SUSCEPTIBILITY OF VITAMIN A-DEFICIENT AND STARVED RATS AND MICE TO A PERORAL INFECTION WITH *SALMONELLA TYPHI-MURIUM**

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(With 2 Figures in the Text)

Green & Mellanby (1928, 1930) were the first to direct attention to a specific relationship between vitamin A deficiency and infection. They studied the occurrence of spontaneous infections in vitamin A-deficient rats and concluded that animals fed on a diet deficient in vitamin A, but complete in all other respects, develop multiple infective lesions and die. Since then vitamin A has become known as the 'anti-infective' vitamin.

A possible explanation for this relationship was provided by the work of Wolbach & Howe (1925), who demonstrated marked changes in the epithelial cells in various tissues in vitamin A-deficient animals. It was assumed that the metaplasia of the epithelial layers destroyed the barrier against the penetration of bacteria into the tissues. Support for this assumption was offered by the experiments of Seidmon & Arnold (1932), showing that *Salmonella typhi-murium* fed to vitamin A-deficient animals were found more frequently and in larger numbers in various organs than in normal rats fed with the same dose of bacteria under the same conditions. The validity of this view was challenged, however, by Lassen (1930) and by Boynton & Bradford (1931), who found that the difference in resistance between vitamin A-deficient and normally fed animals could also be demonstrated if the infection was given intraperitoneally instead of *per os*. Greater penetrability of the mucosa could not, therefore, account for the lowered resistance manifested by the deficient animals. Stryker & Janota (1941) were also unable to find an increased permeability. The mechanism of the lowered resistance remains, therefore, obscure. Fox (1933), in a review of the subject, reached the conclusion that vitamin A functions not as a positive anti-infective agent, but that its deficiency influences general body resistance, or susceptibility.

This view leaves open the question whether the vitamin as such does or does not effect natural resistance either directly or indirectly. More important still, none of the studies mentioned deals with the quantitative aspect of the question, namely, at what degree of avitaminosis does the lowered resistance become manifest? From the hygienic standpoint it is important to know whether the increased susceptibility occurs in the early or late stages of avitaminosis.

It is apparent that the subject requires further study, and we set ourselves the object of obtaining an answer to two questions. At what degree of avitaminosis does increased susceptibility manifest itself? Is the vitamin A deficiency *per se* accountable for the lowered resistance?

METHODS

Rats and mice served as experimental animals and *S. typhi-murium* as the infective agent. The animals were taken from our breeding stock 1 week after they were weaned. The stock food of the rats consisted of a mixture of sprouted wheat, oats and bran, supplemented by milk and seasonal vegetables. For the purpose of the experiments the nursing mothers were deprived of vegetables 2 weeks before the litter was weaned. This reduced the available vitamin A in the food and hence minimal amounts of the vitamin remained in the livers of the weaned rats. The animals used for the experiments weighed 35–50 g. They were placed on a vitamin-free diet of the following composition: alcohol extracted casein, 13 %; rice flour, 65 %; olive oil, 10 %; yeast, 8 %; salt mixture, 4 %; vitamin D, 100 i.u./kg. The control animals received a supplement of 100 i.u. of vitamin A twice a week; Glaxo 'Prepalin' was dissolved in olive oil and 0·1 c.c. fed with a pipette.

The stock food of the mice consisted of whole-wheat bread, sprouted wheat, oats, carrots, beets and milk. Since we found that nursing mice store only minimal quantities of vitamin A, the mothers were not deprived of carrots during nursing. The experimental and the control mice weighed 9–13 g. at the start of the experiments. They received the

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same diet as the rats; the only difference was that the mice received 50 i.u. vitamin A twice a week in 0·05 c.c. of oil.

The strain of *S. typhi-murium*, isolated from a paratyphoid epidemic in guinea-pigs in 1941, was quite virulent for mice and moderately so for rats. The infective dosage finally adopted was based on a large number of preliminary tests; it was graded to give a mild to a moderate infection in normal animals, so that the differences if any between them and the deficient ones would be significant and beyond doubt.

At the time the rats were taken for the experiments they contained between 3 and 10 i.u. of vitamin A per liver. These results were established by killing a number of animals before the start of the experiments, pooling the livers of three or four of them and determining the vitamin A content by the usual procedure (Carr-Price). This initial reserve disappeared from the liver after 1 week on the vitamin A-free diet. Nevertheless, growth still continued normal for the next week or two. During the next 2 weeks growth slackened and then stopped. At this stage no other avitaminotic symptoms were apparent, ceratomalacia and xerophthalmia appeared only in the 6th or 7th week when loss in weight had set in.

In mice the process was quite similar. At the outset the livers contained 1–5 i.u., and these disappeared after a few days. Growth continued normal for 2 weeks, slackened in the third week, stopped in the fourth and loss of weight set in in the fifth. It might be added that at no time did our mice develop xerophthalmia or ceratomalacia. These observations are in accord with those of Beard (1925) and of Pomerene & Beard (1930). Fig. 1 gives the growth curves of mice on a vitamin A-deficient diet.

On the basis of these observations we divided the animals into three classes, or grades of avitaminosis:

Nutritional status	Vitamin A in liver	Weight		Ceratomalacia or Xerophthalmia	
		Rats	Mice	Rats	Mice
I. Euvitaminosis	Present	Normal increase	Normal increase	Absent	Absent
II. Hypovitaminosis: *a*	Absent	,,	,,	,,	,,
b	,,	Slackened	Slackened	,,	,,
III. Avitaminosis: *a*	,,	Stopped	Stopped or decreased	,,	,,
b	,,	Stopped or decreased	Decreased	Present	,,

RESULTS

Effect of avitaminosis on mortality in mice. Thirty females and fifteen males in grade III*a* (see Fig. 1) and an equal number of controls were given *per os* 0·05 c.c. of a 24 hr. broth culture of *S. typhi-murium*, diluted 1:5 with milk. An equal number of avitaminotic animals served as uninfected controls. The animals were kept under observation for 2 weeks and deaths recorded daily. The results are summarized in Table 1. It will be noted that the avitaminotic group reacted more severely than the control. The infection proceeded more rapidly, twenty animals died in 5 days as against ten of the controls, and the total fatality was higher; at the end of 2 weeks only four of the avitaminotic animals remained alive as against sixteen of the controls. In the non-infected vitamin-deficient controls forty-one remained alive at the end of the experiment.

Influence of avitaminosis on the penetration of the infecting organism. In subsequent experiments we attempted to ascertain the course of invasion of the infecting organisms in animals in different stages of avitaminosis. The control group received the basal diet *ad libitum* plus 100 or 50 i.u. vitamin A twice a week. In one experiment a comparison was made between normal and hypovitaminotic mice (stage II*b*), and in another between normal and avitaminotic mice (stage III*a*). The mice were given *per os* 0·05 c.c. of a 24 hr. broth culture diluted 1:1,000,000 in milk. Seven days after the infection the mice were killed and cultures made of the mesenteric glands, liver and spleen. The results are given in Table 2 and indicate that there was no difference in the incidence or in the organ distribution of the infection between normal and hypovitaminotic animals. There were, however, striking differences between the normal and the avitaminotic mice; three times as many mice of the latter group were infected as of the former.

These experiments were repeated using rats. The infection was given when the vitamin A-deficient animals reached grade II*b*. The infecting dose was 0·1 c.c. of a broth culture diluted 1:10 in milk, given perorally. Three days after the infection the animals were killed and cultures made from the organs.

The results correspond with those obtained with mice (see Table 3). Although the average weight of the hypovitaminotic rats was 13 g. less than that of the normal controls, the incidence and distribution of infection was the same in both groups. On the other hand, the avitaminotic animals manifested a markedly lowered resistance both with

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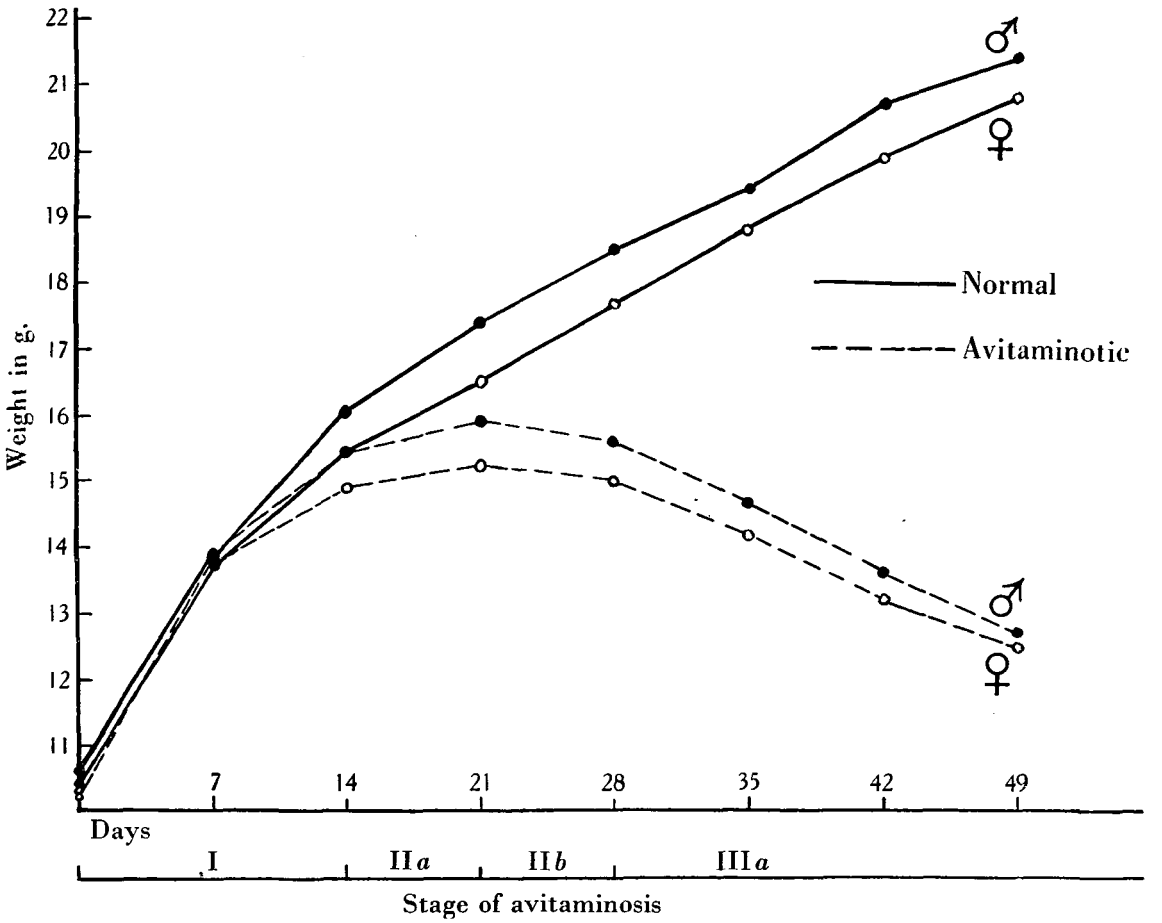

Fig. 1. Growth curves of mice.

Table 1. *Mortality of normal and avitaminotic mice infected* per os *with* [illegible]

Nutritive state	No. of mice	Sex	Weight in g. At start of exp.	Weight in g. At time of infection	Remained alive after days 5	10	14
Vitamin given; infected	30	M.	10·6	18·4	22	10	7
(controls)	15	F.	10·3	18·7	13	10	9
Total	**45**	M.+F.	**10·5**	**18·5**	**35**	**20**	**16**
Avitaminosis (stage III*a*),	30	M.	10·6	14·9	16	6	2
infected	15	F.	10·2	15·7	9	4	2
Total	**45**	M.+F.	**10·5**	**15·8**	**25**	**10**	**4**
Avitaminosis (stage III*a*),	30	M.	10·1	15·3	30	28	27
not infected (controls)	15	F.	10·2	15·6	15	14	14
Total	**45**	M.+F.	**10·1**	**15·4**	**45**	**42**	**41**

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respect to incidence and distribution of the infection in comparison with controls.

It seems, therefore, that diminished resistance sets in only when a severe state of deficiency exists, that is when the animals have begun to eat less and to decline in weight. The question arose, therefore, whether diminished resistance was due specifically to vitamin deficiency or to the associated starvation. To test this we carried out a paired feeding experiment with rats. We used three groups of animals: a control group receiving a full diet, one experimental group receiving a full diet without vitamin A, and a third group receiving the same dose of vitamin A as the control, but fed each day only the weighed amount of food eaten the day before by the vitamin-free animals. The third group received, therefore, an adequate supply of all vitamins, but the quantity of food was limited to that actually consumed by the vitamin A-deficient rats. Fig. 2 shows the average growth curves of the three groups of rats. The infection was given at the time when the rats on the deficient diet reached grade III*a*; the dose was the same as above. The animals were killed on the fourth day and the organs cultured as above.

The results are shown in Table 4. It seems clear that the starved rats did not fare better than the vitamin-deficient ones. Practically all of the rats on the deficient as well as those on the limited basal diet became infected, as against only 50 % of those kept on the normal diet. Even more striking was the difference in the distribution of the infection; in the normal group only four of the fifty rats contained organisms in the liver and spleen as against twenty-nine and thirty in the paired fed and twenty-four in the deficient group. Even more significant is the fact that although the paired fed rats were heavier than the deficient group the incidence and distribution of the infection was the same.

Table 2. *Relative susceptibility of mice in various stages of vitamin A deficiency to a* Salmonella typhi-murium *infection*

Condition of mice	No.	Sex	Weight in g. At start of exp.	Weight in g. At time of infection	No. infected	No. with infections in Liver	Spleen	Mesenteric glands
Normal	10	M.	10·9	17·3	3	1	2	1
Hypovitaminosis	10	M.	10·9	17·0	2	1	0	2
Normal	15	M.	10·4	19·4	4	2	2	1
	10	F.	10·3	19·3	2	3	3	2
Total	**25**	M.+F.	**10·4**	**19·4**	**6**	**5**	**5**	**3**
Avitaminosis	15	M.	10·2	14·4	10	8	7	9
	10	F.	10·3	14·3	8	5	6	6
Total	**25**	M.+F.	**10·2**	**14·4**	**18**	**13**	**13**	**15**

Table 3. *Relative susceptibility of rats in various stages of vitamin A deficiency to a* Salmonella typhi-murium *infection*

Condition of rats	No.	Sex	Weight in g. At start of exp.	Weight in g. At time of infection	No. infected	No. with infections in Liver	Spleen	Mesenteric glands
Normal	10	M.	46	95	5	2	0	4
	15	F.	44	76	9	1	2	7
Total	**25**	M.+F.	**45**	**84**	**14**	**3**	**2**	**11**
Hypovitaminosis	10	M.	45	75	3	1	0	2
	15	F.	44	68	10	2	3	8
Total	**25**	M.+F.	**44**	**71**	**13**	**3**	**3**	**10**
Normal	15	M.	50	123	9	4	4	7
	10	F.	49	102	6	2	1	6
Total	**25**	M.+F.	**50**	**115**	**15**	**6**	**5**	**13**
%					60·0	24·0	20·0	52·0
Avitaminosis	11	M.	49	85	9	8	7	7
	7	F.	48	72	7	4	3	5
Total	**18**	M.+F.	**49**	**80**	**16**	**12**	**10**	**12**
%					88·0	67·0	55·0	67·0

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DISCUSSION

The experiments reported provide an unequivocal answer to the question regarding the relation of vitamin A deficiency to resistance to infection. Rats and mice, kept on a vitamin A-free diet, ultimately reach a state of deficiency which is associated with a markedly increased susceptibility to an enteric infection. In this regard our results correspond with those of various other workers (Verder, 1928; Boynton & Bradford, 1931; Lassen, 1930; McClung & Winters, 1932). The authors cited brought their animals to a state of definite avitaminosis, and there is complete concordance in the results obtained.

Our experiments extend these studies in two directions. On the one hand, they indicate that the rats and mice must reach a marked degree of avitaminosis, manifested by declining weight before a fall of resistance is noted; rats and mice in a state of hypovitaminosis are no less resistant than the control animals kept on an adequate vitamin A-free diet. On the other hand, rats receiving an adequate allowance of vitamin A, but a quantity of food equal

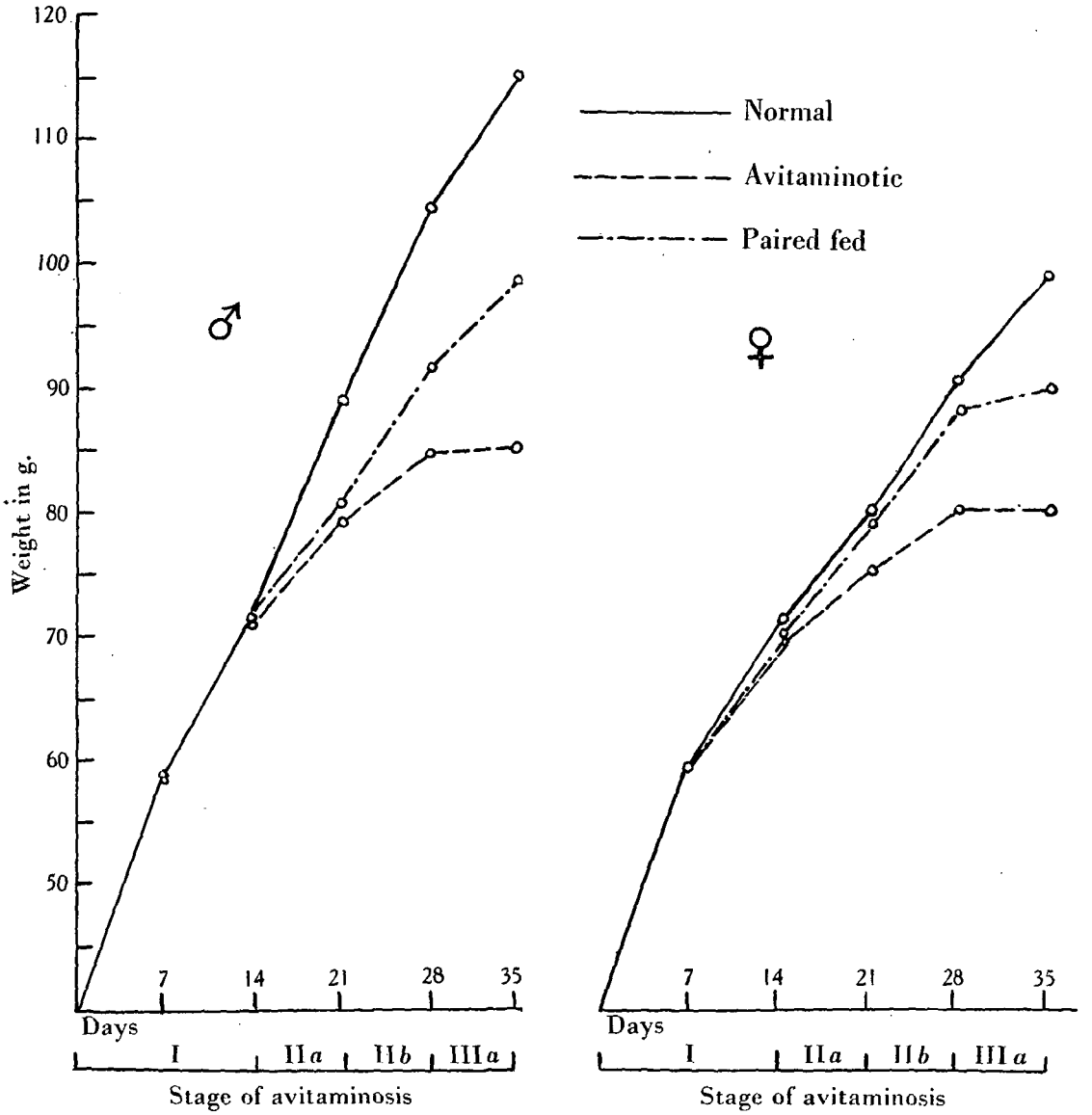

Fig. 2. Growth curves of rats.

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to that eaten by the animals kept on the vitamin A-free ration, manifest the same increased susceptibility to infection as do the avitaminotic animals. These two findings supplement one another. They suggest that the vitamin A as such is not the factor responsible for the lowered resistance, but that the cause is associated with the lowered food intake consequent to the deficiency. In other words, it would seem that the causative factor is starvation, since paired fed rats receiving an adequate amount of vitamin A are as susceptible to the enteric infection as are the corresponding vitamin-deficient animals. These findings cast doubt on the validity of current statements that vitamin A is the 'anti-infective' vitamin.

If starvation is the cause of lowered resistance, then the question is, whether general or specific starvation is involved. It seems possible that the causative factor is protein rather than vitamin deficiency. However, a definitive answer can only be furnished by further experiments which are now in progress.

Table 4. *Relative susceptibility to a* Salmonella typhi-murium *infection of paired fed rats, one group receiving a vitamin A-free diet and the other the same quantity of the same diet with a supplement of vitamin A*

Condition of rats	No.	Sex	Weight in g. At start of exp.	Weight in g. At time of infection	No. infected	No. with infections in Liver	No. with infections in Spleen	No. with infections in Mesenteric glands
Normal control	20	M.	40	121	9	1	2	7
	30	F.	41	100	16	3	2	12
Total	**50**	M.+F.	**41**	**108**	**25**	**4**	**4**	**19**
Avitaminosis (stage III*a*)	20	M.	40	86	19	9	11	16
	30	F.	41	80	29	15	13	26
Total	**50**	M.+F.	**41**	**82**	**48**	**24**	**24**	**42**
Paired fed with avitaminotic group (basal+vitamin)	20	M.	40	100	19	12	14	17
	30	F.	41	90	30	17	16	28
Total	**50**	M.+F.	**41**	**94**	**49**	**29**	**30**	**45**

CONCLUSIONS

1. Mice and rats in a state of hypovitaminosis A are no more susceptible to an oral infection with *Salmonella typhi-murium* than normally fed controls.

2. Mice and rats in a state of avitaminosis A are more highly susceptible than normally fed controls.

3. Paired fed rats receiving an adequate ration of vitamin A, but a total amount of food equal to that consumed by the corresponding animals kept on a diet free of vitamin A, are as susceptible to the enteric infection as the avitaminotic animals. It seems, therefore, that starvation rather than vitamin A deficiency is responsible for the lowered resistance.

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