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FRACTURE REPAIR IN THE SNELL DWARF MOUSE

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Summary.—Snell dwarf mice are deficient in the somatomedin peptides which are mediators of growth hormone action on the skeleton. Tibial fractures in dwarf mice united within 6 weeks, however chondroid differentiation and osteogenesis at the fracture site were retarded in the first few weeks after fracture compared with normal mice.

Administration of bovine growth hormone (5 μg daily) accelerated the repair process and 2 μg thyroxine daily resulted in rapid callus formation and ossification indistinguishable from normal controls.

Normal somatomedin levels are not therefore essential for adequate fracture healing in Snell dwarf mice. The acceleration resulting from growth hormone and thyroxine administration may be due to an increased production of somatomedins locally or systemically or by direct action on connective tissue.

AN EARLY STAGE in fracture repair is the proliferation of osteogenic cells of the periosteum and in the marrow cavity followed by differentiation of cells in some areas to chondrocytes and the formation of cartilagenous callus (Bennett, 1971). During skeletal development, hypersecretion of growth hormone or the administration of growth hormone after hypophysectomy results in increases in chondrogenesis, growth plate width and the synthesis of cartilage matrix proteoglycans (Asling and Evans, 1956; Daughaday and Kipnis, 1966). New bone formation is also enhanced by exogenous growth hormone in adult dogs (Harris *et al.*, 1972). Because of these anabolic actions the role of growth hormone in fracture healing has been the subject of numerous investigations in experimental animals and in man (Nichols, Toto and Choukas, 1968; Koskinen *et al.*, 1978; Misol, Samaan and Ponseti, 1971; Herold, Hurvitz and Tadmor, 1971).

Many of the actions of growth hormone on skeletal tissues are mediated by the somatomedin group of growth promoting peptides (Van Wyk and Underwood, 1978). The hereditary dwarf mouse (Snell strain, Snell, 1929) is deficient in plasma somatomedin as well as in growth hormone, prolactin, thyrotropin and possibly corticotrophin (Sinha, Salocks and Vanderhaan, 1975). Daily administration of thyroxine or growth hormone to these mice raises plasma somatomedin activity and enhances growth (Holder and Wallis, 1977; van Buul-Offers and Van den Brande, 1982).

It has been reported that experimental tibial fractures in Snell dwarf mice do not unite (Hsu and Robinson, 1969). The present investigation was undertaken to investigate this further and to examine the effects on the repair process of exogenous growth hormone and thyroxine.

MATERIALS AND METHODS

Hereditary dwarf mice (Snell strain, dw/dw) and normal litter mates were a generous gift from Dr A. T. Holder, Institute of Child Health, London. They were bred and reared as previously described (Holder *et al.*, 1980). It has been well established that both plasma

growth hormone and somatomedin concentrations are low in these mice (Holder and Wallis, 1977; Holder *et al.*, 1980).

Closed fractures of the right tibia were produced by digital pressure under ether anaesthesia between 10.00–11.00 h. Mice resumed full activity and normal feeding very rapidly thereafter.

Mice (58 Snell dwarfs and 8 litter mates) were used in 4 experiments as follows:

Experiment 1.—Six Snell mice, 12 weeks old at the time of fracture, body weight 9·3 ± 1·3 g. Radiographs were obtained at 3, 6, 7 and 13 weeks postfracture and histology at 6, 7 and 13 weeks.

Experiment 2.—Eighteen Snell mice, 14 weeks old, body weight 10·9 ± 1·2 g at fracture. The mice received exogenous growth hormone, thyroxine or saline daily from the first post-fracture day until killing. Animals were weighed daily. Radiology at 2 weeks and both radiology and histology at 4, 6 and 7 weeks were obtained.

Experiment 3.—Ten Snell mice, 12 weeks old, body weight 9·5 ± 1·1 g at fracture. Histology was obtained at 2, 4, 5, 8 and 12 days post-fracture.

Experiment 4.—Twenty-four Snell mice body weight 8·7 ± 1·2 g and 8 normal litter mates, body weight 16·1 ± 2·8 g at 11 weeks old. Hormones were administered daily to Snell mice for 4 weeks before fracture (at 15 weeks) and thereafter until killing. Animals were weighed daily. Radiology and histology were obtained at 4, 8 days and 2, 3 weeks. The number of mice examined at each interval is shown in the Table.

TABLE.—*Number of mice receiving each treatment at different intervals*

		Snell dwarf mice		
Intervals	Normal Mice	Control	GH	Thyroxine
2d	—	2	—	—
4d	2	4	2	2
5d	—	2	—	—
8d	2	4	2	2
12d	—	2	—	—
2w	2	2	2	2
3w	2	2	2	2
4w	—	2	2	2
6w	—	4	2	2
7w	—	4	2	2
13w	—	2	—	—

Hormones.—Treated animals received a daily (11.00 h, 5 days a week) s.c. injection (0·1 ml) of 50 μg/ml bovine growth hormone (NIH GH 18; 0·81 IU/mg) or 20 μg/ml thyroxine (Sigma). These doses of growth hormone and thyroxine have been shown to have maximum effect at raising plasma somatomedin activity (Holder *et al.*, 1980). Hormones were freshly prepared each week in 0·9% saline. Control animals received 0·1 ml of 0·9% saline.

In each experiment mice were allocated to hormone treatment groups at random. In experiments 2 and 4, each cage contained at least 2 of each treatment.

Histology.—For histological studies, mice were killed at intervals between 2 days and 13 weeks and the fractured limb dissected, fixed in 10% buffered formalin, pH 7·0 and subsequently decalcified in 5% HNO_3 for paraffin embedding. Sections (2–7 μ) were stained with Haematoxylin and Eosin or 0·25% (w/v) toludine blue, pH 4·5.

RESULTS

Untreated mice

It could be seen radiographically that within 6 weeks there was a stable bony union in tibial fractures in Snell dwarf mice which received no hormone treatment (Fig. 1a).

A closer study of the repair process was made histologically beginning at 2 days post-fracture at which time an inflammatory periosteal reaction was evident. Myxoid ground substance could be seen by 4 days and at 8 days (Fig. 2a), there was well marked chondroid differentiation and cartilage formation. Some osteoid trabeculae were visible at 12 days (Fig. 2b) and by 3 weeks (Fig. 2c) new bone with a haemopoietic and fatty marrow was extensive. In these untreated mice islets of cartilage were still present at 3 weeks. Six or 7 weeks after fracture there was well organized trabecular bone and marrow which bridged the fracture site in all the animals which were maintained for this period (Fig. 2d).

The early stages (2–4 days) of periosteal reaction in these untreated dwarf mice was similar to the normal litter mates (data not shown). However, in the normal mice bony trabeculae were already extensive at 8 days (Fig. 3a) and by 3 weeks post-fracture the callous was composed of new bone and haemopoietic bone marrow (Fig. 3b).

Hormone treated mice

Snell dwarf mice which received growth hormone (5 μg) or thyroxine (2 μg) daily

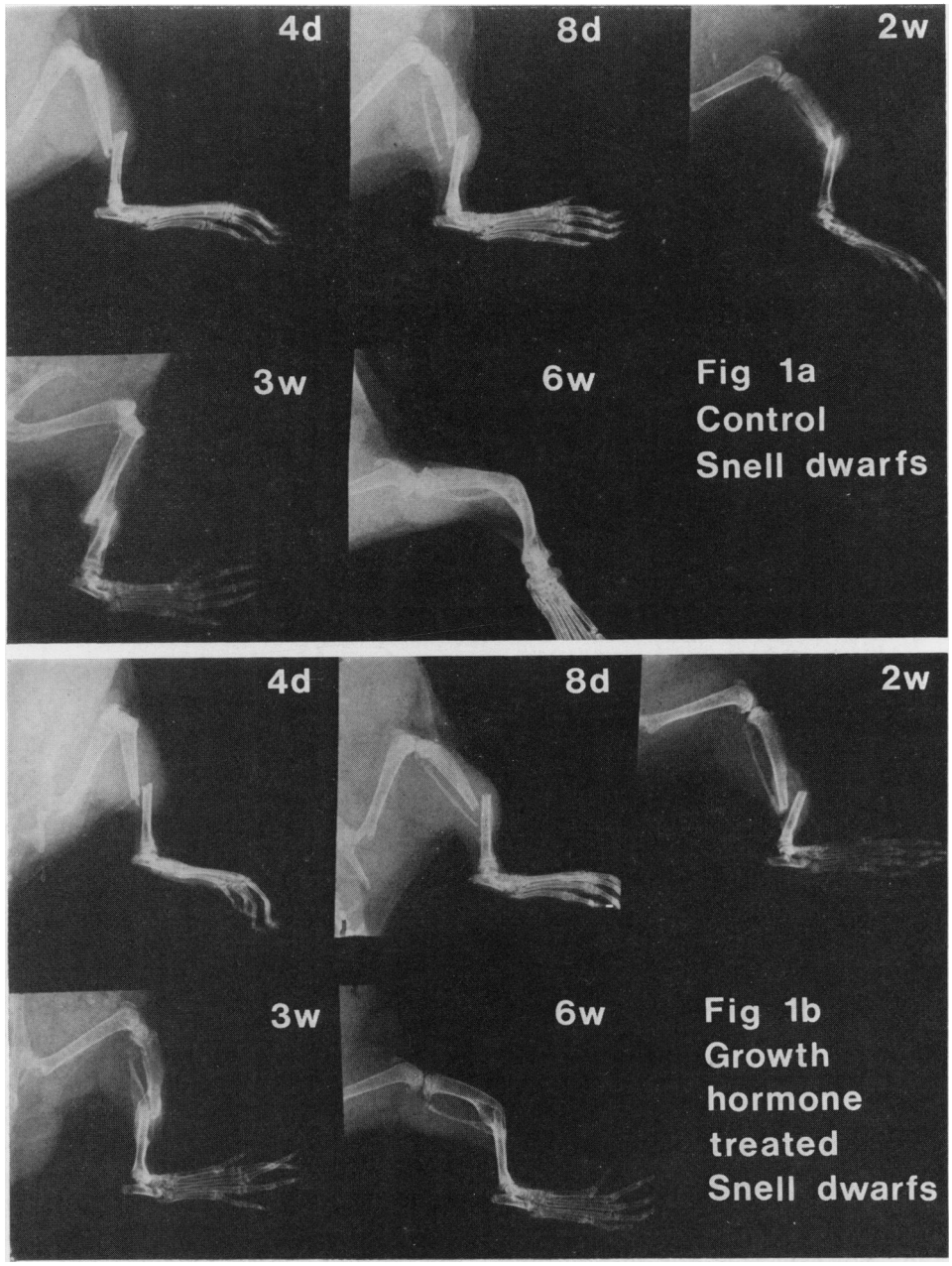

FIG. 1.—Radiographs at 4 days, 8 days and at 2, 3 and 6 weeks after tibial fracture in (a) untreated, (b) growth hormone treated and (c) thyroxine treated Snell dwarf mice.

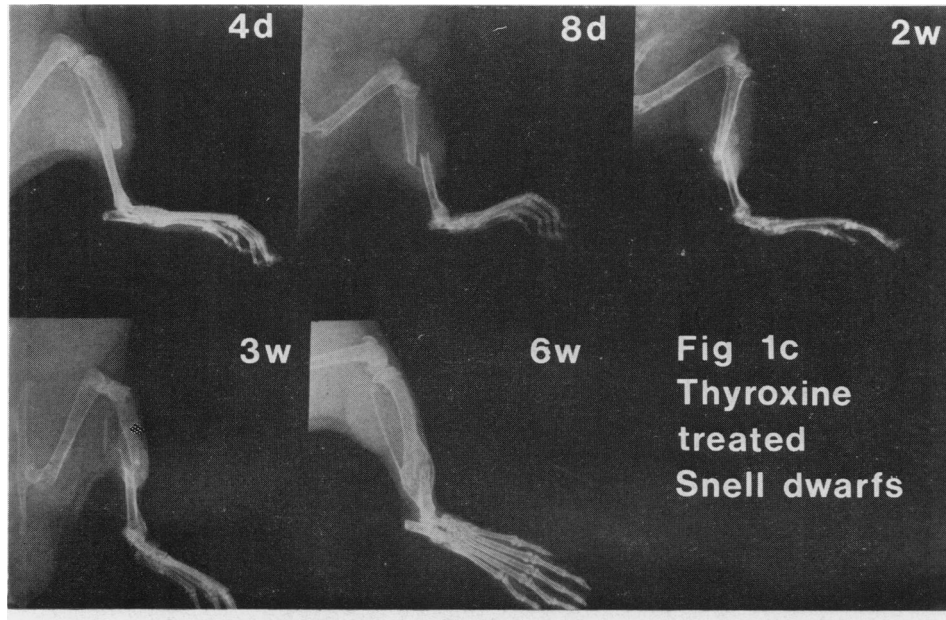

FIG. 1(c)

had a greater weight gain than did control mice (Fig. 4). Radiographically there was no clear difference between the progress of fracture union in the treated and untreated mice (Fig. 1b, c).

Histological examination did, however, reveal differences between the growth hormone and thyroxine treated and untreated mice. In the growth hormone treated group, the cartilagenous callus was better differentiated at 8 days (Fig. 5a) post fracture than was observed in the untreated dwarf mice group although there was little evidence of osteoid formation at this stage. Three weeks post-fracture, new trabecular bone bridged the fracture site. Islets of cartilage remained, (Fig. 5b), however this was substantially less than in the untreated group.

In the thyroxine treated mice, osseous trabeculae similar to those in the normal mice were seen at 8 days (Fig. 6a). Within 3 weeks new trabecular bone and haemopoietic marrow had completely replaced the initial cartilage (Fig. 6b).

DISCUSSION

In the Snell strain of hereditary dwarf mice, growth suppression is evident by 14 days after birth and thereafter the process of skeletal differentiation is greatly reduced (Snell, 1929). This results from a greatly diminished secretion of both growth hormone and thyrotropin leading to exceedingly low levels of plasma somatomedin peptides (Holder and Wallis, 1977; Holder *et al.*, 1980).

The present study shows that there is also a delay in callus and osteoid formtion in the Snell dwarf mice following tibial fracture when compared with normal litter mates. This difference is more noteworthy since the normal litter mates are skeletally much more mature than the corresponding dwarfs (Dawson, 1934). The Snell dwarf mouse therefore resemble the hypophysectomized rat in which osteoblast proliferation in the early stages of repair following tibial fracture (Nichols *et al.*, 1968) is significantly reduced. Somatomedin peptides have a mitogenic

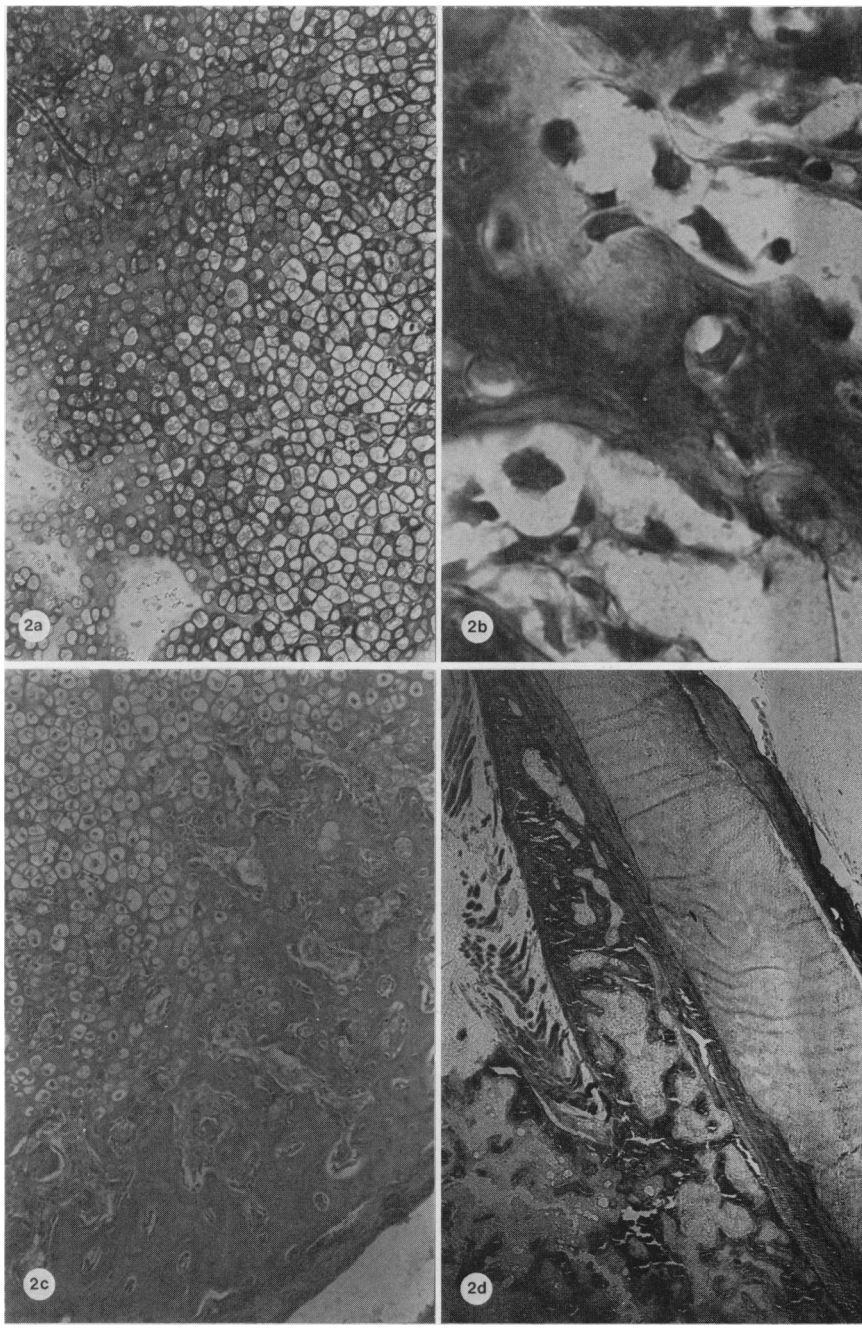

FIG. 2.—Untreated Snell dwarf mouse. (a) 8 days after fracture. Haematoxylin and eosin. Magnification on film 25:1. (b) 12 days post fracture. Toluidine blue. ×83. (c) 3 weeks post fracture. Haemotoxylin and eosin. ×13. (d) 6 weeks post fracture. Toluidine blue. ×5.

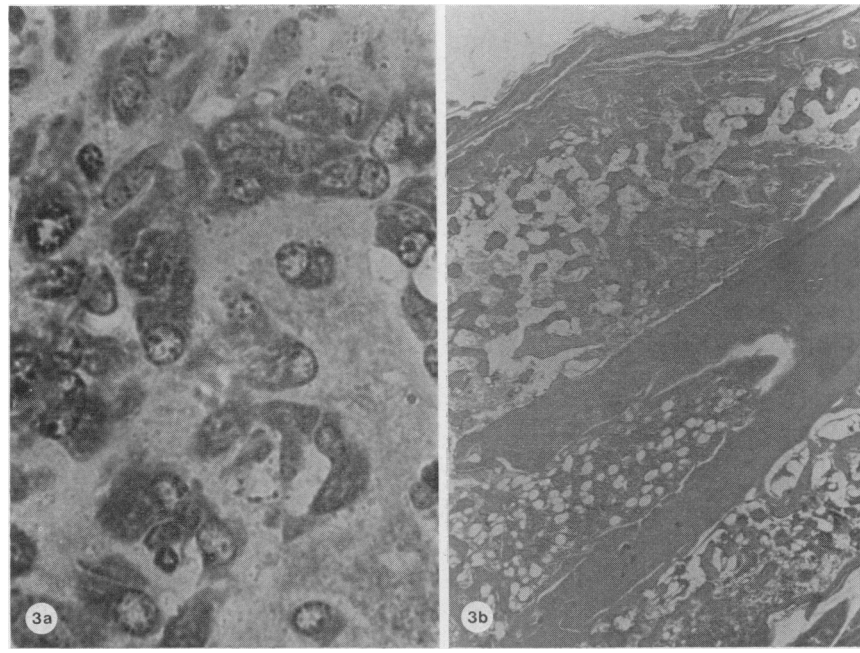

FIG. 3.—Normal mouse. (a) 8 days post fracture. Haematoxylin and eosin. ×83. (b) 3 weeks post fracture. Haematoxylin and eosin. × 6·3.

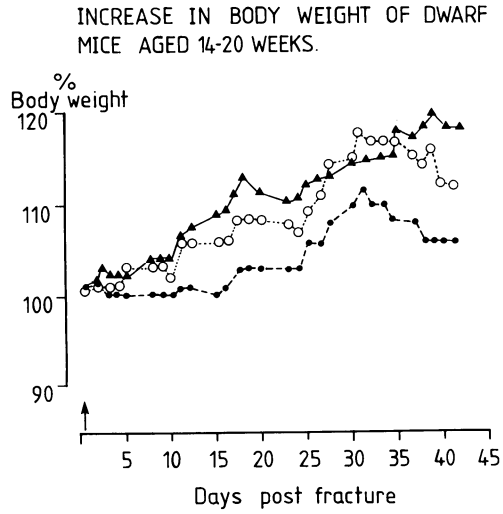

FIG. 4.—Gain in body weight of Snell dwarf mice aged 14 weeks at fracture (↑) treated after fracture with saline (●), growth hormone (○, 5 μg daily) or thyroxine (▲, 2 μg daily). Experiment 2. Each treatment group consisted of 6 animals.

action on both chondrocytes and bone cells *in vitro*, while most experimental evidence has suggested that growth hormone does not. Thus it is possible that the deficiency in plasma somatomedin which is observed in both the Snell dwarf and the hypophysectomized rat may lead to an early delay in the fracture healing process.

The administration of growth hormone or thyroxine promoted only a modest increase in body weight in the Snell dwarf mice compared with other studies (Holder and Wallis, 1977; Van Buul and Van den Brande, 1978; Holder *et al.*, 1980). Nevertheless, both treatments advanced the appearance and maturation of the cartilagenous callus following fracture. The dose of both hormones and the period of administration preceding sacrifice were selected so that plasma somatomedins would be elevated to the same concentration (Holder and Wallis, 1977) in both treatment groups. It is therefore likely that the greater advance in fracture repair which was seen in the thyroxine treated animals, compared to the growth hormone treated group, was due to

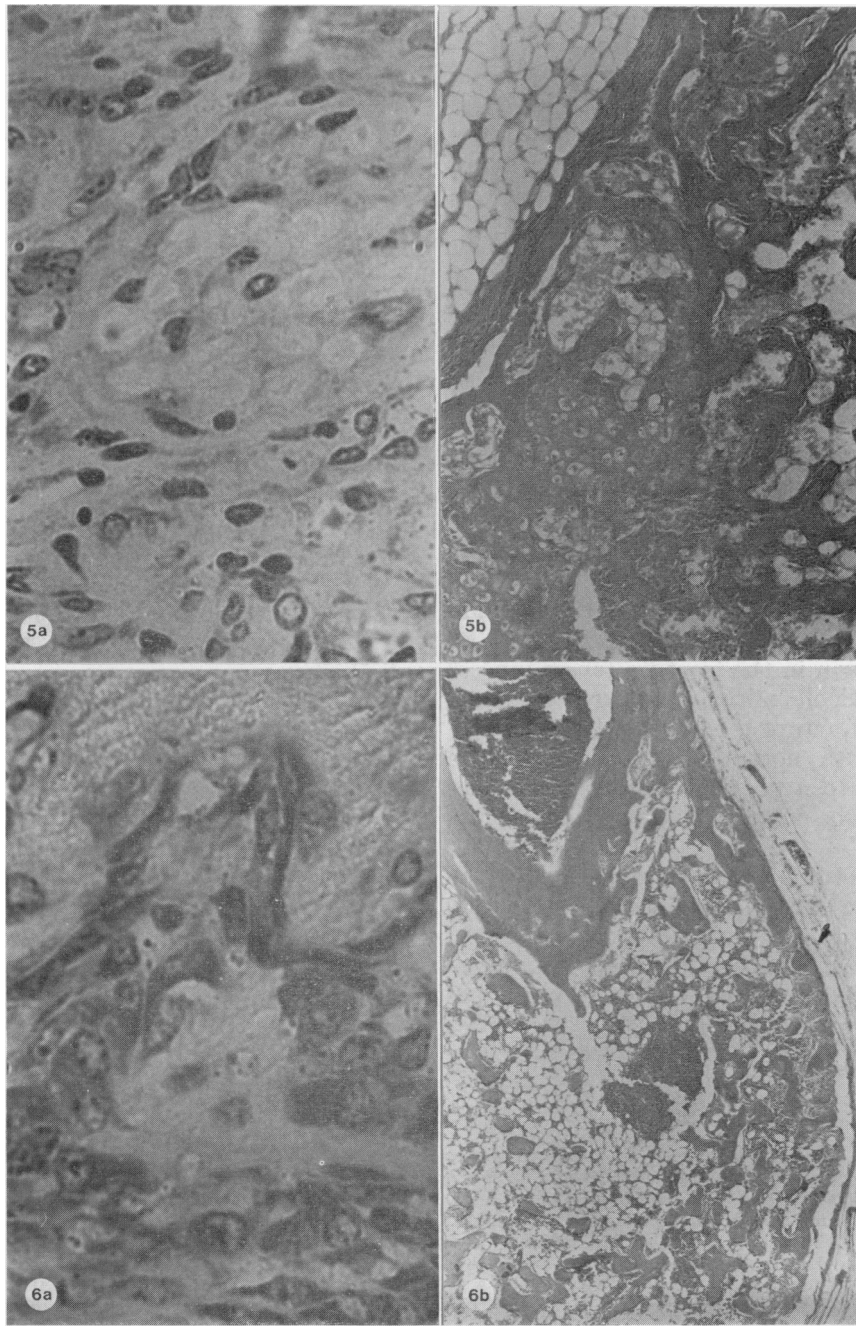

FIG. 5.—Growth hormone treated Snell dwarf mouse. (a) 8 days post fracture. Haematoxylin and eosin. × 83. (b) 3 weeks post fracture. Haematoxylin and eosin. × 13.

FIG. 6.—Thyroxine treated Snell dwarf mouse. (a) 8 days post fracture. Haematoxylin and eosin. × 83. (b) 3 weeks post fracture. Haematoxylin and eosin. × 7.

thyroxine itself or to a synergistic action between thyroxine and the somatomedins.

It has recently been shown (Isaksson, Jansson and Gause, 1982) that local injections of growth hormone into the cartilage growth plate of hypophysectomized rats increases new cell production. This may be due to a stimulated local production of somatomedin peptides as occurs in a number of other tissues. The somatomedins may then have a direct or permissive action on cartilage growth. Clearly an identical situation could exist in cartilagenous callous formation following fracture. However, as the local release of somatomedins *in vivo* cannot be quantified no firm conclusions about the relative roles of growth hormone, thyroxine or the somatomedins can be drawn from the present study.

Clearly, both growth hormone and thyroxine administration promoted an acceleration in the fracture repair process in the Snell mice. In untreated Snell dwarfs, fracture repair was delayed compared with normal litter mates but in contrast to a previous report it was not found to be completely prevented.

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