FORMATION OF A NEW VITAMIN A METABOLITE IN RATS

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Administration of β-carotene-C^{14} to rats is accompanied by the appearance of a radioactive metabolite of vitamin A in the blood, but not in the wall of the small intestine or in the liver.

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In recent years attention has been drawn to metabolites of vitamin A discovered in mammals. These compounds are biologically active and can support growth of young rats kept on a diet deficient in vitamin A [10-12].

In previous investigations the presence of a compound which exhibited vitamin A activity in experiments on rats with avitaminosis A was demonstrated in the blood of man, monkeys, and various other animals in the summer. This compound, which was isolated and named metabolite A, has an absorption maximum at a wavelength of 400 mμ, is not fluorescent in ultraviolet light, and does not form a blue $SbCl_3$-chromogen in the Carr-Price reaction [3].

The object of this investigation was to determine whether metabolite A is formed in rats receiving β-carotene-C^{14}.

EXPERIMENTAL METHOD

β-Carotene-C^{14} was synthesized by a microbiological method from $CH_3C^{14}OOH$ by means of the fungus Phycomycesbleakus lecanus Burgeff (-) by the method of Lilly and co-workers [6].

An oily solution containing from 6 to 150 μg β-carotene-C^{14} with activity of between 13,500 and 327,000 pulses/min was injected through a tube into the esophagus of rats which had been deprived of food for the previous 24 h. The 4 rats of one group received one injection of labeled carotene, and the animals were sacrificed 4, 6, and 8 h later. The 4 rats of the other group received three doses of labeled carotene: the second dose after an interval of 8 h and the third after an interval of 12 h. The rats were all sacrificed 7 h after the last dose of β-carotene-C^{14}. The experiments were carried out in August.

Metabolite A, vitamin A, and β-carotene were determined in whole blood [1], in 5 g of mucous membrane of the small intestine, and in 1 g of liver tissue [2]. Petroleum ether extracts obtained from the non-

TABLE 1. Radioactivity of Metabolite A Isolated from Blood of Rats after Oral Administration of a Single Dose of β-Carotene-C^{14}

Rat. No.	Dose of β-carotene given (μg)	Radioactivity (pulses/min)	Volume of blood (in ml)	Radioactivity of metabolite A (pulses/min)		
				after 4 h	after 6 h	after 8 h
5	10.8	25 400	10	–	–	12.0
6	48.3	118 900	5	11.0	–	–
7	48	108 900	7	11.0	–	–
8	150	327 000	5.5	–	10.0	–

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TABLE 2. Radioactivity of Metabolite A, Vitamin A, and β-Carotene in Tissues of Rats after Oral Administration of 3 Doses of β-Carotene-C^{14}

Rat No.	Dose of β-carotene given (μg)	Radioactivity (pulses/min)	Volume of blood (in ml)	Radioactivity (pulses/min) after 7 h								
				metabolite A			vitamin A			β-carotene		
				blood	intestine	liver	blood	intestine	liver	blood	intestine	liver
5	6.0	13,500	5.0	42.0	Abs	Abs	0	29.0	23.0	Abs	48.0	13.0
6	6.0	13,500	5.0	32.0	"	"	Abs	23.0	16.0	"	43.0	14.0
7	6.0	13,500	6.0	61.0	"	"	10.0	30.0	13.0	"	35.0	15.0
8	6.0	13,500	11.5	99.0	"	"	33.0	26.0	21.0	15.0	50.0	10.0

Note. Abs denotes substance absent on chromatogram; 0 indicates no radioactivity present in the strain.

saponifiable fraction [2] were subjected to thin-layer chromatography on silica (solvent system: isopropanol–petroleum ether–acetone in the ratio of 5:80:15). Metabolite A (Rf 0), vitamin A (Rf 0.45), and β-carotene (Rf 0.95) were eluted with a mixture of isopropanol and petroleum ether in the ratio of 1:3. The resulting eluates were evaporated in a current of nitrogen to a volume of 0.1 ml, transferred quantitatively to a target, and dried, after which their radioactivity was determined on a B-2 apparatus using a BTsA-25-T end-type counter encased in lead. The activity of each sample was measured 10 times for 4 min each, and this was alternated with 10 determinations of the background activity.

EXPERIMENTAL RESULTS

The results given in Table 1 show that 4, 6, and 8 h after a single administration of various doses of labeled β-carotene (from 10.8 to 150 μg with activity increasing from 25,400 to 327,000 pulse/min) the radioactivity of metabolite A in the blood was 10-12 pulses/min, indistinguishable from the background.

Because of the absence of an increase in the radioactivity of metabolite A with an increase in the dose of radioactivity administered with β-carotene, in the next experiments three doses of β-carotene were given, each of 6 μg with activity of 13,500 pulses/min. This ensured continuous absorption of β-carotene-C^{14} over a period of 35 h. In this series of experiments the radioactivity was also counted in stains corresponding to vitamin A and β-carotene on chromatograms of blood and also of homogenates of the small intestine and liver.

During thin-layer chromatography of extracts of whole blood, stains were found with an Rf value of 0, corresponding to the location of metabolite A. Their radioactivity exceeded the background by a statistically significant degree ($P < 0.05$), i.e., from 32 to 99 pulses/min (Table 2).

The blood chromatogram of rat No. 6 had no stain corresponding to vitamin A. Vitamin A (Rf 0.45) was detected chromatographically in the blood of rat No. 5 but radioactivity was absent from it. The β-carotene stain (Rf 0.95) was observed only in material from one rat (No. 8). Investigation of the small intestine and liver demonstrated the absence of metabolite A on the chromatogram, although stains corresponding to vitamin A and β-carotene were present.

Conversion of β-carotene into vitamin A takes place mainly in the wall of the small intestine [8], although in the blood also small quantities of it can be hydrolyzed by carotenase to vitamin A [7]. When β-carotene is given by mouth, the concentration both of it and of vitamin A in the blood reaches a maximum after 7-19 h, and in the liver after 12-24 h [5, 9]. In the case of intraduodenal administration of β-carotene, vitamin A was formed in the small intestine after 1 h [8].

In the present experiments administration of β-carotene-C^{14} led to the appearance of radioactive vitamin A and β-carotene in the tissues. At the same time, radioactive metabolite A was found in the blood. On this basis it can be concluded that metabolite A is a product formed from β-carotene or vitamin A at some stage of metabolism. The fact should be noted that whereas radioactive metabolite A was found in the blood, none was found in the small intestine and liver. Probably metabolite A is formed in the blood or certain other tissues, but the question of whether it is the precursor of vitamin A or a product of its subsequent metabolism is not yet clear. The absence of radioactive metabolite A in the blood in the period up

to 8 h after administration of label and its appearance at much later periods than has been shown for β-carotene and vitamin A (7-19 h) are in favor of the second hypothesis.

Vitamin A was not found in the blood of two rats. The absence of a maximum of absorption at wavelength 328 mμ during spectroscopic investigation of petroleum ether extracts of whole blood from man and various animals has been observed previously [4]. In such cases no blue color developed in the Carr-Price reaction, yet metabolite A was constantly found. As yet no explanation for this fact can be suggested. However, the results described in this paper indicate that β-carotene, administered to rats, is converted not only into vitamin A, but also into its metabolite.

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