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Paper and Cases

Blood Supply of Developing Bone and its Possible Bearing on Malformation of the Limbs and Face in Congenital Hæmangiomatous Disorders

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Circulatory disturbances in bones affect their growth. Striking examples are provided by limb hypertrophy associated with congenital arteriovenous fistulæ, or by cases of congenital dermatological hæmangiomata where abnormal vessels involve not only the dermis but also underlying bones whose growth may be excessive or retarded. As an approach to elucidation of mechanisms whereby vascular disturbances localized to a limb or a part of the face bring about change in bone growth, the following experiments were performed.

Effect of Environmental P_{CO_2} *and* P_{O_2} *on Bone Growth*

An incubator was constructed in which fertilized chick eggs were placed for ten days at 38°C. Partial pressures of CO_2 and O_2 within the incubator were fixed during the incubation period. The fœtal chicks were then removed from their eggs and weighed. Skeletal calcium was extracted with nitric acid and measured by absorption spectrophotometry. Some chicks were rendered transparent and their bones stained red with alizarin (Dawson 1925), so that the length of true bone in humerus, radius, femur and tibia could be measured, as well as total length of these bones including their cartilaginous epiphyses. Nearly 500 eggs were used during this investigation.

The results show that with temperature fixed at 38°C and O_2 kept at atmospheric tension, chick bone growth varies linearly with the P_{CO_2} of incubation. This would readily be transmitted to the chick circulation through the porous eggshell. For every 1% rise in CO_2 content of the respiratory gas inside the incubator, skeletal calcium varied 35% and length of the long bones, on average, by 6%. Histologically, thickness of individual bone trabeculæ and of cortex as a whole was

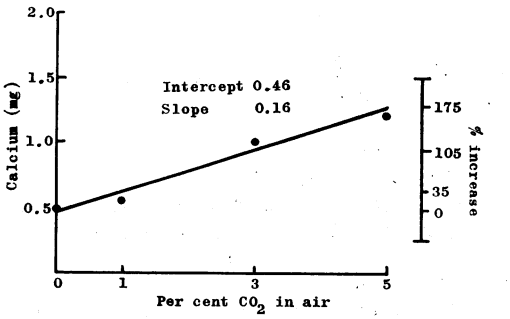

Fig 1 *Regression line showing variation with* CO_2 *tension of calcium content of 60 chick embryos incubated for ten days at 38°C in* CO_2 *and air*

greater in CO_2-incubated bones than in control material (Figs 1, 2).

Doubling O_2 tension by feeding into the incubator a 40% O_2 respiratory gas resulted in a 190% increase in total chick calcium, and an average 15% increase in bone length. When O_2 content of the gas was raised to 70%, skeletal calcium increased by a smaller amount, 147%. Bone length was increased by only 1%, but again bone thickness was histologically increased (Fig 3).

The above results as a whole suggest that bone growth in chicks is particularly sensitive to changes in P_{CO_2} and P_{O_2} of blood supplying the bone, and therefore of tissue fluid bathing the bone-forming cells. In man, lesser changes in these variables may be expected to occur in bone blood, when the osseous circulation is disturbed.

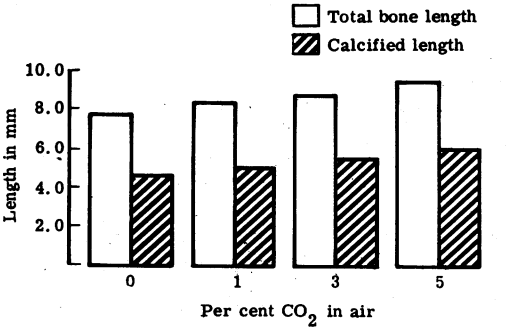

Fig 2 *Effect of* CO_2 *variation on total and true bone length of the tibia. (Alizarin red S data from 40 chick embryos)*

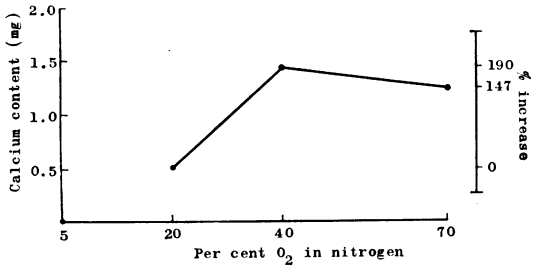

Fig 3 *Effect of hyperbaric O_2 tensions on calcium content of 45 chick embryos incubated for ten days at 38°C*

Vein Ligation and Arteriovenous Anastomosis

Effects on bone growth of femoral vein ligation and experimental arteriovenous fistula were next examined in rats, rabbits and dogs, with special reference to vascular changes including rate of blood flow into the bones and intrabone pH, P_{CO_2} and P_{O_2}. Blood flow rates were measured by arteriolar blockade (Brookes 1971) utilizing ^{59}Fe-labelled microparticles. By comparing radioactivities in the experimental and control (100%) bones, the arterial input to bone was expressed as a percentage. Access to the difficult parameters of pH, P_{O_2} and P_{CO_2} within bone was gained by making bore-holes at various postoperative intervals in the diaphysis of the femur and tibia, and in the metaphyses of these bones adjacent to the knee-joint. Problems of size dictated that mature rabbits and dogs be used for these investigations. Furthermore, it is reasonable to suppose that the pattern and direction of change in the physicochemical characters of bone blood would be of the same type, whether the animal was young or mature. Blood samples were gathered from the bones by means of microhæmatocrit tubes emplaced in the bore-holes, and pH, P_{O_2} and P_{CO_2} of each sample measured, using a Radiometer (Copenhagen) BMSIII apparatus.

Femoral vein ligation: The results showed that there is no sustained change in arterial input to the femur or tibia after high femoral vein ligation in rats, although 20 weeks postoperatively the bones were 3.5% heavier and 2% longer than their controls ($P<0.05$).

Blood sampling in 20 rabbits (5 normals and 3 groups of 5 each at 4, 8, and 24 weeks after vein ligation) gave the following results. In the normals, metaphyseal pH and P_{O_2} were lower and P_{CO_2} was higher than corresponding diaphyseal values ($P<0.05$ for each parameter), and the same applied to the contralateral, control bones. When, however, the 3 selected parameters were compared in the operated and control bones it was apparent that in 'operated' diaphyses pH and P_{O_2} were lower and P_{CO_2} was higher than in contralateral controls ($P<0.05$). Furthermore, in 'operated' metaphyses, pH was lower and both P_{CO_2} and P_{O_2} were higher than in their controls ($P<0.05$) (Table 1). Weights of the 'operated' bones were some 2% higher than their controls. No length change was possible because growth cartilages were no longer present in these mature rabbits.

Table 1

Comparison of pH, P_{O_2} and P_{CO_2} of blood samples drawn from femoral metaphysis of rabbits after left femoral venous ligation. Five animals in each group

Postoperative interval (weeks)	*Function*	*Mean values* *Left (operated)*	*Right (control)*
4	pH	7.514±0.01	7.567±0.04
	P_{O_2} (mmHg)	25.9 ±1.0	23.6 ±1.0
	P_{CO_2} (mmHg)	24.3 ±2.6	23.7 ±0.5
8	pH	7.447±0.03	7.512±0.02
	P_{O_2} (mmHg)	27.4 ±1.6	24.5 ±1.0
	P_{CO_2} (mmHg)	25.5 ±2.2	25.0 ±0.3
24	pH	7.475±0.03	7.508±0.04
	P_{O_2} (mmHg)	53.1 ±5.7	32.8 ±0.9
	P_{CO_2} (mmHg)	25.5 ±1.2	24.5 ±0.3

Table 2

Comparison of blood flow rates in tibiæ of dogs after left femoral arteriovenous anastomosis

No. of animals	*Postoperative interval (weeks)*	*Mean flow rates %* *Left (operated)*	*Right (control)*
2	2	145	100
2	4	194	100
2	8	206	100
2	12	186	100

Arteriovenous fistula: A side-to-side femoral arteriovenous fistula was constructed successfully in 12 growing rats. Twenty weeks postoperatively combined femoral and tibial weight was 20% greater than in controls, and combined length 1.9% greater. Rate of arterial input to the bones was raised considerably, 22% for the femur and 40% for the tibia.

A side-to-side femoral arteriovenous fistula, 1 cm long, was also constructed in 8 mature dogs, which were examined in pairs at 2, 4, 8 and 12 weeks postoperatively. Again, no change in length was possible, nor indeed present, in these mature animals. Blood flow to the bones was, however, considerably increased, on average by 60% in the femur and 83% in the tibia (Table 2). The same dogs before sacrifice also yielded bone blood samples which were analysed for pH, P_{O_2} and P_{CO_2}. The same pattern of change was observed here in the presence of an arteriovenous fistula as in the rabbit material with a femoral vein ligation. pH and P_{O_2} decreased in the diaphysis, and P_{CO_2} was higher than in contralateral controls. In the operated metaphysis pH was lower, and P_{O_2} and P_{CO_2} were both higher (Fig 4, Table 3).

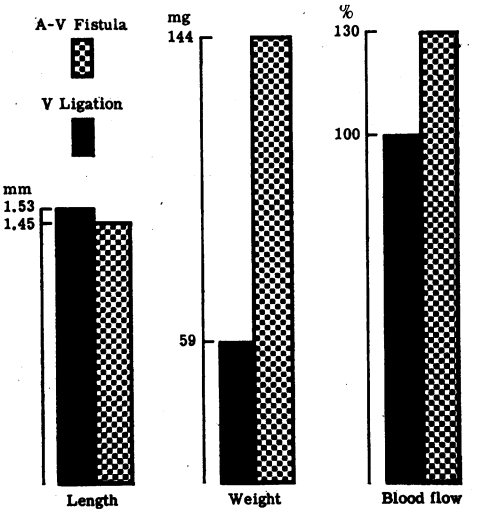

Fig 4 *Effects on length and weight of rat tibia and femur, and blood flow rates to these bones, after femoral vein ligation and femoral arteriovenous anastomosis. The latter increases leg length at the cost of a greatly increased blood supply*

Discussion

The two mammalian examples of circulatory disturbance in a limb, analysed experimentally in this investigation, were selected because a venous impediment in the limb or arteriovenous fistulæ are generally thought to be the cause of bone hypertrophy, especially bone lengthening, in certain cases of congenital hæmangiomata which extend from the dermis to underlying bone. The results show that experimentally both procedures stimulate leg lengthening to the same extent, venous ligation being slightly better in this respect. But whereas venous ligation does not bring about a sustained change in bone blood flow, an arteriovenous fistula increases it to an extent which can seriously affect the myocardium. As an operative treatment for a short limb, femoral vein ligation would therefore appear to be considerably more advantageous than elective arteriovenous anastomosis, which has been recently practised in children.

The two procedures investigated here not only increase the length of bones in immature animals to the same extent, but also the weight of mature bones. Hence, it is apparent that increasing blood flow to bones, as in the case of arteriovenous fistula, is not in itself a stimulus for bone growth. The results on the contrary suggest that it is the physicochemical quality of the blood in bones which provides direct biological control of their growth. Both mammalian experiments resulted in the same pattern of physicochemical change in bone blood. Furthermore, limb venous pressure in arteriovenous fistula is considerably elevated. Both procedures therefore have in common a venous impediment and give rise to similar changes in the internal environment of bones.

Bones grow in length at the metaphyses. The results indicate that here Po_2 and Pco_2 are elevated. The chick experiments reported here demonstrate directly the profound effect exerted on bone growth by elevation in the tension of these gases. The metaphyses in the dogs and rabbits also showed a fall in pH. This causes bone trabeculæ to thicken and promotes bone elongation (Brookes 1971).

In the diaphyses, however, the pattern of change was a fall in pH and Po_2 and a rise in Pco_2. This is explicable on the basis of a transcortical venous shunt from muscle venules into the marrow cavity. The three factors taken together are characteristic of compact bone formation in cortex (Brookes 1971) and account in part for increased bone weight observed after the experimental vascular alterations investigated here.

The results of this enquiry suggest that when dermal hæmangiomata are complicated by limb hypertrophy, then the bones also have an abnormal circulation, with the physicochemical features of adaptation to a venous impediment. Venous obstructions or multiple arteriovenous fistulæ in the limb are two ways in which this may occur. Because bone is a connective tissue, it may be expected that where hæmangiomatous lesions are diffuse and penetrating, not only the bones but also the dermis, fasciæ and intermuscular packing material will exhibit hypertrophy; in addition, there will be a venous change in the internal environment of the limb. On the other hand, where bone atrophy complicates a dermal hæmangioma, for example over the skull, it may again be expected that the atrophic bones have an abnormal circulation. The results of this investigation suggest that depression of Pco_2 and Po_2 figures prominently in the physicochemical retardation of bone growth, and is probably the result of an inhibited arterial supply.

Table 3

Direction of change (indicated by arrow) of physicochemical variables in blood samples drawn from femora and tibiæ after femoral vein ligation and arteriovenous anastomosis

	Venous ligation		*Arteriovenous fistula*	
	Po_2	Pco_2	Po_2	Pco
Femur				
Diaphysis	↓	↑	↓	↑
Metaphysis	↑	↑	↑	↑
Tibia				
Metaphysis	↑	↑	↑	↑
Diaphysis	↓	↓	↓↑	↑

pH was decreased in both surgical situations

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