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GROWTH OF THE RAT FEMUR IN DIFFUSION CHAMBERS

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Growth in the length of 9-day-old rat femurs, isolated and cultivated in diffusion chambers, was studied for a limited period of 16 days. An increment of 40 per cent of the *in vivo* length was observed. It could be established that the elongation was due to chondral growth, of which 40 per cent occurred at the proximal and 60 per cent at the distal end of the bone during the 16 days of observation. When the trochanteric or the head epiphyses with their physeal plates were resected, no statistically significant loss in total length occurred. A small but statistically highly significant biphasic growth due to enchondral ossification was found. Resection of the trochanter produced a widening of the cervico-diaphyseal angle (valgisation) of an average of 11.2°. The findings in this series of isolated femurs are—in a general way—similar to those observed in the living animal.

Key words: rat femur, growth; diffusion chambers

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Various experiments have been conducted in order to understand the mechanisms which govern the activity of the epiphyses situated at the ends of bones (Morgan & Sommerville 1962, Khermosh et al. 1972, Weissman 1974). In these studies, whole animals have been used in *in vivo* experiments. However, it was thought that by depriving the femur of muscle traction, neural stimulation and blood circulation —i.e., by isolating the femur—some more insight into the basic processes involved could be gained. Thus the type of experiment using diffusion chambers, as described below, was developed.

METHODS AND MATERIALS

The experimental set-up is depicted in Figure 1. Nine-day-old rat femurs were dissected free of soft tissue under sterile conditions utilizing microsurgical techniques, care being taken not to damage the periosteum. The prepared bone shafts were pierced through, at their middle, by 5/0 stainless steel wire to serve as a marker. The bones and their various segments were measured with a micrometer fitted to a standard microscope. The bones were placed in the presterilized diffusion chambers which were implanted into the peritoneal cavity of adult male rats. The filter utilized on the diffusion chambers had pores of 0.22 μ diameter.

The host rats were sacrificed on pre-determined days. The diffusion chambers were removed, the bones were extracted, and their various segments were again measured using the method described above.

Nine-day-old rats were selected because on

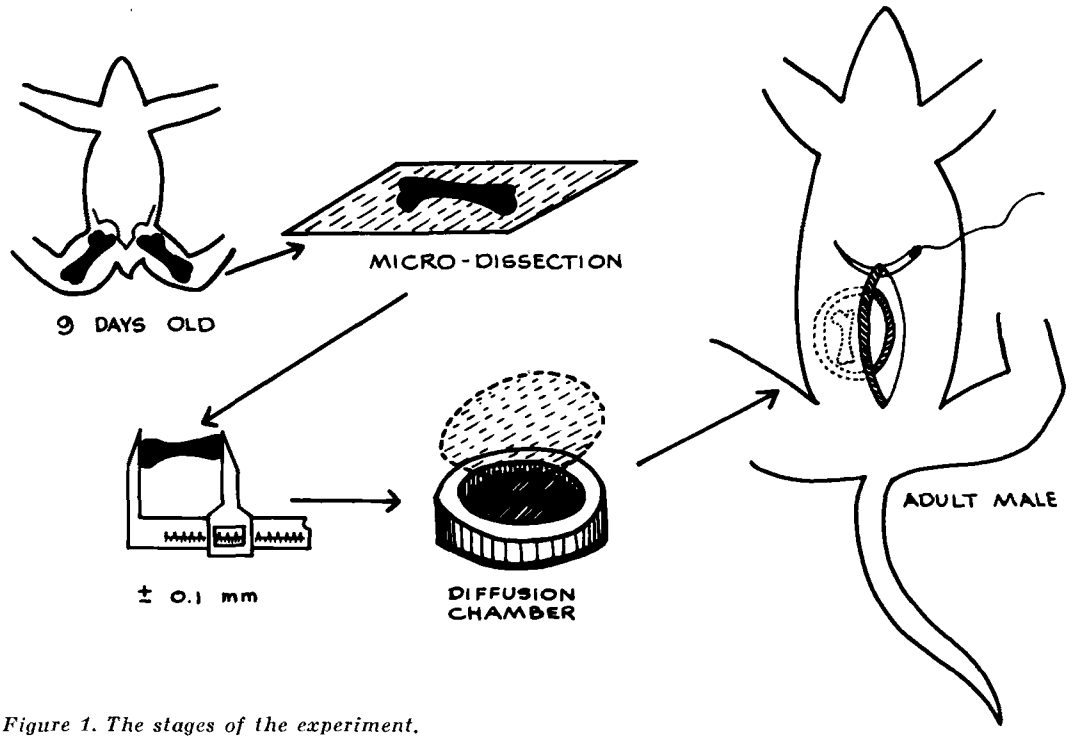

Figure 1. The stages of the experiment.

this day of postnatal life the upper physeal plate of the femur divides into two distinct parts: one for the head and one for the trochanter.

The statistical analysis (paired and simple t-tests) is based on 100 pairs. The right femur from the pair had its trochanter ("T" femur) or its head ("H" femur) resected; the left bone of the pair was left intact ("I" femur), being the intra-experimental control for the operated counterpart. An additional *in vivo* control group was examined: five rats on each predetermined day were sacrificed and the lengths of the femurs were measured. The growth of the *in vivo* femurs was thus noted during the 16 days of the experiment, starting on the ninth postnatal day.

The growth-inhibiting effect of Alizarin Red-S was examined (Harris et al. 1964, 1968). On the eighth postnatal day, i.e., 24 hours before their sacrifice, the young rats were injected intraperitoneally with Alizarin Red-S, 0.01 mg/g weight.

RESULTS

Growth in length

Figure 2 compares the growth increment ($\triangle l$) of the whole femur in diffu-

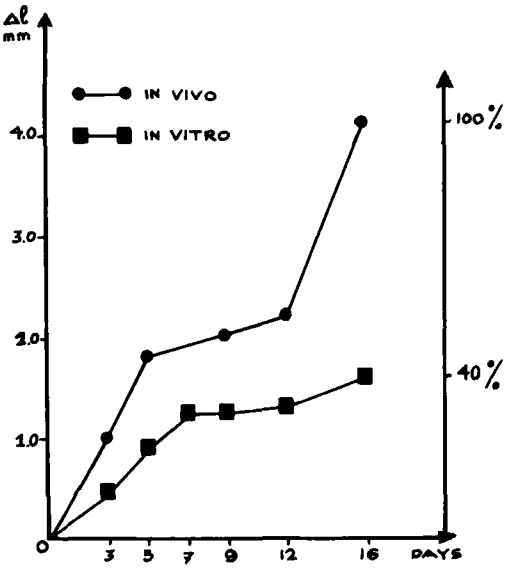

Figure 2. Growth increment ($\triangle l$) of the whole "I" femurs in diffusion chamber—in vitro—*compared to whole intact femurs*—in vivo.

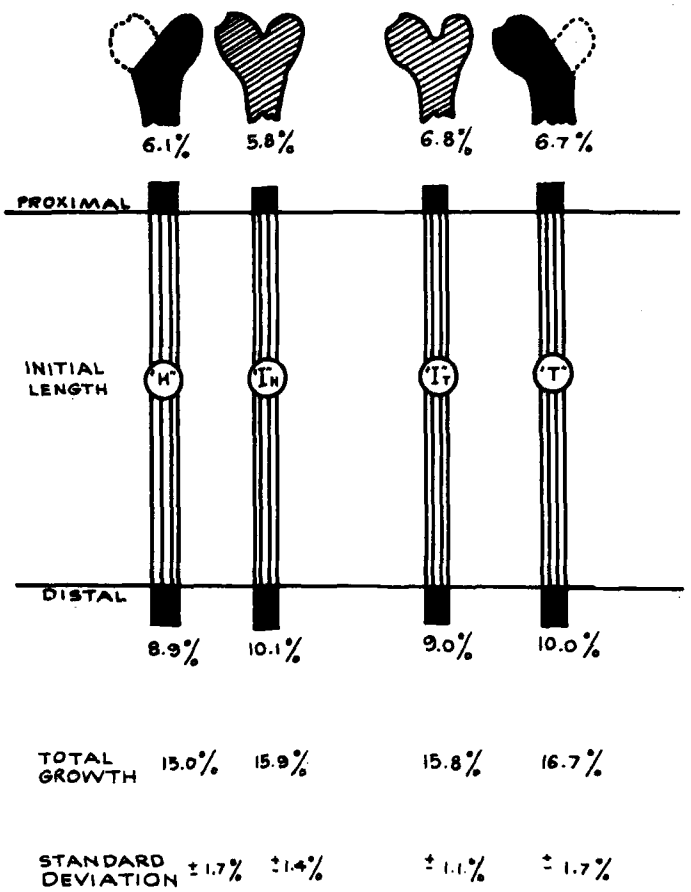

Figure 3. Growth increment of femurs, in percentage, incubated for 16 days in diffusion chambers.

sion chambers under *in vitro* conditions compared with whole femurs grown *in vivo* during the 16 days of the experiment. It shows that whereas the *in vivo* femurs grew 4.1 mm (100 per cent) during the 16 days of the experiment, the *in vitro* femurs grew only 1.6 mm (40 per cent) during the same period.

The graphs in Figure 2 show that there is a great similarity between the two curves, with an initial rise, a plateau and then again an acceleration of growth. These changes in the growth rates are statistically significant to $P<0.001$ (Paired t-test), conforming with other observations on the saltatory nature of biological growth (Swinson 1974). Sixty per cent of the total $\triangle l$ (growth increment) occurred at the distal end and 40 per cent of the total $\triangle l$ at the proximal end of the femur. The same proportion was present in the "T" femurs as well as in the "H" femurs, by the end of 16 days of growth.

Figure 3 summarizes the results in paired femurs. The initial length of the bones was extrapolated to a standard length and the total and differential growth ratios were calculated as percentages. The two columns to the left pertain to "H" femurs (head resected) and to their intact controls ("I_H" femurs). The two columns to the right belong to the intact controls ("I_T" femurs) and the trochanter-resected femurs ("T" femurs). It appears that there was a small difference between the control femurs: "I_T" and "I_H". There is no statistically

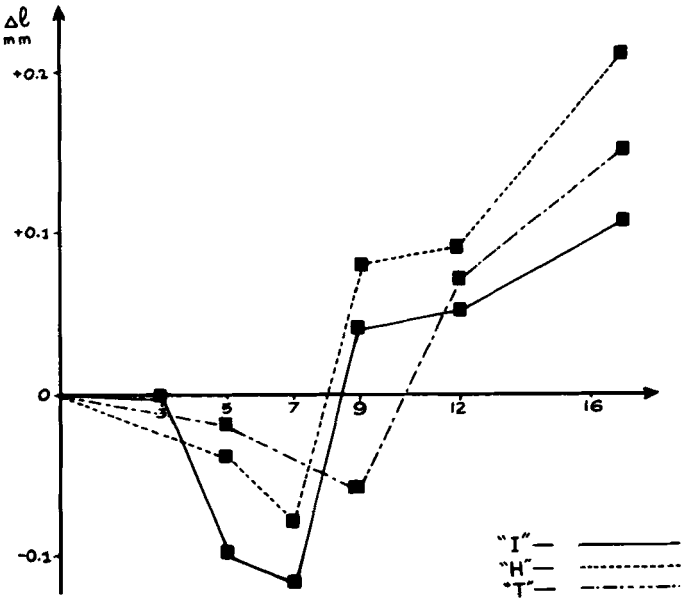

Figure 4. The biphasic growth increment (△ l) of the ossified portion of the bone, in femurs cultivated in diffusion chambers ($P<0.005$).

significant difference in the total length between the "H" femurs and their controls, although a diminished distal and an enhanced proximal lengthening can be noted. The trochanter-resected "T" femurs, which grew more than their controls, also grew more than the "H" femurs. Most of the overgrowth occurred at the distal end; however, the difference is not statistically significant.

Figure 4 shows graphically the elongation of the ossified portion of the bone. Although the measured lengths were small, the differences were statistically highly significant ($P<0.005$ with paired t-test). During the initial 7 days, there is a diminishing in length—negative phase of elongation or a lag period, which is followed by a definite increase which rises steeply with time in a similar pattern for all three types of bones, but which differs significantly in amount.

Neck-shaft angle

During the experiment, we noticed that the "T" femurs showed a persistent and measurable valgisation—i.e., an opening of the cervico-diaphyseal angle as compared with their control "I" femurs. An indirect method of measurement, which compared 18 such pairs, was applied. All of the "T" femurs showed c.d.a. widening with a minimum of 3° in one and a maximum of 27°, with a mean of 11.2°±6°.

Growth inhibiting effect of Alizarin Red-S

The bones exposed to Alizarin Red-S only grew approximately half their initial expected length during the 16 days of the experiment.

DISCUSSION

It was established that the lengthening of the bones was by real growth and not by swelling, as suggested by Fell & Mellanby (1958) and Biggers (1963). This statement is substantiated by the following:

1. The pH, P_{O_2} and P_{CO_2} values of the fluid in the diffusion chambers were similar to the hosts' arterial blood with the pH slightly to the acid side.
2. The *in vitro* growth curve as shown in Figure 2 is similar to the *in vivo* one, the difference being only in the rate

and ultimate length—due probably to unfavourable conditions in the experimental environment.

3. The Alizarin Red-S, which is a known growth inhibitor, caused a 50 per cent decrease in the total lengthening of the bones exposed to it.
4. The soft tissue overgrowth from the areas of muscle insertions showed that there was a favourable environment for cell proliferation in the diffusion chamber.
5. The observed clubbing (enlargement of the epiphyseal cartilages, more than elongation and apposition of the shaft) was similar to the observations of Pratt (1959) in the postnatal changes of rat femurs *in vivo*. He stated that the clubbing is part of this phase of postnatal development and is due mainly to epiphyseal cell proliferation by mitosis and partly to cell enlargement.

The length contribution of the proximal and distal ends of the femur to total length is quite similar to that found by Khermosh et al. (1972) in living rabbits during the entire growth period of the young animals. An analogous difference was found by Andersen et al. (1963) in humans, by following the rate of departure of Harris' lines of growth arrest in children from the physeal growth plates. The similarity of our findings to the *in vivo* developments in different species may suggest that the genetical information predetermining the bone length has a very deep penetration.

Resection of the trochanter or of the head did not produce any (statistically significant) shortening at the end of the observation period. Thus, isolated femurs behave similarly to those in living animals studied by Weissman (1974). However, the isolated femurs did not show a statistically significant enhanced growth of the distal end as did the living animals, even if this end grew more. The relatively short period of observation (16 days in all) may account for this discrepancy.

The widening of the cervico-diaphyseal angle seen in the trochanter-resected femurs is similar to that observed in humans and in experimental animals (Ewald & Hirohashi 1973). Whether it is due to the dynamics of growth, turgor pressure unopposed by other cells damaged by the resection, or due to genetically inherent information in the cells left intact cannot be answered yet.

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