healing. Our results seem to show that it is not concerned with cellular proliferation which is greatest at the lower levels of vitamin C intake where phosphatase activity is least. They are consistent with the suggestion offered in our earlier paper (Fell and Danielli, 1943) "that the phosphatase is connected, directly or indirectly, with the metabolic processes more intimately concerned in the laying down of collagen", but they cannot be regarded as direct evidence of such a connexion. We do not yet know whether this association between phosphatase and the regeneration of fibrous tissue appears in animals other than rodents, and more information is urgently needed on the normal functions of alkaline phosphatase and on the biochemistry of collagen formation. Until more facts are available no definite conclusion can be reached.

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Some Histological Effects of Partial Deficiency of Vitamin C on Healing Processes: The Influence on Bone Repair

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It is well known that total deprivation of vitamin C prevents the repair of bone but the effects of partial deprivation have been less frequently studied. The present experiments began as an attempt to investigate the statement that deprivation of vitamin C leads to the reopening of healed fractures. This has several times been alleged to occur in man; it has twice been claimed to occur in guineapigs, and twice denied. We thought it might occur in guineapigs more readily during a prolonged partial deprivation than in acute scurvy, and that simultaneous deficiency of vitamin D might also be involved.

Fractures were inflicted on the fibulae of guineapigs by means of forceps modelled on those used by Hertz (1936), without open operation. Fourteen days after infliction of the fracture the experimental animals received a diet which was partly deficient in vitamin C, containing 0·5 mg. ascorbic acid daily, or partly deficient in vitamin C and almost completely deficient in vitamin D, or almost completely deficient in vitamin D but adequately supplied with vitamin C. Controls were adequately supplied with both vitamins. Callus formation was well advanced before the experimental diets were first given, but in no case did the callus reopen although the animals were maintained on the diets for periods ranging up to three months.

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In guineapigs with experimental fracture of the fibula and partly deprived of vitamin C, the callus formed was smaller than that produced by normal guineapigs and union of the fractured ends was probably delayed.

The almost complete deprivation of vitamin D did not affect the repair of the fractures and rickets was not induced.

A series of interesting changes occurred in the posterior limbs of many of the animals partly deprived of vitamin C, whether they were deprived also of vitamin D or not. These were porosis and hyperostosis, connective tissue hyperplasia and ankylosis at the knee joint.

Porosis and Hyperostosis

As is usual and well known in vitamin C deficiency, the compact bone became porotic and, in some cases, so much bone was eaten away that in cross sections of the tibia and fibula the old compact bone of the diaphysis was not readily identified. The hyperostosis often appeared in bones which showed also severe porosis; it occurred in the tibia or fibula and usually in both. It consisted of a mass of subperiosteal bone often so wide as to double the original width of the element affected. The new bone was not compact but consisted of rather narrow trabeculae radiating from the periphery of the old bone to the periosteum. There were relatively few cross pieces connecting the trabeculae with one another but a thin peripheral shell was often formed immediately beneath the periosteum.

Connective Tissue Hyperplasia

In many cases, but probably only when hyperostosis was present also, the muscles of the limb were swollen, often greatly so, and felt gelatinous or slimy to the touch. Sections showed an immense hyperplasia of the connective tissue, forming a stroma in which great numbers of cells like fibroblasts were scattered through a loose fibrillar network. This tissue encircled the bones, and extended between the muscles and into them, so that the muscle fibres instead of lying in close contact with one another were separated by this tissue and scattered irregularly in it. In addition, the fasciae, the ligament connecting the tibia and fibula, and the periostea, tended to break down or dissolve into this tissue and, in places, the bone lacked any peripheral boundary other than the limits of the radiating trabeculae of its hyperostosis.

Knee Joint Ankylosis

The knee joints of almost all the animals partly deprived of vitamin C became stiff, being usually fixed in flexion, and attempts to extend the leg obviously caused pain. We have not yet sufficiently studied this condition. It is not caused wholly by muscular contracture because it is not abolished by removal of the muscle. It is due partly to the connective tissue hyperplasia which causes swelling round the joint, making movement difficult, and partly to other pathological changes occurring in and near the joint.

Factors in the Development of the Hyperostosis

The experimental fracture of the fibula was usually followed in animals on a normal diet by the formation of a subperiosteal thickening not of the fibula only but of large areas of the tibia also. If the diet continued

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to contain adequate quantities of vitamin C these thickenings remained slight and might slowly decrease but, if the diet was changed to one partly deficient in vitamin C or, if it was already partly deficient at the time of the fracture, the thickening might increase so greatly as to become a hyperostosis. Since the hyperostosis occurred in no other condition, the partial deficiency of vitamin C was an essential factor in its development.

Hyperostosis occurred occasionally in the uninjured leg of animals in whose other leg the fibula was fractured, and in the legs of animals none of whose bones were injured, but it was far more frequent where an injury had been inflicted. The results of several experiments are given in Table 1.

TABLE 1

EFFECT OF INJURY ON THE DEVELOPMENT OF HYPEROSTOSIS

Hind leg with fracture or attempted fracture			Hind legs intact		
Total no.	No. showing hyperostosis	No. without hyperostosis	Total no.	No. showing hyperostosis	No. without hyperostosis
19	16	3	35	8	27

The conditions which precede the development of a hyperostosis are being investigated histologically. Early stages in the repair of the fracture of the fibula, and of the development of the subperiosteal thickening in the tibia and fibula, were studied in animals kept on a normal diet. During the first few days after the infliction of the injury the fibrous layer of the periosteum became lifted away from the underlying hard substance over large areas of both bones, and the enlarged subperiosteal space was filled with a richly cellular tissue whose cells were or became osteoblastic. From the fourth day callus-like bone began to form in this tissue, some days before its appearance at the site of the fracture itself. This new bone was not deposited as superficial contour lamellae on the surface of the old, but as trabeculae running from the old across the subperiosteal space out to the periosteum. Only a small amount of material has as yet been studied from animals dying soon after the infliction of a fracture during an already existent partial deficiency of vitamin C. It shows that as early as six days after fracture of the fibula the changes just described might already have occurred in both tibia and fibula, there being a subperiosteal zone in which development of bone was already in progress and which differed from that in normal animals in its much greater width.

It is concluded that the diaphyseal thickening, which in animals deprived of vitamin C may amount to hyperostosis, is brought about by the occurrence of ossification in a widened subperiosteal zone, and that this widening is favoured by the mechanical action of the fracture forceps on a periosteum already weakened by the dietary deficiency. The problem is complicated by the occurrence of hyperostosis in three different sets of conditions: when the fracture was made in animals already partially deficient in the vitamin; when the deficiency did not begin until two weeks

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after the infliction of the fracture; when there was no fracture at all. Its occurrence in the first set of circumstances could be, and probably was, attributable to mechanical separation of the periosteum from the bone by the fracture forceps, the subperiosteal space thus created becoming filled with an osteogenic tissue, and the periosteum, in contrast with that of normal animals, failing to re-attach itself. This failure was probably caused by several factors, the lessened ability of the partially deficient animals to form collagen fibres, the pull of muscles on the periosteum, haemorrhage, and oedema beneath it.

In the second group of circumstances, however, the detached periosteum must have at least partly re-established its connexion with the bone during the two weeks before the diet was made partly deficient, but the attachment would be, not to the old compact bone which originally formed the surface of the element, but to the new spongy bone forming the newly developed subperiosteal thickening; it seems reasonable to suppose that this attachment might be weak. It would be further weakened by the slowly developing, hidden scurvy and would tend to be detached by the pull of the muscles, perhaps aided by subperiosteal oedema and haemorrhage.

The occurrence of hyperostosis in uninjured limbs would be brought about by the same set of factors and would be expected to occur less frequently than in the injured limbs, and this was actually the case. The animals were periodically X-rayed in all experiments except one, and to take the photographs it was necessary to anaesthetize them, a procedure which they resisted with violent kicking. It seems likely that separation of the periosteum from the bone may have occurred during these occasional but violent bouts of activity. It may be significant that hyperostosis did not occur in the experiment in which X-ray photographs were not taken.

The Repair of Fractures in Bones with Hyperostosis

A callus was always formed in the animals partially deprived of vitamin C though it was smaller than in normal animals. Little is yet known of the effect of hyperostosis on the callus; it seems that the callus failed to become consolidated into compact bone and retained the trabecular structure seen also in the subperiosteal hyperostosis.

The "Cure" of the Hyperostosis

When animals receiving a diet partially deficient in vitamin C, and showing hyperostosis of the tibia and fibula, were restored to a fully adequate diet for some weeks and then killed, it was found that the thickness of the hyperostosis was scarcely reduced but that there was increased deposition of bone on the trabeculae, tending to make the hyperostosis more compact and less spongy; in addition resorption had begun at the surface of the hyperostosis, osteoclasts participating, and it is possible that the normal form of the bones might, after many months, be restored. Contour lamellae might be deposited on the surface covering up the partly resorbed hyperostosis.

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