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The effect of vitamin A on skeletal tissue cultivated *in vitro*. By Honor B. Fell, *Strangeways Research Laboratory, Cambridge*, and E. Mellanby, *Nutrition Building, National Institute for Medical Research, Mill Hill, London*

The object of this study was to see whether hyper- and hypovitaminosis A acted directly on skeletal tissue grown under the simplified conditions of tissue culture, and if so, what the effects were. The following points will be demonstrated:

Hypervitaminosis A

(1) In one series of experiments, the rudiments of the limb-bones were removed from 6-day chick embryos; at this stage the limb-skeleton consists of very early, unossified cartilage. The cartilages were cultivated by the watch-glass method; one of each pair was placed in normal medium (3 parts plasma : 1 part embryo extract) and the other in medium containing about 1400 i.u./100 ml. of vitamin A. For the first 2–3 days the two sets of explants grew equally well, the diaphysial cartilage cells hypertrophied and periosteal bone was formed, but the experimental rudiments were more slender than their controls. After 4–7 days' cultivation, the bones subjected to hypervitaminosis A became rather flabby, a constriction appeared at the junctions of the shaft with the epiphyses which finally were completely cut off from the diaphysis, and the rudiments began to shrink; the soft tissue surrounding the cartilage continued to grow actively. The controls remained normal and often trebled their length in a week.

(2) The limb-bones of late foetal mice were treated in the same way. These rudiments were well developed when explanted, with a stout shaft of bone containing marrow, and large cartilaginous ends, but they were very susceptible to the action of hypervitaminosis A. After 4 days' cultivation in a medium containing about 2000 i.u./100 ml. of vitamin A, the cartilaginous ends began to shrink rapidly, the bone became greatly rarified but the surrounding soft tissue grew profusely. By the 7th day the bone was reduced to a fraction of its original length and after 10 days it had sometimes vanished completely, leaving only a few crumbs of debris scattered in a sheet of actively growing and migrating cells.

These changes were not associated with cell degeneration, and the more healthy and active the tissue appeared, the more severe were the effects of the hypervitaminosis. Although the action of excessive vitamin A on these bones is destructive, it depends on the viability of the tissues. Thus heating the bones at 45° C. for 15 min. destroys their power to grow and also their susceptibility to excess vitamin A. Heating at 40° C. for 15 min. leaves both actions intact.

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The effect on foetal mouse bones of plasma from a fowl suffering from hypervitaminosis A was compared with that of normal plasma to which pure vitamin A acetate had been added, so that the amount of vitamin A in the two plasmas was approximately the same. The results showed that the 'natural' hypervitaminosis acted on the explants much more slowly than the same degree of 'artificial' hypervitaminosis induced by the pure vitamin A acetate.

It is possible that the different effects of 'natural' and 'artificial' hypervitaminosis A plasmas are due to the different physical conditions of the vitamin A in the plasma. In the 'artificial' plasma much of the added vitamin A can be directly extracted by shaking the plasma with petrol ether. This does not happen in the 'natural' high A plasma, which requires shaking first in 50% alcohol before it is extractable with petrol ether. Most of the vitamin A in the 'artificial' plasma is in the fat-soluble form, whereas under natural conditions the vitamin A is probably in a water-soluble form, and may be in combination with a protein, and this combination has first to be broken down by alcohol before the vitamin A becomes soluble in petrol ether (Dzialoszynski, Mystkowski & Stewart, 1945).

Hypovitaminosis A

The leg-bone rudiments of 6-day chick embryos were cultivated in plasma from vitamin A-deficient fowls. The rudiments from some embryos showed a pronounced effect; chondroblastic hypertrophy and ossification were retarded and the shafts were much shorter than those of corresponding controls in normal medium. These results agree with Wolbach's observations (Wolbach & Bessey, 1941) on the effect of A-deficiency on the limb-skeleton of very young animals. The bone rudiments of other embryos, however, were unaffected by the hypovitaminosis. It is thought that this variation in the response of bones from different chicks may be correlated with the amount of vitamin A stored in the tissues at the beginning of the experiment, and this again with the amount of vitamin A and carotene in the yolk of the original egg. This point is being investigated. It appears as if the younger the animal of one species the greater are the effects of excessive and deficient vitamin-A plasmas on the bones. The effect of hypovitaminosis A on periosteal bone which is so prominent in older growing animals (Mellanby, 1938) has not yet been demonstrated in tissue-culture experiments. The evidence indicates that the vitamin A is a main controlling factor in the development and growth of bone, and that the concentration in the tissues necessary for the proper development in any species of animal must be within fairly narrow limits.

REFERENCES

Dzialoszynski, L. M., Mystkowski, E. M. & Stewart, C. P. (1945). *Biochem. J.* **39**, 63.
Mellanby, E. (1938). *J. Physiol.* **94**, 380.
Wolbach, S. B. & Bessey, O. N. (1941). *Arch. Path.* **32**, 689.