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## Effect of sex on the disappearance of carotene from the alimentary tracts of rats

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After carotene has been ingested by rats, about half of it can usually be recovered in the faeces. During the course of experiments done for other purposes it was observed that the proportion of the carotene that disappeared during its passage through the alimentary tract was greater in male than in female rats, irrespective of dose. The difference was small and, within any one experiment, not usually significant, but a significant difference emerged when the results of the several experiments were pooled. This paper presents the experimental evidence for that finding.

The terms 'absorption', 'digestion' and 'disappearance' have all been used for the difference between the carotene ingested and that excreted. The last is preferred because it involves no assumptions about intestinal destruction (Booth, 1956).

### EXPERIMENTAL

Separately caged piebald rats were given a basal diet devoid of vitamin A and carotenoids, but supplemented in certain experiments by a standard amount of vitamin A. Material containing a known amount of carotene was offered as a single dose. The dose given to each rat was weighed separately, and, within an experiment, the doses were closely similar†: for instance, small dishes (artist's palettes) were weighed, canned

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† The coefficient of variation of weight of one dose was usually <4%.

carrot puree was added to each with a glass tube treated with silicone and used in the manner of a pipette, and each dish was reweighed immediately. The dose was offered at 10 a.m., and the rats quickly consumed it. At 5 p.m. the same day a sheet of paper was placed below each cage, and faeces were collected until 9 a.m. 3 days later, that is from the 7th to the 71st h after dosing. Faeces were softened with water, and carotene was extracted as described below. The amount of carotene that disappeared during its passage through the alimentary tract was found for each rat from the difference between the carotene ingested and that in the faeces, and was expressed as a percentage of the former. The reliability of the technique has been discussed recently (Booth, 1956).

Full details of the methods used for determining carotene and lycopene are published elsewhere (Booth, 1957). Briefly, the vegetable materials or faeces were extracted with a cold mixture of light petroleum, acetone and quinol; acetone was washed out with water automatically, leaving the pigments in light petroleum; carotene was separated from other pigments by chromatography on a mixture of aluminium oxide and anhydrous  $\text{Na}_2\text{SO}_4$ , and was determined in a Unicam photoelectric spectrophotometer. 'Carotene' means total carotene, chiefly  $\beta$ , with some  $\alpha$ .

The experiments differed from one another in various ways, and some of them have already been reported in another form in a previous study (Booth, 1956). They were done at different times of the year, and the ages and weights of the rats differed somewhat between experiments. For instance the mean weight of males varied from 96 g in Exp. 4 to 198 g in Exp. 10, and the mean weight of females varied from 88 to 148 g in the two experiments, although the weights of rats of one sex within an experiment varied only slightly. The vitamin A status differed between experiments, and also within Exps. 5 and 6. The fat content of the diet was usually 14%, but all the rats in Exp. 7, and half the rats in Exp. 9, were given a diet containing 17% fat, whereas the rats in Exp. 8, and half the rats in Exp. 9, were given a diet containing only 5% fat. Arachis oil was the fat supplied. Casein was the principal protein, and it usually made up 18.5% of the basal diet, but in Exp. 10 the percentage was raised to 22 and in Exp. 11 it was lowered to 10.

#### RESULTS

Table 1 shows the summarized results of twelve experiments. A sex difference in percentage disappearance of carotene was observed in the first two experiments. Subsequently, therefore, experiments were so designed as to make it easier to assess statistically the effect of sex differences. In particular the level of the dose of carotene was virtually constant for all the rats within an experiment.

*Interaction between sex and other factors.* Although nutritional status affects the percentage disappearance of carotene (Booth, 1956), analysis of variance showed no significant interaction between sex and vitamin A status, or between sex and fat content of the diet; again in Exps. 1 and 2 there was no evidence that the size of the dose of carotene had any effect on the observed sex difference. Within Exps. 5, 6 and 9 there were differences in experimental conditions. The effects, if any, of these differences were therefore removed from that of the sex difference by appropriate computation

methods. In Exp. 11 the greater disappearance in females than in males suggests an interaction between sex and protein supply, but the difference was not statistically significant. Moreover in Exp. 10 the high-protein diet did not produce a correspondingly high disappearance in males.

Table 1. *Mean percentage disappearance of carotene from the alimentary tracts of male and female rats*

Exp. no.	Source of carotene	Carotene ingested ( $\mu\text{g}$ )	No. of rats		Percentage of carotene in test dose that disappeared*			Nutritional status†	Remarks
			M.	F.	Male rats	Female rats	Difference		
1	Carrot	150-360	15	22	55.5	51.5	4.0	d	Dose varied
2	Carrot, cooked	54-1100	5	5	44.8	41.8	3.0	d	Graded doses
3	Clover, green leaves	120	8	8	61.1 $\pm$ 2.29	59.5 $\pm$ 1.86	1.6	d	
4	Oil solution	178	10	12	74.2 $\pm$ 1.41	72.0 $\pm$ 1.36	2.2	d	
5	Carrot puree	114	12	12	54.2 $\pm$ 1.83	51.6 $\pm$ 2.27	2.6	dr	
6	Carrot puree	115	8	24	53.6 $\pm$ 1.17	52.4 $\pm$ 1.32	1.2	dr	
7	Carrot puree	140	4	8	40.8 $\pm$ 2.44	37.5 $\pm$ 3.3	3.3	n	High-fat diet
8	Carrot puree	140	4	8	34.9 $\pm$ 3.78	30.2 $\pm$ 2.66	4.7	n	Low-fat diet
9	Carrot puree	155	8	8	47.4 $\pm$ 3.83	40.6 $\pm$ 2.81	6.8	r	High- and low-fat diets
10	Carrot puree	150	6	6	26.2 $\pm$ 0.78	24.2 $\pm$ 3.05	2.0	p	High-protein diet
11	Carrot puree	150	6	6	25.0 $\pm$ 2.97	29.7 $\pm$ 3.22	-4.7	p	Low-protein diet
12	Tomato juice	115-210‡	16	16	19.8	17.2	2.6	p	Lycopene
	Total		102	135	Mean difference		2.44 $\pm$ 0.78		

M. = male; F. = female.

\* Mean values with their estimated standard errors. Standard errors are not given for experiments in which the dose varied.

† d = depleted of vitamin A; r = depleted and then repleted with about 400 i.u. vitamin A in halibut-liver oil 4 days before the test; dr = half the rats depleted and half repleted; p = depleted and then partly repleted with 150  $\mu\text{g}$  carotene a week before the test; n = maintained on normal stock diet.

‡ Lycopene.

There were considerable differences in mean disappearances between experiments. For instance, in Exp. 4 the percentage disappearance of carotene given in oil was over 70, whereas the percentage disappearance of lycopene in Exp. 12 was under 20. The present concern is not with the reasons for the difference between experiments, but with the evidence that the sex difference persists through a wide variety of experimental conditions.

*Significance of the difference between sexes.* Although the experiments differed in detail, it is considered valid (Fisher, 1950) to combine them. Within Exps. 1, 2 and 12 the dose varied, but there is no evidence that percentage disappearance was affected by size of dose within the ranges used. Therefore the difference in each of the experiments may be used for computing the mean. Except in Exp. 11, more carotene disappeared in male rats than in female. The arithmetical mean of the twelve differences in percentage disappearance was 2.44; and the standard error of the mean difference, estimated directly from the twelve differences, was 0.78. The mean difference of 2.44 differed significantly from zero ( $P < 0.01$ ).

The mean difference calculated by weighting according to the number of rats used was much the same, namely 2.57. The use of the term  $mf/(m+f)$  to give relatively less weight to experiments with unequal numbers of males ( $m$ ) and females ( $f$ ) gave a weighted mean difference of  $2.62 \pm 0.67$ .

## DISCUSSION

*Detection of small differences.* Within the first two experiments the dose of carotene varied, and in the last experiment a varying dose of lycopene was given. Within each of the other nine experiments each result could be treated as a replicate, and these replicates have been used to estimate the residual variation (or 'experimental error') which, based on 130 degrees of freedom, was 6.5. This value represents the standard deviation of a single observation with one rat in the units used throughout this paper, namely percentage disappearance of the carotene ingested. Few authors give a measure of the precision in their experiments on the 'absorption' of carotene, but where sufficient data are presented for its calculation, the standard deviation of one observation is much greater than 6.5. The lack of precision in other experiments explains why the sex difference has not been established earlier. The greater precision in the work recorded here is mainly due to better control of dose level, more complete extraction of carotene from the faeces, and elimination of 'blank' corrections as previously discussed (Booth, 1956). Arnrich & Morgan (1954) observed a greater intestinal 'removal' of carotene in females than in males in one experiment, but they made no detailed investigation.

*The possible effect of body-weight.* Males are heavier than females, and it is conceivable that the disappearance is greater in large than in small animals, but such a difference has not been observed. Experiments that would test the effect of body-weight independently of age or other factors have not been attempted or designed.

*Physiological significance of the difference.* The small difference in percentage disappearance between the sexes in itself has no great importance. Its interest lies in its connexion with another, but opposite, effect. Of a given large dose of carotene, the amounts stored as vitamin A in the livers of female rats were greater than those in males (Booth, 1950, 1952). A possible explanation considered at that time was that females absorb more than males. Since they apparently absorb less, the difference in absorption offers no explanation, but even masks some of the real difference in storage. It is true that Moore & Sharman (1950) and Moore, Sharman & Ward (1951) observed a greater storage of vitamin A in the kidneys of males than of females, but the difference was not seen immediately after a single dose of vitamin A (Booth, 1952); moreover, the slow increase in the kidneys was accompanied by depletion of the liver.

## SUMMARY

1. Some of the experiments made previously (Booth, 1956) have been extended and the results have been used to study the effect of sex on the 'absorption' of carotene.
2. Rats of both sexes subsisting on a carotene-free basal diet were given a single dose of carotene. The amount of carotene excreted in the faeces of each rat was

determined, and the percentage of the ingested carotene that disappeared during its passage through the alimentary tract was calculated from twelve experiments with a total of 237 rats.

3. In eleven experiments the amount of carotene that disappeared was greater in males than in females; in one experiment only was the difference in the opposite sense. The mean of the twelve differences in percentage disappearance was  $2.44 \pm 0.78$ .

4. There was no evidence that such factors as the nature of the vehicle in which the carotene was given, the proportions of the dietary components, or the vitamin A status of the rats, significantly affected the differences observed between the sexes.

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## The effect of ascorbic-acid deficiency on the concentration of acid mucopolysaccharides in guinea-pig skin and cartilage

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Previous histological studies have indicated that mucopolysaccharides are affected during scurvy in guinea-pigs. The evidence about the precise effect of ascorbic-acid deficiency on the metabolism of the mucopolysaccharides is, however, somewhat conflicting. A diminished content of acid mucopolysaccharides was found by Meyer (1944) in the collagen, and by Penney & Balfour (1949) in the early stages of healing wounds, in scorbutic as compared with normal guinea-pigs. Studying healing wounds, Bunting & White (1950) noted that mucopolysaccharide material persisted longer in scorbutic than in normal animals. Bradfield & Kodicek (1951) found mucopolysaccharide to be more abundant round the 'precollagen strands' of old scorbutic wounds than of non-scorbutic wounds (see also Kodicek & Loewi, 1955). Gersh & Catchpole (1949) concluded that acid mucopolysaccharides were depolymerized in the healing wounds of scorbutic guinea-pigs.

In studies with  $^{35}\text{S}$ , Friberg & Ringertz (1954) found that the uptake of radioactive sulphate by guinea-pig tissues was reduced during scurvy. They suggested that it was