Suppression of Tuberculin Reaction in the Scorbutic Guinea Pig

In a recent publication from this laboratory it was noted that vitamin C deficiency in guinea pigs prevented induction of experimental allergic encephalomyelitis as well as development of the tuberculin reaction[1]. Replacement of the scorbutic diet with a normal stock diet four weeks after deprivation began and three weeks after inoculation of the challenge vaccine containing brain and killed *Mycobacterium butyricum* in water-in-oil emulsion permitted subsequent development of tuberculin sensitivity although the guinea pigs failed to develop clinical or histological signs of experimental allergic encephalomyelitis. It was important to determine the exact basis for the animals' failure to respond to tuberculin by development of a skin reaction because of the bearing this information might have on the induction of experimental allergic encephalomyelitis. Did absence of vitamin C prevent the end organ response (development of a positive skin reaction to tuberculin or, in the case of experimental allergic encephalomyelitis, the inflammatory lesions in the brain), or was the vitamin essential to the primary immunological response to the injected antigen(s)?

It is known that a generalized state of delayed hypersensitivity can exist in animals which fail to give a positive skin reaction; for example, tuberculous rabbits may lose their cutaneous hypersensitivity without decrease or loss of cellular hypersensitivity[2]. The present communication shows that the skin of the scorbutic animal is capable of responding to tuberculin if the delayed hypersensitivity has been induced prior to deprivation of vitamin C. Thus we can assume that the inhibition of the response comes from interference with the actual induction of delayed hypersensitivity rather than interference with the skin's reactivity.

Disease-free, mixed-colour, male guinea pigs, bred in a closed colony in the Animal Production Section at the National Institutes of Health, were used for all experiments described. Their weights were 400–500 gm. They were obtained from a stock colony one week prior to inoculation of tuberculin and maintained on the diet specified. All animals were maintained in large general-purpose cages, six to a cage, on a bed of sawdust and cedar shavings.

Tuberculin sensitivity was induced in the guinea pigs by one intracutaneous injection of 0·1 mgm. *Mycobacterium butyricum* (Difco) in 0·1 ml. of an emulsion made up of one part phenol–saline (0·5 per cent phenol–0·9 per cent sodium chloride), one part melted 'Aquaphor' (Duke Laboratories), and two parts mineral oil (Fisher Scientific Co.).

The 'normal diet' consisted of stock diet for guinea pigs (N.I.H. Formula *A*) plus a daily supplement of greens, cabbage and kale. Vitamin C-deficient diet was supplied by Nutritional Biochemical Corporation. The guinea pigs on the 'scorbutic diet plus vitamin C' received 50 mgm. ascorbic acid (Lederle) daily in 0·1 ml. intraperitoneal injection. All animals received tap water *ad lib.*

Tuberculin sensitivity was measured as mm. induration 24 hr. after intradermal injection of 0·1 ml. of second-strength tuberculin (0·05 mgm./ml.).

All animals were placed on their respective diets, as indicated under diet I in Table 1, for a week prior to inoculation with *M. butyricum*. The skins were tested two weeks after inoculation and, after these tests, the guinea pigs were placed on their second diet. The second skin test was performed three weeks after institution of the second diet.

Table 1. Suppression of Tuberculin Response in the Guinea Pig by Vitamin C Deficiency

Diet I	Diet II	No. of animals	First tuberculin response mm. induration (mean ± *S.D.*)	Second tuberculin response mm. induration (mean ± *S.D.*)
Normal	Normal	6	10·7 ± 2·1	12·8 ± 2·1
Normal	Scorbutic	8	10·3 ± 2·3	11·9 ± 1·4
Scorbutic plus vitamin C	Scorbutic	6	11·0 ± 4·4	9·3 ± 2·9
Scorbutic	Scorbutic plus vitamin C	5	0·4 ± 0·2	7·6 ± 2·0

Guinea pigs were put on diet I one week prior to injection with Freund's complete adjuvant (0·1 mgm. *Mycobacterium butyricum* ID). Two weeks after the injection they were challenged with 5 μgm. tuberculin and the skin reaction observed 24 hr. later (first response to tuberculin). Each group was then placed on diet II, and after three more weeks again challenged with 5 μgm. tuberculin (second response to tuberculin).

As shown in Table 1, the animals on the vitamin C-deficient diet, when inoculated with *M. butyricum*, failed to respond to tuberculin as in the previous investigation[1]. Also, as had been observed previously, restoration of vitamin C to these animals resulted in subsequent development of sensitivity to tuberculin. In the present series of animals, the second tuberculin test was carried out only three weeks after the vitamin C supplementation was instituted. This was possibly too short a time for the animals to recover completely from their scorbutic state, since the skin reactions observed were somewhat less pronounced than the reactions of animals on the normal diet. There was no difference in the first tuberculin response of animals on a normal diet and the response of animals on the scorbutic diet plus vitamin C injections. Removal of vitamin C from the diet after the hypersensitive state had been induced did not alter significantly the tuberculin response (line 2 in Table 1).

From these results it would appear that the vitamin C-deficient guinea pig is able to respond dermatologically to tuberculin once tuberculin hypersensitivity has been induced. Failure of the induction of the delayed hypersensitivity phenomenon itself would appear to be the best explanation for the absence of response to tuberculin in those animals which were on the vitamin C-deficient diet at the time they were inoculated with *M. butyricum*.

Peter S. Mueller
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Age Differences in Cyclic Motor Reaction

Different manifestations of time judgment in man, becoming still more important with the advent of flights into space, have been examined by a vast number of psychologists as well as psychiatrists[1], and last but not least by physiologists, including those of the Pavlovian school[2]. They used methods of estimation of presented intervals, production and reproduc-