CLXXII. GROWTH AND VITAMIN A DEFICIENCY¹.

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THE idea that vitamin A has a specific effect in promoting growth has been prevalent since the earliest days of vitamin work. Thus when accumulating evidence indicated that a fat-soluble accessory factor was necessary for satisfactory growth in rats, Hopkins [1912] suggested that the missing factor was one "concerned solely with growth," and Osborne and Mendel [1913] sought to discover the factor which is "peculiarly essential for growth." Even after the fat-soluble factor had been differentiated into two components, and it had been shown that absence of the antirachitic component D might also be a limiting factor in growth [Steenbock and Nelson, 1923], the tendency still persisted to regard component A as the "growth-promoting vitamin," and references to its activity in this respect have abounded in the literature up to the present day.

Although the early experimental evidence may have given some justification for assuming that the fat-soluble factor had a specific effect on growth, further research on the rôle of vitamin A in nutrition has indicated that the pathological conditions arising from deficiency of this vitamin are of more importance than any apparent cessation of growth that may occur. A few workers have realised the significance of these observations, and have protested against the continued use of the term "growth vitamin" for vitamin A [*e.g.* Steenbock *et al.*, 1924-25; Green and Mellanby, 1928], but the idea is still widely accepted. Thus, in the monograph on the "Vitamins" recently published by the Medical Research Council [1932], it is stated that "the biological estimation of vitamin A is generally based on its growth-promoting property," that "in a curative test, the animals are allowed to exhaust their reserves of vitamin A, as indicated by cessation of growth," and that "the timely administration of adequate supplies of vitamin A is soon followed by a resumption of growth." Still more recently we find papers published in Germany and France, bearing in their titles, with reference to vitamin A, the phrases "Das Vitamin des Wachstums" and "Le Facteur de Croissance" [Kuhn and Brockman, 1933; Busson, 1933].

Although there is abundant evidence of the fall in weight which eventually occurs in the absence of this vitamin, no proof has been given of the generally accepted assumption that this loss in weight indicates cessation of growth. The weight of an animal is dependent partly on the weight of its skeleton, but to a large extent also on the weight of its organs, and on the amount of muscle and fat which cover the skeletal framework. The weight is thus very readily affected by variations in the health and state of nutrition of the animal. On the other hand, size, stature or length is determined almost entirely by the amount of skeletal development. It is a commonplace clinical observation that a pathological condition may be accompanied by loss of weight, and further, that in

¹ A preliminary outline of this work¹ has already been published [Orr and Richards, 1934].

spite of loss in weight a child during a long illness may increase considerably in height. It is thus obvious that failure to increase in weight is not necessarily synonymous with failure to "grow." It cannot therefore be claimed that loss of weight indicates cessation of growth unless it can be demonstrated that the animal has ceased to increase in length as well as in weight, and that skeletal growth has ceased. The investigations here recorded were therefore undertaken to ascertain, by measurements of body length in the live animal and of bone length *post mortem*, whether growth as determined by increase in length of the skeleton, and in particular of the long bones, does in fact cease in vitamin A deficiency when the animal ceases to increase in weight.

EXPERIMENTAL.

The *basal diet* used (Diet L) was that recommended by Sampson *et al.* [1932], except that a small amount of potassium iodide was added, and vitamin D was supplied as radiostol instead of by irradiation of cotton-seed oil. This diet, which contains a high percentage of yeast (20 % of the dry substances), proved eminently satisfactory. It seemed to provide a sufficiency of all dietary factors other than vitamin A, since rats fed on the diet, supplemented by an adequate dose of vitamin A concentrate, showed normal weight curves throughout the period of 12–13 weeks covered by the experiment. In certain cases our tests involved the addition of a supplement of dried separated milk to the basal diet. Twelve rats on this diet (L+M) served as negative controls, the absence of vitamin A from both the basal diet and the added dried milk being indicated by the weight curves, which were typical of those obtained on diets containing no vitamin A.

1. *Live measurements.*

(a) *Measurements of tail growth in rats receiving a small dose of vitamin A.* Some preliminary tests were carried out in which increase in the length of the tail was used as an indication of growth. Measurements of the length of the tail were made twice weekly on a series of rats which had received from weaning a small dose of vitamin A in addition to the basal diet L. The supplement given was 0.075 g. of "fortified milk," *i.e.* dried separated milk to which had been added before evaporation a vitamin A concentrate. The small amount of

Table I. *Growth of tail in rats receiving an inadequate dose of vitamin A.*

Each measurement is the average of three successive determinations made on dates 3–4 days apart, and each weight given is the average of the weights on the dates of measurement.

Period ...	Length of tail in cm.				Weight in g.			
	1	2	3	4	1	2	3	4
Rat 2581	16.5	16.7	16.8	—	151	143	123	—
2582	16.8	16.9	17.0	17.2	156	144	147	146
2612	—	*14.4	14.6	14.8	—	139	139	136
2636	15.5	15.9	15.9	16.3	136	137	131	133
2638	*12.5	13.4	13.5	13.7	154	165	159	148
2640	15.6	15.9	16.5	16.8	140	156	158	138
2585	15.1	15.4	15.9	16.1	108	112	109	106
2596	14.3	14.9	15.3	—	116	110	106	—
2603	13.9	14.6	15.0	15.0	115	120	121	117
2607	14.0	14.4	14.5	—	85	87	71	—
2634	15.1	15.6	15.9	16.3	104	104	103	105
2635	14.1	15.0	15.3	15.6	110	114	118	117

* Tip of tail missing.

vitamin A potency retained in the evaporation of the "fortified milk" sufficed to prolong the lives of the rats very considerably in comparison with the negative controls, and the tail measurements could thus be continued for some time. The tail was measured from base to tip by holding it firmly, without actual stretching, along the edge of a measuring rule, 2 or 3 readings being taken. The results are shown in Table I, which gives measurements and weights for periods of approximately 10 days each. Although the degree of accuracy obtainable by this method is not high, comparison of the successive weights from period to period with the measurements of tail length for the corresponding periods shows that there is in each case a distinct increase in tail length after increase in weight has ceased.

(b) *Measurements of body length and tail length in rats receiving no vitamin A.* In the second series of live measurements comparison was made between animals receiving no vitamin A (Diet L or Diet L+M) and positive controls which received Diet L supplemented by an ample dose of vitamin A concentrate (Diet L+A). The dose given was 4 mg. per rat daily, the amount being based on the blue unit value of the concentrate at the beginning of the experiment (700 c.p. units). The rats available, which were of the black-and-white (Lister) strain, were divided as far as possible into sets of three litter-mates of the same sex, each rat of a set receiving one of the three diets L, L+M or L+A, after weaning at the age of 23 days.

In order to increase the accuracy of the measurements, the animals were anaesthetised once a week with a mixture of nitrous oxide and oxygen, and measurements were made both of tail-length, in the manner already described, and of the total length from nose to tail. The total length was measured by means of a board with an upright end-piece against which the nose of the rat was held. The body and tail were straightened out, and the position of the tip of the tail was marked on the graph paper with which the board was covered, two or three readings being taken. It is to be noted that the length of the body alone is not given accurately by the difference between the two sets of measurements thus obtained, since in measuring the total length the tail was more relaxed than when it was held along the rule for direct measurement. The data have therefore been used to construct for each animal two independent growth curves¹ based on length of tail, and total length of body + tail, as measured under these conditions. A number of these growth curves, along with the weight curves¹ of the rats in question, are shown in Fig. 1. It will be seen that while there is no very great increase in length during the period of flattening of the weight curve, there is at the same time no indication of any "cessation of growth." The curves of tail-length and total length are simply of the nature of continuous slow growth curves. It is to be noted, moreover, that by the time the weight curves of the deficient rats are beginning to fall off, the growth curves, though not the weight curves, of the positive controls have already begun to flatten considerably, *e.g.* Rats 3220 and 3231 (Fig. 1 *b*). Further, if the weight of the vitamin A-deficient rat has been well maintained until shortly before death, *e.g.* Rats 3257, 3378 and 3314 (Fig. 1 *a*), it will be seen that the growth curves are practically identical with those of the positive controls. Thus these animals have reached a moribund condition through vitamin A deficiency before there has been any marked slackening in the rate of growth.

The curves for Rat 3289 (Fig. 1 *b*) which received an inadequate dose of vitamin A after a "depletion" period, show that considerable growth took place for a period of 25 days after the weight began to fall, while the curves for

¹ These are graphs of the observations, not statistically fitted curves.

Rat 3265, which suffered severely from the anaesthetising, provide an example of retardation of growth occurring in the presence of ample vitamin A. (It is to be noted that this retardation of growth was accompanied by a very pronounced reduction in food consumption, and that the subsequent rise in both weight and

Fig. 1a. Weight and growth curves for rats receiving no vitamin A. In each set of 3 curves, the top, middle and bottom curves represent weight, total length and tail length respectively.

Fig. 1b. Weight and growth curves for rats receiving inadequate or adequate doses of vitamin A. Note the pathological retardation of growth in Rat 3265, in spite of the presence of ample vitamin A.

In Figures 1, 3 and 4, D=died; KD=killed, moribund; K=killed, not moribund.

growth curves coincided with a return to normal food intake.) It is clear therefore that it is not the presence or absence of vitamin A in itself that is the determining factor in the continuation of growth as estimated by increase in length. Growth in this sense does not cease when vitamin A is absent, or present in inadequate amounts, and on the other hand the rate of growth may be very considerably retarded in presence of adequate vitamin A, if the animal is in a pathological condition from some other cause.

2. Skeletal growth.

As it is obviously impossible to measure bones accurately during the life of the animal, an indirect method based on *post mortem* measurements was adopted to ascertain whether the bones continued to grow in length after increase in weight ceased. If weight be a measure of growth, then we should expect to find a relationship between the length of the bones and weight, and if growth ceases in vitamin A-deficient rats when increase in weight has ceased, then the bones

Table II. *Weights and bone lengths of positive controls: i.e. rats receiving basal diet + adequate vitamin A.*

No. of rat	Sex	Wt. when killed (g.)	Age when killed (days)	Lengths of bones		
				Femur cm.	Tibia cm.	Humerus cm.
3461	♀	81	44	2.38	2.85	1.91
3503	♀	81	44	2.32	2.73	1.86
3462	♀	96	51	2.59	2.98	2.02
3502	♀	98	44	2.45	2.87	1.93
3512	♀	100	44	2.50	2.92	2.00
3275	♀	103	66	2.61	3.04	2.02
3445	♀	107	51	2.60	3.00	2.06
3494	♀	109	44	2.50	2.88	1.94
3453	♀	111	44	2.53	2.98	1.98
3451	♀	113	51	2.69	3.10	2.10
3499	♀	115	44	2.58	2.97	2.03
3510	♀	117	44	2.67	3.06	2.09
3174	♀	118	74	2.69	3.12	2.09
3410	♀	119	58	2.71	3.15	2.10
3507	♀	120	44	2.59	3.00	2.04
3394	♀	120	58	2.68	3.05	2.11
3274	♀	121	73	2.67	3.09	2.05
3438	♀	126	51	2.73	3.09	2.16
3326	♀	132	57	2.78	3.20	2.18
3395	♀	133	58	2.79	3.19	2.19
3428	♀	135	51	2.77	3.16	2.17
3416	♀	135	58	2.79	3.24	2.13
3329	♀	137	59	2.74	3.17	2.10
3284	♀	137	74	2.78	3.18	2.12
3362	♀	141	65	2.98	3.39	2.32
3161	♀	145	73	2.79	3.24	2.21
3437	♀	148	58	2.81	3.27	2.21
3309	♀	148	67	2.82	3.18	2.17
3285	♀	148	74	2.94	3.41	2.22
3454	♀	149	50	2.89	3.30	2.26
3429	♀	149	58	2.91	3.30	2.29
3255	♀	152	75	2.79	3.20	2.16
3266	♀	155	80	2.82	3.26	2.19
3443	♀	155	51	2.91	3.32	2.22
3312	♀	158	73	3.00	3.39	2.32
3170	♀	161	87	2.78	3.18	2.18
3169	♀	164	81	2.91	3.32	2.25
3153	♀	164	89	2.97	3.39	2.28
3176	♀	165	79	2.88	3.29	2.24
3377	♀	166	75	2.98	3.40	2.28
3431	♀	167	58	2.93	3.35	2.28
3231	♀	170	85	2.92	3.31	2.26
3291	♀	172	65	2.88	3.35	2.19
3200	♀	172	87	3.04	3.46	2.36
3277	♀	178	82	3.05	3.50	2.32
3142	♀	178	103	2.96	3.41	2.36
3216	♀	198	83	3.06	3.51	2.35
3177	♀	199	93	3.18	3.59	2.44
3236	♀	201	64	3.05	3.48	2.30
3212	♀	206	86	3.24	3.69	2.49
3220	♀	208	89	3.12	3.50	2.34
3379	♀	217	77	3.30	3.71	2.52
3210	♀	219	86	3.28	3.75	2.49
3331	♀	221	75	3.13	3.54	2.40
3147	♀	230	80	3.28	3.64	2.51
3224	♀	232	87	3.20	3.64	2.46
3225	♀	241	87	3.23	3.66	2.47
3173	♀	247	94	3.22	3.64	2.50
3204	♀	248	84	3.29	3.72	2.53
3366	♀	252	109	3.50	3.90	2.70

should correspond in length with the highest weight attained by the rat, *i.e.* they should be approximately the same as might be expected in a normal rat of that weight. On the other hand, if the bones continue to grow after the animal has ceased to increase in weight, the ratio of the length of the bones to the maximum weight of the animal will be greater for the rats deprived of vitamin A than for those on adequate diet.

Bone measurements. The animals used for bone measurements comprised not only those on which live measurements had been carried out, but also many other similar sets of 3 rats on the diets L, L+M and L+A respectively. A number of the sets were killed off after 3, 4, 5 and 6 weeks on the various diets. In the remaining sets each of the rats on deficient diet was allowed to become moribund before being killed, and the positive control of the set was killed along with the second of the deficient rats. A *post mortem* examination was carried out on all the rats. Thereafter the right femur, tibia and humerus were dissected and cleaned, and the lengths of the fresh bones were measured by means of sliding callipers with vernier scale, the results being expressed in centimetres to two decimal places.

Correlation between bone length and weight in normal animals. For the determination of the relationship between bone length and body weight in normal animals under the experimental conditions, data were available for 60 positive controls representing 29 litters. These data are presented in Table II, any controls in which pathological condition had caused abnormalities in the weight curve being omitted. It will be observed that considerable variations occurred in the rates of growth of these controls. This is frequently the case with rats bred specially for vitamin work, since the common practice of restricting the vitamin content of the mother's diet with the object of "reducing the reserve stores of vitamin" in the young, undoubtedly tends to produce subnormal and variable litters. Since the vitamin A-deficient rats are in general growing at a slower rate than the controls, it is essential, before making comparisons of bone lengths in the two series, to ascertain whether in the normal series the weight or the age of the animal has the greater influence in determining bone length.

A statistical analysis of the data in Table II showed that the correlation between weight and length of bone is high, the correlation coefficients (*r*) for femur, tibia and humerus being 0.95, 0.96 and 0.93 respectively (Table III).

Table III.

Characters	Correlation ratios		Correlation coefficients	
	Positive controls	V.A.D. rats	Positive controls	V.A.D. rats
T W	0.952	0.951	0.950	0.948
F W	0.968	0.968	0.958	0.966
H W	0.938	0.951	0.933	0.948

W=Body weight T=Tibia F=Femur H=Humerus V.A.D.=Vitamin A-deficient

Length of bone and age are less highly correlated. In the case of the tibia "*r*" was found to be 0.95 for bone length and weight, as against *r* = 0.80 for bone length and age. The graphs in Fig. 2, in which bone length has been plotted for the 60 positive controls both against weight and age of rat, indicate that for the femur and humerus also the correlation between bone length and weight is much closer than that between bone length and age.

The best fitting curves of bone lengths on weights¹ were then determined in order to find the theoretical weights for each length of bone in the control series. These theoretical weights were used as standards with which the data for the

¹ See next page for equations for the curves.

deficient animals were compared, in order to determine whether in animals suffering from avitaminosis-A the normal relationship between bone length and

Fig. 2. Normal rats. Graphs showing closer correlation of bone length and weight than bone length and age.

body weight had been maintained. The standard curves are shown in Fig. 3, and the corresponding numerical data in Table IV.

1. The equations for the curves are:

$$\text{Tibia parabola: } y = 89.554 - 120.528x + 42.720x^2,$$

$$\text{Femur parabola: } y = 235.025 - 224.247x + 68.200x^2,$$

$$\text{Humerus parabola: } y = -141.697 + 35.400x + 44.270x^2,$$

where

y = predicted weight of rats in g.

and

x = length of bone in cm.

Table IV.

Tibia length cm.	Weight		Femur length cm.	Weight		Humerus length cm.	Weight	
	Control g.	V.A.D. g.		Control g.	V.A.D. g.		Control g.	V.A.D. g.
2.75	81.17	—	2.35	84.68	—	1.85	75.31	—
2.85	93.04	85.74	2.45	94.99	85.24	1.95	95.67	88.82
2.95	105.77	90.69	2.55	106.67	93.61	2.05	116.92	103.46
3.05	119.35	97.59	2.65	119.70	102.15	2.15	139.05	117.41
3.15	133.78	106.44	2.75	134.11	110.88	2.25	162.07	130.67
3.25	149.07	117.23	2.85	149.88	119.79	2.35	185.97	143.23
3.35	165.21	129.97	2.95	167.41	128.88	2.45	210.76	155.11
3.45	182.21	144.66	3.05	185.50	138.15	2.55	236.44	166.29
3.55	200.06	—	3.15	205.36	147.61	2.65	263.00	176.78
3.65	218.76	—	3.25	226.58	—	—	—	—
3.75	238.32	—	3.35	249.17	—	—	—	—
3.85	258.74	—	3.45	273.12	—	—	—	—

Fig. 3a.

Fig. 3b.

Fig. 3a. Weight of rat on length of femur. — Control rats. ---- V.A.D. rats.

Fig. 3b. Weight of rat on length of tibia. — Control rats. ---- V.A.D. rats.
1 S.E. = Curve of standard error. 2 S.E. = Curve of twice standard error.

Fig. 3c. Weight of rat on length of humerus. — Control rats. ---- V.A.D. rats.
1 S.E. = curve of standard error. 2 S.E. = Curve of twice standard error.

Application to vitamin A-deficient rats. The method of using the standard curves may be illustrated by two examples. For rat 3201 ♀ (Diet L+M), the lengths of femur, tibia and humerus were 2.81, 3.24 and 2.18 cm. respectively. From the standard curves the predicted weights for these bone lengths should be 143.3, 147.3 and 146 g.; i.e. rat 3201 had the skeletal development of a rat of 145.5 g. in the control series, while the highest weight attained was only 117 g. Similarly rat 3203 ♂ (Diet L), had bone lengths of 3.12, 3.50 and 2.36 cm., which should correspond with weights of 199, 190.8 and 188 g. Thus this animal, for which the highest weight recorded was 144 g., had the bone lengths of a rat of 192.6 g. By interpolating these values in the weight graphs, we obtain a graphical representation of bone growth as contrasted with weight in the deficient rats.

Fig. 4a. Weight curves and bone growth curves for rats receiving no vitamin A, showing the continuation of skeletal growth after weight increase has ceased.

Figs. 4 and 5 show the contrast between weight and bone growth for representative experimental animals, the corresponding numerical data being given in Tables V and VI. Fig. 4 a gives the graphs for 24 rats which were allowed to die or become moribund on the vitamin A-deficient diet. Both sexes are included, and the periods on the experimental diet vary from 42 to 70 days. The graphs have been arranged in groups in descending order of weight as estimated from skeletal development, the average weights for successive rows being 165.3, 145.2, 135.4 and 109.0 g. It will be seen that when vitamin A is completely absent from the diet, the discrepancy between bone growth and weight increase

is universal in its occurrence, whether the animals are well or poorly developed, whether death occurs suddenly, very soon after the first drop in weight, *e.g.* rats 3149, 3155 and 3171, or whether there is a fairly prolonged flattening of the weight curve, *e.g.* rats 3228, 3156, 3146, 3229 and 3166. Even in animals killed before reaching moribund condition, after only 4–5 weeks on the diet, the disproportion between bone length and body weight is already apparent (Fig. 4 *b*).

Fig. 4*b*. Curves showing early divergence of bone growth from weight. For the 12 rats above, which received no vitamin A, the average time of divergence is 14.4 days.

Fig. 5*a*. Divergence of bone growth from weight in rats receiving an inadequate dose of vitamin A. The arrow indicates the point at which vitamin A dosage was begun.

Fig. 5*b*. Divergence of bone growth from weight in rats receiving an adequate dose of vitamin A, but suffering from some pathological condition.

The course of the graphs seems to indicate that divergence between growth and weight may begin early, the average time of divergence in the 12 examples given being 14.4 days.

In Fig. 5 *a* are given the curves for 5 rats which, after varying periods (21–46 days) on the vitamin A-free diet, were given a small dose of vitamin A in the form of "fortified milk." In these rats, with their prolonged survival periods, the difference between bone growth and weight is marked. Thus in Rat 3365 we find that a practically constant weight has been maintained for 9 weeks, while the bones have continued to increase far beyond the length normally associated with this weight.

Fig. 5 *b* shows graphs for a series of 5 rats which received ample vitamin A, but which were in a pathological condition from varying causes, as indicated in

the diagram. The same disproportion between body weight and bone growth is apparent in these cases as was found in the animals suffering from vitamin A deficiency.

Disproportion between bone growth and body weight may apparently be produced by varying types of nutritional deficiencies, *e.g.* by restriction in the amount of food [Aron, 1911], mineral salt deficiency [Clarke and Smith, 1933], deficiency of vitamin A or vitamin B [Quinn *et al.*, 1929]. These last authors state that in these vitamin deficiencies there was less retardation of increase in skeletal measurements than in body weight, but they do not give their numerical data in a form which facilitates comparison with the values for their normal controls.

Statistical analysis of the data for the vitamin A-deficient rats. In order to have some estimate of the probable error of the weights calculated from the observed bone lengths, a statistical analysis has been carried out of the data for the 24 rats included in Table V, which were allowed to die or become moribund on the vitamin A-deficient diet. It was found (Table III) that these vitamin A-deficient rats were correlated to the same degree as the control rats, so far as weight of rat and length of bone are concerned. The curves of bone lengths on weights for the deficient rats are shown along with those of the control rats in Figs. 3 *a, b, c* and the corresponding numerical data in Table IV. In each case the predicted weights for length of bone of the deficient rats are less than the predicted weights for length of bone of the control series. Those values for vitamin A-deficient rats outside twice the standard error of the values for the control series may be regarded as quite significantly less than the controls. It

Table V. *Relation of actual weight to weight predicted statistically from bone lengths in vitamin A-deficient rats.*

			Mean predicted weight = 138.74 g. Mean actual weight = 114.04 g. Difference = 24.7 g.			Standard error of difference = 5.55 Ratio = 4.45					
(1) V.A.D. rats (No.)	(2) Diet	(3) Period on diet days	(4) Actual lengths of bones			(5) Weights predicted from lengths of bones			(6) Av. of predicted weights g.	(7) Max. wt. of rat g.	(8) D = Dif- ference in wt. (6)-(7) g.
			Femur cm.	Tibia cm.	Humerus cm.	Femur g.	Tibia g.	Humerus g.			
3160 ♀	L + M	47	2.46	2.86	1.94	96	94	94	94.67	81	13.67
3167 ♀	L + M	47	2.45	2.90	1.95	95	99	95.7	96.57	84	12.57
3173 ♀	L	45	2.60	3.02	2.04	113	115	115	114.33	91	23.33
3257 ♀	L + M	52	2.59	3.00	2.00	111.8	112	106.3	110.03	96	14.03
3166 ♀	L	62	2.61	3.05	2.03	114.2	119.3	113	115.50	98	17.50
3171 ♂	L	46	2.65	3.11	2.07	119.7	127.7	121	122.80	104	18.80
3150 ♀	L	65	2.77	3.15	2.12	137.2	133.8	132	134.33	107	27.33
3229 ♀	L	70	2.74	3.18	2.09	132	138.2	126	132.07	108	24.07
3209 ♂	L + M	63	2.80	3.24	2.12	142	147.2	132	140.40	111	29.40
3155 ♂	L + M	42	2.75	3.16	2.10	135.5	135.2	128	132.90	113	19.90
3222 ♂	L	58	2.78	3.23	2.11	139	146	130	138.33	113	25.33
3151 ♀	L	66	2.82	3.22	2.14	145	144.2	136.5	141.90	114	27.90
3201 ♀	L + M	56	2.81	3.24	2.18	143.3	147.3	146	145.53	117	28.53
3223 ♂	L	48	2.81	3.22	2.10	143.3	144.2	128	138.50	117	21.50
3146 ♀	L + M	60	2.78	3.25	2.15	139	149	139	142.33	119	23.33
3208 ♂	L + M	63	2.88	3.27	2.18	155	152	146	151.00	119	32.00
3156 ♂	L	64	2.89	3.33	2.21	156.5	162	152	156.83	120	36.83
3230 ♀	L	58	2.76	3.21	2.11	136	143	130	136.33	120	16.33
3205 ♂	L + M	56	2.86	3.28	2.18	151	153.7	146	150.23	126	24.23
3148 ♂	L + M	56	2.92	3.31	2.22	161.5	158.5	155	158.33	127	31.33
3381 ♂	L	47	2.94	3.30	2.21	165	157	152	158.00	130	28.00
3228 ♂	L + M	64	2.92	3.38	2.18	161.5	170	146	159.17	136	23.17
3149 ♂	L	49	2.98	3.34	2.26	172.2	163.8	165	167.00	142	25.00
3203 ♂	L	61	3.12	3.50	2.36	199	190.8	188	192.60	144	48.60
									Mean = 138.74	Mean = 114.04	

Table VI. *Relation of actual weight to weight predicted statistically from bone lengths in rats suffering from pathological condition due to vitamin A deficiency or other cause.*

(1) No. of rat	(2) Diet	(3) Period on diet days	(4) Actual lengths of bones			(5) Weights predicted from lengths of bones			(6) Av. of predicted weights	(7) Max. wt. of rat	(8) Difference in wt. (6)-(7)
			Femur cm.	Tibia cm.	Humerus cm.	Femur g.	Tibia g.	Humerus g.	g.	g.	g.
(a) 3448 ♂	L	28	2.55	2.98	1.99	106.7	109.8	104.1	106.9	94	12.9
3449 ♂	L + M	28	2.61	3.02	2.06	114.4	115.2	119.1	116.2	101	15.2
3444 ♀	L	28	2.45	2.87	1.94	95.0	95.5	93.6	94.7	82	12.7
3447 ♀	L + M	28	2.49	2.95	1.97	99.5	105.8	99.8	101.7	81	20.7
3435 ♀	L	28	2.59	2.97	2.00	111.7	108.4	106.2	108.8	97	11.8
3440 ♀	L + M	28	2.56	2.99	2.03	107.9	111.1	112.6	110.5	94	16.5
3441 ♀	L	35	2.69	3.10	2.10	125.3	126.5	127.9	126.6	107	19.6
3434 ♂	L	35	2.80	3.22	2.18	141.8	144.4	145.9	144.0	119	25.0
3415 ♂	L	35	2.67	3.10	2.07	122.5	126.5	121.3	123.4	107	16.4
3414 ♂	L + M	35	2.63	3.06	2.06	117.0	120.8	119.1	119.0	94	25.0
3408 ♀	L	35	2.54	2.97	2.00	105.4	108.4	106.2	106.7	88	18.7
3393 ♀	L	35	2.71	3.13	2.13	128.2	130.8	134.6	131.2	108	23.2
(b) 3206 ♀	F.M.	82	2.87	3.31	2.18	153.2	158.7	145.9	152.6	116	36.6
3270 ♂	F.M.	89	3.15	3.51	2.38	205.4	192.8	193.3	197.2	157	40.2
3288 ♀	F.M.	97	3.00	3.40	2.28	176.1	173.6	169.2	173.0	143	30.0
3364 ♀	F.M.	73	3.03	3.37	2.29	181.7	168.6	171.5	173.9	136	37.9
3365 ♂	F.M.	86	2.95	3.33	Lost	167.0	161.9	—	164.5	122	42.5
(c) 3158 ♂	L + A	63	3.05	3.45	2.35	185.5	182.2	186.0	184.6	158	26.6
3154 ♀	L + A	66	2.93	3.38	2.24	163.5	170.2	159.7	164.5	140	24.5
3265 ♀	L + A	63	2.96	3.42	2.30	168.8	177.0	173.9	173.2	156	17.2
3232 ♀	L + A	70	3.07	3.43	2.35	189.4	178.7	186.0	184.7	169	15.7
3280 ♀	L + A	84	2.98	3.39	2.32	172.4	171.9	178.7	174.3	135	39.3

will be noted that the result for the humerus is less significant than those for the femur and tibia. It may be that this fact is associated with the greater activity of the hind-limbs as compared with the fore-limbs in the rat.

In Table V the "standard" weights corresponding to the observed bone lengths are compared with the maximum weights actually attained by the vitamin A-deficient rats. In each case the actual weight was found to be distinctly less than the standard weight. The table shows the predicted weight, the actual maximum weight attained and the difference in weight for each vitamin A-deficient rat. The standard error of the difference between the two means was found to be 5.55. The difference between the two means was $138.74 - 114.04 = 24.7$ g., which is 4.45 times the standard error and 6.6 times the probable error. These figures indicate clearly that the deficient rats are significantly less in weight than the controls for the actual lengths of bone of the vitamin A-deficient series. In other words, length of bone in the vitamin A-deficient series is significantly greater than in controls of the same weight, showing that the bones have continued to increase in length after increase in weight has ceased.

With regard to the 12 rats in Table VI, which were killed after 4-5 weeks on the deficient diet, the differences between predicted weights and actual weights are naturally not very large in most cases. Although these differences may possibly not be statistically significant, nevertheless when taken in conjunction with the data for the older rats, they may be regarded as showing that the divergence between bone growth and body weight tends to show at an early stage.

Food consumption and retardation of growth in vitamin A deficiency. It is clear both from the live measurements and from the measurements of bone length that growth in the sense of increase in length has not ceased in animals suffering from vitamin A deficiency, and although in most cases the rate of growth has been considerably retarded in the deficient rats, this is no justification

for assigning to vitamin A a specific influence on growth. Even with a diet fully adequate in quality, the rate of growth of an animal is largely dependent on the amount of food consumed, and we have found that the food consumption of rats on the vitamin A-free diet is much below that of the positive controls receiving vitamin A. This is shown graphically in Fig. 6, which gives weight and food con-

Fig. 6. Food consumption and weight graphs for pairs of litter-mates of the same sex, one rat on vitamin A-free diet (—), and the other on the same diet supplemented by vitamin A (....). The amount consumed has been averaged for periods of 6 days, except in the final period, which varies from 1 to 5 days. The food was given as a stiff paste formed into balls weighing 24 g. Similar balls left beside the cages overnight and weighed at the same time as the residues were found to weigh 22.0–22.3 g., with an average of 22.2 g. for 11 determinations. The weight of the residues was therefore subtracted from 22.2 g., and the food consumption was thus found approximately in terms of this dried paste, of which the actual moisture content was found by analysis to be 33 %.

sumption diagrams for 12 pairs of rats, each pair consisting of a positive control and a litter-mate on deficient diet. The food consumption records do not cover the whole period from weaning but were begun at varying stages of the deficiency, as shown in the diagram, and continued until death.

A study of the graphs shows that food consumption is practically identical for the control and deficient rats during the period when the graphs of weight are in close agreement (*e.g.* during the first 6–12 days of the food records in the first

4 pairs of rats), and that the beginning of divergence in the weight curves coincides with a corresponding divergence in the amount of food intake. Even in rats receiving vitamin A, the food intake is influenced very considerably by their physical condition. Thus for rat 3232 ♀, in which the lungs were affected, the food intake remained practically constant for 28 days at approximately 14 g., while the intake for a more normal female control, *viz.* 3377, rose steadily in the same period from 13 to nearly 18 g. Similarly in rat 3265, which suffered severely from gassing and showed a very sharp drop in weight, the food intakes in the first 3 periods were less than those for the two litter-mates receiving no vitamin A (pairs 11 and 12), and the subsequent rise in the weight curve, and also in the growth curves as previously noted, was accompanied by a return to normal food intake.

The daily food consumption has been recorded graphically for rat 3265 to illustrate the very close correspondence found between weight fluctuations and food intake. Our findings in this respect agree with those of Irwin *et al.* [1930], who showed that in vitamin work variability in weight gain was largely due to variations in total food intake. Sampson *et al.* [1932] found that in vitamin A deficiency the decrease in food intake could offer only a partial explanation of the change in rate of growth. It is to be noted, however, that they calculate food consumption per 200 g. body weight and estimate growth by increase in weight. Our work has shown that in pathological cases, such as occur in vitamin A deficiency, weight cannot be regarded as an accurate index of the growth of the animal. Hence any comparison of food intakes and metabolism on the basis of body weight may lead to fallacious conclusions.

The data we have recorded indicate clearly that the faster growth attained by the rats receiving vitamin A depends to a large extent on their greater food consumption, and that the appetite of all the rats is determined largely by their state of health. If an animal falls into ill-health, even when it is receiving vitamin A, appetite fails and the rate of growth slows down. Similarly in animals deprived of vitamin A the pathological conditions caused by this deprivation are responsible for the diminished appetite, the consequent fall in weight and the slower rate of growth.

The existence of pathological conditions in vitamin A deficiency has been noted by most workers, but they occur much earlier than has been generally supposed. Recently Thatcher and Sure [1932] found that "71 % of the animals deficient in vitamin A showed metaplastic changes before decline of body weight or complete cessation of growth took place." About one-third of their animals were killed after 33–40 days on the diet; the remainder at a later date. We have found that the pathological condition begins even earlier than this. In animals killed after being only 21 days on the diet, such conditions as ulceration of the stomach or caecum, and inflammation of the caecum or small intestine, especially the duodenum, could be detected macroscopically in over 70 % of the cases.

It would appear that the animal's capacity for storing reserves of vitamin A is much less than is generally supposed, and that the preliminary or "depletion" period, during which it is assumed to be using up these reserves is in reality the time taken for the outward manifestation of symptoms of disease which may be already far advanced. The relationship between pathological conditions and the weight responses obtained in the curative method of vitamin A assay is discussed in detail in a subsequent paper [Richards and Simpson, 1934]. There is little doubt that the diversity of these conditions in the experimental animals after the so-called "depletion" period accounts for most of the discrepancies encountered in such assays.

CONCLUSIONS.

A survey of the above experimental data indicates that there is no ground for ascribing any special growth-promoting activity to vitamin A. The characteristic loss of weight occurring in its absence does not indicate cessation of growth as has been generally assumed but is a sign of the onset of disease. Vitamin A is merely one of the many dietary components, known and unknown, that are essential for optimum development and growth in the organism. In this sense only is vitamin A a "growth factor," and its claim to the title is no greater than that of any other indispensable dietary constituent. Its power of preventing disease remains beyond question, although the term "anti-infective" vitamin proposed by Green and Mellanby [1928] has not met with general approval as an accurate definition of its function. It seems wiser for the present, in the words of Mendel [1932] "to stress the indefinite function of the vitamin in preserving health and vigour, rather than to herald any specific action against definite microbiotic enemies."

SUMMARY.

1. Measurements of tail length and total body length made at intervals on rats suffering from avitaminosis-A have shown that increase in length continues when increase in weight has ceased.

2. *Post mortem* measurement of the limb bones has shown that the relationship between body weight and bone length established for the positive controls is not maintained in the vitamin A-deficient animals. The bones have continued to increase in length while the animal has been losing weight. The weight curve is thus not a true index of skeletal growth.

3. The results recorded here indicate that the most important rôle of vitamin A in nutrition lies in its power of preventing the onset of pathological conditions, which are the cause of the loss of weight that occurs in vitamin A deficiency.

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