

Discussion

Mr. P. B. Croft (Department of Biochemistry, Oxford): In the work which has been done in Oxford in the last four years (Clark, Peters and Rossiter, 1943, 1945; Croft and Peters, 1945), it was first established by carefully controlled experiments that in rats there is an increased excretion of nitrogen in the urine after burns, which is maintained for periods up to at least ten days after the burn, and is accompanied by a corresponding loss of weight. This occurs on a diet containing 10 per cent. casein and 10 per cent. dry yeast as the source of protein, supplying the equivalent of 14.4 per cent. total protein, but the loss is almost prevented by increasing the casein content of this basal diet to 18.2 per cent. These findings are in general agreement with observations made previously and since, upon human patients suffering from severe burns (Lucido, 1940; Taylor, Levenson, Davidson, Browder and Lund, 1943; Co Tui, Wright, Mulholland, Barcham and Breed, 1944).

We considered first the hypothesis that the increased nitrogen excretion in the urine might be due to the raiding of the tissue protein molecules for one or a few essential amino-acids specially needed in the replacement of the destroyed areas of skin, with the elimination as urea of the nitrogen from the unwanted amino-acids. We tried the effect on the nitrogen loss of supplementing the basal diet with various amino-acids, particularly methionine. The synthetic amino-acids were kindly made for us by the Ministry of Supply. Quite briefly, a supplement of 1 per cent. of methionine almost completely eliminated the loss of nitrogen whereas other amino-acids did not. One curious feature was that a mixture of eight essential amino-acids, containing as much methionine as was present in the 8 per cent. casein addition in the higher protein diet, was not nearly as effective as this increased protein, or as a 1 per cent. supplement of methionine. At present we consider this to be connected with the relative rates of supply of amino-acids to the liver by absorption from the gastro-intestinal tract.

It is natural to think that the beneficial effect of methionine might be exerted because of its high sulphur content, or of some methylating or other role exerted by it in the liver. Du Vigneaud (1942) has reviewed recently the problem of the relationships of methionine, cysteine and choline, and has discussed the significance of the labile methyl group. He suggests a possible path for the conversion of methionine to cysteine, with cystathionine as one of the intermediate products. Du Vigneaud and colleagues have demonstrated the cleavage of cystathionine to cysteine in tissue slices and have made progress in isolating from the liver an enzyme system involved in this change. In investigating this idea, we can say that *l*-cysteine is not effective in preventing the nitrogen loss after a burn, and hence the effect cannot be due merely to the supply of the necessary sulphur for the replacement of the destroyed skin protein (Croft and Peters, 1945). The second possibility is now under examination in Oxford, but it should be pointed out that the more recent work on burns, both in this country and in America, does not support the idea that there is extensive liver damage after burns, now that treatment with tannic acid has been discontinued.

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Dr. B. S. Platt (Medical Research Council Human Nutrition Research Unit, National Hospital, Queen Square, London, W.C.1) commented as follows on slides which he showed to illustrate how, on the legs of

TABLE I
CORRELATION BETWEEN THE INTAKE OF CERTAIN DIETARY FACTORS AND THE OCCURRENCE OF SKIN LESIONS IN THREE DIFFERENT PARTS OF THE WORLD

	Newfoundland	West Indies (Barbados)	West Africa (Gambia)
No. of persons examined	868	192	50
Composition of population sample	Mixed ages and sexes	Schoolchildren both sexes	Schoolchildren both sexes
Occurrence of ulcers and ulcer scars on legs	Rare	Common, 30 per cent.	Common, 56 per cent.
Occurrence of dry skin	Rare, 3 per cent.	Common, over 50 per cent.	Common, 54 per cent.
Occurrence of crackled skin on legs	Rare	Fairly common, 14 per cent.	Common, 54 per cent.
Evidence of deficiency of vitamins of the B ₃ group	Common	Fairly common	Rare
Evidence of vitamin C deficiency	Common	None	None
Daily intake of nutrients: Protein g.	High, mixed 88.4	Low 42	Probably low, mainly of plant origin
Riboflavin mg.	1	0.75	No quantitative data*
Nicotinic acid mg.	15	7	„†
Ascorbic acid mg.	48	69	„

* Probably better than in Barbados in view of amounts of fermented foods eaten (Platt and Webb, 1946).

† Probably satisfactory, partly for the same reason as above and partly because of consumption of groundnuts.

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Chinese apprentices in Shanghai factories, ulcers developed in skins showing evidence of malnutrition: Besides malnutrition the factors of trauma and infection are involved. In the examples shown it is probable that the lesion began as a result of the bites of bed bugs on poorly nourished skins and that the bites became infected.

The nature of the malnutrition present in the skins in which these chronic ulcers develop is a question. The "crackled skin" has an appearance similar to that seen in mild pellagra, and this type of skin lesion might be associated with insufficiency of vitamin B₂ factors in the diet. It should be recalled, however, that, in the earlier work on the biological value of proteins, false values were obtained probably because the proteins were impure and were mixed with B₂ vitamins. It seems likely that at the present time there may be a similar confusion between the clinical evidence of vitamin B₂ deficiency and of insufficiency of certain essential amino-acids. Some data obtained in three different parts of the world are set out in Table 1. They suggest that the underlying malnutrition in chronic ulcer of the skin of the legs may prove to be due to an insufficiency of certain amino-acids.

In view of these observations, the prevalence of ulcers might not be related to the intake of B₂ vitamins but it appears to be associated with the level of protein intake. Some estimations already made with West Indian diets indicate a methionine content of about one-quarter of that of a European diet.

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Dr. D. C. Wilson (10 Parks Road, Oxford): Dr. Platt has shown interesting photographs of rough skins and ulcerations among colonial peoples. Similar skin lesions have been studied by various nutritional workers in India in communities existing on ill balanced and inadequate dietaries. Dr. John Lowe, who was working on leprosy at the Calcutta School of Tropical Medicine, drew my attention to a pellagra-like condition of the skin which is not primarily a manifestation of leprosy but occurs among lepers in certain Indian asylums. Leprosy in India is a disease of the rice eating districts and is seldom seen in areas where wheat is the staple cereal. While investigating more than one thousand lepers in different asylums I found the condition of the skin, described by Dr. Lowe, in those asylums which admitted more patients than their finances could support and where the feeding was very inadequate. In neighbouring asylums, more favourably situated financially, where some milk was included in the dietary, this roughness of the skin was rarely seen. In asylums further north where wheat was provided for leper patients, the pellagra-like condition of the skin was not found although the rations did not contain milk.

Dr. B. S. Platt replied: I recently saw under the care of Dr. E. Muir at Chacachacare a group of lepers who also, like the lepers referred to by Dr. Wilson, had pellagra-like lesions of the skin. They had changes also in the tongue and eyes which I have associated with vitamin B₂ deficiency. Dr. Muir and I agreed after a consideration of these that the nature of the leprosy process in these tissues was likely to accentuate the effects of

insufficiency of vitamin B₂ 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 B₂ 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.