insufficiency of vitamin B_2 factors, thereby giving rise to definite clinical evidences of deficiency which might not otherwise have been manifest. The observation of Dr. Wilson that a pellagra-like condition of the skin did not occur among lepers whose diet included milk, or where wheat was substituted for rice, suggests also that the condition might have been due in part to vitamin B_2 deficiency, or it may be another example of insufficiency of certain essential amino-acids.

Some Histological Effects of Partial Deficiency of Vitamin C on Healing Processes: The Influence on Phosphatase Formation in Experimental Skin Wounds*

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In a previous investigation (Fell and Danielli, 1943) it was found that the regenerating connective tissue in skin wounds of the rat, unlike the normal dermis, contained alkaline phosphatase. The distribution of the enzyme was studied by the histochemical method of Gomori (1939), which can be applied to ordinary paraffin sections of material fixed in alcohol. This technique renders areas of phosphatase activity black or grey, while tissue containing little or no phosphatase remains colourless.

The phosphatase activity of the rat wounds during the later stages of healing was closely associated with the development of collagen fibres which suggested that the phosphatase might be concerned in the formation of these fibres. In view of this possible correlation it seemed interesting to investigate the phosphatase activity of healing wounds during deprivation of vitamin C, since it has long been known that the poor healing of wounds in scurvy is due mainly to the imperfect development of collagen fibres in the scar.

It was, therefore, decided to study the effect of different degrees of vitamin C deficiency on the phosphatase activity and differentiation of scar tissue. For these experiments guineapigs were used which, unlike rats, cannot synthesize their own vitamin C. Standard wounds were made under ether anaesthesia in the dorsal skin with a rotating cylindrical knife 6 mm. in diameter; the method has been described by Dann, Glücksmann and Tansley (1941).

We have already reported to the Society for Experimental Biology the effect of acute vitamin C deficiency on the phosphatase activity of such wounds, and it is desirable to summarize these unpublished results before an account is given of new experiments on the effect on the enzyme of different levels of vitamin C intake.

In the *normal* guineapig the uninjured skin is almost devoid of phosphatase and only the hair follicles, sebaceous glands and occasional capillaries give a strong reaction by Gomori's (1939) method. The phosphatase activity of healing skin wounds in the non-scorbutic guineapig

* A detailed account of this work will be published elsewhere.

develops in essentially the same way as in similar wounds in the rat, but more slowly. There are accordingly two peaks of phosphatase activity, one due to invasion of the wound by the strongly reacting leucocytes, which reaches a maximum during the first few days after operation and then declines, and a second associated with the differentiation of collagenous fibrous tissue, which usually has its maximum between the 21st and 28th day and diminishes during the final stages of healing. As in the rat, the phosphatase reaction is weakest at the base of the wound where contraction is greatest and regeneration least, and is most intense near the surface where contraction is least and regeneration greatest.

In *acute deprivation* the healing process is very different. The wound becomes fully epithelialized but is filled by a large dome-shaped mass of fibroblasts with a sparse, irregular network of very abnormal intercellular material bearing little resemblance to the profuse, neatly orientated collagen fibres of the controls; the tissue is very oedematous and haemorrhagic. Preparations made by Gomori's technique show that, while the first peak of phosphatase activity due to the invading leucocytes is almost or quite as pronounced as in the controls, the second peak, normally associated with the development of fibrous tissue, is absent or negligibly slight and, even after 24 days, the scar gives an almost negative phosphatase reaction, although the enzyme is present in the hair follicles and sebaceous glands of the uninjured skin.

Guineapigs kept at different levels of *partial deprivation* provided an interesting graded series of scars intermediate between those of the normal and of the acutely deficient animals. For these experiments 31 animals were divided into groups of 3 to 7 and, after a preliminary period of 9 days without vitamin C, were dosed daily with 0·5, 0·7, 1·0, 2·0, 5·0 or 10 mg. ascorbic acid. All operations were made on the 24th day of partial deprivation and the animals were killed 28 days later, *i.e.*, after 9 days of acute deficiency and 52 days of partial deficiency. To control the possible effect on healing of the 9-day period of acute deficiency, an additional group received 10 mg. of ascorbic acid daily from the beginning of the experiment; the operations were made at the same time as in the other groups and the animals were killed 28 days later. Since all the animals were killed 28 days after operation, the observations on phosphatase refer to the second peak of activity, normally associated with the differentiation of collagen.

The average gains in weight from the beginning of the experiment with the different doses of vitamin C showed a graded response according to the amount of ascorbic acid given. It should be pointed out that the number of animals in each group was not sufficient to provide statistical evidence of the significance of these differences, but the gradation in weight-gains agreed well with the clinical and histological findings described below.

In all the animals of the group receiving 10 mg. ascorbic acid daily, healing of the wounds, as indicated by granulation and contraction, began in the first week. In the groups receiving 5 mg. or less, however, healing was delayed to a variable extent and, in the guineapigs receiving from 0·5 to 2·0 mg., was accompanied by intense swelling, which could not be due to infection alone, slight suppuration being observed in one animal only. Severe adhesions developed at the base of the wound, which became

more intense during the next two weeks, so that at autopsy it was quite difficult to remove the skin in the area of injury.

In the group receiving 10 mg., and not previously subjected to deprivation, all the wounds were epithelialized during the second week. Among those previously deprived all the wounds in the groups receiving 10 or 5 mg. were fully epithelialized during the third week but, with the lower doses of ascorbic acid, the process was not complete until the 4th week. Severe adhesions were formed in all groups receiving doses up to 1 mg. and, even after 4 weeks, haemorrhages in the wound were seen with doses up to 2 mg.

There was no significant histological difference between the scars of the groups receiving 0·5, 0·7 and 1·0 mg. As in acute deprivation the wound was filled by a spongy, poorly differentiated, haemorrhagic tissue in which the cells had no orderly arrangement. The intercellular material, though much less abundant than in the fibrous control scars, was rather more plentiful and slightly less abnormal than in acute deficiency. The phosphatase reaction of many of the scars was stronger than any observed in the acutely deficient animals, but it was nevertheless very feeble compared with that of the corresponding controls.

A marked superiority was seen in the scars of the group receiving 2 mg. They were smaller than those produced with the 0·5, 0·7 and 1 mg. doses, though still abnormally large; the intercellular fibres were more numerous and better differentiated, and the tissue had a much more orderly structure. All showed a considerable but subnormal phosphatase activity.

The scars of the group receiving 5 mg. were almost normal and gave an intense phosphatase reaction. There was no significant difference between the two groups having 10 mg.; healing was far advanced in both and the phosphatase reaction, though strong, had probably passed its peak, being slightly less than in the set with 5 mg. The results show clearly that with different degrees of vitamin C deficiency the amount of phosphatase activity in the scar was correlated with the degree of differentiation. There was a marked difference between the effect of a daily dose of 1 mg. vitamin C, at which level differentiation was poor and phosphatase activity slight, and that of 2 mg., at which differentiation approached the normal, and phosphatase activity, though much less than at higher levels of dosage, was nevertheless considerable. The optimum dose of vitamin C, assessed by its action on rate of healing, on differentiation and on phosphatase activity, was, however, not less than 5 mg. and possibly even more. These findings agree well with the critical doses of vitamin C found necessary for the repair of other types of tissue and for producing a scar of normal tensile strength. Thus, Hines, Larere, Thomson and Cretzmeyer (1944) found that 2·5 mg. was the minimum daily dose required for the repair of muscle tissue. Kuether, Telford and Roe (1944) noted that a daily dose of 5 mg. completely prevented pathological changes in the teeth, and Bourne (1943) showed that the phosphatase reaction of regenerating osteoid tissue was strong at a dosage of 2 mg., slight at 0·5 mg. and absent in a completely scorbutic animal; this author (Bourne, 1944) found also that wounds attained a normal tensile strength at a dosage of 2 mg.

The question arises as to what part the phosphatase plays in wound

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.