THE INFLUENCE OF *SALMONELLA TYPHI-MURIUM* INFECTION IN RATS ON VITAMIN A METABOLISM*

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Since Green & Mellanby (1928, 1930) reported that vitamin A protected the body against infection, this problem has been studied by various authors (Verder, 1928; Lassen, 1930, 1931; Seidmon & Arnold, 1931–2; Boynton & Bradford, 1931; McClung & Winters, 1932; Stryker & Janota, 1941). In general, the results showed that A avitaminotic animals were more susceptible to spontaneous or experimental infections than controls kept on a normal diet. However, in so far as we know, no experimental work has been done on the inverse problem—the effect of infection on vitamin A metabolism.

As a part of a programme for the reinvestigation of the relation of vitamins to infection and resistance, we attempted to determine whether an enteric infection influences vitamin A metabolism. The experiments were designed to ascertain the vitamin A requirements, the storage capacity of the liver, and the resorption and conversion of carotene during the infective process.

METHODS

Moore (1931), and Baumann, Riising & Steenbock (1934), showed that the storage of vitamin A in the liver was a satisfactory measure of vitamin A saturation, and we adopted this as a basis for our work. In order to start from a known base-line, the diet of the rats used in these experiments was adjusted so that, before starting the experiment, the animals were brought to a state of complete liver depletion without any manifestation of avitaminosis. To achieve such depletion promptly, the nursing mothers were deprived of vegetables 2 weeks before the litter was weaned, thus reducing the vitamin A in the diet. Repeated tests showed that at the time of weaning the liver of the litter rats contained only 3–10 i.u. vitamin A. This small amount disappeared completely in 2–6 days, if the young rats were put on a vitamin A-free diet after they were weaned. Such depleted rats were ready for the tests.

As soon as liver depletion was achieved, the rats were given an intraperitoneal inoculation of *Salmonella typhi-murium*. The infective dose used was 0·5 c.c. of a 24 hr. broth culture diluted 1:20 with saline. Some of the animals died in the course of the experiment, but the majority developed a severe infection from which they ultimately recovered. The tests on vitamin metabolism were made during the period of illness when the liver was most severely involved. We thus tried to simulate a condition comparable to that existing in man during a severe typhoid infection. The rats were kept on a complete diet lacking only vitamin A until the third day after the inoculation; thereafter given amounts of vitamin A or carotene were fed. The vitamin A was given in 0·1 c.c. olive oil *per os*, and the carotene in the form of weighed quantities of carrots. The requisite amounts were given either daily or every other day, but always at the same time and in the same quantities as to the non-infected controls kept on the same diet and under the same conditions. The vitamin or carotene administration was discontinued 2 days before the end of the experiment, when the rats were sacrificed for the liver examination.

The livers were prepared for the vitamin A determinations according to Lindquist's method (1938), and the vitamin A was determined colorimetrically by the method of Carr-Price. The carrots and the faeces were prepared according to the Peterson, Hughes & Freeman (1937) modification of Guilbert's (1934) technique, and the carotene determined colorimetrically by the azobenzene method of Kuhn & Brockmann (1932). The faeces were collected daily and stored in the ice box and the combined material examined every 2–3 days.

EXPERIMENTS

The course of the infection in the rats was quite severe and lasted about 2 weeks. Its progress during the first 2 weeks was manifested by retardation of growth and in the second week by about 25 % fatality. Thereafter, recovery set in and gains in weight were registered. The fatality varied from one experiment to another, but the general course of the infection remained quite constant. Rats sacrificed during the first 2 weeks of the infection showed greatly enlarged, oedematous livers (see

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Tables 1, 2). In the control rats the average ratio of liver to body weight was 4·0 %; in the infected rats the proportion at first increased steadily and then returned to that of the control rats. The following are average figures obtained at different intervals after infection:

	5 days after	infection	5·8 %
8–10	,,	,,	7·0 %
14	,,	,,	5·9 %
17–22	,,	,,	4·9 %

Cultures were made from all inoculated rats to make sure that the specific infection had actually developed. In all cases a positive spleen culture was obtained.

collected and the amount of carotene excreted in the stools determined.* The results are summarized in Table 2 and show that in general, irrespective of the amount of carotene given, the amount of vitamin A found in the livers of the non-infected controls was about twice as high as that in the corresponding infected groups. This difference was noted at all stages of the infection up to 17 days. A summary of the data detailed in Table 2 is given in Table 3.

A statistical analysis of the data indicates that the differences obtained were significant; the probability of a chance occurrence of these differences ranges, for the various levels of carotene given, between 0·05 and $<0·01$.

Table 1. *Influence of intraperitoneal infection with* Salmonella typhi-murium *on the resorption and storage of vitamin A*

No. of exp.	Total amount of vitamin A given during the exp. i.u. per 10 g. body weight	Duration of exp. days	No. of rats	Weight in g. Start of exp.	Weight in g. End of exp.	Liver Weight g.	Liver Weight % of body weight	Liver Vitamin A i.u. per liver
				Non-infected rats				
1*a*	60	8	6	52	76	3·4	4·5	8·0
2*a*	60	8	4	57	72	3·0	4·2	14·6
3*a*	60	8	4	60	82	3·7	4·5	15·0
4*a*	60	8	4	43	64	2·9	4·5	9·2
5*a*	160	22	4	64	110	4·0	3·6	57·0
				Infected rats				
1*b*	60	8	6	52	63	4·7	7·5	10·0
2*b*	60	8	4	59	68	5·5	8·1	14·5
3*b*	60	8	4	58	71	5·4	7·6	15·8
4*b*	60	8	4	43	57	3·2	5·6	10·0
5*b*	160	22	4	63	90	3·9	4·3	53·0

The effect of infection on vitamin A storage in the liver. In the first series of experiments the rats were given specific quantities of vitamin A and the amount stored in the liver compared with that in a comparable control group. The results are summarized in Table 2. It will be noted that irrespective of the quantity of vitamin given, the total amount recovered in the liver was the same in the infected as in the uninfected groups. The infected animals gained less in weight than did the controls and had much larger livers, but the total quantities of vitamin A stored, which imply also the amounts used by the body, were the same in both groups. The infection had, therefore, no effect on vitamin A consumption or storage.

The effect of infection on resorption and conversion of carotene. As in the preceding experiments depleted rats were infected and they, as well as uninfected controls, were fed on varying quantities of carotene. At varying intervals the rats were killed and the amounts of vitamin A in the livers determined. During the experiment the faeces were

There are two possible explanations of these findings. The lower vitamin A reserve in the infected animals may be due either to poor absorption of the carotene or to poor conversion. The stool analyses for carotene show (see Tables 2, 3) that the non-infected control rats excreted 39–62 % of the carotene ingested, amounts corresponding to those found by De (1937); while the infected rats excreted somewhat larger proportions of the amount ingested (45–74 %). These differences, though small, are statistically significant. However, even though the difference in the quantity of carotene excreted is significantly higher in the infected than in the non-infected rats, the actual differences in the amounts excreted are not large enough to account

* Faeces of rats not receiving carotene contain a pigment resembling carotene. This pigment was found and studied by Kemmerer & Fraps (1938) and Russell, Taylor, Walker & Polskin (1942). The daily quantity excreted is equivalent to 0·5–2γ of carotene. This quantity was deducted from the results in estimating the daily excretion of carotene.

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for the markedly lower storage of vitamin A in the livers of the former. The lesser storage of vitamin A in the infected animals must, therefore, be due chiefly to impaired conversion of carotene to vitamin A. Carotene as such was not found in either normal or infected rats. Since the first series of experiments indicate that there was no increased consumption of vitamin A during infection, it seems that in the infected animals the carotene is absorbed by the body, but that only part of it is converted into vitamin A; what happens to the remainder is problematical.

DISCUSSION

The experiments reported above establish three points: (*a*) rats infected with paratyphoid and given vitamin A by the mouth store as much of this vitamin as do non-infected controls; (*b*) rats so infected and fed with carotene store about half as much vitamin

Table 2. *Influence of intraperitoneal infection with* Salmonella typhi-murium *on the resorption of carotene and its storage as vitamin A in the liver*

No. of exp.	Total amount of carotene given during exp. per 10 g. body weight	Duration of exp. days	No. of rats	Weight in g.		Carotene in faeces, % of amount given	Liver		
				Start of exp.	End of exp.		Weight		Vitamin A i.u. per liver
							g.	% of body weight	
				Non-infected rats					
6*a*	44	5	4	41	56	59	2·1	3·7	20·0
7*a*	44	5	4	45	61	58	2·3	3·8	15·0
8*a*	44	5	4	44	61	41	2·2	3·6	20·0
9*a*	66	8	4	39	55	54	2·0	3·6	16·5
10*a*	66	8	4	38	55	52	2·2	4·0	16·0
11*a*	66	8	4	40	59	39	2·1	3·6	19·0
12*a*	66	8	4	55	76	—	3·6	4·7	14·8
13*a*	66	8	4	55	75	—	2·7	3·6	12·4
14*a*	132	10	4	45	71	43	2·9	4·1	52·0
15*a*	132	10	4	45	68	59	2·9	4·3	60·0
16*a*	132	10	4	45	71	52	2·9	4·1	48·0
17*a*	132	17	4	45	81	—	3·1	3·8	37·0
18*a*	176	14	4	48	83	49	3·5	4·2	70·0
19*a*	176	14	4	48	84	62	3·8	4·5	75·0
20*a*	176	14	4	46	79	42	2·8	3·5	72·0
21*a*	180	9	4	45	75	60	3·2	4·3	78·0
22*a*	180	9	4	45	70	43	3·0	4·3	84·0
23*a*	180	9	4	45	72	59	2·9	4·0	91·0
				Infected rats					
6*b*	44	5	5	41	48	61	2·6	5·4	12·0
7*b*	44	5	5	45	54	65	3·4	6·3	12·0
8*b*	44	5	5	44	55	53	3·2	5·8	14·0
9*b*	66	8	5	39	51	55	3·5	6·9	8·3
10*b*	66	8	5	39	51	65	3·2	6·5	9·0
11*b*	66	8	5	40	52	50	3·1	6·0	12·1
12*b*	66	8	4	57	70	—	5·2	7·4	9·8
13*b*	66	8	4	55	60	—	5·2	8·7	7·7
14*b*	132	10	5	45	58	45	3·7	6·4	29·0
15*b*	132	10	5	45	60	55	3·2	5·3	31·0
16*b*	132	10	5	45	58	69	3·4	5·9	24·0
17*b*	132	17	5	45	61	—	3·3	5·4	20·0
18*b*	176	14	5	48	71	56	3·8	5·4	37·0
19*b*	176	14	5	48	74	54	4·5	6·1	39·0
20*b*	176	14	5	46	61	59	3·2	5·2	33·0
21*b*	180	9	4	45	50	71	3·5	7·0	50·0
22*b*	180	9	5	44	54	53	4·3	8·0	54·0
23*b*	180	9	5	44	57	67	4·4	7·7	64·0

Mean carotene excretion: normal rats: 51 %; standard deviation: 8·0 %; standard error: 2·1 %.
Mean carotene excretion: infected rats: 58 %; standard deviation: 7·6 %; standard error: 4·0 %.
Probability that the difference in the means is a matter of chance is 0·02.

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A as the non-infected controls; (*c*) the infected rats excrete somewhat larger proportions of the carotene fed to them than do control rats, but the difference is not enough to account for the striking differences in the quantities of vitamin A stored in the liver. It seems, therefore, that resorption is only to the same degree as do normal individuals. In the *typhi-murium* infection the liver is seriously involved and apparently this injury reduces markedly its capacity to convert carotene to vitamin A. The inference from these findings to vitamin A metabolism in typhoid infection in man

Table 3. *Summary of Table* 2

		Non-infected rats			Infected rats				
			Liver			Liver		Ratios	
No. of exps.	Duration of exp. days	Carotene in faeces, % of amount given	Weight as % of body weight (*a*)	Vitamin A i.u. (*b*)	Carotene in faeces, % of amount given	Weight as % of body weight (*c*)	Vitamin A i.u. (*d*)	Liver weights *c/a*	Liver vitamin A *d/b*
6– 8	5	53	3·7	18	63	5·8	13	1·57	0·72
9–13	8	48	3·9	16	59	7·1	9	1·82	0·56
14–16	10	51	4·2	53	56	5·9	28	1·40	0·53
17	17	—	3·8	37	—	5·4	20	1·42	0·54
18–20	14	51	4·1	72	54	5·6	36	1·37	0·50
21–23	9	54	4·2	84	64	7·6	56	1·80	0·67

slightly impaired while the conversion mechanism is seriously damaged.

An analogue to the impaired absorption is found in the work of Heymann (1936), who found that children suffering from infectious ailments excrete a larger proportion of the carotene fed than do healthy children. A suggestive explanation of our finding is offered by the work of Greaves & Schmidt (1935*a*), who demonstrated that bile is necessary for carotene absorption. It may well be that in the infected rats bile excretion is below normal.

The more important aspect of the problem is the reason for the relative inability of infected rats to convert carotene to vitamin A. Again, Greaves & Schmidt (1935*b*) showed that livers poisoned with phosphorus could not convert carotene to vitamin A. Monceaux (1938*a*, *b*) reports that patients with liver damage cannot convert carotene, fed in abundance, is obvious. It seems clear that until the contrary is shown to be the fact, typhoid patients should receive their vitamin A ration as vitamin and not in the form of carotene.

CONCLUSIONS

Young rats infected intraperitoneally with *Salmonella typhi-murium* develop a severe infection, one of the manifestations of which is involvement of the liver.

Such infected rats receiving vitamin A by the mouth store the same quantities as do non-infected controls.

If such infected rats receive carotene by the mouth the amount of carotene excreted is somewhat higher, and the amount of vitamin A stored in the liver markedly lower, than in controls.

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