Reprinted from
GHANA MEDICAL JOURNAL
Vol. 11 *No.* 1 *pp.* 71—82, *March* 1972

RETINOL IN CHOLESTEROL BIOSYNTHESIS

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Introduction

Relationship between vitamin A and cholesterol has been reported [1–4]. Wiss *et al.* [5] observed a reduction in cholesterol synthesis during vitamin A deficiency. Kinley and Krause[6] noted a decrease in elevated serum cholesterol of atherosclerotic human patients following oral adiminstration of high levels of vitamin A. The same was also reported in chickens and in rats[7–9]. Misra[10] observed an increase in tissue cholesterol with intramuscular administration of high doses of vitamin A.

Experimental

The experiment described below was originally designed to investigate the effects of carotenaemia (produced by consuming palm oil) on various tissues of the chick**. The object of the study was to investigate long term offects, if any, of high intake of carotenoids in oil (palm oil) or its equivalent in preformed vitamin A in oil. The present paper reports part of the findings of the original study.

Animals and Diet. Chickens and rats were used for the experiment. These were divided into three groups. Protein and caloric values of the diets fed each group of animals were identical, as also was the proportion of oil in each diet, but they differred in the composition of the oil.

Fifty day old male chickens obtaining from Sterling Poultry Products Limited, Grt North Rd.,Welwyn, Garden City, Berts, were housed under a brooder and were fed diet 41B[11]. At twenty one days of age, thirty-six healthy chickens weighing between 162 and 212gm. were chosen and distributed into three groups of twelve chickens. The average weight was approximately the same for each group. The chickens were housed by groups in raised wire cages and were fed the experimental diets. They were given free access to water. The remaining chickens were housed together as a fourth group and were kept on the basic diet.

In making the experimental diets, oil was added to diet 41B. Since the extra oil diluted the vitamin constituents of this diet, extra vitamins based on the animal's requirements[12] were added to give a basic diet. The vitamins added per Kg. of diet were as follows:

Vitamin A . . .	1000 Units
Cyanocobalamin .	40 μg
Nicotinic acid . .	20 mg
Vitamin K . . .	0.82 mg
Pteroylmonoglutamic acid	2.0 mg
Pantothemic acid .	30 mg
Cholecalciferol . .	400 Units
Vitamin E . . .	0.14 mg.
Pyridoxal . . .	8.0 mg
Choline Chloride .	1,2000 mg
Biotin	0.4 mg.

The experimental diets fed to the chickens were prepared as follows: Diet I was made by mixing 150 gm of arachis oil with 850 gm of the basic diet to make 1kg diet. The vitamin A content was approximately 5000 IU per Kg. diet.

A precisely weighed quantity of retinol palmitate (commercially made retinol palmitate in arachis oil, concentration 50,000 IU per gm), equivalent to the vitamin A value*** of the palm oil used in Diet III was diluted to 150gm with arachis oil. This was mixed with 850 gm of the basic diet to give Diet II. The vitamin A amounts added to Diet II ranged from 157,000 to 273,000 IU per Kg. diet.

Diet III was prepared by mixing 150 gms of red palm oil with 850 gms of the basic diet to make one Kg diet. (Red palm oil is obtained from the fruit of the palmtree Elacis Guineensis. The carotenoid content**** of the

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**This study was done with a World Health Organisation Fellowship.

***Vitamin A value was obtained by dividing the carotenoid values by 0.6/W.H.O., 1950 Expert Committee on Biological Standardization. W.H.O. Tech. Rep. Series No. 3:4.

****Total carotenoid content of the palm oils were determined by the saponification and extraction method recommended by Moore[13].

samples of red palm oils used to make diet III varied from 706 to 1091 μg/gm). The total carotenoids added ranged from 94,000 to 164,000 μg/Kg.

Daily records were kept of the food intake of each group and the chickens were weighed twice a week, except for the last five weeks of the experiment when the remaining chickens were weighed weekly. Up to eight weeks of age enough heating was provided in the room to keep the temperature of the environment above 60° (64–94°F). At eight weeks of age, the groups were transferred into separate sections of rooms with saw dust on the floor and lights were left in the room all the time. The chickens were allowed enough space for free movement.

After 60 and 120 days on the experimental diets, four chickens were randomly chosen from each group because of earlier deaths, some groups killed at a particular age consisted of only three chickens. The animals to be killed were starved for 18 to 24 hours, anaesthetized under chloroform and killed by decapitation. The remaining chickens were kept on the experimental diets for a total of 170 days when they were also killed. A fourth group of three chickens fed on the basic diet with no additional oil, was also killed at this time.

Eighteen male hooded weanling rats were kept on diet 41B for seven days. On the eighth day, the rats weighing 52–79 gms. were distributed into three groups of six rats each. The average weight was approximately the same for each group. The rats were housed in raised wire cages and were put on the experimental diets I, II or III, the same diets as prepared for the chickens except that 200 gms. of the various oils were mixed with 800 gms of the basic diet. Water was provided all the time. Vitamin A contents of the rat experimental diets were 5000 IU/Kg for Diet I, Diet II had additional 236,000 to 364,000 IU pre-formed vitamin A per Kg and from 141,000 to 218,000 μg total carotenoids were added per Kg diet in the form of palm oil to Diet III. Daily food intake was recorded per group and the rats weighed twice a week. After 60 days on the experimental diets, three rats randomly chosen from each group were killed. The remaining rats were killed after a total of 130 days on the experimental diets.

Collection and Treatment of Samples. Freely flowing blood samples were collected from all animals into heparinized tubes, before they were killed. Livers and kidneys were immediately removed, cleaned of all extraneous fatty tissue, rinsed in chilled distilled water, blotted dry and weighed. Plasma, liver and kidney samples were immediately frozen at −20°C till needed for the determination of total and free cholesterol, total ester and alcohol vitamin A.

Plasma total and free cholesterol were determined using the method by Searcy and Bergquist[14]. Plasma total vitamin A was analysed by the method discribed in the "Methods of Biochemical Analysis"[15]. Vitamin A ester and alcohol separation and determination were done by the method of Eden [16]. Tissue lipids were extracted by a modification of the method of Nishida *et al.*[17] A 1:1 ratio of ethanol to acetone was used instead of the recommended solvents. Aliquots of the lipid extract were used for the determination of total and free cholesterol employing the same method as used for the plasma.

The presence of Vitamin A in large quantities in the tissue samples was later discovered to give similar colour reactions with the ferrous sulphate acetic acid reagent used for the total cholesterol determination. A correction was therefore, made to the tissue cholesterol determination. Using a vitamin A acetate standard solution, the optical density for a known amount of vitamin A reacted with ferrous sulphate acetic acid + concentrated sulphuric acid reagent was determined at 490 mμ. The correction was then made by calculating the optical densities due to vitamin A contained in the aliquots of lipid extracts taken for cholesterol determination. These were subtracted from the optical density readings obtained for total cholesterol + vitamin A present in the aliquots taken. The optical density due to cholestrol alone was then obtained.

Vitamin A was extracted from the tissues using a modification of the method by Ames *et al.* as set up in the "Method of Biochemical Analysis"[15]. Precisely weighed one to two gm. portions of tissue were ground to dryness with anhydrous sodium sulphate in a mortar. This mixture was quantitatively transferred into a centrifuge tube. Ten to 20 ml of light petroleum ether (40–60°) were added and the contents mixed using a vortex stirrer. The tubes were centrifuged at low speed for 5 minutes and the supernatants carefully transferred into 100 ml volumetric flasks. The residue broken up with a glass rod and the extraction repeated three times.

TABLE 1*a*. *Mean Relative and Absolute weights of livers and kidneys of the chickens and rats killed at the various intervals.*

CHICKEN GROUPS

	I *oil*	II *oil + vit. A*	III *Palm oil*	IV *No oil*
Batch killed at 60 days	3	4	4	—
	M ± SD gm	M ± SD gm	M ± SD gm	gm
Body wt. at death	968.6	1066.0	1016.2	—
Liver weight	19.7	21.8	20.0	—
Liver wt./kg body wt.	20.3±1.1	20.4±1.7	20.2±2.1	—
Kidney weight	7.2	7.8	7.0	—
Kidney wt./kg body wt.	7.4±0.5	7.3±0.7	6.9±0.6	—
Batch killed at 120 days	4	4	3	—
Body weight at death	2503.7	2712.2	2694.6	—
Liver weight	41.7	37.6	35.6	—
Liver wt./kg body wt.	16.7±0.5	14.0±2.4*	13.3±0.3**	—
Kidney weight	14.0	12.0	11.4	—
Kidney wt /kg body wt	5.6±0.6	4.4±0.7*	4.2±0.1**	—
Batch killed at 170 days	4	4	3	3
Body weight at death	3123.7	3365.7	2956.6	2966.6
Liver weight	42.4	37.7	34.9	31.8
Liver wt./kg body wt.	13.7±1.2	11.2±0.4**	11.8±1.3*	10.7
Kidney weight	14.1	11.9	13.3	11.9
Kidney wt./kg body wt.	4.6±1.1	3.5±0.2*	4.4±0.9	4.0

TABLE 1*b*.

RAT GROUPS

Groups	Batch killed at 60 days			Batch killed at 130 days		
	I *oil*	II *oil+vit. A*	III *p/oil*	*I oil*	*II oil+vit. A*	*III p/oil*
Number of rats	3 gm	3 gm	3 gm	3 gm	3 gm	3 gm
Body weight	244.0	263.6	224.6	366.0	414.6	410.3
Liver weight	8.08	8.43	7.04	10.67	11.84	11.76
Liver wt/100gm body wiehgt	3.31	3.19	3.14	2.92	2.87	2.87
Kieney weight	1.85	1.70	1.57	2.34	2.43	2.60
Kidney wt/100gm body weight	0.76	0.65	0.70	0.64	0.59	0.65

The extracts were combined with the previous ones in the volumetric flasks and the contents finally made to volume with petroluem ether and tightly stoppered. Total ester and alcohol vitamin A were determined using methods as for plasma.

Statistical analyses and presentation of data were done by personal communication with Mr. Lowy, Maitre de recherche au C.N.R.S. Paris.

Notation:– * represents a statisticlaly significant difference ($P \leq 0.05$).

** stand for a highly significant difference ($P \leq 0.01$).

These notations are used throughout the paper whenever differences have been checked statistically and found to be significant.

Results

There were no differences between the chicken groups with regard to food intake up to 120 days. After 120 days, Group III chickens decreased their daily average intake. Group II maintained their average intake while Group I continued to gradually increase their intake. The average vitamin A intake calculated from the daily average food intake records were from 150 to 600 IU for Group I; an additional 5800–30,000 IU vitamin A per day was ingested by Group II chickens and Group III had 3 500 to 21 300 μg additional carotenoids per day.

Livers taken from Group II chickens at 120 days and those from both Group II and III chickens at the end of the experiment were pale (muddy pink). The group II livers were distinctively much paler than those from Group III. Table I shows mean body weight at death and the mean relative and absolute weights of liver and kidneys of the chicken groups killed at the various intervals.

For the rat groups, food intake paralleled growth. With group II and III ending the experiment with slightly higher daily average intake and slightly higher mean body weights than Group I. Corresponding daily vitamin A intake were 40–95 IU for Group I; 2 500 to 8 400 IU additional vitamin A for Group II and 1 500 to 5 200 μg additional carotenoids for Group III. Group II rats also exhibited muddy pink livers at 60 and 130 days. Mean body, liver and kidney absolute and relative weights of the rats are also shown in Table I*b*.

Plasma, liver and kidney total cholesterol cholesterol ester* and free cholesterol values for the chickens and rats are presented in figure 1. Total vitamin A, vitamin A ester and vitamin A alcohol values for plasma, liver and kidneys are shown for the chickens in Table 2*a*. Plasma samples from the rat groups were insufficient for vitamin A determination to be made. Vitamin A levels in liver and kidneys for the rats are shown in Table 2*b*.

Discussion

The levels of retinol needed to cause acute poisoning in rats have been indicated in experiments by Moore and Wang[18] as ranging from 40,000 to 90,000 IU per day. When the vitamin is administered paranterally, 50,000 IU has been taken as the average needed[19]. Nieman and Klein Obbink[20] considered that oral toxicity begins at 25,000 IU per day for rats. It has been generally accepted that toxicity levels are to a great extent related to body weight. Rodahl[21] reported that toxic signs developed when doses ranged from 50 to 130 IU per gram of body weight per day. He cited loss of apetite, emaciation haemorrhages, fractures and bone changes as some of the toxic manifestations.

The levels of retinol ingested by both the experimental groups of chickens and rats in this study when expressed per gram of body weight vary from 31.5 to 8.0 IU per day for the chickens and 37 to 20 IU for the rats. The retinol intake therefore at no time of the experiment reached levels needed for toxic signs to develop. This was confirmed by the fact that the most obvious toxic signs of hypervitaminosis A, loss of apetite, emaciation etc. were not observed in the animals fed additional retinol/or carotenoids. The amounts of retinol ingested by both groups II and III animals, however, were 40 and 60 times respectively higher for the chickens and the rats than the retinol requirement of their species. This, therefore, could be considered sub-toxic or physiologically tolerable levels.

Squibb[22], Bring *et al.*[9] observed a decrease in liver weight with high doses of vitamin A intake. The same was also confirmed in this study with the physiologically tolerable doses fed. Both the livers and kidneys of the Group II and III animals were observed to be significantly lower in weight than those of the control groups. (Table I*a*.).

*Cholesterol ester values were obtained by subtracting free cholesterol from the total cholesterol values.

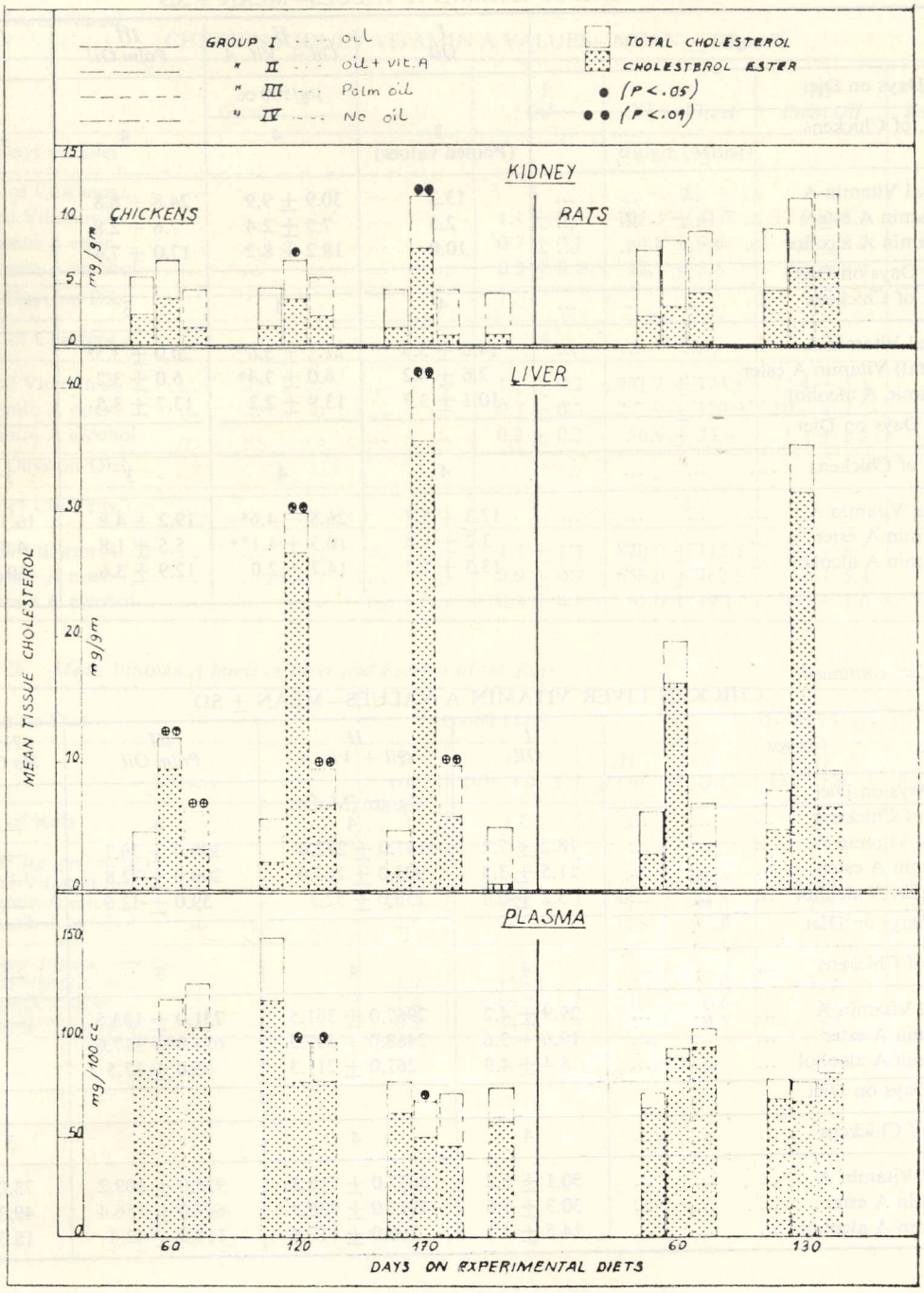


FIG. 1. *The effects of feeding vitamin A or carotenoid (palm oil) on tissue cholesterol levels.*

TABLE 2*a* CHICKEN PLASMA VITAMIN A VALUES—MEAN ± SD

Groups	*I* *Oil*	*II* *Oil + Vit. A*	*III* *Palm Oil*	*IV*
60 Days on Diet		μg/100 cc		
No. of Chickens	3 (Pooled values)	4	4	—
Total Vitamin A	13.3	30.9 ± 9.9	24.8 + 8.8	
Vitamin A ester	2.5	7.5 ± 2.4	7.6 + 2.8	
Vitamin A alcohol	10.0	18.2 ± 8.2	17.0 + 7.4	
120 Days on Diet				
No. of Chickens	4	4	3	
Total Vitamin A	14.0 ± 3.3	21.7 ± 5.6*	20.0 ± 3.5*	
(Total) Vitamin A ester	2.6 ± 2.2	6.0 ± 3.4*	6.0 ± 3.2	
Vitamin A alcohol	10.1 ± 3.7	13.9 ± 2.2	13.7 ± 3.5	
170 Days on Diet				
No. of Chickens	4	4	3	3
Total Vitamin A	17.3 + 5.7	26.8 + 4.6*	19.2 + 4.8	16.3
Vitamin A ester	3.2 ± 1.3	10.3 ± 4.1**	5.5 ± 1.8	4.8
Vitamin A alcohol	13.3 ± 5.7	14.7 ± 2.0	12.9 ± 3.6	10.7

TABLE 2*a*. *continued*.

CHICKEN LIVER VITAMIN A VALUES—MEAN ± SD

Groups	*I* *Oil*	*II* *Oil + Vit. A*	*III* *Palm Oil*	*IV* *No Oil*
60 Days on Diet		μg/gm (Moist)		
No. of Chickens	3	4	4	—
Total Vitamin A	18.2 ± 7.2	1147.0 ± 252.0	308.0 ± 36.7	—
Vitamin A ester	11.5 ± 3.3	903.0 ± 267.0	200.0 ± 72.8	—
Vitamin A alcohol	5.2 ± 0.8	132.0 ± 52.5	59.0 ± 32.6	—
120 Days on Diet				
No. of Chickens	4	4	3	—
Total Vitamin A	29.9 ± 4.2	2967.0 ± 361.5	731.0 ± 133.5	—
Vitamin A ester	19.6 + 3.6	2488.0 + 507.5	624.0 + 117.6	—
Vitamin A alcohol	8.4 ± 4.9	267.0 ± 211.3	65.0 ± 17.5	—
170 Days on Diet				
No. of Chickens	4	4	3	3
Total Vitamin A	50.1 ± 6.2	5677.0 ± 718.3	915.0 ± 109.2	73.0
Vitamin A ester	30.3 ± 8.6	4911.0 ± 938.9	647.0 ± 156.4	49.3
Vitamin A alcohol	14.5 ± 3.3	260.0 ± 117.8	114.0 ± 42.4	18.3

TABLE 2*a. continued.*

CHICKEN KIDNEY VITAMIN A VALUES—MEAN ± SD

Groups	*I Oil*	*II Oil + Vit. A*	*III Palm Oil*	*IV Ro Oil*
60 Days on Diet		μg/gm (Moist)		
No. of Chickens...	3	4	4	—
Total Vitamin A	1.1 ± 0.3	105.3 ± 41.7	12.6 ± 2.3	—
Vitamin A ester	0.7 ± 0.1	84.8 ± 9.6	6.5 ± 2.7	—
Vitamin A alcohol	0.3 ± 0.2	12.7 ± 5.5	2.2 ± 0.4	—
120 Days on Diet				
No. of Chickens...	4	4	3	—
Total Vitamin A	1.1 ± 0.2	341.7 ± 124.1	12.4 ± 5.5	—
Vitamin A ester	0.7 ± 0.3	272.9 ± 120.9	10.6 ± 4.2	—
Vitamin A alcohol	0.2 ± 0.2	56.9 ± 33.6	1.4 ± 1.2	—
170 Days on Diet				
No. of Chickens...	4	4	3	3
Total Vitamin A	1.3 ± 1.3	820.0 ± 115.7	7.9 ± 8.5	2.1
Vitamin A ester	0.9 ± 0.7	658.0 ± 162.5	4.8 ± 5.4	1.2
Vitamin A alcohol	0.3 ± 0.1	92.0 ± 19.1	1.3 ± 1.0	0.4

TABLE 2*b. Mean Vitamin A levels in Liver and Kidneys of the Rats.*

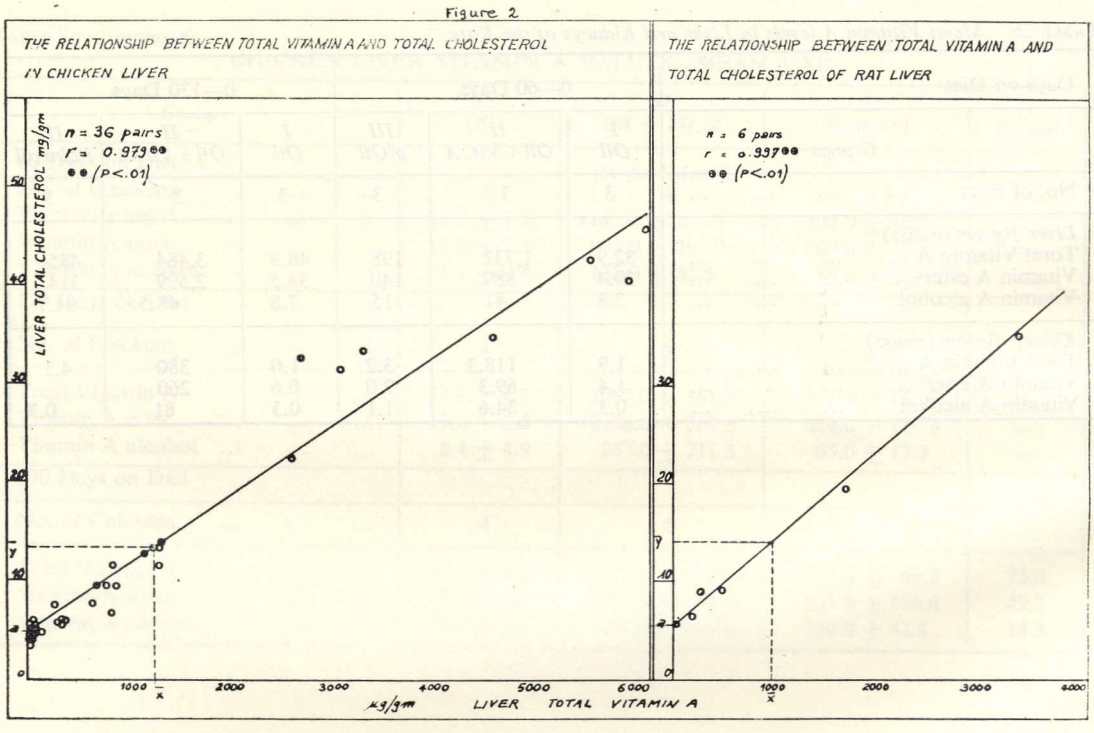
Days on Diet	0—60 Days			0—130 Days		
Groups	*I Oil*	*II Oil+Vit. A*	*III p/Oil*	*I Oil*	*II Oil+Vit. A*	*III Palm oil*
No. of Rats	3	3	3	3	3	3
Liver μg/gm (moist)						
Toral Vitamin A	32.9	1,732	198	48.9	3,484	485
Vitamin A ester	29.4	889	140	34.5	2,599	314
Vitamin A alcohol	2.8	41	15	7.8	48.5	91.7
Kidney μg/gm (moist)						
Total Vitamin A	1.9	118.3	3.2	1.0	380	4.1
Vitamin A ester	1.4	69.3	2.0	0.6	260	
Vitamin A alcohol	0.3	34.6	1.1	0.3	81	0.7

High plasma cholesterol levels resulting from high fat feeding in the absence of dietary cholesterol has been reported.[19, 20] Others, however, were unable to demonstrate any increased cholesterol synthesis in animals fed considerable amounts of lard and or other fats.[25, 26] Figure 1 (170 days) reveals that, in the present work, addition of fat to the diet of Group I chickens did not affect the cholesterol levels in plasma, liver and kidneys of this group as compared with Group IV animals who had no extra fat added to their diet. The cholesterol levels obtained for these two groups compare favourably with values reported for chickens elsewhere[24]. The control rats, (Figure 1) however, showed tendencies towards slight increases in liver and kidney cholesterol levels with age. Whether this was due to the increased fat feeding or not could not be accessed since a fourth rat group was not included on a diet with no additional oil.

Dietary cholesterol has been repeatedly shown according to March and Biely [24] not to effectively promote hyper-cholesterolaemia and accumulation of tissue cholesterol unless the diet likewise contains a high level of fat. It has been shown from various reports[25, 26] and been confirmed in this study that the addition of oil alone to the diet did not significnatly affect the cholesterol levels in the tissues of the animals. The cholesterol levels in this study however, were strinkingly increased in the tissues of both the chicken and the rat groups only when moderately high levels of retinol or its precursor (carotenoids) were fed in addition to the high fat diets (Figure 1.). This therefore, brings retinol or its precursor into the same position as cholesterol, in that when fed in moderately high levels with fat, they both lead to accumulation of tissue cholesterol as brought to light in this study.

That the amount of retinol added to the high fat diet was responsible for the amount

Figure 2



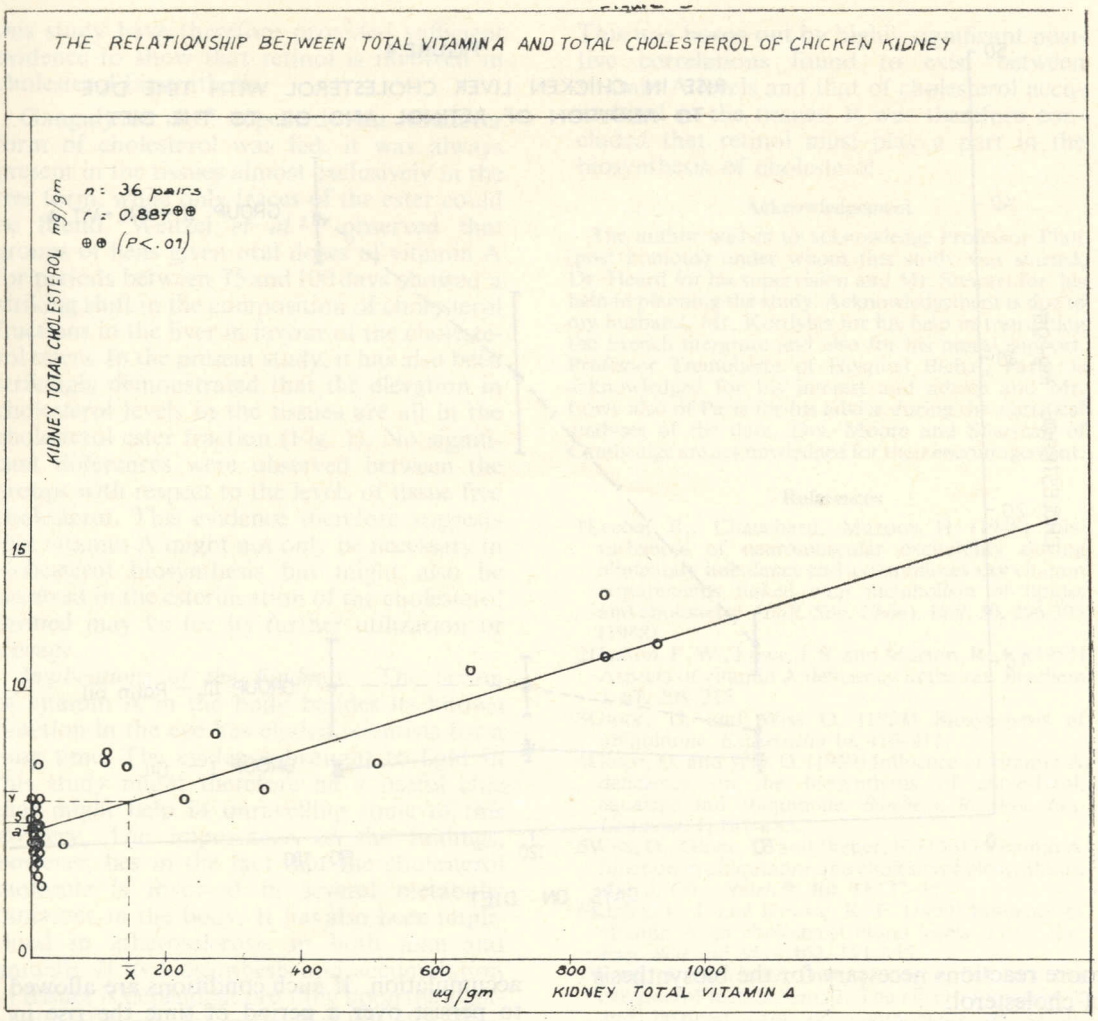


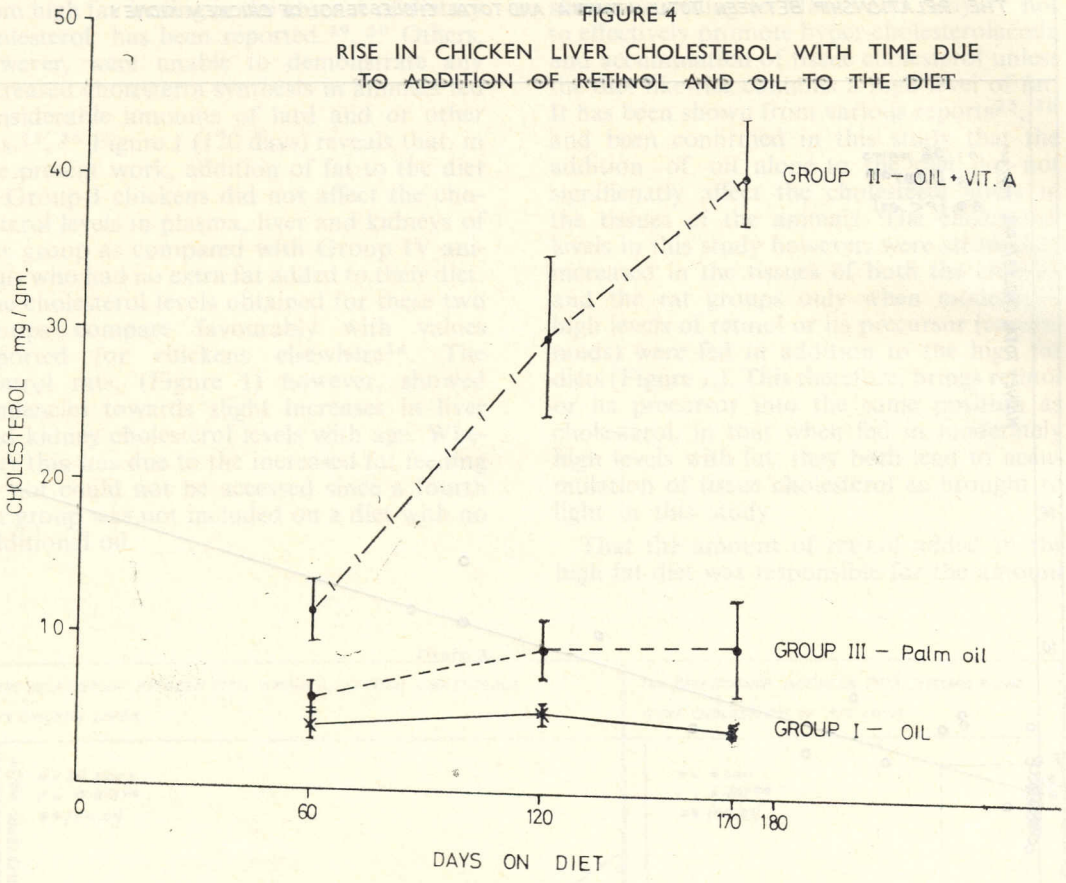
FIG. 3.

of cholesterol produced and accumulated in the tissues can again be seen in figure 1, where cholesterol levels in the livers and kidneys of the animals fed pre-formed vitamin A are two to four fold the amount showed by the carotenoid groups with their slow rate of conversion of carotenoids to vitamin A, and several times the amount seen in the control groups which did not have any extra vitamin A added to their high fat diets. The rises in the tissue cholesterol levels can again be shown to be dependent on the retinol levels by the fact that cholesterol was accumulated in higher levels in the livers where vitamin A is mostly stored than in the kidneys with their lower rate of storage. This, therefore, reveals a definate relationship between vitamin A and cholesterol which is confirmed in figures 2 and 3 as highly significant positive correlations existing between total cholesterol levels and that of the vitamin A stored in the livers and kidneys respectively. There is an almost 1 to 10 ratio existing between the level of vitamin A stored and the amount of cholesterol accumulated in these tissues.

Relationship between vitamin A and cholesterol has long been reported [1–4]. Wiss *et al.* [3–5] indicated that the incorporation of mavelonic acid into squalene and ubiquinone is clearly increased during vitamin A deficiency whereas the biosynthesis of cholesterol is depressed. They observed a clear trend towards normalization in cholesterol biosynthesis within a few hours after vitamin A was added and the authors therefore suggested that vitamin A might be involved in one or

FIGURE 4

RISE IN CHICKEN LIVER CHOLESTEROL WITH TIME DUE TO ADDITION OF RETINOL AND OIL TO THE DIET



more reactions necessary for the biosynthesis of cholesterol.

Deuel[19] wrote that not only animal fats which contain cholesterol per se, are a source of this sterol in the animal, but also that vegetable fats provide cholesterol because the acetate molecules are available as a result of oxidation of fatty acids. The feeding of additional fat to the experimental animals would therefore provide a source of acetate molecules that could be used as fundamental units for cholesterol biosynthesis. If the suggestion that vitamin A is involved at a metabolic level in cholesterol biosynthesis is assumed, then one would expect that when both fat (acetate precussor) and vitamin A are provided in doses excessive of the animals requirement, then the acetate vitamin A cholesterol reaction would be favoured and an increase in cholesterol biosynthesis observed as an increase in cholesterol accumulation. If such conditions are allowed to persist over a period of time the rise in cholesterol accumulation should also be evident with time. In the present study such striking rises in the accumulation of cholesterol have been demonstrated in both the chicken and the rat groups fed extra vitamin A and oil in the diet. Figure 4 compares the rise in cholesterol levels in the chicken livers with time. The cholesterol values for group II rose from 11 to 29 to 40 mg per gm of tissue from 60 to 120 to 170 days respectively. The rats fed diet II had a rise from a mean of 19.8 mg/gm at 60 days to 35.2mg cholesterol per gm of liver at 130 days (Figure 1). The livers containing high levels of cholesterol demonstrated typical cholesterol fatty livers. It can therefore be concluded that physiologically tolerable levels of vitamin A fed with high fat diets enhance the biosynthesis of cholesterol. The findings of

this study have therefore provided sufficient evidence to show that retinol is involved in cholesterol biosynthesis.

Ganguly *et al.*[27] reported that whatever form of cholesterol was fed, it was always present in the tissues almost exclusively in the free form, while only traces of the ester could be found. Weitzel *et al.*[28] observed that groups of hens given oral doses of vitamin A for periods between 75 and 100 days showed a striking shift in the composition of cholesterol fractions in the liver in favour of the cholesterol esters. In the present study, it has also been strikingly demonstrated that the elevation in cholesterol levels in the tissues are all in the cholesterol ester fraction (Fig. 1). No significant differences were observed between the groups with respect to the levels of tissue free cholesterol. This evidence therefore suggests that vitamin A might not only be necessary in cholesterol biosynthesis but might also be involved in the esterification of the cholesterol formed may be for its further utilization or storage.

Implications of the Findings. The action of vitamin A in the body besides its known function in the eye has eluded scientists for a long time. The evidence brought to light in this study might therefore be a useful clue that might help in unravelling some of this mystery. The importance of the findings, however, lies in the fact that the cholesterol molecule is involved in several metabolic functions in the body. It has also been implicated in atherosclerosis in both man and animals. If its biosynthesis and accumulation in tissues is dependent not only upon the availability of excess fat in the diet but also upon the availability of vitamin A as brought to light in this study, then the findings of this study could be said to have opened avenues for further investigation in vitamin A in association with fat (butter, margarine, animal fats) in the diet and their relationship to cholesterol, cholesterol hormones and atherosclerosis.

Summary

The feeding of physiologically tolerable doses of carotenoids or their equivalent in pre-formed vitamin A in association with a high fat diet has provided sufficient evidence to show that tissue cholesterol can be accumulated by the feeding of such diets. The amounts of cholesterol accumulated in livers and kidneys of experimental animals fed these diets were found to be dependent on the amount of retinol stored in these tissues. This was borne out by highly significant positive correlations found to exist between vitamin A levels and that of cholesterol accumulated in the tissues. It was therefore concluded that retinol must play a part in the biosynthesis of cholesterol.

Acknowledgement

The author wishes to acknowledge Professor Platt (post humous) under whom this study was started, Dr. Heard for his supervision and Mr. Stewart for his help in planning the study. Acknowledgement is due to my husband, Mr. Kordylas for his help in translating the French literature and also for his moral support. Professor Tremolieres of Hospital Bichat, Paris, is acknowledged for his interest and advice and Mr. Lowy also of Paris for his advice during the statistical analyses of the data. Drs. Moore and Sharman of Cambridge are acknowledged for their encouragement.

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