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## EXPLANATION OF PLATE

Pl. 1. London's cannula, protective Perspex dome and collecting bottle.

## A Demonstration of the Conversion of Carotene into Vitamin A in Conscious Rats

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There is now considerable evidence that in animals carotene is converted into vitamin A in either the lumen or the wall of the intestine, most probably in the latter. This has been proved in many ways by experiments both in vivo and in vitro on a variety of animals (Glover, Goodwin & Morton, 1947, 1948; Goodwin & Gregory, 1948; Wiese, Mehl & Deuel, 1947; Mattson, Mehl & Deuel, 1947; Thompson, Ganguly & Kon, 1947, 1949; Mattson, 1948; Krause & Pierce, 1948; Thompson, Coates & Kon, 1950; Lane, 1950). In particular, Goodwin & Gregory (1948) showed that carotene, introduced into the intestine of conscious goats provided with an abomasal or duodenal fistula and a thoracic duct fistula, gave rise to increased amounts of vitamin A in the lymph but no carotene.

Although the conversion in the rat's intestine has been well established, it has not been confirmed in conscious animals by the method applied to goats and since, primarily for another purpose, the intestinal lymphatics of a large number of rats were being cannulated, the opportunity was taken of using some of the animals to follow the changes in the vitamin A content of the lymph after oral administration of  $\beta$ -carotene. These experiments were in progress when Coates, Thompson & Kon (1950) gave a preliminary report of the results of their extensive investigation carried out along similar lines. Our own was then discontinued, but the results obtained are briefly reported here because they completely confirm those of the Reading investigators, which are published in full in the paper just preceding this one (Thompson, Braude, Coates, Cowie, Ganguly & Kon, 1950).

## EXPERIMENTAL

*Preparation of rats.* The intestinal lymphatic vessel was cannulated with fine polythene tubing in an aseptic operation under ether anaesthesia by the technique devised by Bollman, Cain, Grindlay & van Hook (1948). In their original description

of the operation Bollman *et al.* show the superior mesenteric artery as lying close beside the hepatic lymphatic; it does in fact lie beside the main intestinal lymphatic, and in rats weighing less than 250 g. it may be almost impossible to cannulate the lymphatic vessel satisfactorily; either the artery is damaged or the lymphatic vessel is torn, which leads to subsequent leakage of the lymph. In larger rats the artery lies more to one side of the lymphatic vessel and the plastic tube can readily be introduced and tied in position with a fine ligature without damaging the artery.

After recovering from the anaesthetic the rats were placed in a cage of the type described by Bollman & van Hook (1948) and maintained in a constant-temperature cabinet at 25° during the experiment. It was found desirable to leave about 25 cm. of plastic tubing outside the animal so that when it was filled with lymph, slight suction was exerted upon the lymphatic vessels. Once flow began it usually continued without clotting for at least 1-2 days. The report (Mann, Mann & Bollman, 1949) that haemorrhages occur in cannulated rats when lymph drainage continues for more than about 24 hr., and that the haemorrhagic tendency can be prevented by administration of vitamin K, was confirmed.

After the operation the rats, which had previously been maintained on rat cubes supplied by Lever Bros. and Unilever Ltd., were kept without food for 24 hr. and then dosed by stomach tube with 0.5 ml. of refined arachis oil in which had been dissolved a known amount of crystalline carotene (a mixture of about 90%  $\beta$ - and 10%  $\alpha$ -carotene); in different experiments the amount varied from 100 to 120  $\mu\text{g.}/\text{ml.}$  arachis oil. In control experiments the rats were dosed only with arachis oil. The lymph, collected for 18-22 hr. before dosing and for 6-8 hr. afterwards, was examined for vitamin A by the antimony-trichloride test in conjunction with a Beckman spectrophotometer, according to the method previously described (Goodwin & Gregory, 1948).

## RESULTS

The results (Table 1) clearly indicate that after  $\beta$ -carotene dissolved in arachis oil had been given, there was a marked rise in the concentration of vitamin A in the

Table 1. *The effect of carotene administered orally on the vitamin A content of the intestinal lymph of conscious rats*

Dose	Lymph					
	Before administration of dose			After administration of dose		
	Period of collection (hr.)	Volume collected (ml.)	Vitamin A (i.u./100 ml.)	Period of collection (hr.)	Volume collected (ml.)	Vitamin A (i.u./100 ml.)
Arachis oil only, 0.5 ml.	18	9.6	42	6	5.5	44
	23	12.4	26	6	8.9	29
$\beta$ -carotene, 50-60 $\mu\text{g.}$ in 0.5 ml. arachis oil	22	9.6	26	5	7.5	68
	22	12.9	43	6.25	5.1	51
	22	2.8	87	8	3.6	121
	19	6.9	59	8	8.4	76
	22	5.3	34	7	5.3	51
	22	9.7	40	7	5.7	132
	18	9.2	53	7	8.2	60

intestinal lymph. No such rise was observed when arachis oil alone was given. Further, it is important to note that in no experiment did carotene appear in the lymph in detectable quantities.

## SUMMARY

1. The oral administration of carotene to conscious rats with the intestinal lymphatic vessel cannulated resulted in a marked increase in the vitamin A content of the lymph, clearly indicating the conversion of carotene into vitamin A in either the intestine or intestinal wall.

2. No carotene was ever observed in the lymph during these experiments.

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