CLVIII. THE INFLUENCE OF VITAMIN A DEFICIENCY ON MALE RATS IN PAIRED FEEDING EXPERIMENTS.

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The object of this investigation was to determine whether the changes produced in body weight and in the deposition of fat in male rats on vitamin A-deficient diets are due to this deficiency alone or to the accompanying reduced appetite; and also to study the changes in weight of the sexual and endocrine organs.

The following experiments, therefore, supplement those published in a previous preliminary paper [Sampson, Dennison and Korenchevsky, 1932].

In the preliminary paper observations were made on 2 rats on vitamin A-deficient diet and on 2 control rats on a complete diet, paired in regard to food intake and other conditions with the respective rats on the vitamin A-deficient diet.

In spite of the decreased appetite during the period of cessation of growth the rats on vitamin A-deficient diet (– A diet) consumed a larger amount of food per 200 g. of body weight than did those on the complete diet. At the same time one unit of the vitamin A-deficient food consumed by the rats produced a much smaller increase in body weight than the same unit of complete diet. The latter fact was perhaps correlated with the simultaneously increased nitrogen metabolism which was observed. It was suggested that these facts give the chief explanation of the checking of growth produced by the direct deficiency of vitamin A irrespective of appetite since, during this period, the absorption from the alimentary canal did not show any significant changes.

We were unable to find in the literature any record of the use of the paired-feeding method in investigations of vitamin A deficiency, or, consequently, any accurate estimations by weight of changes in fat deposition, or in sexual or endocrine organs due directly to vitamin A deficiency.

It is known that, in general, fat deposition is decreased in animals kept on a vitamin A-deficient diet. It is also known that, in such animals, the actual weight of the testes is less than in those on unlimited complete diet. Histological examination reveals degeneration of the seminiferous tissue [see references by Mason, 1930].

A critical review of these references will be given in a subsequent paper elsewhere, when dealing with the histological changes observed in the sexual and endocrine organs of the rats on which the experiments described in this paper were performed.

Technique.

The experiments were performed on 63 male rats belonging to 12 litters. They were reared on the stock diet used at the Lister Institute, described by Smith and Chick [1926]. The litters were placed on the experimental diets at the following ages:

Litters 6 and 7 on the 7th day.
Litters 1, 2, 3, 5, 10, 11 and 12 on the 21st to 26th day.
Litters 4, 8, 9 on the 27th to 33rd day.

The duration of the experiment was:

Litter 8—24 days.
Litters 1, 2, 3, 6, 7 (except rats 30 and 32, 28 days)—9 days.
Litters 10, 12—31 to 35 days.
Litters 5, 11—39 to 42 days.
Litter 4—52 days.

The final ages and the weights of the rats are given in Table III.

In Exps. I and II, the diet was given *ad libitum* to all the rats. In the remaining ten experiments the rats were paired. One member of each pair received a diet deficient in vitamin A only, the other member a diet adequate in all respects. On the vitamin A-deficient diet the food intake gradually decreases. This may affect not only the body weight but also the weight of the organs and their structure and function. To discriminate between the effect of decreased food intake and that of the vitamin A deficiency, it is necessary that the paired control animal should receive the adequate diet in the same quantity as was consumed by the litter mate on the deficient diet. This procedure, adopted in this and the previous investigations [Sampson *et al.*, 1932] did not necessitate any considerable reduction in the food intake of the control rats since the pairs were killed on the day on which the vitamin A-deficient litter-mate showed a marked loss of appetite, which sometimes also resulted in a loss of body weight. Owing to these precautions the paired control rats were kept in good health throughout the whole experiment. In order to make a comparison, in some experiments a third litter-mate was allowed the complete diet *ad libitum*.

The period required for exhaustion of the vitamin A reserves in the young rats may be shortened by substituting a vitamin A-deficient diet for the usual diet at the 17th day of lactation—the time when the young begin to consume the stock food directly. Of course, the mother is also consuming this deficient diet from this period. By this method, as was shown by Korenchevsky [1922], the effects of the deficiency may be accentuated. In an endeavour to obtain

extreme changes the mothers and the young of the litters Nos. 6 and 7 were placed on a vitamin A-deficient diet from the 17th day of lactation.

The composition of the – A diet was as follows: caseinogen (heated) 180, starch (wheat) 500, cotton-seed oil (irradiated) 150, salt mixture 50, yeast (dried) 183, lemon juice 50, water 700 parts by weight.

The control animals received the same diet supplemented with daily doses of 20 to 100 mg. (according to the weight of the animal) of cod-liver oil (Fairbank Kirby). To keep the caloric intake of the paired litter-mates equal, the rats on vitamin A-deficient diet received an equal amount of hydrogenated cotton-seed oil.

In Exps. I and II, however, the control animals received a paste in which the cotton-seed oil was replaced by 120 parts of butter and 30 parts of cod-liver oil. All the rats of these two litters also received 6 drops of wheat-germ oil daily to supply an extra amount of vitamin E and a small though inadequate amount of vitamin A, so that the vitamin A deficiency in litters 1 and 2 was much less complete than in litters 3 to 12. Vitamin E was supplied in the latter experiments by hydrogenated cotton-seed oil, since according to Evans and Burr [1927] this fat, when fed in the amount given above, is a satisfactory and sufficient source of the antisterility vitamin.

The diet in all the experiments, except IX, was given in the form of a paste. In Exp. IX the rats were given a diet of the same composition but without the addition of water. It was hoped that a more accurate weighing of the dry residue would thus be obtained. Unfortunately the vitamin A-deficient rats during the period of pronounced deficiency scattered this dry diet badly and the collection of the residue was much more difficult than with the paste.

At the same time the accurate estimation of food eaten by the deficient rats was of the utmost importance since this amount determined the amount of diet to be given to the paired control on the following day.

The chief difficulty was to determine the amount of the residue of the paste since, by the end of 24 hours when the residue was collected, some of the water in the paste had evaporated.

In order to prevent this evaporation as much as possible and also to prevent the scattering of the food the paste was firmly pressed into heavy metal cups. Though helpful, this precaution was insufficient, so that it was necessary to introduce a correction for the evaporation of water.

The amount of evaporation of water from the diet was determined in the following way. Weighed samples of the paste in quantities equal to the amount of food given to the rats and to the amount of residue usually left were placed on the same shelves as those on which the rat-cages were kept. At the end of 24 hours the samples were reweighed and the amount of water evaporated estimated. At frequent periods during the investigation observations of the evaporation were made and on the average the loss of water was found to be nearly constant, probably owing to the fact that the room temperature was kept practically constant.

Table I. *Average evaporation of water from the food residue at the end of 24 hours.*

Weight of sample g.	Loss of water by evaporation g.
1 to 2	0·5
2 to 5	1·0
5 to 10	2·0

The loss of water was on the average as given in Table I.

These figures indicate that to the weight of paste uneaten at the end of 24 hours and partially dried, from 0·5 to 2 g. must be added according to the weight of the residue to offset the evaporation of water.

In order to estimate the accuracy of this method of correction for evaporation the data of the food consumption obtained in this way were compared with the food consumption calculated on the basis of the nitrogen intake in the experiment described in the previous paper [Sampson, Dennison and Korenchevsky, 1932]. The results are summarised in Table II.

Table II. *The relation of the quantity of paste consumed, estimated by weighing the paste and residue and correcting for evaporation, to 1 g. of the actual intake of nitrogen, for the different periods of the experiments on rats 1756 and 1757.*

Period		Corrected paste intake	Nitrogen intake by analysis	Relation of paste intake to 1 g. N intake
1756	I	127	2·70	47·0
	II	113	2·46	45·9
	III	110	2·45	44·9
	IV	122	2·52	48·4
	V	120	2·51	47·8
	VI	105	2·19	47·9
	VII	46	1·02	45·1
1757	I	138	2·93	47·1
	II	137	2·93	46·7
	III	116	2·51	46·2
	IV	114	2·38	47·9
	V	100	2·12	47·2
	VI	120	2·54	47·2
	VII	78	1·72	45·3
			Average	46·7

If the method were perfect 1 g. of nitrogen would always have corresponded exactly to the same amount of paste, for which the nitrogen content was found, by analysis, to have been constant throughout the experiment. Fluctuations, however, were observed, the lowest figure being 44·9 and the highest 48·4, with an average of 46·7. Since the maximum deviation from the average did not exceed 3·9 % this method of correction may be considered to be quite satisfactory.

To the rats mentioned in Table II the paste was given in a semi-liquid form, while to all other rats it was given in the form of a dough-like paste. In these latter cases, therefore, some scattering of the food always occurred and consequently the possible error was greater. Great care was taken,

however, to collect accurately the scattered food and the scattering was considerably reduced by pressing the ration firmly into the metal cups to which it adhered quite well.

For the interpretation of the changes in the thyroid the consideration of the amount of iodine present in the diet is of importance. The vitamin A-deficient diet used differed in iodine content from the complete diet since the cod-liver oil given to the rats on the complete diet contains iodine, while cotton-seed oil does not. Therefore some of the rats of litters 10, 11 and 12 (marked with an asterisk, Table III) were given 7 and 30 γ of iodine per day dissolved in cotton-seed oil. Both these doses, in any case, are larger than the maximum amount of iodine in the daily quantity of cod-liver oil given to the rats [see Orr and Leitch, 1929, p. 88].

All rats were weighed at least twice weekly, and examined for symptoms of vitamin A depletion. At the onset of xerophthalmia the rats were weighed daily. The experiments were continued for 24 to 52 days in each case until xerophthalmia was obvious or until loss of appetite led to slower gain in, or loss of, body weight. The rats were killed by coal gas, pairs being killed simultaneously.

For dissecting and weighing the organs the technique proposed by Korenchevsky [1930; 1932] was used. Thus the intra-abdominal fat and penis were weighed fresh, while the testes, prostate with seminal vesicles, thyroids, adrenals, pituitary and thymus glands were weighed after remaining in Allen's modification of Bouin's fixative for 48 hours. The fixed material was subsequently used for histological study.

Experimental results.

Body weight and appetite.

The age and the initial and final weights of the rats are given in Table III. The abbreviations for the diets which are employed in the tables are also used in the text (see footnote to Table III). Since the −A diet used in the experiments contains a large amount of vitamin B in the form of dried yeast, the rats on this diet suddenly lose their appetite, usually only a few days before death. As mentioned above, on the day when a considerable loss of appetite and of body weight occurred the pairs were killed. The gain or loss in weight during the last day is given, to indicate the condition, *i.e.* retarded growth, cessation of growth or fall in weight at this time.

It should be noted that of the 34 rats on the − A diet, loss of weight, generally small, occurred in 19 rats only, whilst xerophthalmia was noted in all except the rats of litter 1. The initial weights of the rats of this litter on −A diet were greater than those of any other rats on this diet, and the gain in weight was continuous up to the day of killing. This shows that at killing vitamin A reserves were still present in these rats.

Table III. *Body weight of rats in g.*

	CoU diet			CoP diet			− A diet				
		Weight			Weight			Weight		Gain or loss	Final age
Litter no.	Rat no.	Initial	Final	Rat no.	Initial	Final	Rat no.	Initial	Final	during last day	in days
1	1	58	289	—	—	—	3	56	259	+ 4	62
	2	58	289	—	—	—	4	61	257	+ 3	62
2	5	40	231	—	—	—	8	38	185	− 5	58
	6	36	254	—	—	—	9	40	179	+ 3	58
	7	35	237	—	—	—	10	38	180	− 5	58
3	—	—	—	11	30	146	13	34	119	− 7	56
	—	—	—	12	31	147	14	35	130	+ 3	56
	—	—	—	—	—	—	15	33	119	+ 3	56
4	—	—	—	16	48	214	18	50	145	− 9	84
	—	—	—	17	48	205	19	48	149	−16	85
5	20	46	249	22	45	251	24	45	195	+ 3	68
	21	38	264	23	40	222	25	41	149	− 8	68
	—	—	—	—	—	—	26	43	180	− 3	68
6	—	—	—	27	38	123	28	38	121	− 1	55
7	—	—	—	29	38	150	31	40	130	+ 1	55
	—	—	—	30	35	87	32	35	68	− 7	49
	—	—	—	—	—	—	33	36	109	± 0	55
8	34	37	95	—	—	—	37	35	57	−10	51
	—	—	—	35	43	91	38	42	87	± 0	51
	—	—	—	36	35	78	39	39	73	− 4	51
9	40	38	165	42	39	134	44	41	130	− 1	62
	41	39	165	43	41	148	45	43	130	− 4	59
10	—	—	—	46	42	145	49	51	142	+ 1	64
	—	—	—	—	—	—	50	51	115	−10	61
	—	—	—	47	48	152	51*	48	135	− 3	64
	—	—	—	48	44	133	52*	48	112	−11	61
11	—	—	—	53	50	185	55	50	155	− 9	67
	—	—	—	—	—	—	56	47	164	+ 2	67
	—	—	—	54	43	190	57*	52	165	± 0	67
	—	—	—	—	—	—	58*	49	147	− 5	67
12	—	—	—	—	—	—	60	55	123	− 1	63
	—	—	—	—	—	—	61	47	128	+ 2	63
	—	—	—	59	49	148	62*	49	122	+ 2	63
	—	—	—	—	—	—	63*	44	101	+ 3	63

Note. In the tables of this paper the following abbreviations are used: CoU = complete unlimited diet; CoP = complete paired diet, *i.e.* given in the same amount as is consumed by the paired rat on the vitamin A-deficient diet; − A = vitamin A-deficient diet.

In columns CoP and − A the numbers in the same line indicate paired animals.

* These rats received iodine.

Since both in litter 1 and litter 2 rats on the deficient diet received small amounts of vitamin A in the wheat-germ oil, these two litters are considered separately from the other 10 litters in all our averages and interpretations.

The weight and the appetite of the rats must be considered simultaneously. The actual food intake and the intake per 200 g. of body weight are given in Table IV. As can be seen from the figures in this table small discrepancies exist in most pairs between the amounts of food eaten by the rats of a pair. In Table IV in each of the litters in which the paired feeding method was used, *i.e.* litters 3 to 12, the figures of the paired − A-rat are placed below those of its paired control (CoP)-rat, *e.g.* in litter 3 in the column "Actual food

Table IV. *Daily average of food intake in g., actual and per 200 g. of body weight; and average daily gain of each rat per 10 g. of actual food intake.*

Litter no.	Diet	Actual food intake	Intake per 200 g. body weight	Gain per 10 g. of actual food intake
1	CoU	33·1, 30·4 — —	37, 34 — —	1·9, 2·1 — —
	−A	27·1, 25·1 — —	34, 31 — —	2·0, 2·1 — —
2	CoU	26·8, 27·9, 26·0 —	42, 41, 39 —	2·0, 2·2, 2·1 —
	−A	22·3, 20·4, 20·7 —	39, 36, 34 —	1·7, 1·8, 1·8 —
3	CoP	16·8, 16·2 — —	39, 37 — —	2·0, 2·0 — —
	−A	16·0, 16·5, 14·6 —	38, 40, 40 —	1·5, 1·6, 1·7 —
4	CoP	17·6, 17·6 — —	26, 27 — —	1·8, 1·7 — —
	−A	16·3, 17·7 — —	27, 29 — —	1·1, 1·1 — —
5	CoU	24·1, 26·4 — —	35, 39 — —	2·0, 2·0 — —
	CoP	23·7, 21·4 — —	36, 34 — —	2·1, 2·0 — —
	−A	23·6, 20·2, 22·6 —	37, 37, 37 —	1·5, 1·3, 1·4 —
6	CoP	11·5 — — —	32 — — —	2·2 — — —
	−A	16·9 — — —	43 — — —	1·4 — — —
7	CoP	16·3, 10·4 — —	37, 37 — —	2·0, 1·8 — —
	−A	17·0, 10·1, 14·3 —	40, 36, 37 —	1·6, 1·2, 1·5 —
8	CoU	12·6 — — —	41 — — —	1·9 — — —
	CoP	12·1, 11·4 — —	34, 36 — —	1·7, 1·6 — —
	−A	12·2, 11·5, 10·6 —	36, 36, 36 —	1·5, 1·2, 0·9 —
9	CoU	19·2, 20·5 — —	40, 41 — —	1·9, 2·0 — —
	CoP	15·7, 16·6 — —	38, 38 — —	1·8, 2·1 — —
	−A	16·5, 18·3 — —	37, 39 — —	1·6, 1·5 — —
10	CoP	16·0, — 17·2, 16·5	34, — 32, 35	1·8, — 1·7, 1·7
	−A	17·4, 16·6, 17·1, 15·6	35, 32, 32, 33	1·5, 1·2, 1·5, 1·3
11	CoP	19·7, — 19·8, —	32, — 33, —	1·8, — 1·9, —
	−A	20·1, 20·2, 21·0, 18·8	34, 36, 36, 33	1·3, 1·5, 1·4, 1·3
12	CoP	— — 15·0, —	— — 32 —	— — 1·9 —
	−A	15·7, 15·7, 14·7, 12·7	33, 36, 34, 35	1·2, 1·5, 1·4, 1·3

Note. Each figure relates to a rat in the order of the respective numbers of the rats, which can be ascertained by reference to the corresponding litters and groups in Table III.

intake" the figures for the respective pairs are 16·8 and 16·0; 16·2 and 16·5; in the next column they are 39 and 38; 37 and 40 and so on.

When there is a slight excess in the amount of food eaten by the control paired rats as compared with that eaten by the −A rats it is chiefly due to differences in intake on the first and last days of the experiment. On the first day of the experiment the control paired rat may eat more than the deficient rat since actual pairing does not start till the second day when the control rat receives the same amount as was eaten the previous day by the −A rat. On the last day of the experiment, in most cases, the food was completely consumed by the control rats, while the deficient rats might eat very little.

When the amount of food eaten by the control rats is slightly less than that eaten by the −A rats, it is due, in some cases, to giving a little less food to the CoP rats. This was done in order to be certain that the control rats did not receive more than the experimental rats and in order to increase the effect of limitation of the diet. Care was taken, however, not to injure the healthy condition of the control rats by such adjustments. In other cases

the smaller intake of the CoP rats was due to their refusal to consume the diet completely. As is well known some rats dislike synthetic diets. A consideration of these CoP rats on reduced intake as compared with their pairs is of especial importance in our conclusions.

Of those rats receiving the complete unlimited diet, the body weight, in about half of the individuals, was only slightly greater than that of the rats on a complete but paired diet (Table III, litters 5, 8 and 9). This shows that, because of the precautions mentioned above, the limitation of the diet of the paired rats was very small (see Table IV), except in those cases in which the rats disliked the diet and, therefore, left part of it unconsumed.

A comparison of the paired rats reveals that the gain in weight of the rats on CoP diet is greater than that of the rats on −A diet. In a few cases, rats 27, 35, 36 and 42, this difference is small, from 2 to 9 g., but in all the other CoP rats it is much greater, being from 12 to 30 g. in 9 rats and from 31 to 74 g. in the remaining 6 rats. At the same time it can be seen from Table IV that in litters 3 to 12 the food intake of the rats on CoP diet was, in most cases, the same as that of the rats on −A diet or even less. This is evident in the figures for actual food intake but is accentuated when the intake is calculated per 200 g. of body weight. For litters 1 and 2, in which the vitamin A deficiency was only partial, the increase in weight produced by one unit of food complete or deficient in vitamin A was also calculated. The results obtained are given as "Gain per 10 g. of actual food intake," in the third column of Table IV. In all the rats which showed vitamin A deficiency, even in those of litter 2, the gain in weight produced by one unit of vitamin A-deficient diet was, without exception, less than that produced by the same unit of complete diet. In litter 1, in which, as was mentioned above, the depletion of vitamin A was incomplete, this difference in rate of growth per unit of food intake of −A as compared with CoP diet did not occur. The absence of this change in this litter only emphasises its presence in all the other litters. In litters 3 to 12 the actual daily average of food intake for the rats on CoP and deficient diets was 16·4 and 16·6 g. respectively; the average daily food intake per 200 g. of body weight was 34 and 36 g. respectively and the average daily gain in weight per 10 g. of actual food intake 1·9 and 1·4 g. respectively. It is worthy of note that the gain in weight per 10 g. of food intake is nearly the same in the rats on complete diet, in litters 5 and 9, both when consumed *ad libitum* (CoU diet) and when adjusted in quantity to that consumed by the deficient rats. In litter 8 the gain in weight on CoU diet was greater.

In litters 5, 8 and 9, in which all three groups are represented, the body weights in groups CoP and −A are on the average about 11 % and 26 % respectively less than in group CoU. In other words, roughly two-fifths of the deficiency in weight of −A rats in these experiments was due to decreased appetite and three-fifths was due to the direct influence of vitamin A deficiency.

These data also indicate that in order to obtain the same increase in body weight it is not sufficient to ingest the same unit of food. Only if vitamin A is present is this so, otherwise the food ingested in the same quantity will produce a smaller increase in body weight and presumably in the growth of the animal.

Fat deposition.

As judged by the average weights of retroperitoneal and testicular fat in litters 2 to 12, the weight of the fat was less in the −A rats than in those on complete diet (Table V). In the three litters 5, 8 and 9, in which all three

Table V. *Average weight of body fat of rats in each group of each litter* (*g.*).

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	9·68	6·69	±0·69	—	—	—	7·75	6·01	±0·16
2	6·23	5·34	+0·25 −0·42	—	—	—	2·60	2·87	+0·26 −0·17
3	—	—	—	4·55	6·21	±0·43	2·92	4·77	+0·41 −0·79
4	—	—	—	8·64	8·20	±2·16	3·60	4·88	±1·41
5	9·18	7·17	±0·44	6·01	4·95	±2·02	3·24	3·65	+0·43 −0·85
6	—	—	—	2·96	4·80	±0	2·85	4·70	±0·00
7	—	—	—	1·97	3·11	±0·76	1·42	2·58	+0·82 −1·05
8	1·07	2·25	±0·00	0·77	1·78	±0·53	0·53	1·02	+0·26 −0·28
9	4·87	5·90	±0·09	2·53	3·54	±0·85	2·08	3·19	±0·27
10	—	—	—	3·53	4·91	+1·03 −0·54	1·84	2·94	+0·64 −0·68
11	—	—	—	7·33	7·81	±0·39	4·58	4·47	+1·48 −1·45
12	—	—	—	4·25	5·74	±0	1·84	3·07	+0·41 −0·78

groups are represented, the percentage deficiency of fat per 200 g. of body weight in the CoP group and in the −A group, in relation to the CoU group, and in the −A group in relation to the CoP group, was calculated.

It was found that on the average in these three litters (1) the fat deposition was decreased by about 31 % when the amount of complete diet was adjusted to that consumed by the −A rats; (2) it was decreased by about 26 % in the rats on vitamin A-deficient diet as compared with those which consumed, on an average, the same, or even slightly less, amounts of food rich in vitamin A; (3) the deficiency of fat in the −A group in relation to the CoU group was on the average about 49 %. In litter 2 this decrease was 46 %. Therefore, about three-fifths of the deficiency of fat deposition was due to the decrease in appetite and two-fifths to the direct influence of the vitamin A deficiency. In litters 3 to 12 the percentage deficiency of fat in the

rats in the −A group in relation to those in the CoP group was 23, 40, 26, 2, 17, 21, 40, 40, 33 and 47; on the average 29 %, which is nearly the same as the 26 % obtained with the three litters mentioned above. The unusually small deficiency of 2 % occurred in litter 6, in which the control rat in the CoP group ate much less than his litter-mate in the −A group (see Table IV).

Certainly the decrease of stores of fat in the body will vary with the period of time at which the −A rats are killed. If, however, as was the case in our experiments, the rats are killed in a more or less satisfactory nutritional condition, with xerophthalmia well developed, but a few days before or at the beginning of the marked fall in weight about three-fifths of the average deficiency will be due, at this period of vitamin A deficiency, to the decreased food intake, and about two-fifths of the deficiency to the direct influence of vitamin A deficiency irrespective of the appetite.

Testes.

In general the actual weight of the testes in −A groups was smaller than that of the rats on complete diet. In litters 5, 8 and 9 the weights of the testes per 200 g. of body weight (Table VI) were slightly greater (about

Table VI. *Average weight of testes of rats in each group of each litter (g.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	3·00	2·08	±0·02	—	—	—	2·81	2·18	±0·07
2	2·66	2·21	+0·06 −0·04	—	—	—	2·31	2·55	+0·43 −0·41
3	—	—	—	1·97	2·68	±0·09	1·95	3·13	+0·13 −0·05
4	—	—	—	2·30	2·20	±0·03	1·86	2·53	±0·06
5	2·42	1·89	±0·10	2·55	2·17	±0·19	1·78	2·00	+0·42 −0·50
6	—	—	—	1·69	2·75	±0·00	1·17	1·94	±0·00
7	—	—	—	1·55	2·57	±0·18	1·32	2·90	+0·54 −0·58
8	1·16	2·45	±0·00	1·17	2·79	±0·26	1·05	2·91	+0·20 −0·12
9	1·84	2·23	±0·02	1·73	2·45	±0·01	1·76	2·70	±0·18
10	—	—	—	1·80	2·52	+0·10 −0·13	1·67	2·68	+0·49 −0·33
11	—	—	—	2·54	2·71	±0·10	1·97	2·52	+0·88 −0·70
12	—	—	—	1·88	2·54	±0·00	1·36	2·30	+0·22 −0·34

13 %) in the group CoP than in the CoU group. In the −A group in relation to the same group CoU, the average increase was about the same, namely 15 %.

Although the actual weight of the testes of the rats on vitamin A-deficient diet was always less than that of the rats on CoP diet, when calculated per

200 g. of body weight, the change was not constant. There was an increase of 6 to 17 % in 6 litters, a decrease of 7 to 9 % in 3 litters, of 30 % in litter 6. Taking into consideration the fact that in litters 1 and 2 there was also an increase of 5 % and 15 %, and that, therefore, in most litters the testes of rats on vitamin A-deficient diet weighed more per 200 g. of body weight than those on CoP diet, it must be admitted that, as judged by the weight of the organs, in relation to the same unit of body weight, vitamin A deficiency seems to indicate hypertrophic rather than atrophic changes in the testes. This was not expected since, histologically, in all the testes of the above rats on vitamin A-deficient diet, degenerative or atrophic changes were found. No degeneration was found in the testes of the rats on the paired complete diet. These results will be communicated in detail later. An explanation, however, may be found in the fact that macroscopically these testes appeared to be oedematous, being soft and semi-transparent as compared with the testes of the control rats which were of firm consistency and of milky colour. Oedema of the testes would explain their greater weight.

Penis.

Table VII shows that in all the litters except 1 and 6, the penis, per 200 g. of body weight, was on the average about 21 and 24 % heavier in rats on −A diet than in rats on CoU or CoP diets respectively. In litter 1 there was practically no change, while in litter 6 the penis was smaller in the −A rats.

Table VII. *Average weight of penis of rats in each group of each litter (mg.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	267	185	+25	—	—	—	283	180	± 3
2	219	182	± 1	—	—	—	199	219	+ 9 − 7
3	—	—	—	160	218	± 1	162	263	+48 −45
4	—	—	—	180	172	± 9	191	260	±10
5	224	175	± 4	210	178	±11	184	214	+28 −19
6	—	—	—	142	230	± 0	117	193	± 0
7	—	—	—	98	166	± 1	104	208	+33 −28
8	70	147	± 0	64	151	± 0	67	187	+17 − 9
9	160	194	±24	131	187	±13	143	219	±12
10	—	—	—	160	230	±20	165	260	±20
11	—	—	—	170	180	± 1	175	220	+20 −10
12	—	—	—	150	200	± 0	145	240	+20 −10

Litter 6 was exceptional, owing to the great difference in food intake between the control and −A rats (see Table IV) as was mentioned previously. The influence of vitamin A deficiency on the weight of the penis can be ascribed to the direct action of the deficiency since there was no significant change between the CoU and CoP groups (per 200 g. of body weight).

Prostate with seminal vesicles.

As can be seen from Table VIII decreased food intake caused a decrease in weight of the prostate with seminal vesicles, both in the actual figures and per 200 g. of body weight (compare litters 5, 8 and 9, CoU and CoP groups). Per 200 g. of body weight, on the average, this decrease was about 19 %.

Table VIII. *Average weight of prostate and seminal vesicles of rats in each group of each litter (mg.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	1129	782	± 20	—	—	—	1189	922	± 22
2	888	738	+ 7 − 9	—	—	—	583	644	+113 −131
3	—	—	—	434	592	± 24	439	716	+243 −236
4	—	—	—	838	803	±129	887	1206	± 28
5	1257	975	±159	1066	906	± 72	878	1012	+ 99 − 52
6	—	—	—	269	438	± 0	262	434	± 0
7	—	—	—	216	346	± 71	203	388	+ 75 − 51
8	156	328	± 0	90	216	± 45	115	313	+ 32 − 50
9	561	680	±147	399	568	± 34	581	889	±166
10	—	—	—	457	635	+112 − 86	519	827	+ 84 − 69
11	—	—	—	701	747	± 17	747	951	+129 −194
12	—	—	—	466	629	± 0	388	649	+138 − 94

A comparison of the figures calculated per 200 g. of body weight of the groups on CoP and −A diets shows that the direct influence of vitamin A deficiency was towards an increase in weight of these organs, on the average by 32 % (with a maximum of 57 % and a minimum of 12 %). This influence was absent only in litter 6. Since the influence of decreased food intake is opposed to that of vitamin A deficiency the influence on the weight of these organs would probably depend on the relative action of these two factors.

Adrenals.

No influence of decreased appetite or of vitamin A deficiency on the adrenals could be observed from the results obtained (Table IX) when the

Table IX. *Average weight of adrenals of rats in each group of each litter (mg.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	42·0	29·0	±0·3	—	—	—	42·4	32·8	± 1·8
2	39·9	33·3	±3·8	—	—	—	31·2	34·4	+ 3·6 − 2·2
3	—	—	—	44·3	60·1	±2·0	39·7	65·1	+22·6 −11·6
4	—	—	—	48·3	46·1	±2·6	41·9	57·1	± 5·7
5	46·9	36·5	±0·8	48·1	40·6	±0·3	37·1	43·2	+ 8·6 − 7·8
6	—	—	—	31·6	51·4	±0·0	28·1	46·4	± 0·0
7	—	—	—	44·2	76·3	±6·2	34·8	70·7	+14·6 −10·6
8	33·6	70·7	±0·0	30·9	73·2	±1·6	26·3	75·8	+27·3 −19·3
9	42·5	51·5	±1·8	40·7	58·0	±3·9	29·3	45·4	± 0·2
10	—	—	—	42·9	60·1	+7·0 −6·9	31·6	50·4	+ 7·0 − 3·7
11	—	—	—	46·5	49·6	±1·0	38·2	45·9	+ 3·1 − 2·5
12	—	—	—	44·0	59·4	±0	29·4	49·0	+ 3·7 − 3·8

weights were calculated per 200 g. of body weight; the glands weighing, on the average, 57·5 mg. in the CoP group and 54·9 mg. in the −A group. The actual weights in all the litters except litter 1 were smaller in the −A group.

Thyroids.

A comparison of groups CoP and CoU in Table XI shows that decreased food intake produced in most of the rats of litters 5, 8 and 9 a more or less pronounced decrease in weight of the thyroids, both in the actual figures (on the average, 22 %) and when calculated per 200 g. of body weight (on the average 13 %). On −A diet the weights of the thyroids, in most cases in the actual figures, but always when calculated per 200 g. of body weight, were greater than those of the controls, on an average by 43 %.

This diet did not include iodine. Iodine was given to a pair of rats in each of the litters 10 and 12, in the daily amount of 7 γ, and to one pair of litter 11 in the daily amount of 30 γ. As can be seen from Table X, in these three litters the thyroids of the −A groups again weighed more than those of the controls when no supplementary iodine was given, but the same or even less when iodine was added to the diet.

Table X. *Average weight of thyroids of rats in each group of each litter (mg.).*

	CoU diet			CoP diet			−A diet			
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.		Iodine
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	content
1	28·4	19·7	±0·9	—	—	—	31·6	24·5	±1·3	−
2	25·3	20·9	+1·2 −1·0	—	—	—	25·0	27·5	+1·9 −1·6	− −
3	—	—	—	18·2	24·8	±2·4	21·6	35·1	+2·7 −1·5	− −
4	—	—	—	25·2	24·0	±0·6	24·8	33·7	±0·9	−
5	32·0	24·9	±1·5	24·5	20·8	±0·9	29·6	34·3	+4·7 −5·3	− −
6	—	—	—	13·0	21·1	±0·0	25·6	42·3	±0·0	−
7	—	—	—	15·4	26·2	±0·6	16·3	30·9	+5·0 −6·2	− −
8	16·0	33·6	±0·0	11·6	27·0	±1·0	16·0	43·9	+4·8 −6·1	− −
9	25·3	30·6	±5·0	20·9	29·7	±3·4	26·9	41·1	±7·7	−
10	—	—	—	19·9	27·7	+4·0 −2·2	23·0	36·0	±2·8	−
							17·5	28·6	±2·1	+
11	—	—	—	21·5	22·8	±2·3	22·7	28·5	±0·9	−
				—	—	—	19·0	24·4	±0·9	+
12	—	—	—	22·2	30·0	±0·0	20·1	32·6	±1·8	−
				—	—	—	14·4	23·4	±2·6	+

This indicates that the hypertrophy of the thyroids observed on our basal −A diet was produced, not by vitamin A deficiency, but by iodine deficiency. The control rats on CoU and CoP diets received sufficient iodine in the cod-liver oil which was given, this being one of the foods richest in iodine. Cotton-seed oil does not contain iodine in an amount detectable by micro-analysis.

Hypophysis.

In nearly all the litters, as can be seen from Table XI, the actual weight of the hypophysis was less in the −A and CoP rats than in those on complete unlimited diet. When, however, these weights are calculated per 200 g. of body weight, no significant or constant changes are observed, the weights being on the average 9·2 mg. and 8·7 mg. in the CoP and −A groups respectively.

Thymus.

No significant or constant changes were obtained in the weight of the thymus per 200 g. of body weight as a result of decrease in food intake in these experiments (Table XII).

In the rats on −A diet as compared with those on the CoP diet the weight of thymus per 200 g. of body weight in two (litters 8 and 12) of the 9 litters was not appreciably changed, whilst in the remaining seven it was less, on the average, by 23 % (maximum 38 %, minimum 12 %). The atrophic changes of the thymus in our experiments seem, therefore, to be related to the direct influence of vitamin A deficiency.

Table XI. *Average weight of pituitary of rats in each group of each litter (mg.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	10·5	7·3	±0·5	—	—	—	9·6	7·5	±0·4
2	8·3	6·9	+0·4 −0·8	—	—	—	6·7	7·4	+0·2 −0·5
3	—	—	—	6·7	9·1	±0·4	5·8	9·4	+0·4 −0·7
4	—	—	—	7·2	6·9	±0·6	5·4	7·3	±0·4
5	9·1	7·0	±0·4	8·7	7·3	±0·7	6·3	7·2	+1·2 −1·3
6	—	—	—	5·2	8·4	±0·0	4·8	7·9	±0·0
7	—	—	—	6·6	13·0	±1·9	5·3	11·6	+7·2 −4·3
8	5·2	10·9	±0·0	5·0	12·0	±1·8	3·0	8·3	±0·1
9	6·8	8·3	±0·2	6·0	8·4	±0·2	7·1	10·9	±0·7
10	—	—	—	6·3	8·8	+0·2 −0·1	5·0	8·1	+1·2 −1·6
11	—	—	—	8·0	8·5	±0·1	6·1	7·7	+0·6 −1·2
12	—	—	—	6·8	9·2	±0·0	5·1	8·6	+1·4 −0·7

Table XII. *Average weight of thymus of rats in each group of litters nos. 4 to 12 (mg.).*

	CoU diet			CoP diet			−A diet		
		Per 200 g. body wt.			Per 200 g. body wt.			Per 200 g. body wt.	
Litter no.	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation	Actual wt.	Wt.	Deviation
1	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	—	—	—	317	302	± 8	182	249	± 76
5	580	453	±16	478	488	± 5	278	340	± 15
6	—	—	—	283	459	± 0	223	368	± 0
7	—	—	—	351	606	±51	200	374	+ 69 −105
8	226	475	± 0	160	378	±22	134	364	+ 54 − 49
9	377	457	±29	372	528	±13	235	360	± 44
10	—	—	—	159	225	+24 −41	125	198	+ 23 − 21
11	—	—	—	370	394	±26	268	341	+ 78 −123
12	—	—	—	227	307	± 0	184	309	+ 52 − 40

Iodine in −A diet.

We have already discussed the effect of addition of iodine to the −A diet on changes in the thyroid gland. No appreciable differences were noted, when iodine was included in the −A diet, in the changes above described in the other endocrine organs. Therefore, only in the case of the thyroid gland (Table X) in litters 10, 11 and 12 are the figures given separately for the groups fed on −A diet with and without the addition of iodine.

No influence was noted on the changes in body weight, gain per unit of food intake, or in fat deposition, or sexual organs.

Discussion.

It is clear from the data given above that, in rats kept on a vitamin A-deficient diet, two main factors are operating in influencing the organs and their functions.

The first is the decreased appetite, which, although directly caused by vitamin A deficiency, is in itself the cause of several secondary changes, which are entirely due to the effect of the decreased food intake.

The second factor is some other direct influence of vitamin A deficiency on the organs and their functions. In the animals kept on vitamin A-deficient diet the decreased food intake seemed to produce greater changes in fat deposition than in general body weight. For, while in our rats roughly 2/5 of the deficient gain in body weight was due to the decreased food intake, about 3/5 was caused by some other direct influence of the deficiency. In the fat deposition on the other hand these proportions were reversed, decreased food intake causing 3/5 of the deficiency in the body fat. This observation is in agreement with the general principle that with decreased food intake chiefly the carbohydrate and fat stores are affected first.

Measurements of body weight do not always represent the changes in growth of the animals, or the same degree of growth. In our young growing animals, however, these changes in weight roughly indicate the changes in growth. Thus in the following, with certain reservations, what is said about body weight also applies to the growth of the animals.

That the gain in weight is influenced to a considerable degree by the direct action of the deficiency is shown in all the litters in which vitamin A was depleted (*i.e.* litters 2 to 12). In every case one unit of the deficient diet produced a much smaller increase in body weight than the same unit of diet rich in vitamin A.

This property of vitamin A relating to building of tissue can be defined as an "anabolic principle." An increase in body weight and in growth of the animals is produced by this and by "the appetite producing" principle of this vitamin. In the absence of this "anabolic" principle, as is indicated by the results of our previous papers, the food consumed and absorbed is not deposited in the body normally or to a normal extent. The increased nitrogen

metabolism also testifies to this inability of the vitamin A-deficient organism to utilise the food normally for building new tissue.

In most cases the organs investigated were also under a twofold influence: that of the "appetite producing principle," *i.e.* of decreased food intake, and that of some other direct action of vitamin A deficiency.

With the sexual organs the unexpected result was noted of an increased weight, per 200 g. of body weight, of testes and notably of penis and prostate with seminal vesicles in most of the rats on −A diet as compared with those of the rats on CoP diet. In the case of the testes the oedematous condition would explain this increase, for histologically degenerative and atrophic changes were found in the seminiferous tissue which were completely absent from the testes of rats on CoP diet. In any case the changes in the testes did not affect the weights of the prostate with seminal vesicles or of the penis.

The decrease in food intake did not produce any significant changes in the weight (per 200 g. of body weight) of the penis, decreased the weight of the prostate with seminal vesicles and in some cases slightly increased the weight of the testes.

The thyroids in rats on −A diet were found to be hypertrophied. The −A diet, used by us, however, was deficient in iodine. When, in three litters, iodine was added to the −A diet no such hypertrophy was obtained. This change was therefore due to the iodine, rather than to the vitamin A, deficiency. In some rats decreased food intake on complete diet produced a more or less pronounced decrease in weight of the thyroids.

A direct influence of vitamin A deficiency on the thymus gland was evident as was shown by the decrease in weight of the gland.

No constant or significant changes were found in the adrenals and hypophysis.

Summary.

1. Using 63 male rats belonging to 12 litters a study was made of the influence of vitamin A deficiency on the body weight, fat deposition and the weight of the endocrine organs in order to distinguish the secondary changes produced by the decreased appetite from those produced by some direct influence of the deficiency.

2. Both these factors were found to be concerned in the changes produced in the organs and their functions.

3. At the stage of vitamin A depletion at which the rats were killed, about 2/5 of the deficiency in body weight would seem to be due to the decreased food intake, while 3/5 would seem to be caused by some other direct influence of the deficiency.

4. These proportions were reversed for the fat deposition.

5. In these experiments, in agreement with the previous results, it was found that one unit of the vitamin A-deficient diet produced a much smaller increase in body weight than was produced by the same unit of complete diet.

6. Therefore, vitamin A seems to have, in addition to an "appetite producing" principle, an "anabolic" principle, both of which are essential for building of the tissues, for increase in body weight and for growth of the animal.

7. When the weight of the organs is calculated per the same unit of body weight the following changes in the sexual organs were noticed. In most of the rats on vitamin A-deficient diet the weights of the testes and especially of the penis and of the prostate with seminal vesicles were greater than those of rats kept on a paired complete diet. The testes of the rats on the deficient diet seemed to be oedematous. The decreased food intake produced no noticeable effect on the penis, decreased the weight of the prostate with seminal vesicles and in some cases slightly increased the weight of the testes.

8. Reduction in the weight of the thymus was observed in most of the rats on the deficient diet and seemed to be due to the direct influence of vitamin A deficiency.

9. No significant changes were found in the weights of the adrenals or of the hypophysis.

10. The thyroids were hypertrophied only in the rats kept on the vitamin A-deficient diet which were used in the experiments. In rats receiving iodine in addition to this diet this change in the weight of the thyroids did not occur. It was, therefore, due to iodine deficiency.

11. No influence of iodine on any of the other changes caused by vitamin A deficiency was noticed.

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