C H A P T E R V

General Summary

CHAPTER V

GENERAL SUMMARY

The skeleton was till recently considered to consist of metabolically inert material and its sole function was believed to be to provide physical support to the body. Recent studies on turnover rates of calcium in the bone and activities of certain enzymes in the bone show the same to be an active site of metabolism.

Studies carried out by Fell, Biggers and others have shown the feasibility of using the tissue culture technique for a study of bone tissue which has been cultivated *in vitro* in natural as well as synthetic media. The use of this technique is becoming increasingly popular as it enables the elimination of host influences. In most of the studies on bone cultures, however, histological rather than biochemical criteria have been used for assessing the metabolic state of the cultures.

The present studies were designed to assess the biochemical state of bone cultures cultivated *in vitro* in synthetic medium and relate the same to data obtained on growth and chemical composition. The results of *in vitro*

studies were compared with those of parallel studies carried out *in vivo*.

Ten day old chick embryonic tibiae were cultivated *in vitro* in a completely defined medium (M 858) and data obtained at different stages of cultivation on (a) the chemical composition of the cultures with regard to calcium, phosphorus, nitrogen, hexosamine, citrate and moisture content and (b) glucose utilization and lactic and keto acid production by the cultures which were measured by changes in the composition of the medium with regard to those constituents. Comparative data on the former were obtained for embryonic tibiae grown *in vivo*.

Tibiae cultivated *in vitro* grew and calcified as indicated by increase in length, wet and dry weights, calcium, phosphorus, nitrogen and hexosamine linearly with time. The utilization of glucose in the medium was high for 4 days and 34% of glucose utilized was accounted for as lactic acid released suggesting the predominance of glycolysis. A comparison of the composition of tibiae cultivated in medium 858 for four days with that of tibiae grown *in vivo* for the same period showed that the growth achieved *in vitro* was smaller. However, the rate of increase in length and weight was essentially constant

for four days. Calcification and utilization of glucose were also maintained for this period of time. Citric acid on the other hand was found to disappear after explantation in the cultures.

Next, studies were carried out on chemical composition and activities of key enzymes of glycolysis, TCA cycle and hexose monophosphate shunt as well as certain other enzymes in five different areas of hen tibia, viz., periosteum, cartilage, epiphyseal head, cortical shaft and bone marrow. The enzymes studied were: α-glucan phosphorylase, lactate dehydrogenase, citrate synthase, aconitate hydratase, NADP-isocitrate dehydrogenase, glutamine synthetase, hexosamine synthetase, aspartate-2-oxoglutarate aminotransferase, glucose-6-phosphate dehydrogenase and fumarate hydratase. Chemical composition was studied with regard to the parameters mentioned earlier, viz., length, wet weight, dry weight, calcium, phosphorus, nitrogen, hexosamine and citric acid.

The cortical shaft, cartilage and epiphyseal head contained more calcium, phosphorus, and hexosamine and less nitrogen than periosteum and bone marrow. The former also constituted the calcified area of the bone. Amongst the three calcified areas, viz., cortical shaft

cartilage and epiphyseal head cortical shaft contained more calcium and phosphorus and less nitrogen than epiphyseal head and cartilage. It also contained the most citric acid, Although periosteum and bone marrow are not part of the calcified area of the bone, they did contain appreciable amounts of calcium and phosphorus.

The activity of lactate dehydrogenase was found to be of a much higher order than that of enzymes of the TCA cycle suggesting again the predominance of glycolysis in bone tissue. The activities of aconitate hydratase, NADP-isocitrate dehydrogenase and fumarate hydratase were highest in bone marrow and periosteum and lowest in cortical shaft.

Glutamine synthetase activity was detected only in bone marrow suggesting that it may be the source of glutamine required for the formation of hexosamine by hexosamine synthetase in epiphyseal head.

Activities of most of the above enzymes were measured in tibiae from 10-day and 14-day-old chick embryos and 10-day-old chick embryonic tibia cultivated *in vitro* for four days. All the enzymes studied except glutamine synthetase were found to be active in tibiae grown *in vitro* and the activities were found to compare with those of tibiae

grown *in vivo* when considered on a wet weight basis.

The increase in enzyme activity during cultivation *in vitro* was proportional to that in nitrogen content, but the latter was very much greater during growth *in vivo*. The percentage of extractable nitrogen also differed during growth *in vitro* and *in vivo* (64% and 43%). These differences showed that the form in which nitrogen was deposited differed during growth *in vitro* and *in vivo* .

Glutamine synthetase was absent in all three cases.

The results of these investigations demonstrate that tissue culture is a useful method to study the physiology and biochemistry of bone. Appreciable growth and calcification were achieved by tibiae cultivated *in vitro*. It is believed that greater growth and calcification may be possibly obtained by some modifications in the method used such as leaving the periosteum intact, addition of citric acid to the culture medium and perhaps using a gas phase of humidified air gassed with 5% carbon dioxide.