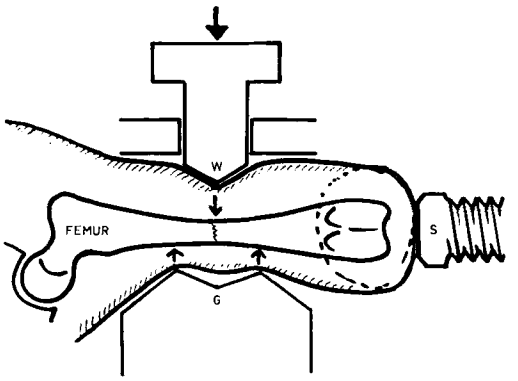

Figure 1. Standardized left femoral fractures were obtained by three-point loading of the thigh between wedge (W) and groove (G). A constant level above the flexed knee joint was ensured by the screw (S).

Mean weight at the start of the experiment was 187 g with S.D. = 8 g.

A closed, standardized mid-diaphyseal fracture of the left femur (Figure 1) was made under ether anaesthesia. Immediate weight-bearing was allowed without immobilization of the fracture.

Indomethacin suspension 0.4 mg/ml (1.1 mmol/l) (diluted from Indocid commercial suspension, Merck Sharp & Dohme, Haarlem, The Netherlands) and placebo (Vehiculum, Indocid suspension, MSD) was given by stomach tube in doses of 1 ml once daily. The resultant dosage of indomethacin was about 2 mg/kg/day, depending on the exact weight of the animal. The first dose was given immediately following fracturing of the left femur, and the medication was continued until sacrifice of each animal.

Sixty-one animals were used for extensometric measurements, and 60 animals for bending-strength testing of the fractured femurs on days 6, 9, 12, 18 and 24, respectively, after fracture (Table 1). Three animals in the placebo-treated group were excluded from the bending-strength experiment: one animal died accidentally during the experiment, in one the fracture was in the wrong place and in one animal the callus tumour was unintentionally injured during dissection. Radiological examination of the fractured limbs was made on 89 of the animals, randomly selected. A total of eight animals was used for histological study of the fracture healing on days 9, 12, 18 and 24 of the experiment.

At sacrifice, blood was collected in heparinized tubes by cannulation of the inferior caval vein during laparotomy under ether anaesthesia. After having reached room temperature, the blood was centrifuged for 30 minutes at 150 × *g*. Plasma samples were collected and stored at — 20° C until spectrofluorometric indomethacin analysis (Gribnau et al. 1973) was performed. The animals were killed by severing of the aorta.

Front and side radiographs were made of the fractured limb, either on the day before sacrifice or immediately after exarticulation of the limb at the hip joint. Measurements were made on the lateral view radiographs as illustrated in Figure 2. The ventral angulation of the distal fragment relative to the proximal one was measured to the nearest 5° using a protractor. The distance between the fracture line and the knee joint was measured to the nearest millimetre.

The fractured femur with the callus tumour was dissected free of soft tissue. The specimens to be used for mechanical strength testing were kept wrapped in moist 0.9 per cent saline dressings at room temperature until tested. The testing was done within 2 hours after the dissection.

To assess the load-deformation characteristics, the equipment illustrated in Figure 3 was used. Both ends of the femur preparation were clamped in a hydraulic strength tester (Lorentzen & Wettre tensile strength tester type 7-1/1, AB Lorentzen & Wettres Maskinaffär, Stockholm, Sweden) fitted with a strain gauge (HBM Kraftaufnehmer Type U 1, Hottinger Baldwin

Table 1. Methods of investigation and number of rats in the different groups.

Experimental methods	Placebo			Indomethacin			Total	
	start	end	x-ray	start	end	x-ray	start	included in material
Extensometric	31	31	19	30	30	18	61	61
Bending momentum	30	27	22	30	30	24	60	57
Histology	4	4	3	4	4	3	8	8
Total	65	62	44	64	64	45	129	126

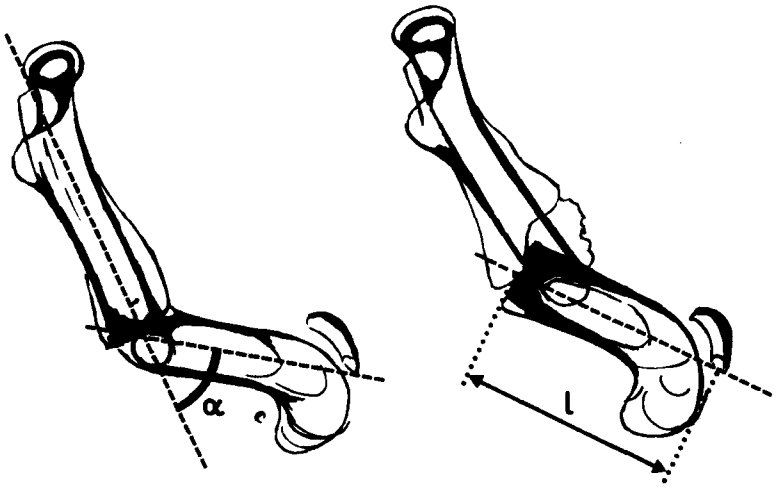

Figure 2. Measurements made on lateral view radiographs. α: ventral angulation of the distal fragment relative to the proximal one (left). l: distance between the fracture line and the knee joint (right).

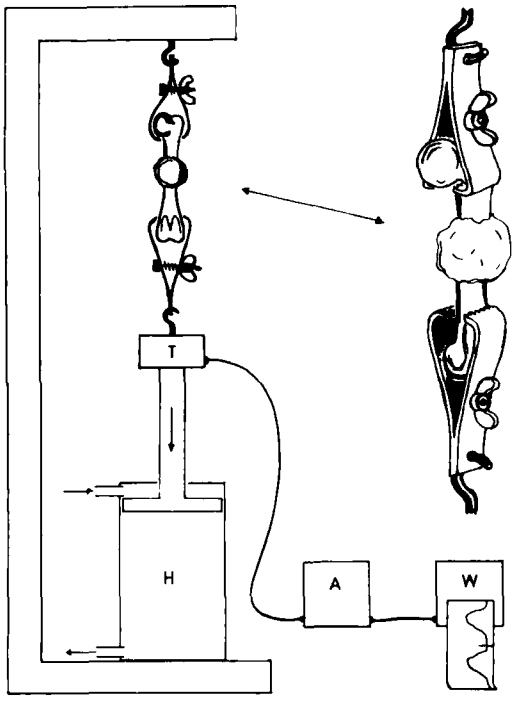

Figure 3. Equipment to assess the load-deformation relationship. Tensile force is exerted by the hydraulic cylinder (H), measured by the load transducer (T) and recorded by the writer (W) via a signal amplifier (A).

Messtechnik, Darmstadt, W. Germany) connected via an amplifier (HBM Messverstärker KWS/T-5) to a recorder (Gould Brush 220 writer, Gould Inc. Instruments Systems Division, Cleveland, Ohio, USA). The equipment was calibrated with known weights before use. The load was recorded continuously during constant rate elongation (0.4 mm/s). Maximal tensile strength and elastic stiffness were calculated from the graphs (Figure 4).

Bending-strength testing was done by measuring the moment needed to bend the distal femur fragment ventrally relative to the proximal fragment (Rø, in preparation). The proximal end of the femur preparation was clamped immediately above a horizontal disc mounted on a vertical axis (Figure 5), the centre of the callus tumour situated in the extension of this axis. The disc could be turned by pulling a thin and flexible steel wire attached to the circumference of the disc. The other end of the wire was connected to the plunger of the hydraulic tensile strength tester via the strain gauge. The torque was conveyed to the distal femur fragment by means of a vertical cam mounted on the disc. The force used was recorded continuously during a 90° turn of the disc at a speed of 6°/s. This procedure usually caused the callus tumour to break, thus each femur preparation could be measured only once.

The femur preparations to be used for histological study were fixed in buffered formole (Vittali 1970). Following decalcification in

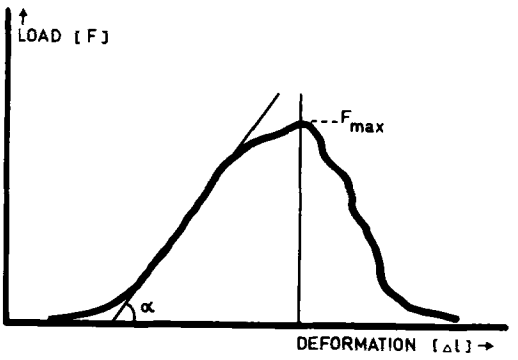

Figure 4. Schematic load-deformation curve as obtained by testing femur-callus specimens. F_{max} = maximal load = maximal tensile strength. Tan (α) = elastic stiffness.

Decalc (Bethlehem Trading, Gothenburg, Sweden), each femur with its callus tumour was transsected in a sagittal plane along its long axis. After being photographed the medial half of each preparation was embedded in paraffin. Histological sections were cut at 6 μm and stained with haematoxylin/eosin.

Statistical significances were evaluated by the Wilcoxon rank sum test (Wilcoxon 1947) and the Wilcoxon-Van Elteren block test for grouped data (Høyland & Walløe 1975). Differences were considered significant when $2\alpha < 0.05$. Unless otherwise stated, results are given as medians ± one absolute deviation.

RESULTS

There was no significant difference in weight gain between the indomethacin-treated and placebo-treated groups. The rats tolerated the fracture well, both groups resuming full activity in a couple of days.

Macroscopic examination

Measurement of the distance between the fracture and the knee joint (Figure 2) was made in 101 animals, randomly selected. The mean distance was 13.4 mm with S.D. = 1.3 mm.

During dissection of the fracture area, several distinctive features were noted. In the placebo-treated animals, a well-developed callus tumour produced good connection between the fragments as early as 6 days after fracture, and only small remnants of the fracture haematoma could be seen embedded in the callus tissue. In contrast, the connection between the fragments in the indomethacin-treated animals was less stable, and large fracture haematomas were, as a rule, encountered until 12 days after fracture. These were thin-walled and fluctuating, and contained thin, dark red fluid.

Firm fracture union was observed 18 to 24 days after fracture in the control animals, whereas most of the fractures in the indomethacin-treated animals

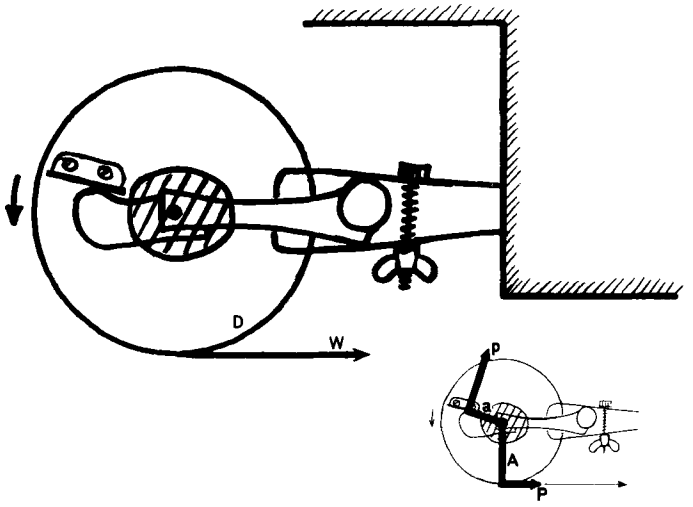

Figure 5. Bending moment measuring device. The disc (D) is turned by pulling the wire (W), connected to the hydraulic tensile strength tester via the strain gauge. Insert (right) shows that the moment measured (P.A.) equals the moment needed to bend the distal femur fragment ventrally relative to the proximal fragment (p.a).

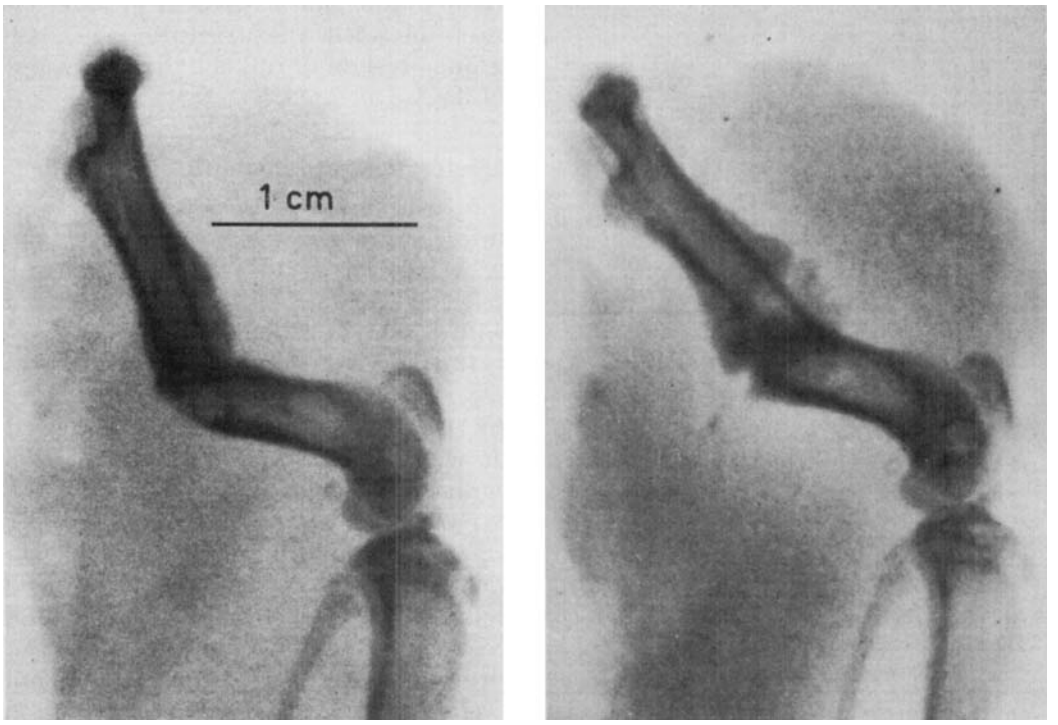

Figure 6. Lateral view radiographs of fractured femurs, 24 days after fracture. There is a smaller amount of mineralized callus, and the angulation between the fragments is more pronounced in the indomethacin-treated animal (left) than in the placebo-treated animal (right).

were still unstable at the end of the experiment. Broad cartilaginous collars around both fracture ends were found in these animals.

Table 2. Ventral angulation of the distal femur fragments relative to the proximal fragments as seen on lateral view radiographs. (Median ± 1 absolute deviation)

	n	Degrees
Placebo	44	25.0 ± 10.1
Indomethacin	45	40.0 ± 12.0
	89	

The difference between the groups is highly significant ($2\alpha < 0.0002$).

X-ray examination

Radiological signs of continuity between the fragments, while present in the control animals, were scarce in the indomethacin-treated animals. Signs of callus mineralization appeared later and were less pronounced in the indomethacin-treated than in the placebo-treated animals. The angle between the longitudinal axes of the fragments in lateral view (Figure 2) was larger in the indomethacin-treated group (Figure 6; Table 2). This difference between the two groups was highly significant ($2\alpha < 0.0002$). A scatter diagram of the individual values (Figure 7) shows that this angle tended to increase with time after fracture in the indomethacin-treated animals (linear correlation coefficient $r = 0.43$), whereas no such relationship was discernible in the placebo-treated group of rats ($r = -0.08$).

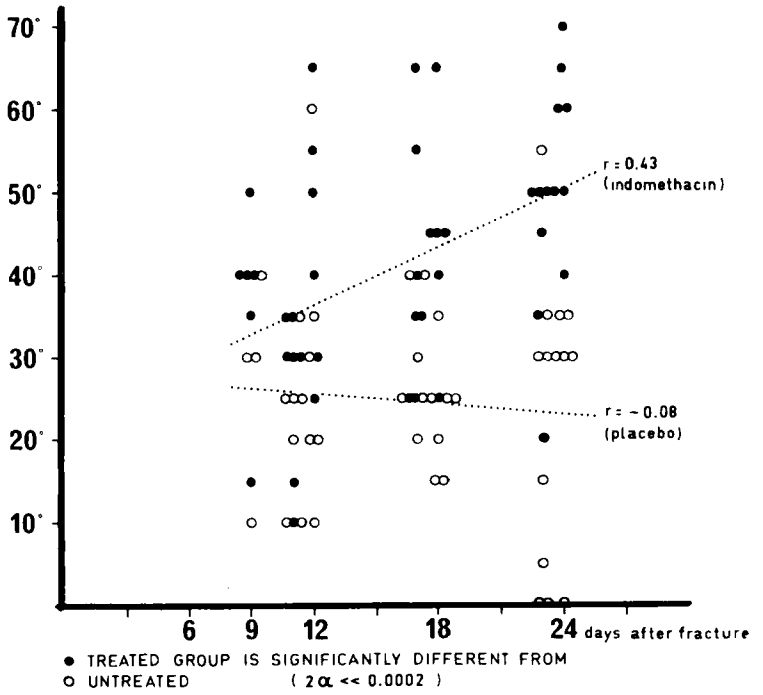

Figure 7. Scatter diagram of the angles between femur fragments on lateral view radiographs (see Figure 2, α).

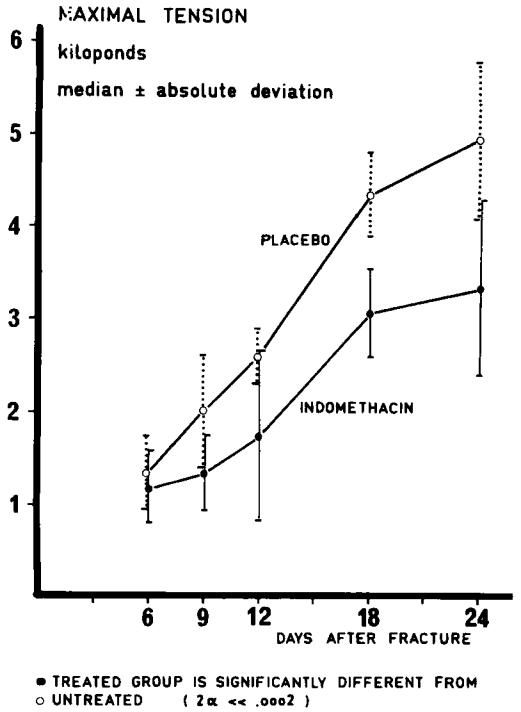

Figure 8. Maximal tensile strength of femur fractures.

Mechanical strength testing

Both the maximal tensile strength (Figure 8) and the elastic stiffness (Figure 9) were significantly lower throughout the experiment in the indomethacin-treated animals than in the placebo group (Table 3). The maximal moment required to bend the distal fragment ventrally relative to the proximal fragment was found to be significantly lower in the indomethacin-treated rats on days 9 and 12 after fracture (Figure 10; Table 3), whereas on days 18 and 24 after fracture no significant difference was found between the groups.

Pathological anatomy

Macroscopic examination of the transsected decalcified femur preparations showed in all the placebo-treated animals compact callus tumours without any visible central cavity. Indomethacin-treated animals, however, had at all times sampled a central, transverse, slit-

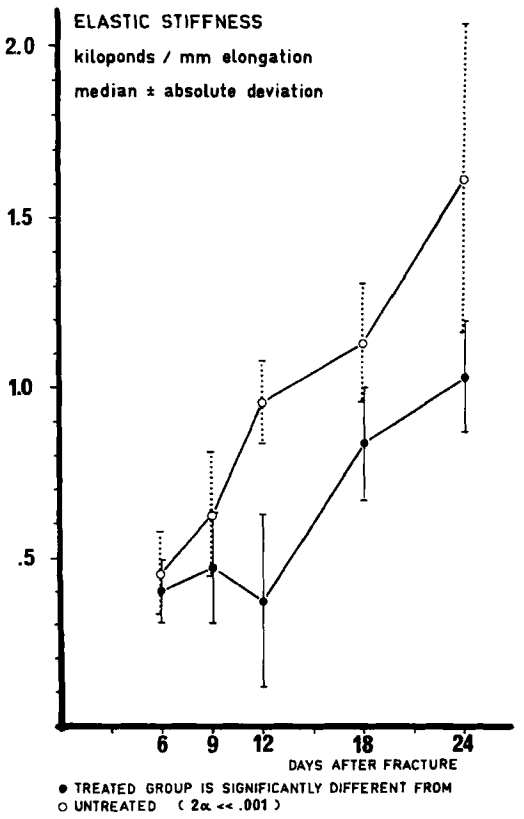

Figure 9. Elastic stiffness of femur fractures.

like cavity between the ends of the fracture fragments (Figure 11). In preparations taken on days 9 and 12, this cavity was lined by irregular, fuzzy walls which on days 18 and 24 were seen to be smooth and lined by a cartilage-like tissue.

Histological examination of the femur specimens from indomethacin-treated animals showed at all stages of healing a lesser degree of osteoid and bone callus formation than did control specimens from the placebo-treated animals. At 24 days, newly formed cartilage and bone in the placebo-treated animals bridged the fragments (Figure 12 b), whereas no such bridging had taken place in the indomethacin-treated animals.

The slit-like cavity between the ends of the femur fragments of indomethacin-treated animals was lined by fibrous tissue and fibrin on days 9 and 12. Later this cavity became smooth-walled by organization of the fibrous tissue and by local formation of cartilage, on days 18 and 24 closely resembling pseudarthrosis formation (Figure 12 a).

Indomethacin analysis

Plasma levels of indomethacin were determined in 48 randomly selected animals that were killed 3 to 21 hours after the last drug administration (Table 4). The highest indomethacin concentrations were found 6 hours after drug administration and were in the range 3.9–8.5 μg/ml with a median of 6.2 μg/ml. From 16 to 21 hours after drug administration

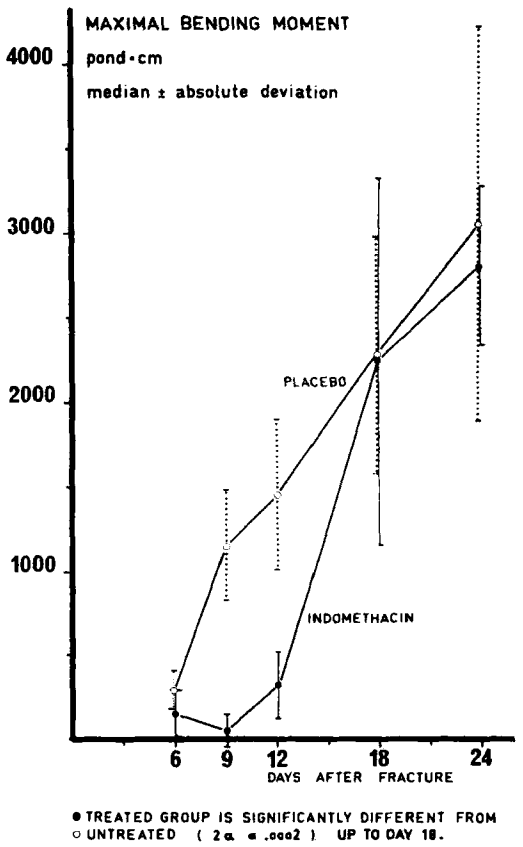

Figure 10. Maximal moment required to bend distal fragment ventrally with respect to proximal fragment.

Table 3. Results of mechanical testing of fracture healing in rats receiving placebo (P) or indomethacin 2 mg/kg/day (I). (Median ± 1 absolute deviation)

Days after fracture	Extensometric testing: Maximal tension (kp) n	P	I	Elastic stiffness (kp/mm) P	I	Bending moment (pond cm) n	P	n	I
6	6	1.36±0.42	6 1.19 ±0.40	0.46±0.12	0.40 ±0.09	6	289± 112	6	146 ± 141
9	6	2.03±0.62	6 1.36*±0.41	0.62±0.18	0.47 ±0.17	4	1155± 329	6	44*± 102
12	6	2.60±0.29	6 1.74 ±0.91	0.96±0.12	0.37*±0.25	5	1455± 446	6	314*± 208
18	6	4.36±0.46	6 3.07*±0.49	1.13±0.18	0.84*±0.17	6	2283± 696	6	2246 ±1080
24	7	4.95±0.85	6 3.36*±0.95	1.62±0.45	1.03 ±0.16	6	3060±1164	6	2810 ± 472
WVE			$\overset{*}{2\alpha} < 0.0002$		$\overset{*}{2\alpha} < 0.0002$				$\overset{*}{2\alpha} = 0.002$

WVE: Wilcoxon-Van Elteren test for grouped data.
*: Indomethacin-treated group significantly different from placebo-treated group ($2\alpha < 0.05$).

the levels were found to be decreasing from 1.4 μg/ml (range 1.3–1.6 μg/ml) to 1.0 μg/ml (range 0.4–1.6 μg/ml).

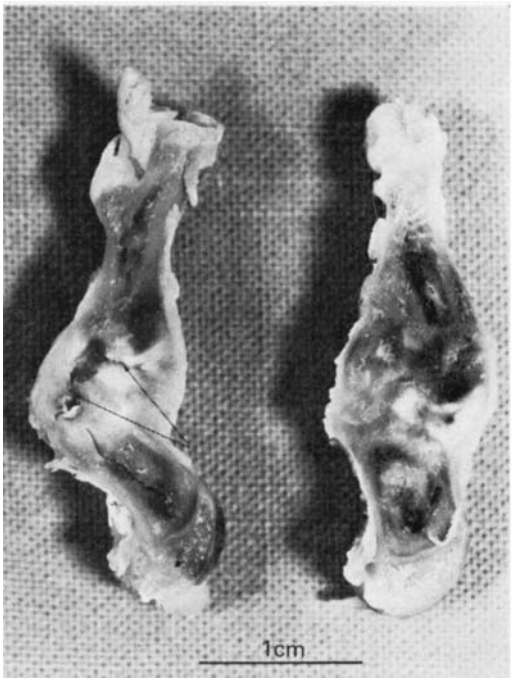

Figure 11. Fractured left femur from indomethacin-treated (left) and placebo-treated (right) animals, 24 days after fracture, sagittally sectioned. Note slit-shaped interfragmental cavity (arrows) lined with cartilaginous tissue in the indomethacin-treated fracture. The placebo-treated fracture was clinically stable.

DISCUSSION

The present investigation indicates that an oral indomethacin dosage of 2 mg/kg/day seriously impairs the healing of closed, non-immobilized femoral fractures in rats. Such fractures usually unite within 3–4 weeks (Hulth & Olerud 1964, Lindholm et al. 1970).

The dose of indomethacin used in this investigation is within the limits of tolerance for rats (Phelps et al. 1968). An anti-inflammatory action is observed in rats with as small a dose as 0.015 mg/kg/day in acute inflammation (Phelps et al. 1968). Apparently higher doses of indomethacin are required to obtain inhibition in models of chronic inflammation, as it has been claimed that a dose of 0.1 mg/kg/day will enhance the collagen synthesis in cotton pellet granulomas in the rat (Kulonen & Potila 1975). According to Winter (1965) indomethacin in a dose of 2 mg/kg/day, which is the dose chosen for the present investigation, produces between 30 and 40 per cent inhibition in the cotton pellet rat granuloma test.

It is desirable that the conclusions drawn from these experiments on autopsy specimens should reflect the properties of the fracture healing tissues *in vivo*. The mechanical strength of frac-

Figure 12. Photomicrographs of detail from fracture site, indomethacin-treated (a) and placebo-treated (b) animal, 24 days after fracture. Inserts show the localization of the areas reproduced (arrows) with reference to Figure 11. (Haematoxylin-eosin.)

Table 4. Plasma levels of indomethacin at time of sacrifice.

Hours after last drug administration	Indomethacin concentration μg/ml Median ± 1 absolute deviation
3	3.7 ± 1.6
6	6.2 ± 1.5
16	1.4 ± 0.1
18	1.4 ± 0.3
19	1.4 ± 0.4
19.5	1.3 ± 0.4
20	0.9 ± 0.3
21	1.0 ± 0.4

ture repair has often been evaluated in terms of maximal tensile strength. To reach this value, however, it is usually necessary to exceed the range of elastic deformation. When the range of plastic deformation is entered, the deformation causes lasting damage to the tissues tested. Consequently, it appears to be most appropriate to perform measurements within the range of elastic deformation. Within this range, measurement of the elastic stiffness has been recommended (Viidik 1973). This is taken as the slope of the linear part of the load-deformation curve (Figure 4).

Since the forces used in tensile testing systems are more or less the opposite of the forces acting *in vivo* (i.e. muscle tone and weightbearing), such models appear to have limited relevance as tests of fracture healing. As a consequence, a method was developed to obtain measurements of the capacity of the fractured femurs to resist bending (Rø, in preparation). These bending moment measurements were included in the present study

as a supplement to the tensile tests, and served to demonstrate the considerable difference in fracture stability between the indomethacin-treated and placebo-treated animals on days 9 and 12 after fracture (Figure 10). On days 18 and 24 the large cartilaginous collars which developed around the fracture ends in the indomethacin-treated animals probably were an obstacle to the bending procedure. This may explain why the bending moment measurements were not significantly different between the groups on these days.

The radiological and mechanical tests demonstrated that the fracture repair strength was considerably lowered by indomethacin administration. Furthermore, the changes found in the macroscopic and histological examinations closely resembled those seen in pseudarthrosis formation. This finding is quite remarkable, since it indicates that indomethacin not only delays the fracture healing process, but causes the process of repair to be qualitatively altered. It is thus probable that while new bone is produced in the placebo-treated animals, connective tissue and cartilage dominate at the fracture site of the indomethacin-treated animals. This may well account for the considerable differences in mechanical properties of the fractures in indomethacin- and placebo-treated animals. It should be noted, however, that it would be necessary to continue the study for a considerable length of time in order to assess whether pseudarthrosis would occur. Lindholm et al. (1970) have shown that abnormal mobility can be maintained in tibial fractures in rats for as long as 48 days after fracture by daily manipulation under narcosis. When left undisturbed, however, the resultant large cartilaginous callus tumour will mineralize and proceed to consolidation in a very short time. A similar effect might be encountered after discontinuation of indomethacin.

The fracture trauma is followed by an aseptic inflammation which initiates the process of fracture repair. The effect of indomethacin demonstrated in the present study may, consequently, be caused by an interference with the inflammatory process. If this is so, other potent anti-inflammatory drugs may also affect fracture healing in this way.

It has been postulated that the effects of the nonsteroidal anti-inflammatory drugs are due to inhibition of the prostaglandin synthetase activity (Vane 1971). The role of prostaglandins in bone formation has not been fully established, but it has been reported that prostaglandin E_2 inhibits bone collagen synthesis dose-dependently (Raisz & Koolemans-Beynen 1974). Indomethacin dosage inhibited bone callus production in the present study, whereas increased bone formation would have been expected if this drug influenced fracture healing through prostaglandin E_2 synthetase inhibition.

Collagen synthesis in experimental rat granulomas is inhibited by indomethacin in the dose used in the present study (Winter 1965). It has recently been shown, however, that collagen synthesis studied *in vitro* is greater in fracture callus tumour tissue from rats given indomethacin 2 mg/kg/day than in corresponding tissue from placebo-treated animals (Rø & Sander, in preparation). It is thus not probable that indomethacin delays the fracture healing process by inhibiting the callus collagen synthesis. Since osteoid and mineralized bone tissue formation were sparse in indomethacin-treated animals both in the present study and in a preceding study (Sudmann 1975), these findings suggest that the effect of indomethacin on fibroblastic activity is qualitatively different from its effect on primitive, disordered osteoblastic activity.

Large fracture haematomas were observed in the indomethacin-treated ani-

mals. This is in accord with the anticoagulant effect of nonsteroidal anti-inflammatory drugs, and most of these drugs furthermore induce a moderate degree of fibrinolysis *in vitro* (Famaey et al. 1975). Since the use of anticoagulants has been reported to inhibit fracture healing (Stinchfield et al. 1956), and the fracture haematoma according to Ham & Harris (1971) is thought to be an obstacle to the fracture healing process, these factors may have contributed to the delayed fracture healing in the present study.

A possible relationship between indomethacin medication and joint destruction has been reported (Coke 1967, Arora 1968, Rubens-Duval et al. 1970, Desproges-Gotteron et al. 1971, Solomon 1973, Hauge 1975). It has been presumed that this effect is primarily brought about by the analgetic action of the drug, the state of diminished sensibility predisposing to trauma in the subarticular bone. Sudmann (1975) has recently postulated that inhibition of the healing of spontaneous microfractures in the subchondral cancellous bone in weight-bearing joints may be of importance in the pathogenesis of the indomethacin-induced arthropathy. This contention is supported by the present study, provided that indomethacin when given in clinical doses interferes with the normal fracture healing in man as it does in rats in the dose used here.

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