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EFFECT OF MARSILID ON DIAPHYSEAL LAMELLAR RABBIT BONE

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INTRODUCTION

THESE INVESTIGATIONS followed the observation by one of us that the quality of bone in patients who had received Marsilid for long periods of time appeared to differ at the operating table from that of patients who had not received the drug. This series of experiments resulted and were approached with certain questions:

1) Is there an effect of Marsilid on rabbit bone? If so, is the effect exerted on bone formation, on bone resorption or on the state of already present bone?

2) Is there an effect of tetracyclines on rabbit bone?

3) Is there any mutual synergism or opposition between Marsilid and tetracyclines?

4) Does Marsilid affect the amount of tetracycline deposited in rabbit bone in vivo?

It is interesting to note that for the most part our experiments did not yield answers to these questions, although there were some relevant findings.

The work embraces three separate experiments performed sequentially, the latter experiments to some degree being designed on the basis of experience gained from the earlier ones. In the first experiment Marsilid was given for prolonged periods. In the second, Marsilid was given with orally administered tetracycline for an intermediate length of time. In the third, Marsilid and cortisone were given in toxic doses for brief periods, concomitantly with tetracycline.

Attention in these experiments was limited to lamellar bone formation in the diaphyses of selected long bones in New Zealand white rabbits. Other effects of the drugs on rate of growth, rate of gain in weight, enchondral ossification, and so forth were ignored, with minor exceptions which will be noted.

EXPERIMENTS

Experiment I

Ten three-month old rabbits were divided, two serving as controls and eight as experimental animals. The latter group was given 10 mg/kilo Marsilid daily by gavage for three months. Then all animals were sacrificed, their ages then being six months. All were healthy. There was no gross or histological evidence of liver damage in either group.

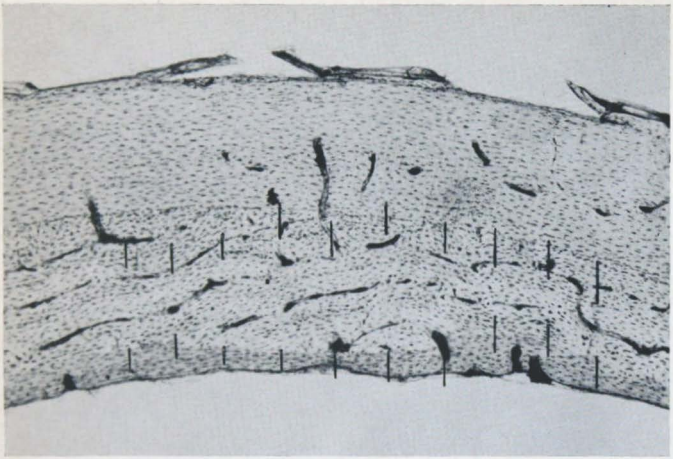

Figure 1

Undecalcified cross section tibia, 6 months old rabbit, treated with Marsilid 10 mg/kilo/day for 90 days. Periosteum at top, endosteum below.

A) Note the zone of micropetrosis, appearing as the clearer bone between the series of vertical India ink bars. This condition results from occlusion of canaliculae with mineral and is accompanied or preceded by death of the osteocytes depending on these canaliculae for nutrition.

B) Note lack of internal reconstruction of cortex and lack of Haversian systems. This bone in rabbits at this age normally contains myraid Haversian systems. The defect illustrated is a selected, extreme example. In a larger series (Experiment II) the depression of remodelling was about 40% of the activity observed in controls.

STATISTICS: Fresh, undecalcified, fuchsin-stained cross sections were made from the middle third, and junctions of the thirds, of the tibia and femur of one hind limb.[2,4] From the same bones of the other hind limb longitudinal sections were prepared. Eighty sections were suitable for observation.

OBSERVATIONS: A) There was considerable micropetrosis[5] in the Marsilid treated group and little in the two control animals. This was interpreted as a possible osteocytotoxic effect requiring confirmation by repetition of, and enlargement of, the experiment (Figure 1).

B) There was a decrease in internal cortical remodelling, noted on reviewing the material after the data from Experiment II were available. By internal remodelling is meant the production of resorption spaces in the confines of the cortex, and subsequent filling of these spaces with Haversian systems (Figures 1, 2b, and 3).

C) An increase of dead osteocytes, in the Marsilid treated group of animals, was present in the micropetrotic bone. Since osteocyte death appears to be the prelude to development of micropetrosis, the two observations could be related.

Experiment II

Fifty-two six-week old rabbits were divided into two groups, a control and an experimental group. All were given tetracycline orally by gavage for an initial period of two weeks, followed by two weeks without tetracycline, followed by another two weeks on tetracycline. This multiple-band tetracycline labelling, and in vivo labelling of bone with the tetracycline group of antibiotics resulting from the reports of Milch, Rall, and Tobie, is discussed in detail elsewhere.[6,7,10,11]

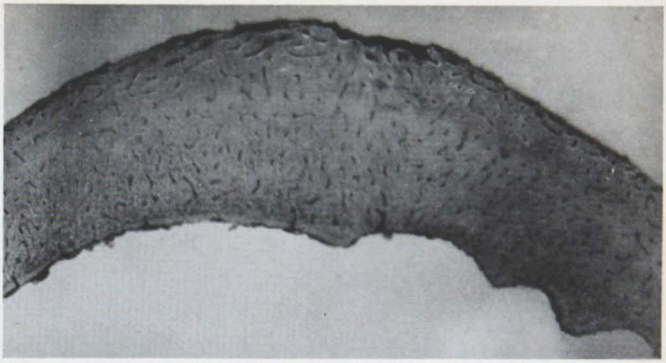

Figure 2a

A) Cross section M/3 tibial crest from a rabbit on Marsilid for 6 weeks. Experiment II. Periosteum is at the top. Blue-light microfluorescence. Although tetracycline was given twice for a total of 4 weeks only traces of it are visible as bright spots near the periosteum.

Note the lack of resorption spaces which are normally present to some extent in this part of the tibia. There has been less Haversian system replacement of the cortical interior than is normally found.

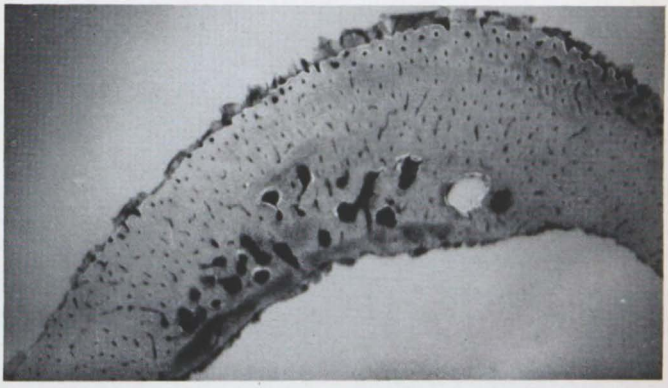

Figure 2b

B) Similar bone, location and experiment as in Figure 2A but from a control animal which did not receive Marsilid. A cluster of resorption spaces, some actively being filled with new Haversian bone lies in the cortical interior near the endosteal (lower) surface. There is also more remodelling going on close to the periosteum than observed in Figure 2A. The occasional bright lines seen on the periosteal and resorption space surfaces are tetracycline fluorescing by blue-light technique. The presence of the drug proves the presence of active mineralization of bone matrix.

As in Figure 2A, the extreme cases have been photographed.

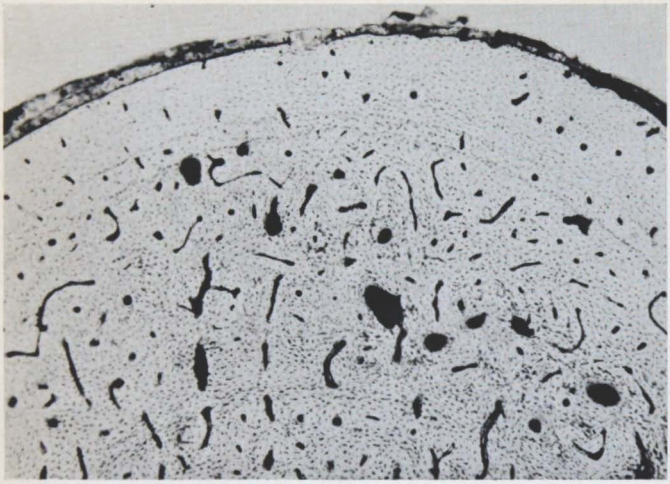

Figure 3

Cross, undecalcified section through middle third of tibia of control rabbit. Experiment II. Compare with Figure 1. Some resorption spaces may be seen as enlarged, black areas. In the actual section osteoid seams (Pre-bone of Lacroix and his school) may be seen, indicating new bone formation is in progress. There is considerable Haversian reconstruction present, almost all of the small, circular black areas being Haversian canals seen in cross section.

This section represents the average, rather than the extreme appearance of normal rabbit tibia of the age noted in the text.

Marsilid, 3 mg/kilo/day, was given to the experimental group for the duration of the experiment.

STATISTICS: There were 26 control and 26 experimental animals. Sections were made as described in Experiment I from the tibias, femurs, and in addition from humerus and rib. Over 800 sections were suitable for study.

OBSERVATIONS: A) It was noted that internal remodelling of the bones was retarded, meaning that there was less Haversian reconstruction of the cortex, in the animals receiving Marsilid. In an effort to quantitate this observation the number of resorption spaces within the cortices was counted in a series of sections. There were 738 resorption spaces in 23 cross sections from the Marsilid group and 980 in 19 sections from the control group. This is an average of 32 per section in the experimental, and 52 per section in the control, groups.

This finding was not anticipated. We believe it is real rather than a statistical fluke and it is worthy of confirmation by others (Figures 2a, 2b, and 3).

B) Administration of tetracycline was accompanied by an unequivocal decrease in either the rate of formation of new osteoid, or the rate of its mineralization, or both. This was true in the controls and the Marsilid treated group.

Recall that the duration of the periods when tetracycline was given and the duration of the intervening period were the same. If bone growth rate were linear, the labelled bands would be of similar width and the same as the width of the unlabelled band between them.

What was actually seen was also not anticipated: In eight animals the thickness of the initial tetracyline band averaged 50 microns; of the second tetracycline band 30 microns;

but of the unlabeled band between them 300 microns. It is unknown whether this effect is a direct result of the tetracyclines, or an indirect effect of this or some other factor in the experimental design.

C) It was tentatively observed that Marsilid decreased the rate of circumferential lamellar apposition. An average of 381 microns was added to the outside diameter of the femur in two control animals and 270 microns in six Marsilid treated animals over a two week period. This sounds better than it is because the scatter in individual cases was too large to permit confidence in the meaning of the averaged values.

D) There was no significant difference in the amount of micropetrosis noted in the control and Marsilid treated animals in this experiment. The duration of the experiment was admittedly less but one has more confidence in a negative result involving study of 52 animals than from a positive result involving only ten. In addition, the animals in the present experiment were still immature at time of sacrifice, while those in experiment one were mature. Micropetrosis appears to be a common feature of adult rodent bone.

E) The information sought for when this experiment was designed could not be obtained. Mutual effects of tetracycline and Marsilid could not be evaluated because oral labelling proved to be a completely unsatisfactory method of labelling rabbit bone. In only eight of the 52 animals could enough tetracycline be seen in the sections to permit measurements. The equipment used was orthodox and efficient: a Zeiss (Oberkochen) 200 watt high pressure mercury source used with Zeiss barrier filters. Russe in vienna has experienced the same difficulty with labelling rabbits.[12] No such difficulties have been noted in labelling man, rats, or dogs.[6]

Experiment III

Six week old rabbits were again used, a triple band design was again selected, the period between tetracycline labels being a week, the labels being intraperitoneally injected tetracycline, 200 mg/kilo, given on the first day of three successive weeks.

Six of these animals were placed on a toxic dose of Marsilid, 25 mg/kilo/day for a week. Three animals were placed on 50 mg/kilo/day of cortisone acetate for a week and six animals served as controls. Since no tetracycline was administered during the actual times the other drugs were given there was no question of mutual effects.

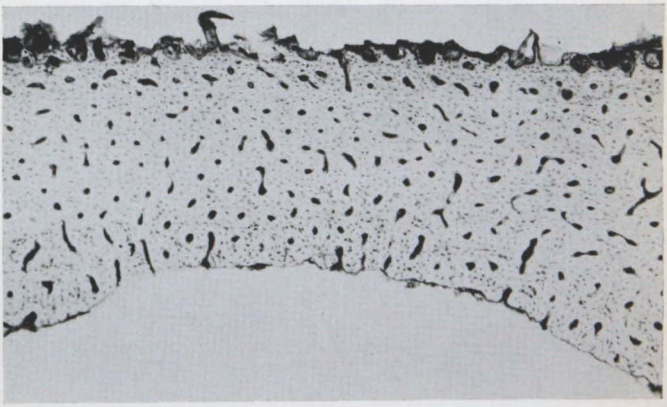

Figure 4

Undecalcified cross section tibia of rabbit given 50 mg/kilo of cortisone during its last week of life, as described in Experiment III. Bright field. Periosteum is at top.

Note irregular periosteal surface which appears to be the result of resorption. Note that most of the remainder of the cortex (which was formed before cortisone was started) exhibits much Haversian reconstruction.

STATISTICS: Two of the Marsilid group died during the experiment while receiving this drug. The remaining animals were sacrificed from one to nine days after ending the experiment and sections made from bones as described in Experiment II.

OBSERVATIONS: A) The two Marsilid treated animals that died prematurely exhibited liver damage grossly and histologically.

B) In the surviving Marsilid treated animals there was an increase in death of osteocytes, but only in bone formed during the experiment. This distribution of osteocyte death is different from that seen in micropetrosis and as the result of increase in age.

C) In the cortisone treated animals sacrificed immediately after stoppage of the drug, there was an almost total arrest of new bone formation with an accompanying lack of osteoid seams and decrease in the amount of fuchsin permeable bone (Figure 4). By way of explanation it may be noted that recently formed bone is fuchsin permeable because it has not had time to mineralize to the point that diffusion of the dye is effectively prevented. With increasing time after formation bone normally becomes fuchsin impermeable. When new bone formation is arrested, no new fuchsin permeable material is formed but that already present continues to mineralize, so that after a sufficient period of time little or no fuchsin permeable bone will be observed.[3]

D) If cortisone treated animals are not sacrificed until a week or so after stoppage of the drug there is a distinct "bounce" in new bone formation during this week and very active periosteal appositional activity may be observed, as though nature were trying to makeup for lost time.

E) Intraperitoneally injected tetracycline produces intense labels easily observed by ultraviolet[9] or blue-light microfluorescence.[6,11] However, the formation of new periosteal bone is a very irregular, sporadic process in rabbits with the result that often a single dose of tetracycline does not show up on sections: no bone was actively being formed when the drug was given (Figure 5).

F) In Marsilid treated animals sacrificed at the conclusion of the experiment there was little active periosteal bone formation observed, few osteoid seams, and little fuchsin permeable bone.

G) In Marsilid treated animals sacrificed a week or more after conclusion of the experiment very active periosteal new bone formation was apparent, indicating that there was a "bounce" in bone formation just as was seen in the cortisone treated, but less numerous, group of animals (Figure 6).

Conclusions

Subject to independent confirmation the following statements about this work appear reasonable:

1) Marsilid seems to inhibit internal cortical reconstruction in rabbit diaphysis. The histology suggests that this is the result of interference with the initial phase of reconstruction: the formation of resorption spaces.

2) There is no definite evidence that Marsilid in reasonable doses exerts any effect on osteocytes. There is definite evidence that in toxic doses Marsilid has an osteocytotoxic effect on the osteocytes in recently formed bone.

3) Toxic dosages of Marsilid produce bone effects similar to those produced by cortisone. It is therefore possible that the observed effects of Marsilid were the result of an endogenous increase in cortisone secretion resulting from the stress of the experiment. It also is possible that Marsilid in some manner potentiates the effect of endogenously secreted corticoids.

4) The rebound acceleration in bone formation noted in Experiment III has been observed by other investigators, notably Collins, Garrett, and Johnston[1] using

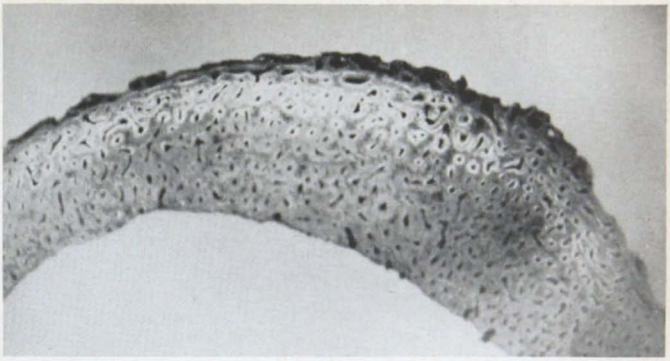

Figure 5

Undecalcified cross section tibia of rabbit given 25 mg/kilo of Marsilid in the last week of its life. Blue-light fluorescence. Periosteum is at the top. The bright lines are flurescing tetracycline and identify the sites of active mineralization of new matrix during the 3 weeks prior to sacrifice.
Note the irregular periosteal surface, especially at the right, similar to but not as marked as that illustrated in Figure 4. Only two of the 3 tetracycline bands are identifiable. The morphology suggests that the third band is the missing one. Since this band is also missing in *most* of the controls no conclusions may be drawn.

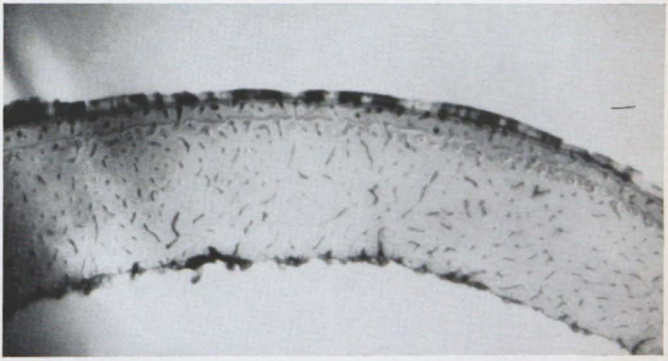

Figure 6

Undecalcified cross section tibia from a rabbit given Marsilid 25 mg/kilo for 7 days, but not sacrificed until 9 days after stoppage of the drug. Microfluorescence. Periosteum at top.
The top irregular layer is periosteum.
Just under this layer lies a thin lamina of more darkly staining bone. This layer is fuchsin permeable and is the result of intense new bone forming activity following stoppage of the drug. Compare with Figure 5. *None* of the 3 tetracycline labels are present in this bone!

calcium. It has been variously interpreted by investigators who did not have access to direct histological observation.

5) Rabbits appear to be a poor animal for study of rate of bone formation due to their unpredictable, irregular rate of bone formation. There appears to be an adverse effect of tetracycline on rabbit bone formation which has not been noted in other mammals after birth and has not been noted in man.

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