Metabolism of C^{14} Labelled Beta-Carotene in the Rat.* (23285)

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Our previous studies on the metabolism of C^{14} labelled beta-carotene have indicated that in the rat it is largely converted to a non-saponifiable substance or substances(1). At that time it was not possible to determine if all radioactivity of the non-saponifiable material resided in vit. A because no method was available for the isolation and purification of vit. A from this fraction. Since then, a method has been developed for isolation and purification of vit. A from the non-saponifiable extracts of liver tissue(2). With this technic it appeared worthwhile to continue our investigation on the metabolism of beta-carotene.

Method. Our previous commercial source of radioactive carotene was not available so it was necessary to develop a technic to produce it. Such carotene was produced by growing *Phycomyces blakesleeanus* in a suitable C^{14} labelled acetate medium.† Two samples of labelled C^{14} carotene were prepared. Sample No. 1 contained 1010 μg of beta-carotene with an $E^{1\%}_{1\ cm}$ of 2430‡ and a total radioactivity of 65,000 cpm. Sample No. 2 contained 1120 μg of beta-carotene with an $E^{1\%}_{1\ cm}$ of 2470 and a total radioactivity of 35,000 cpm. Each sample was prepared and fed to rats numbered 1 and 2 respectively, as previously described(1). Immediately after feeding, rats were placed in a metabolism chamber without food and water. The CO_2 was collected according to procedure of Skipper *et al.*(3). Both rats were sacrificed 24 hours after feeding the carotene. The non-saponifiable material was extracted from the liver and from the total extrahepatic tissue according to the method of Hjarde(4). The tissue extracts were freed from carotene by chromatographic procedures of Glover *et al.*(5). The carotene-free non-saponifiable extracts are referred to as CFNS fractions. After non-saponifiable material was extracted, the fatty acids were removed from the saponified extracts by acidifying them with 2N HCl, extracting with petroleum ether, and washing the latter extracts with distilled water. These washings were combined with the remainder of the saponified extracts and labelled "water soluble" fraction. The digitonin-precipitable sterols were isolated from the CFNS fraction according to the procedure of Kelsey(6). Vit. A was isolated from liver CFNS material by the procedure of Powell *et al.*(2). The water soluble fraction was oxidized and the CO_2 collected as described by Skipper *et al.* (3). Radioactivity of the various fractions was measured in a Tracerlab windowless gas flow counter and corrected for background. All CO_2 collected was counted in the form of $BaCO_3$ and corrected for self absorption. Vit. A content of various CFNS fractions was determined according to technic of Glover *et al.*(7). Due to technical difficulties, it was not possible to purify and count the vit. A in total extrahepatic tissues. Extinction of petroleum ether solutions was measured at 450 mμ and, accepting the $E^{1\%}_{1\ cm}$ for beta-carotene as 2500, the amount of carotene was calculated.

Results. From Table I it may be noted that the material in feces, urine, and intestinal contents contained approximately 50% of the total radioactive carbon fed. The total non-saponifiable material from these sources amounted to an average of 30% of the dose fed. Therefore, about 70% of the carotene was assumed to be absorbed. The carotene content of the non-saponifiable fraction from feces of rat No. 1 was 340 μg and from rat No. 2 was 360 μg. The fatty acids from feces, urine, and intestinal contents accounted for about 20% of the dose fed.

Table I summarizes the distribution of C^{14} from the labelled carotene in the tissue and

* This work was supported in part by Atomic Energy Commission contract.

† Unpublished.

‡ These "E values" were measured in a light petroleum ether (b.p. 30-40°C) solution at 450 mμ.

TABLE I. Distribution of C^{14} in Rats after Oral Administration of C^{14} Labelled β-Carotene.

	Total c.p.m. in sample		% of dose absorbed*	
	Rat 1	Rat 2	Rat 1	Rat 2
Fed	65,000	35,000		
Feces, urine, and intestinal contents	35,000	16,000		
Non-saponifiable	21,000	10,000		
Fatty acids	14,000	6,000		
H_2O soluble	—	—		
Total extrahepatic tissue	13,200	10,850	44.0	57.1
CFNS	9,700	7,050	32.3	37.1
Fatty	3,500	3,800	11.7	20.0
H_2O soluble	—	—	—	—
Total liver	1,425	872	4.8	4.6
CFNS	765	599	2.6	3.1
Vit. A	425	310	1.4	1.6
Sterols	60	39	.2	.2
Rest	280	250	.9	1.3
Fatty acids	510	173	1.7	.9
H_2O soluble	150	100	.5	.5
Expired CO_2	1,420	1,020	4.7	5.4
Recovered	51,045	28,742		
Non-saponifiable	31,465	17,649		
Fatty acids	18,010	9,973		
H_2O soluble	150	100		
CO_2	1,420	1,020		
Unaccounted for	13,955	6,258		

* Absorbed dose = Administered dose − (amt in feces, urine, and intestinal contents).

fractions examined. In both rats the highest amount of radioactivity resided in the CFNS fraction of extrahepatic tissue. Some activity was present also in the fatty acid fractions. The only "water soluble' fraction found to contain radioactivity was from the liver. About 1.5% of the absorbed C^{14} was found in liver vit. A. The combined activity in the CFNS and fatty acid fraction from extrahepatic and liver tissue accounted for an average of about 55% of the absorbed C^{14}. Approximately 5% of the absorbed C^{14} was recovered in the expired CO_2. Thus, about 60% of the absorbed C^{14} was accounted for. The average recovery for the total dose fed was about 80%.

The distribution of C^{14} in the 3 different fractions of the CFNS material from liver is such that vit. A composes 50% of the total activity of the fraction, the digitonin-precipitable sterols are only slightly active, the remaining non-saponifiable fraction is about as active as the vit. A fraction. The 2 latter fractions were examined and found to contain no vit. A according to accepted colorimetric or spectrophotometric technics.

Each rat liver contained approximately 3000 μg of vit. A, and the extrahepatic tissue approximately 150 μg of the vitamin.

Discussion. It is evident that C^{14} from rats fed labelled beta-carotene enters into compounds other than vit. A. These findings support the suggestion of Glover and Redfearn (7) and others(8) that carotene is not entirely converted into vit. A and that it might be directed into other metabolic pathways.

It is interesting to note that considerable radioactivity existed in the fatty acid fraction from feces, urine, and intestinal contents, but no detectable activity was found in the water-soluble fraction from this material. Also, it is of interest that of all the animal tissue, the only water soluble fraction that contained radioactivity was from liver tissue.

The presence of only about 5% of the absorbed C^{14} in expired CO_2 is much lower than Laughland(9) reported for the chick and rat. Failure to account for a large amount of C^{14} from absorbed carotene is in disagreement with our earlier findings where almost 100% of absorbed C^{14} was accounted for in the CFNS fraction. Two explanations seem plausible. One is that the position of labelling of the C^{14} in the carotene molecule may have been different in the 2 sources of carotene. The source of carotene in our earlier investigation was from algae which was supposedly uniformly labelled, and in the present study, from fungi where the type of labelling is unknown. Secondly, it is possible that since the animals used contained larger amounts of vit. A than the previous ones, carotene was not needed to supply the animal's need for vit. A, and hence it was directed into secondary metabolic pathways. Studies seeking support for both of these suggestions are now under investigation.

Summary. Feeding of C^{14} labelled carotene resulted in the deposition of approximately 1.5% of absorbed C^{14} into liver vit. A. In the total animal an average of about 15% of the absorbed C^{14} went into fatty acids, and 40% into non-saponifiable material. An av-

erage of about 5% of the absorbed C^{14} was found in the expired CO_2. Approximately 40% of the absorbed C^{14} remains to be accounted for.

The authors wish to thank Drs. V. G. Lilly, H. L. Barnett, and Mrs. Ruthann Folmer, West Virginia University, for assistance in production of labelled beta-carotene.

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Received May 24, 1957. P.S.E.B.M., 1957, v95.

Hypoglycemic Effect of D-Ribose in Man. (23286)

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Ribose 5-phosphate occupies an important position as an intermediate in the oxidative or pentose phosphate pathway of glucose metabolism. In both plant and animal tissues ribose 5-phosphate forms fructose 6-phosphate(1) which may be readily converted to glucose. Recently Agranoff and Brady(2) have isolated an enzyme from mammalian liver capable of phosphorylating D-ribose to form ribose 5-phosphate, thus permitting ribose to enter into this pathway. The conversion of ribose to glucose has been demonstrated by Katz *et al.*(3) who found that ribose 1-C^{14} incubated with rat liver slices yielded glucose labeled in a pattern which agreed with predictions from known reactions. Intravenous infusion of known glycogenic sugars such as D-galactose(4) and D-fructose(5,6) have been shown to cause an increase in blood glucose levels in normal man. Occurrence of increases in blood glucose levels after infusion of the pentoses D-xylose and L-arabinose has been reported from this laboratory although no effect was observed after D-lyxose and D-arabinose administration(7). Bruck and Rapoport(8) have reported that D-galactose infusions cause a lowering of blood glucose levels in galactosemic individuals. Except for a single observation that infusion of D-fructose may have a glucose lowering effect (9) no sugars have been observed to cause decreases in blood glucose levels following infusion into normal man. This paper reports that following infusion of D-ribose into normal man, decreases in blood glucose levels occur despite the fact that this sugar is susceptible to conversion to glucose via known metabolic pathways.

Methods. The D-ribose used was obtained from the Pfanstiehl Co., Waukegan, Ill. By the criteria of optical rotation and paper chromatography, the sugar was authentic D-ribose. Fifteen infusions of D-ribose were performed in 5 fasting normal males, (ages 18-21 yrs.) 3 diabetics (ages 23, 31 and 53 yrs.) whose last insulin dose (NPH + crystalline) was given 24 hours prior to study and one 48 yr. old subject with liver disease proved by liver biopsy. D-ribose was autoclaved as a 7.5% solution and found sterile and pyrogen-free prior to use. In 13 studies, 10 to 20 g of D-ribose were infused intravenously over 10-25 minutes. Blood specimens were obtained from an indwelling plas-

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