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The Transport of Carotenoids, Vitamin A and Cholesterol Across the Intestines of Rats and Chickens

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(*Received* 16 *June* 1958)

During his classical studies on the isolation and characterization of carotenoids from the tissues of animals, Zechmeister (1937) noticed that different species of animals are able to store different carotenoids selectively. The animals were broadly classified into four groups according to the nature of the carotenoids in their tissues: thus cows have mostly β-carotene, birds mostly hydroxy carotenoids, man and frogs all carotenoids, while most mammals have none. This has subsequently been confirmed by Goodwin (1950) and by Ganguly, Mehl & Deuel (1953). Ganguly, Krinsky, Mehl & Deuel (1952) and Ganguly *et al.* (1953) have postulated that the presence or absence in the tissues of animals of receptor proteins capable of specifically combining with the carotenoid concerned might be the reason for the wide variation in the distribution of carotenoids as related to species. Although Zechmeister & Tuzson (1934, 1935) and Ganguly *et al.* (1953) were able to conclude from the available indirect evidence that this is due to selective absorption in the intestine itself, very little direct evidence was available. Moreover, the exact mechanism and the exact site of selection in the intestine remained obscure.

The selectivity in the absorption of lipids is not confined to carotenoids alone. It has long been known that a similar situation is encountered in the absorption of sterols, since animals do not absorb plant sterols efficiently. Recently Glover & Green (1957) have suggested the possible presence in the intestinal cells of lipoproteins capable of combining stereo-specifically with the particular sterol to facilitate its absorption. By the use of techniques similar to those of Glover & Green (1957) it is demonstrated in the present communication that highly specialized mechanisms exist in the mucosal cells of animal intestines for selective admission of the carotenoids into their tissues.

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MATERIALS AND METHODS

Light petroleum (b.p. 40–60°; Burmah-Shell) was allowed to stand over an aqueous solution of potassium permanganate for a few days, washed with water, dried over calcium chloride and double-distilled. A.R. acetone was obtained from British Drug Houses Ltd. and A.R. chloroform from Merck. Ethanol was kept over solid potassium hydroxide for 7 days and double-distilled. Antimony trichloride was obtained from Thomas Tyrer Co. Ltd., London; alumina from Merck; Celite 535 from the Johns-Manville Co. Ltd., London; magnesium oxide from British Drug Houses Ltd.; synthetic vitamin A from Hoffman-La-Roche, Basle; and crystalline cholesterol from British Drug Houses Ltd.

β-Carotene. Fresh carrots were minced and saponified at room temperature in brown bottles and exhaustively extracted with light petroleum. The concentrated extracts

were passed twice through alumina columns, from which the β-carotene band was eluted with 4% (v/v) acetone in light petroleum. The solution was finally chromatographed through a magnesium oxide–Celite (1:1) column with 4% (v/v) acetone in light petroleum. The main β-carotene band was eluted from the extruded column with ethanol–light petroleum (1:1) and was finally dissolved in light petroleum. It showed the typical β-carotene spectrum in the same solvent (Fig. 1*a*) and was not purified any further.

Lycopene. This was similarly extracted from saponified tomato pulp and purified by the same procedure. No further purification was attempted and the product gave the typical lycopene spectrum (Fig. 1*b*).

Lutein. This was extracted from saponified fresh maize leaves and was purified as described by Ganguly *et al.* (1952).

All manipulations were carried out in subdued light. The carotenoids were stored in solution in light petroleum in brown bottles in a refrigerator and suitable portions were withdrawn at the time of each experiment. Given amounts of vitamin A or cholesterol and α-tocopherol were dissolved in 10 ml. of light petroleum; refined groundnut oil was then added and the light petroleum evaporated under reduced pressure, the last traces of it being removed in a stream of nitrogen. Similarly, the carotenoids were obtained in solution in fat by adding α-tocopherol and refined groundnut oil to the stock solutions of carotenoids.

Animals. Male rats of this Institute's strain (usually 4–6 months old) were starved for 24 hr., fed with 0·5 ml. of the oily solution of the substance by means of a stomach tube and killed 60–90 min. later by heart puncture. Chickens of both sexes and of unknown breed and weighing about 0·8–1 kg. were obtained from the local market and kept for about 2 weeks on a diet poor in carotenoids, consisting of polished rice, casein and yeast, in order to free the intestines of any carotenoids that might have been present. The birds were then starved for 24 hr., dosed with 2 ml. of oily solution through a stomach tube, and killed by heart puncture 150–180 min. later. Preliminary experiments showed that at these particular intervals the lipids fed were present in the small intestines in sufficient concentrations for active absorption.

Preparation of tissue fractions

The small intestines were immediately removed and the contents washed out with cold physiological saline. The mucosal cells were then scraped off with the blunt end of a scalpel as described by Glover & Green (1957). Both the intestinal muscles (that part of the small intestine left after the removal of the mucosae) and the mucosae were collected in separate beakers previously placed in crushed ice, and weighed. The mucosal cells were then homogenized in 10 vol. of cold 0·25 M-sucrose in a Potter–Elvehjem homogenizer. The homogenate, after being filtered through flannel, was fractionated into nuclear, mitochondrial, microsomal and supernatant (cell sap) fractions according to the method of Schneider & Hogeboom (1950) in an International refrigerated centrifuge with a high-speed head attachment. In a preliminary study of the distribution of enzymes among the cell fractions in both species the bulk of the succinic dehydrogenase and alkaline phosphatase activities was located in the mitochondrial and microsomal fractions respectively. No attempt was made to separate the creamy layer, which was added to the supernatant fraction. The homogenate and the cell fractions were made up to volume with 0·25 M-sucrose. The whole procedure was carried out at low temperature as quickly as possible.

Extraction of lipids and determination of carotenoids, of free and esterified vitamin A and of free and esterified cholesterol

Suitable portions of the intestinal contents, muscles, whole homogenate and cell fractions were extracted with ethanol and light petroleum in a Waring Blendor as described by Thompson, Ganguly & Kon (1949). The complete spectra in the visible region of the carotenoids were measured in a Beckman spectrophotometer Model DU after chromatography through alumina columns, and their concentrations calculated by using $E^{1\%}_{1\,cm.}$ values of 2600, 3470 and 2520 for β-carotene, lycopene and lutein at $\lambda_{max.}$ 450–452, 470–472 and 444–446 mμ respectively in light-petroleum solution. Vitamin A ester and vitamin A alcohol

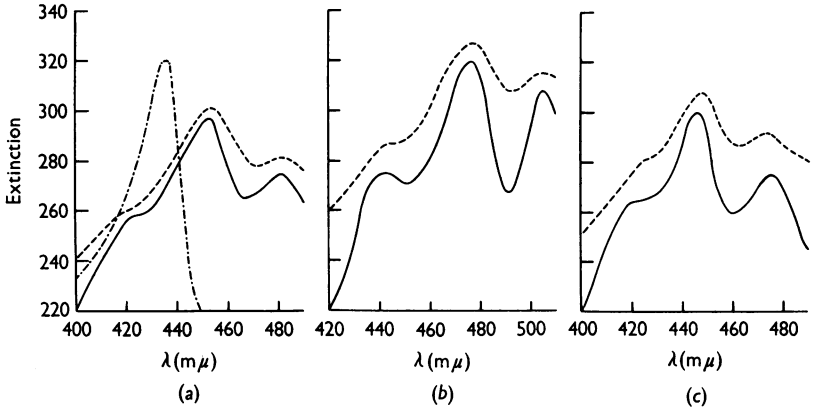

Fig. 1. Spectra of carotenoids [(*a*) β-carotene; (*b*) lycopene; (*c*) lutein] in light petroleum before and after feeding. – – – –, Carotenoids extracted from tissues after feeding; ——, original carotenoids; –·–·–, unidentified pigment in chickens after feeding β-carotene.

and free and esterified cholesterol were determined as described by Krishnamurthy, Seshadri Sastry & Ganguly (1958*b*).

Two species of animals, the rat and the chicken, were chosen. Both convert β-carotene into vitamin A in the intestine, but they differ greatly in their capacity for admitting different carotenoids into their tissues: rats reject all of them, while chickens absorb mainly hydroxy carotenoids. Three types of fat-soluble compounds, namely carotenoids (β-carotene, lycopene and lutein), vitamin A (free, acetate and palmitate) and cholesterol (free, acetate and palmitate), were used. The carotenoids were selected in such a way that one of them (β-carotene) is a hydrocarbon provitamin A, another (lycopene) a hydrocarbon with no provitamin A activity, and the third (lutein) a dihydroxy carotenoid without biological activity. The other two types of fat-soluble compounds (vitamin A and cholesterol) are absorbed by both species of animals. The animals were killed at the time of active absorption after dosing, and the mucosal cells of the small intestines were fractionated by the differential centrifuging procedure of Schneider & Hogeboom (1950). If the lipid fed follows the pathway suggested in Frazer's (1946) hypothesis one would expect to recover it from the supernatant fraction (cell sap) of the cell homogenate. Its presence in the supernatant fraction does not rule out the possibility of its having entered the cell, but if it were found in the particulate materials of the cell this would indicate that during absorption it is transported across the cell not in solution in triglycerides.

RESULTS

Absorption of vitamin A and cholesterol

Vitamin A. Irrespective of the form of vitamin A fed both the ester and the alcohol were present in the intestinal contents, mucosae and muscles of both species (Table 1). The supernatant fraction contained most of the ester, whereas the alcohol was distributed more diffusely among the fractions of the mucosal-cell homogenate. The pattern of distribution of the two forms of vitamin A in their livers also is of a similar nature (Krishnamurthy *et al.* 1958*b*; Krinsky & Ganguly, 1952).

Cholesterol. The results obtained on feeding free cholesterol, its acetate or its palmitate are presented in Table 2. Whatever form of cholesterol was fed, it was always present almost exclusively in the free form both in the mucosal cells and in the muscles, while only traces of the ester could be found. The free cholesterol was largely concentrated in the microsomal fractions of the mucosal cells of both species. The intracellular distribution of cholesterol seems to be similar whether liver cells of rats (Schotz, Rice & Alfin-Slater, 1953; Spiro & McKibbin, 1956), or of chickens (Krishnamurthy *et al.* 1958*b*), or the mucosal cells of rabbits (Glover & Green, 1957), rats or of chickens (present study)

Table 1. *Distribution of vitamin A ester and vitamin A alcohol among the muscles and contents and within the mucosal cells of the small intestines of rats and chickens*

For each experiment individual birds were dosed with 2 ml. of oily solutions containing 15 mg. of vitamin A alcohol, acetate or palmitate and 0·5 mg. of α-tocopherol. Individual rats were dosed separately with 0·5 ml. of oily solutions containing 3 mg. of vitamin A alcohol, acetate or palmitate and 0·2 mg. of α-tocopherol. Tissues from three rats were combined for each experiment. Values are expressed as μg./g. and as the mean of three separate experiments. The vitamin A values obtained in the control animals were negligible. H, Whole homogenate; N, nuclear fraction; Mt, mitochondrial fraction; Mc, microsomal fraction; S, supernatant (cell sap) fraction; tr, trace (detectable but too low for estimation).

			Form of vitamin A fed					
			Alcohol		Acetate		Palmitate	
	Fractions		Ester	Alcohol	Ester	Alcohol	Ester	Alcohol
Chicken	Mucosal cells	H	20·0	3·2	31·0	4·4	9·6	1·6
		N	1·9	0·5	2·9	0·6	1·1	tr.
		Mt	2·4	0·6	3·8	0·7	0·5	0·2
		Mc	2·8	1·0	5·4	1·2	0·5	0·5
		S	12·5	1·3	20·0	1·8	7·3	1·0
	Muscles		7·7	1·6	10·1	1·33	8·3	0·8
	Contents		275·0	2200·0	3100·0	285·0	2500·0	90·0
Rat	Mucosal cells	H	17·0	6·2	28·5	11·0	20·0	9·2
		N	1·2	0·8	2·2	0·9	1·0	0·5
		Mt	2·2	1·1	2·5	2·4	1·5	1·0
		Mc	2·4	1·4	3·6	2·6	2·0	1·5
		S	12·0	2·4	20·1	5·4	16·1	5·8
	Muscles		12·0	3·7	15·5	5·9	10·0	3·5
	Contents		630·0	1920·0	1580·0	380·0	1020·0	447·0

Table 2. *Distribution of free and esterified cholesterol among the muscles, contents and within the mucosal cells of the small intestines of rats and chickens*

Individual chickens and rats were used for each separate experiment. The chickens received 80 mg. of free cholesterol, its acetate or palmitate and 0·5 mg. of α-tocopherol in 2 ml. of oil and rats received 10 mg. of the cholesterol and 0·2 mg. of α-tocopherol in 0·5 ml. of oil. The animals not given cholesterol received the oil and tocopherol alone. Values are expressed as mg./g. and as the mean of three separate experiments. For abbreviations see Table 1.

			Form of cholesterol fed							
			None		Free		Acetate		Palmitate	
	Fractions		Free	Ester	Free	Ester	Free	Ester	Free	Ester
Chicken	Mucosal cells	H	2·60	0·15	2·61	0·08	2·60	0·12	2·50	0·14
		N	0·20	tr.	0·42	tr.	0·23	tr.	0·21	tr.
		Mt	0·50	tr.	0·57	tr.	0·51	tr.	0·52	tr.
		Mc	1·50	tr.	1·52	tr.	1·40	tr.	1·50	tr.
		S	0·25	0·13	0·11	0·08	0·45	0·12	0·25	0·13
	Muscles		2·50	0·12	2·95	0·03	2·85	tr.	2·80	0·03
	Contents		tr.	tr.	21·00	48·00	24·00	16·00	31·00	18·00
Rat	Mucosal cells	H	1·55	0·05	1·53	0·16	1·60	0·06	1·62	0·10
		N	0·18	tr.	0·20	tr.	0·10	tr.	0·10	tr.
		Mt	0·35	tr.	0·35	tr.	0·30	tr.	0·40	tr.
		Mc	0·90	tr.	0·85	tr.	0·95	tr.	0·95	tr.
		S	0·11	0·05	0·09	0·13	0·15	0·05	0·10	0·08
	Muscles		1·57	0·04	2·07	0·04	1·95	tr.	1·85	0·03
	Contents		tr.	tr.	3·23	0·37	2·00	0·79	3·01	0·82

are examined. Another striking feature of cholesterol absorption is that its concentration in the mucosal cells remains almost steady after feeding, while that in the intestinal muscles can be increased only with difficulty. Large amounts of both forms of cholesterol could be found in the intestinal contents (in rats about 3 mg. out of 10 mg. fed and in chickens about 40 mg. out of 80 mg. fed) which would prove that sufficient amounts were present to ensure active absorption. The appearance of both forms of cholesterol in the intestinal contents, irrespective of the form fed, would seem to confirm the presence of both hydrolytic and esterifying enzymes in the intestine.

Absorption of carotenoids

In Table 3 the results of administration of β-carotene, lycopene and lutein are summarized. After the feeding of β-carotene only traces were found in the muscles or mucosae of the rat. The small amounts in the mucosae were mostly in the supernatant fraction, while the particulate materials of the cells were essentially free of it. The vitamin A formed from the β-carotene was largely esterified and was distributed within the cells in the same manner as after the administration of preformed vitamin A. These data seem to agree with the observations of Greenberg (1957) that the conversion of carotene in the intestine is probably by an extracellular mechanism, because the β-carotene seems unable to penetrate the mucosal cells. The traces of it found in the supernatant fraction probably came from the lipids mechanically adhering to the intestinal wall after the contents had been washed out.

β-Carotene suffered the same fate in chickens, except that the extracts of the mucosae and of the muscles contained an unidentified pigment that stayed on an alumina column on elution of β-carotene with 4 % (v/v) acetone in light petroleum. This pigment was eluted with 8 % (v/v) ethanol in light petroleum and gave a spectrum different from that of β-carotene with $\lambda_{max.}$ 434 mμ in light petroleum (Fig. 1*a*). Assuming that its extinction values are the same as those of β-carotene in light petroleum, the average concentrations of β-carotene and of this pigment were 2·2 and 0·9 μg./g. respectively in the mucosae, with traces and 0·72 μg./g. respectively in the intestinal muscles.

The distribution of lycopene was similar to that of β-carotene, and no unusual pigment was formed from the lycopene in either species. Lutein, however, gave a completely different picture. In rats its distribution was similar to that of β-carotene or lycopene, but in chickens the lutein was present in large concentrations both in the muscles and in the mucosae. Within the mucosal cells it was concentrated in the mitochondrial and microsomal particles. The lutein of the cells and of the muscles was in the free form [as only 8 % (v/v) ethanol in light petroleum was able to elute it from an alumina column] and neither lutein nor lycopene underwent any appreciable change, since their spectra were identical with those of the original

Table 3. *Distribution of carotenoids among the muscles and contents and within the mucosal cells of the small intestines of rats and chickens*

For each experiment individual birds were dosed with 2 ml. of oily solutions containing 15 mg. of β-carotene, lycopene or lutein and 0·5 mg. of α-tocopherol. Individual rats were dosed separately with 0·5 ml. of oily solutions containing 3 mg. of β-carotene, lycopene or lutein and 0·2 mg. of α-tocopherol. Tissues from three rats were combined for each experiment. Values are expressed as μg./g. and as the mean of three separate experiments. The values for carotenoids in the control animals were nil. Reisolated β-carotene was determined from the 4% acetone fraction after chromatography through an alumina column. For abbreviations see Table 1.

			Substance fed				
			β-Carotene				
	Fractions		β-Carotene	Vitamin A ester	Vitamin A alcohol	Lycopene	Lutein
Chicken	Mucosal cells	H	2·20	1·80	0·60	0·70	20·70
		N	tr.	tr.	tr.	tr.	3·40
		Mt	tr.	tr.	tr.	tr.	5·10
		Mc	tr.	tr.	tr.	tr.	10·00
		S	2·00	1·50	0·50	tr.	3·50
	Muscles		tr.	0·70	tr.	tr.	13·30
	Contents		2400	Not estimated		5875	3483
Rat	Mucosal cells	H	1·00	2·60	0·50	tr.	2·80
		N	tr.	tr.	tr.	tr.	tr.
		Mt	tr.	tr.	tr.	tr.	0·30
		Mc	tr.	tr.	tr.	tr.	0·90
		S	1·00	2·60	0·40	tr.	1·80
	Muscles		tr.	4·00	0·50	tr.	0·90
	Contents		2200	Not estimated		2490	2900

pigments (Fig. 1*b* and *c*). In a separate experiment (not shown in Table 3), where a mixture of lycopene and lutein in equal proportions (8·0 mg. of each) was given to chickens, only lutein was taken up preferentially by the particulate materials of the mucosal cells and it was present in the intestinal muscles also. The concentration of lutein in both the mucosae and muscles of the intestine were the same as those after feeding only 15·0 mg. of lutein. On opening the small intestines of both species after treatment with the carotenoids, in chickens treated with lutein the villi could be seen, even with the naked eye, to be uniformly coloured yellow, whereas in all other cases the coloured areas were only small and scattered. These patches were probably caused by fatty materials, mechanically held and containing the carotenoids in solution.

Stability of carotenoids in isolated intestines

The experiments *in vivo* with the carotenoids described above do not rule out the possibility of preferential destruction of the carotenoids. Experiments *in vitro* were therefore planned to study the relative stability of the three carotenoids in the isolated intestines of the two species. The animals were killed after starving them for 24 hr., and the small intestines were immediately taken out and cut into 20 cm. segments. Into the segments of the rat intestine 0·5 ml. of oil containing 2·0 mg. of β-carotene, lycopene or lutein and 0·2 mg. of α-tocopherol, and into the segments of chicken intestines 1 ml. of oil containing 6·0 mg. of the particular carotenoid and 0·5 mg. of α-tocopherol, were introduced, and they were then incubated for 90 and 180 min. respectively at 37° in physiological saline. The experiment was carried out in triplicate and analysis of the carotenoids after incubation showed that the concentrations had altered only a little or not at all.

DISCUSSION

Vitamin A, cholesterol and carotenoids are all fat-soluble compounds; yet their behaviour during absorption from the intestine does not seem to be identical. Whether vitamin A alcohol, acetate or palmitate was given, it appeared in the intestinal muscles and in the mucosal cells largely as ester, and most of the ester of the cell was recovered from the supernatant fraction of the cell homogenate. This finding is in general agreement with the earlier reports of Thompson *et al.* (1949), Thompson *et al.* (1950) and of Glover, Goodwin & Morton (1948) where it has been demonstrated that vitamin A in the intestinal wall and in mesenteric lymph is mostly esterified.

The picture was quite different with cholesterol. In all the trials where no cholesterol, free cholesterol or its acetate or palmitate was fed, it was

present in the mucosal cells and in the muscles in the free form and was concentrated in the microsomal fraction of the mucosal cells. Also the absolute values of the free cholesterol could not be increased by any appreciable amounts in the tissue fractions after these treatments. Kim & Ivy (1952) believe that only free cholesterol can enter the cell, and according to Glover & Green (1957) free cholesterol is transported across the cell by a mechanism of simultaneous adsorption and desorption on lipoproteins of the cell. Both suggestions seem to be compatible and our results are in agreement with them since, whatever the form fed, cholesterol appeared mostly in the free form in the intestinal tissue fractions and its concentration could hardly be increased even after heavy dosing.

The mechanism of absorption of carotenoids appears to be different from that of vitamin A or of cholesterol. β-Carotene and lycopene were found only in traces in the mucosae and in the intestinal muscles of both species. In the rat, the concentrations of lutein were similar to those of the other two carotenoids, whereas in the chicken it appeared in marked concentrations in the muscles and in the particulate materials such as the mitochondria and the microsomes of the mucosal cells.

It might be argued that the provitamins A are converted into vitamin A during the process of absorption. This cannot, however, always be the case, because cows are known to absorb (unchanged) a provitamin A, β-carotene, in preference to other biologically inert carotenoids (Gillam & Kon, 1940). Moreover, it should follow that inert carotenoids would be absorbed with equal efficiency. But the results of this study demonstrate that, although neither lutein nor lycopene is a provitamin A, only lutein is able to penetrate the mucosal cells of chickens, while neither of them can enter the mucosal cells of the rat. The preferential absorption of lutein by chickens might be due to the presence of the hydroxyl groups offering anchorage. Rats, however, reject this pigment.

Another possibility is that animals possess efficient enzyme systems that selectively destroy the carotenoids; the recent findings of Bieri (1955), however, rule out this possibility: normally rabbit tissues do not contain any carotenoids, whereas chickens contain both hydroxycarotenoids and traces of carotenes. But once β-carotene, dispersed in water with Tween 40 (polyoxyethylene sorbitan monopalmitate), is introduced intravenously into these animals, it continues to circulate in the blood unchanged for a long time (Bieri, 1955). Our experiments *in vitro* on the stability of carotenoids in isolated intestines of rats and chickens have demonstrated also that no such mechanisms for selective destruction exist in their intestines. It would thus appear that selective absorption of one carotenoid from a mixture is not the result of preferential destruction of the others.

Ganguly *et al.* (1952, 1953) had earlier proposed that the species difference in the absorption and deposition of different carotenoids might be due to the presence of specific receptor proteins in animal tissues. More recently Glover & Green (1957) have pointed out that during absorption from the intestine the sterols are thrown out of fat solution, to be absorbed at the molecular level by stereospecific lipoproteins of the mucosal cells. The results presented here indicate that carotenoids also are similarly thrown out of fat solution during absorption. Here again, it is possible that the intestinal cells of the chicken contain stereospecific lipoproteins capable of combining specifically with lutein at the molecular level and thereby allowing its entry into the animal. More compelling evidence in favour of such a mechanism is that after oral administration of the three carotenoids to rats and chickens, the villi of chickens receiving lutein become uniformly yellow, whereas in all the other experiments they remain almost colourless.

There seems to be general agreement on the specificity of association of lipoproteins with various lipid components; as mentioned above, Glover & Green (1957) believe such a phenomenon to obtain in sterols. Krishnamurthy, Mahadevan & Ganguly (1958*a*) have discussed the specific nature of association of vitamin A ester and vitamin A alcohol with different lipoproteins in rat liver. Krinsky, Cornwell & Oncley (1958) have clearly demonstrated that in post-absorptive human blood different lipoproteins are associated with vitamin A ester, vitamin A alcohol and carotenoids, whereas the chylomicra are essentially free of these lipid compounds. They also have emphasized the highly specific nature of the association of the lipoproteins with such compounds. The findings of this study, when considered with those of the Harvard workers, strongly indicate that during absorption from the intestine the lipids under consideration are separated for transport by specific lipoproteins, whereas the triglycerides are absorbed as chylomicra.

Lutein exists in combination with proteins in chicken blood (Ganguly *et al.* 1952). In chicken liver also it is present in a similar combination with lipoproteins, because it can be fully extracted from the liver homogenate with diethyl ether only after protein denaturation (unpublished results). In the present study, the lutein was not fully extractable with diethyl ether from mucosal-cell homogenates unless the protein was denatured by ethanol (results not included in the text). The pattern of intracellular distribution of lutein in both liver and mucosae of chickens seems to be similar. It is

thus possible that a lipoprotein stereo-specific for lutein exists in dynamic equilibrium among the various tissues of chickens and helps in the transport of lutein across the mucosal cells and in its subsequent distribution among the different tissues. Similarly it is suggested that, in the cow, mainly β-carotene is absorbed, because of the presence of a stereo-specific lipoprotein that selectively combines with β-carotene, whereas in most other mammals carotenoids are not absorbed because of the absence of suitable lipoproteins.

SUMMARY

1. Chickens kept on a low-carotenoid diet and normal adult rats were starved for 24 hr., and dosed with oily solutions of vitamin A alcohol, its acetate or palmitate, free cholesterol, its acetate or palmitate, β-carotene, lycopene or lutein. The rats were killed 60–90 min. later and the chickens after 150–180 min. The intestinal contents were washed out and, after being separated from the intestinal muscles, the mucosal cells were homogenized and fractionated into nuclear, mitochondrial, microsomal and supernatant (cell sap) fractions.

2. In all trials with vitamin A the latter was present mostly as ester in both the intestinal muscles and in the mucosal cells, and this ester could be recovered almost completely from the supernatant fraction of the cell homogenate.

3. It was immaterial whether no cholesterol, free cholesterol, its acetate or palmitate was fed; cholesterol was present predominantly as the free compound both in the muscles and in the mucosal cells, and the microsomal fraction of the mucosal cells contained most of the free cholesterol. The concentration of free cholesterol in the microsomal fraction could not be changed, whereas that in the muscles increased slightly after heavy dosing.

4. Except for lutein in chickens, all carotenoids were found only in traces in both intestinal muscles and in mucosae. In chickens lutein was found in high concentration in the intestinal muscles and in the mucosal cells; in the latter it was concentrated in subcellular particles such as mitochondria and microsomes.

5. The role of stereospecific binding by lipoproteins in the absorption, transport and storage of carotenoids is discussed with special reference to species differences in the selective absorption of different carotenoids.

This work was supported by a research grant from the Indian Council of Medical Research and the instruments used were gifts from the Rockefeller Foundation. We are grateful to Voltas Ltd., Bombay, and Hoffman–La-Roche, Basle, for a gift of synthetic vitamin A used in these experiments.

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