LXXIII. THE EFFECT OF ADDING VITAMIN A TO A RACHITOGENIC DIET.

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It is generally held that a direct relation exists between growth-rate and intensity of rachitic lesions in the human subject. Thus Hess [1929] writes, "In general, it may be stated that the more rapid the growth, the greater the tendency to the development of rickets....Animal experiments confirm this general point of view....All investigators who have used the rat as the experimental animal have reported similar experiences." There are abundant references in the literature to "spontaneous healing," and this has in very many cases been associated with a diminished food intake and a retarded rate of growth.

Recently Coward and Cambden [1929] made a special investigation of this matter. They made use of a modified McCollum line test, and did not find any significant correlation between intensity of rickets and failure to grow. Their results were in contradiction to those reported by Bills, Honeywell and MacNair [1928], who remarked "It is imperative to discard all rats which lose even as little as 1 g." Bills also used the line test technique; whereas the curative period in Coward's experiments was 10 days, that in Bills's investigations was only 5. The experiments of Coward, of Bills and of other investigators who have reported results in either sense, were not primarily designed to investigate the particular point under discussion; their conclusions have been drawn from a considerable body of experimental evidence bearing mainly on different points. Bills has recently re-affirmed the view expressed in the earlier paper [Bills, Honeywell, Wirrick and Nussmeier, 1931], and has repeated the exact words of the sentence already quoted.

The following investigation was planned and concluded before the publication of the paper last mentioned. It was designed with the specific object of investigating directly the relationship between increase in growth and intensity of rickets.

The matter is of considerable importance in the assay of vitamin D. The rachitogenic diet most used in this country is Steenbock's 2965. In this the only source of vitamin A is yellow maize. It is known that maize varies in its vitamin A content, the white-cap variety being practically devoid of it [*e.g.* Steenbock and Coward, 1927; Hauge and Trost, 1928]. There is even no reason for assuming that all samples of yellow-cap maize are equally potent

sources of vitamin A. It might therefore happen that a particular batch of diet 2965 was actually deficient in the growth-promoting properties associated with vitamin A (or carotene). If this batch were being used in the comparison of a sample of cod-liver oil with irradiated ergosterol, the presence of vitamin A in the former might bring about markedly greater growth in the animals receiving it than in those receiving the irradiated ergosterol. If this increased growth were associated with greater intensity of rickets, the comparison of antirachitic potency would be thereby completely invalidated. Accordingly the investigation here described was undertaken to determine whether the daily addition of fresh carrot to diet 2965 affected the degree of rickets produced by the diet (*a*) when no source of vitamin D was added and (*b*) in the presence of two different levels of vitamin D supplement.

Thirty-one animals were divided into three groups, the first receiving no source of vitamin D, the second a very small daily dose, in one drop of olive oil, and the third eight times as much as the second, dissolved in the same weight of the same olive oil. The doses administered in the two cases were approximately 0.1 and 0.8 Medical Research Council units daily; no great stress is laid upon the absolute amount of vitamin D administered, but it is definitely known that the two dosages were in the ratio of 1 : 8, since they were made up from the same preparation of irradiated ergosterol. Each group was divided into two sub-groups, in one of which each animal received 1 g. of fresh carrot daily. Although no assay of vitamin A (or carotene) was carried out on the samples of carrot used, similar samples purchased at the same time were found to be ample in daily doses of 1 g. to restore growth to animals "run out" on a vitamin A-free diet. In view of the unanimity of the literature on the subject, it can safely be assumed that the animals receiving the supplementary doses of carrot were receiving adequate supplies of vitamin A.

The experimental period was 5 weeks; the animals were all kept in separate cages. One hind-leg and one fore-leg of each animal were taken for extraction and ashing, and photographic records were kept of the "lines" from the other tibia of each animal. In general the appearance of the lines was quite consistent with the figures obtained by chemical analyses. The criterion of rickets used in these experiments has been the percentage of ash in the moisture- and fat-free bones, [A]. This figure is closely related to the "rachitic index," used by Chick and Roscoe [1926], which is the ratio of the ash to the non-fatty organic matter in the bone. This last figure, denoted R, is related to [A] and to R.I. (the rachitic index) in the following way:

$$\text{R.I.} = \frac{[A]}{R} = \frac{[A]}{100 - [A]}$$

When the value of [A] is 50, the R.I. is clearly 1.

Records were kept of the initial and final weights of all the experimental animals. As far as possible animals of the same sex and from the same litter (for which it is proposed to use the term isogenic animals) were distributed over the various dosages administered.

The actual increases in weight appeared to bear no relation to the weights of the animals at the beginning of the experimental period, but rather, as was to be expected, to show some correlation with the individual litters. Since the intensely inbred London strain of Wistar rat was used throughout, the differences can hardly have been genetic, and are probably associated with differences of prenatal nutrition and fat-soluble vitamin storage.

The value for [A] was obtained on the bones after they had been extracted with alcohol to constant weight. We have found this to be an essential precaution, since the size and porosity of different bones are the main factors affecting the ease with which the fat and moisture can be removed from them. We have also found that subsequent ether extraction is unnecessary since it never causes a further loss in weight of more than 1 or 2 mg. The bones were ashed in a muffle at dull red heat, and were also weighed a second time after a second heating, although the latter never produced any significant change in weight.

Table I shows the distribution of litters, animals, and doses, the value found for [A] and for the increase in growth during the experimental period, δM (in brackets).

Table I. *Values for [A] and δM (in brackets) for 31 animals from 3 litters.*

Litter		Vitamin D					
		No vitamin D		0.10 unit		0.80 unit	
		No carrot	Carrot	No carrot	Carrot	No carrot	Carrot
1534	7 ♂	40.9 (48)	45.5 (62)	42.3 (60) 37.0 (47)	39.8 (52)	44.4 (59)	46.5 (45)
	7 ♀	34.2 (41)	34.2 (33)	34.8 (42)	38.7 (44) 46.4 (32)	40.1 (47)	45.1 (43)
1529	7 ♀	33.5 (22)	32.8 (31) 32.3 (38)	40.1 (38)	42.3 (40)	41.5 (38)	44.2 (35)
1386	6 ♂	38.2 (74)	36.4 (72)	38.8 (78)	28.4 (63)	41.6 (80)	48.4 (86)
	4 ♀	33.4 (44)	31.7 (51)	39.1 (58)	—	36.1 (45)	—
Averages		36.0 (46)	35.5 (48)	38.7 (54)	38.7 (46)	40.7 (54)	46.1 (52)
		35.8 (47)		38.7 (50)		43.4 (53)	

DISCUSSION OF RESULTS.

It appears that the batch of diet 2965 used in these tests was an adequate source of vitamin A, under the conditions of the experiment, since a daily supplement of 1 g. of carrot did not appear to bring about a significant change in growth-rate. The average increases for all animals during the whole 5 weeks were:

without carrot (7♂, 9♀) 51
with carrot (6♂, 9♀) 48

The difference between the averages is not more than could be accounted for by the slightly different sex-ratio and other normal variations and is certainly not significant.

It also appears that there is no correlation whatever, under the conditions and within the limits of this experiment, between growth-rate and severity of rickets, as chemically assayed and corroborated by the line test technique. Fig. 1 shows [A] plotted against δM for all the animals.

Fig. 1.

Without carrot.		With carrot.	
○	without vitamin D.	◊	without vitamin D.
●	with 0.1 unit vitamin D.	◐	with 0.1 unit vitamin D.
◻	with 0.8 " "	◑	with 0.8 " "

The carrot appears to have exercised no antirachitic effect when given unsupplemented at a daily level of 1 g. There is a suggestion that higher, and nearly prophylactic, doses of vitamin D are made more effective in the presence of increased vitamin A (or carotene), but the number of animals used was insufficient, and the differences in bone-ash percentage too fluctuating, for this to be anything more than an indication. Nevertheless, all four relevant pairs of isogenic animals show this effect, and its significance seems probable. Steenbock *et al.* [1930], amongst others, have called attention to the absence of evidence that "the various factors operative in bone production act independently and consistently without one modifying the influence of the other."

SUMMARY.

1. Addition of 1 g. of fresh carrot daily to the diet of animals on Steenbock's diet 2965 did not significantly affect growth-rate, whether in the absence or presence of vitamin D.

2. No evidence was found, under the conditions of the experiment, for any correlation between severity of rickets and rate of growth.

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