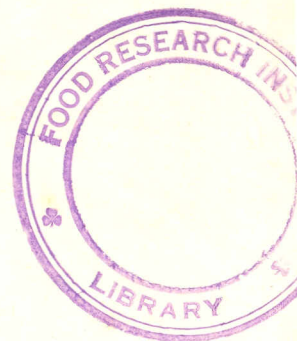


RETINOL IN CHOLESTROL BIOSYNTHESIS: EFFECT ON LIPID METABOLISM

By

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Introduction

Josephs¹ reported hyperlipemia with high intake of vitamin A as well as with carotenoids. Van Bruggen and Struamfjord², observed that long-term administration of large doses of vitamin A to patients raised their plasma cholesterol and phospholipid levels. Pallotta and Krause³ observed a rise in serum phospholipid levels with administration of vitamin A. Misra⁴ reported that plasma cholesterol levels were lowered and phospholipid levels elevated with the administration of vitamin A. The administration of vitamin A has also been reported to result in increased deposition of fat in the liver and also in various other organs.⁵⁻¹⁰ Misra⁹ observed an increase in the glyceride fraction of the liver lipids of rats given excessive doses of vitamin A. Singh *et al*¹⁰ reported increases in hepatic cholesterol but found no change in phospholipid levels with high intake of vitamin A. Kordylas¹¹ provided sufficient evidence to show that accumulation of tissue cholesterol is dependent on vitamin A.

Experiment: 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 the long term effects, if any, of high intake of carotenoids in oil (palm oil) or its equivalent in preformed vitamin A in oil. This paper reports a section of the findings of the original study.

Chickens and rats were used for the experiment. The chickens were divided into three groups of 12 chickens and the rats into three groups of 6 rats. Protein and caloric values of diets fed to each group were identical, as also was the proportion of oil in each diet, but they differed in the composition of the oil. Group I chickens received a diet made of 150 gms of arachis oil and 850 gms of basic diet. The vitamin A content was approximately 5000 IU per kg diet. Group II chickens were given 850 gm of the basic diet mixed with 150

gms arachis oil to which was added a precisely weighed retinol palmitate equivalent to the vitamin A value of the red palm oil in diet III. The vitamin A content ranged from 157,000 IU to 273,000 IU per kg. diet. Diet III given to group III chickens was made by mixing 150 gm of red palm oil with 850 gm of the basic diet to make 1 kg diet. The total carotenoid content of this diet ranged from 94,000 to 164,000 μg per kg diet.

The rat groups were given the same diet as prepared for the chickens except that 200 gms of the various oils were mixed with 800 gms of the basic diet.

The animal groups were given the experimental diets over a period of time. After 60, 120 and 170 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. A fourth group of three chickens kept on the basic diet with no additional oil, was killed with the last batch at the end of the 170 days. Three rats from each of the groups were also killed at 60 days, the remaining rats were killed at the end of 130 days. Detail description of the diet and treatment of the animals are as previously reported¹¹.

Collection and Treatment of Samples: Animals to be killed were starved for 18 to 24 hour and were anaesthetized under chloroform. Freely flowing blood samples were collected from all animals into heparinized tubes before they were killed by decapitation. 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 lipids, phospholipid phosphorus, total cholesterol and

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free cholesterol, also total, ester and alcohol vitamin A. Glyceride values were obtained by subtracting total cholesterol and phospholipid values from the total lipid values. Cholesterol ester values were obtained by subtracting free cholesterol from the total cholesterol values.

Methods

Plasma lipids were extracted using a modification of the method recommended in the "Methods of Biochemical Analysis"¹². Acetone-absolute ethanol (1:1) mixture¹³ was substituted for the recommended solvents and the extraction method carried out as directed. Determination of the plasma total lipids was done using the gravitational method employed by Leveille and Sauberlich¹⁴. Lipid phosphorus for plasma was determined by the method of Zilversmit and Davies¹⁵. To obtain phospholipid values the lipid phosphorus results were multiplied by 25 (1:25) being the ratio of phosphorus in the phosphatide contained in an individual lipid¹⁶. Plasma total and free cholesterol were determined using the method by Searcy and Bergquist¹³. Total Vitamin A were analyzed by the method described in the "Methods of Biochemical Analysis"¹⁷. Vitamin A ester and alcohol separation and determination were done by the method of Eden¹⁸. Tissue lipids were extracted by a modification of the method of Nishida *et al.*¹⁹ A (1:1) ratio of ethanol and acetone was used instead of the recommended solvents.

For the determination of tissue lipid phosphorus, 5ml. aliquot of the tissue lipid extract was transferred into a test tube, evaporated to dryness in a hot water bath²⁰ and the determination of the phosphorus content of the dry lipid was carried out as for plasma. Aliquot of the tissue lipid extract were used for the determination of total and free cholesterol employing the same methods as used for plasma. Correction was made for vitamin A interference with the ferrous sulphate reagent used in the determination of tissue cholesterol. This was done as previously reported¹¹. Methods used for tissue vitamin A determination was as reported¹¹.

Statistical analyses and presentation of data were done by personal communication with Mr. Lowy, Maitre de recherche au C.N.R.S. Paris; Director adjoint a l'Ecole Pratique des Hautes Etudes—Paris.

Notation:—*—Represents a statistically 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

The Vitamin A intake calculated from the daily average food intake records of the chickens was 150 to 600 IU for Group I; additional 5,800 to 30,000 IU per day was ingested by Group II, and Group III chickens had 3,500 to 21,300 μg additional carotenoids per day. Corresponding daily intake of Vitamin A for the rats 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.

Tables 1 (a), (b) and (c) show values obtained for total lipids, phospholipid, total cholesterol, free cholesterol, cholesterol ester and glyceride levels for plasma, liver and kidney respectively, for the chickens. Lipid values for the rat groups are shown in Table 2. Histograms corresponding to the distribution of the various lipid fractions except cholesterol are shown for both chicken and rats in Figure 1. The cholesterol histograms have been previously published¹¹. Total Vitamin A, Vitamin A ester and alcohol levels of plasma, liver and kidneys are as previously reported¹¹.

Discussion

Effect of Vitamin A on Plasma lipids:

Fatty acids originate from depot tissue and they circulate in the blood not in the free state but bound to albumin. The fatty acid-albumin complex releases its fatty acid at the sites of utilization which include liver, muscle, heart and many other tissues²¹. The liver has the capacity to dispose of these fatty acids (a) by oxidation and (b) by esterification and incorporation into triglycerides which are resecreted mostly in very low density lipoproteins²². According to Steinberg²², elevated plasma levels of Free Fatty Acids, and the consequent elevated rate of uptake of the free fatty acids by the liver, lead in a rather direct manner to an increase in the rate of production and secretion of lipoproteins in the form of triglycerides.

A rise in plasma triglyceride level will therefore be in accordance with the above reactions. In figure 1, at 170 days, Group I chickens fed additional fat in the diet show higher plasma total lipids and plasma triglyceride levels than observed in Group IV

TABLE 1a: The effects of feeding vitamin A or Carotenoids (Palm Oil) on the mean values \pm SD of Plasma Lipids of Chickens.

Groups	I Oil	II Oil + Vit. A	III Palm Oil	IV No Oil
mg/100 CC	M \pm SD	M \pm SD	M \pm SD	M
<i>60 days on diet</i>				
Total lipids... ..	(3) 1/ 2 183.0 \pm 170.2	2 156.0 \pm 318.4	1 969.0 \pm 159.9	—
Phospholipids	196.3 \pm 19.2	168.3 \pm 32.1	206.9 \pm 48.4	—
Total cholesterol	99.0 \pm 6.2	118.7 \pm 32.2	127.5 \pm 36.4	—
Free cholesterol	14.7 \pm 5.0	19.5 \pm 5.7	21.0 \pm 12.3	—
Cholesterol ester 2/	84.3 —	99.2 —	106.5 —	—
Glycerides 3/	1 887.7 —	1 869.0 —	1 634.6 —	—
<i>120 days on diet</i>				
Total lipids... ..	2 225.0 \pm 177.9	1 900.0 \pm 54.0*	(3) 1 808.0 \pm 101.0**	—
Phospholipids	203.8 \pm 36.5	179.0 \pm 41.4	199.4 \pm 21.7	—
Total cholesterol	150.0 \pm 42.7	97.0 \pm 11.7	97.0 \pm 11.5*	—
Free cholesterol	33.5 \pm 9.3	19.0 \pm 3.3	18.0 \pm 2.2	—
Cholesterol ester 2/	117.2 —	78.0 —	79.0 —	—
Glycerides 3/	1 870.5 —	1 624.0 —	1 511.6 —	—
<i>170 days on diet</i>				
Total lipids... ..	2 094.0 \pm 235.8	1 838.0 \pm 101.0*	(3) 1 858.0 \pm 87.8	(3) 1 817.0
Phospholipids	186.1 \pm 6.0	147.2 \pm 9.8	225.1 \pm 1.6	206.5
Total cholesterol	78.0 \pm 4.2	68.0 \pm 5.0*	72.0 \pm 11.1	75.0
Free cholesterol	16.0 \pm 4.7	18.0 \pm 6.1	27.0 \pm 13.6	17.0
Cholesterol ester 2/	62.0 —	50.0 —	45.0 —	58.0
Glycerides 3/	1 829.9 —	1 622.8 —	1 560.0 —	1 535.5

* (p < 0.05) ** (p < 0.01)

1/ Four birds per batch except when noted in brackets.

2/ Cholesterol ester obtained by sub-tracting free cholesterol values from total cholesterol.

3/ Glycerides obtained by sub-tracting total cholesterol + phospholipids from total lipid values.

chickens which had no additional oil in their diet. This finding show that the feeding of the extra fat to the control group I caused a rise in the triglyceride level in their blood. Since the same dietary levels of fat were fed to the other experimental groups of animals as given to the control group I, it would be expected that the plasma total lipids and plasma triglyceride levels in these groups would also be of the same magnitude as observed in this group.

Table 1(a) and Fig. 1, however, demonstrate that apart from an increase in the plasma total lipids, observed in group II animals killed at 60 days, neither the group III animals nor the group II birds remaining on the experimental diets after 60 days, showed any elevation in their plasma total lipids and glyceride levels, although those of the group I animals remained high throughout. The addition of Vitamin A or carotenoids to the groups II and III experimental diets, therefore, may have

prevented the plasma lipids from rising although these groups also received the same level of dietary fat.

The total plasma lipids and glyceride levels observed at the end of the experiment for groups II and III animals were of the same order as the values obtained for group IV chickens which received neither extra oil nor additional vitamin A in the diet (Table 1a, Fig 1, 170 days). Groups II and III rats also showed lower plasma total lipids and plasma triglyceride levels than the control group I animals killed at 130 days, (Table 2 and Fig. 1.). These results therefore suggest that the ingestion over a period of time of the moderately high doses of vitamin A in the diet may have counteracted the tendency of high dietary fat in causing a rise in the plasma lipids.

Effect of Vitamin A on Tissue lipids

Rat hepatic total lipids and triglycerides

TABLE 1b: The effects of Feeding Vitamin A or Carotenoids (Palm Oil) on the mean values \pm SD of Liver Lipids of chickens.

Groups	I Oil	II Oil + Vit. A	III Palm Oil	IV No Oil
mg / gm (moist)	M \pm SD	M \pm SD	M \pm SD	M
<i>60 days on diet</i>				
Total lipids... ..	(3) 1/ 69.0 \pm 16.2	76.5 \pm 7.4*	70.1 \pm 11.5	—
Phospholipids	22.7 \pm 2.5	25.8 \pm 3.9	24.4 \pm 3.3	—
Total cholesterol	4.3 \pm 1.1	11.8 \pm 1.9**	6.0 \pm 1.0**	—
Free cholesterol	3.4 \pm 0.0	2.8 \pm 0.6	3.0 \pm 0.6	—
Cholesterol ester 2/	0.9 —	9.0 —	3.0 —	—
Glycerides 3/	42.0 —	37.8 —	39.4 —	—
<i>120 days on diet</i>				
Total lipids... ..	69.6 \pm 6.9	93.9 \pm 17.6*	(3) 74.3 \pm 8.5	—
Phospholipids	26.0 \pm 3.2	26.0 \pm 5.1	30.9 \pm 4.3	—
Total cholesterol	5.2 \pm 0.7	29.9 \pm 5.1**	9.4 \pm 1.8**	—
Free cholesterol	3.2 \pm 0.7	3.4 \pm 1.0	2.9 \pm 1.1	—
Cholesterol ester 2/	2.0 —	26.5 —	6.5 —	—
Glycerides... ..	38.4 —	35.0 —	33.3 —	—
<i>170 days on diet</i>				
Total lipids	64.4 \pm 6.4	109.6 \pm 12.5**	(3) 73.1 \pm 1.0*	66.6
Phospholipids	24.5 \pm 1.7	24.9 \pm 1.4	24.8 \pm 3.0	25.5
Total cholesterol	4.3 \pm 0.2	40.4 \pm 3.8**	9.6 \pm 3.3**	4.7
Free cholesterol	3.1 \pm 0.4	4.9 \pm 0.4	4.0 \pm 0.7	4.4
Cholesterol ester 2/	1.2 —	35.5 —	5.6 —	0.3
Glycerides 3/	35.6 —	38.6 —	37.8 —	36.4

* ($p < 0.05$) ** ($p < 0.01$)

1/ Four birds per batch except when noted in brackets.

2/ Cholesterol ester obtained by sub-tracting free cholesterol values from total cholesterol.

3/ Glycerides obtained by sub-tracting total cholesterol + phospholipids from total lipid values.

have been reported to increase with oral administration of high levels of vitamin A^{9,10}. In the present study, Table 2, and Fig. 1 also confirm these findings, they show that hepatic total lipids, and hepatic triglyceride levels of both groups II and III rats were increased with the intake of the physiologically tolerable doses of either vitamin A or carotenoids fed. Rat kidneys, at 130 days, also showed the same tendency. The chicken groups II and III also had significant increases in hepatic total lipids than the control groups. Their liver glyceride levels were however, not appreciably affected in any way by either vitamin A or carotenoid administration. The values obtained were similar for all three groups and compare favourably with the level obtained for Group IV chickens left on the basic diet. (Table 1b and Fig. 1). The lack of decreases in the glyceride levels in the livers

of the experimental animal groups II and III, who showed lower plasma glyceride levels with vitamin A and high fat intake, support the view that synthesis and (or) secretion of lipoprotein is not impaired with high intake of vitamin A.¹⁰

Kidney phospholipid levels measured were more or less the same for the experimental groups of both chickens and rats as for the control groups. The values obtained for the experimental chickens, however, were slightly lower than observed for Group IV chickens left on the basic diet (Fig. 1). Liver phospholipid levels remained almost the same for all groups at all the experimental periods. This is in accordance with the findings of Singh *et al.*¹⁰ who also found no changes in hepatic phospholipid levels with vitamin A administration. However, strikingly high levels of cholesterol were observed in the livers and

TABLE 1c. *The effects of Feeding Vitamin A or Carotenoids (Palm Oil) on the mean values \pm SD of Kidney Lipids of chickens.*

Groups mg / gm				I Oil	II Oil + Vit. A	III Palm Oil	IV No Oil
				M \pm SD	M \pm SD	M \pm SD	M
60 days on diet				(3)	1/		
Total lipids...	59.5 \pm 7.5	64.8 \pm 11.4	74.7 \pm 7.6	—
Phospholipids	—	—	—	—
Total cholesterol	5.4 \pm 0.5	6.6 \pm 1.8	4.3 \pm 1.7	—
Free cholesterol	3.0 \pm 0.6	3.0 \pm 0.9	2.9 \pm 1.6	—
Cholesterol ester	2/	2.4	3.6	1.4	—
Glycerides	3/	—	—	—	—
120 days on diet						(3)	
Total lipids...	64.9 \pm 8.5	72.5 \pm 12.6	77.7 \pm 19.6	—
Phospholipids	—	—	—	—
Total cholesterol	4.6 \pm 1.0	6.9 \pm 1.3*	5.3 \pm 1.5	—
Free cholesterol	3.0 \pm 1.0	3.2 \pm 0.8	3.0 \pm 0.5	—
Cholesterol ester	2/	1.6	3.7	2.3	—
Glycerides	3/	—	—	—	—
170 days on diet						(3)	(3)
Total lipids...	61.1 \pm 14.4	78.4 \pm 12.8	59.6 \pm 2.8	46.1
Phospholipids	19.7 \pm 1.4	21.4 \pm 1.1	19.7 \pm 1.5	25.1
Total cholesterol	4.3 \pm 0.7	11.7 \pm 1.5**	4.3 \pm 0.5	5.0
Free cholesterol	3.1 \pm 0.9	4.0 \pm 0.9	3.6 \pm 0.7	4.1
Cholesterol ester	2/	1.2	7.7	0.7	0.9
Glycerides	3/	37.1	44.5	35.6	34.0

* ($p < 0.05$) ** ($p < 0.01$)

1/ Four birds per batch except when noted in brackets.

2/ Cholesterol ester obtained by sub-tracting free cholesterol values from total cholesterol.

3/ Glycerides obtained by sub-tracting total cholesterol + phospholipids from total lipid values.

kidneys of these same experimental animals. This has already been reported elsewhere¹¹. Table 1b, 1c, and 2 show the tissue cholesterol values obtained.

Vitamin A and Fatty Acid Oxidation

Evidence has been provided above to suggest that the prolonged administration of physiologically tolerable doses of vitamin A or carotenoids prevented the tendency of high dietary fat in causing increases in plasma total lipids and triglyceride levels. The reaction of vitamin A in preventing such a rise has been shown not to be attributable to a decrease in lipoprotein synthesis and (or) secretion in the liver.

Besides the incorporation of fatty acids into lipoproteins for secretion as triglycerides into the blood, the liver is also known to have the capacity to dispose of fatty acids by oxidation. If therefore, there is an increase in fatty acid oxidation with vitamin A administration, this could cause a decrease in the amount of fatty acids being incorporated into lipoproteins for

secretion as triglycerides. Such an increase in fatty acid oxidation, could explain the lack of increase in the plasma triglyceride levels observed with vitamin A or carotenoid administration during high fat intake. This explanation therefore may suggest that vitamin A administration could cause an increase in oxidation of fatty acids.

Fatty acid oxidation is known to give rise to acetate molecules and these acetate molecules are also known to act as precursors for cholesterol biosynthesis. If the suggestion that vitamin A administration may cause an increase in fatty acid oxidation is true, and that this in turn would provide acetate precursors for cholesterol biosynthesis, then one would expect to find evidence of such a reaction. The animals fed additional vitamin A or carotenoids gave such striking evidence by showing high accumulation of cholesterol in their tissues, while at the same time they demonstrated lower plasma triglyceride levels, than observed in the control groups, whose plasma triglyceride levels remained high while

TABLE 2. *The effect of feeding Vitamin A or Carotenoids on the lipid fraction levels of Plasma, Liver and Kidney of the rat. (Pooled values).*

Days on diet Groups	0-60			0-130		
	I Oil	II Oil + Vit. A	III Palm Oil	I Oil	II Oil + Vit. A	II Palm Oil
No of Rats	3	3	3	3	3	3
PLASMA mg/100cc						
Total Lipids	—	—	—	1,850	1,625	1,675
Phospholipids	—	—	—	181.3	146.3	167.8
Total Cholesterol	72	95	105	79	77	89
Free Cholesterol	7	5	9	9	9	6
Cholesterol Ester 5/	65	90	96	70	68	83
Glyceride 4/				1589.7	1401.7	1418.2
LIVER mg/gm (moist)						
Total Lipids	76.2	99.2	89.6	80.5	117.3	106.6
Phospholipids	25.8	29.3	27.8	26.0	26.3	28.3
Total Cholesterol	6.0	19.8	6.6	7.9	35.2	9.1
Free Cholesterol	3.2	3.6	3.1	3.2	3.9	2.9
Cholesterol Ester	2.8	16.2	3.5	4.7	31.3	6.2
Glycerides	44.4	48.4	55.2	46.6	52.3	69.2
KIDNEY mg/gm (Moist)						
Total Lipids	90.5	85.2	86.5	101.0	106.7	120.3
Phospholipids	21.5	21.0	21.8	22.3	23.3	23.8
Total Cholesterol	7.7	8.3	9.0	9.3	11.9	12.0
Free Cholesterol	5.5	5.3	5.8	5.0	5.7	6.2
Cholesterol Ester	2.2	3.0	4.0	4.3	6.2	5.9
Glycerides	61.3	55.9	55.7	69.4	71.5	84.5

4/ Glyceride values were obtained by subtracting total cholesterol + phospholipid from the total lipid values
5/ Cholesterol ester values were obtained by subtracting free cholesterol from the total cholesterol values.

their tissue cholesterol levels remained unchanged. This evidence can therefore be used to support the suggestion that vitamin A administration could cause an increase in fatty acid oxidation in order to provide necessary acetate precursors for cholesterol biosynthesis which has been suggested to be one of the main reactions of vitamin A¹¹. Singh *et al*¹⁰, also provided evidence to show that administration of vitamin A causes mobilization of fatty acids from depot tissue, presumably for presentation to the sites of oxidation for the production of acetate molecules.

It is suggested that in cases where such fatty acids are not so readily available, one may suggest that lipids may then be mobilized from various other vital sites of the body to satisfy the need for oxidation. Such an action could then explain the well known effect namely, emaciation which occurs during vitamin A poisoning. The continuous mobilization and oxidation of such lipids from specific sites might eventually effect permeability of certain tissues and cells in these sites. Such an action may then cause blood cells to escape into

the surroundings and this may explain haemorrhagic manifestations known to occur with vitamin A toxicity.

Effect of Cholesterol Accumulation on Tissue lipids

As previously mentioned, the administration of physiologically tolerable doses of vitamin A or carotenoids caused increased storage of lipids in the livers and kidneys of the animal groups given the experimental diets. (Table 1b, c and Fig. 1, Table 2). Examination of the lipid components making up the total lipids reveals that, accumulation of cholesterol in these tissues was responsible for the high lipid values obtained for groups II and III animals, since without the increased cholesterol values, tissue lipid levels are more or less the same for all groups. Cholesterol accumulation in these tissues therefore may be said to have led to the increased levels of tissue lipids. This fact can be supported by highly significant positive correlations found to exist between total lipids and total cholesterol values for both the chicken and the rat livers

Figure 1.

THE EFFECTS OF VITAMIN A OR CAROTENOID (PALM OIL) FEEDING ON TISSUE LIPID LEVELS

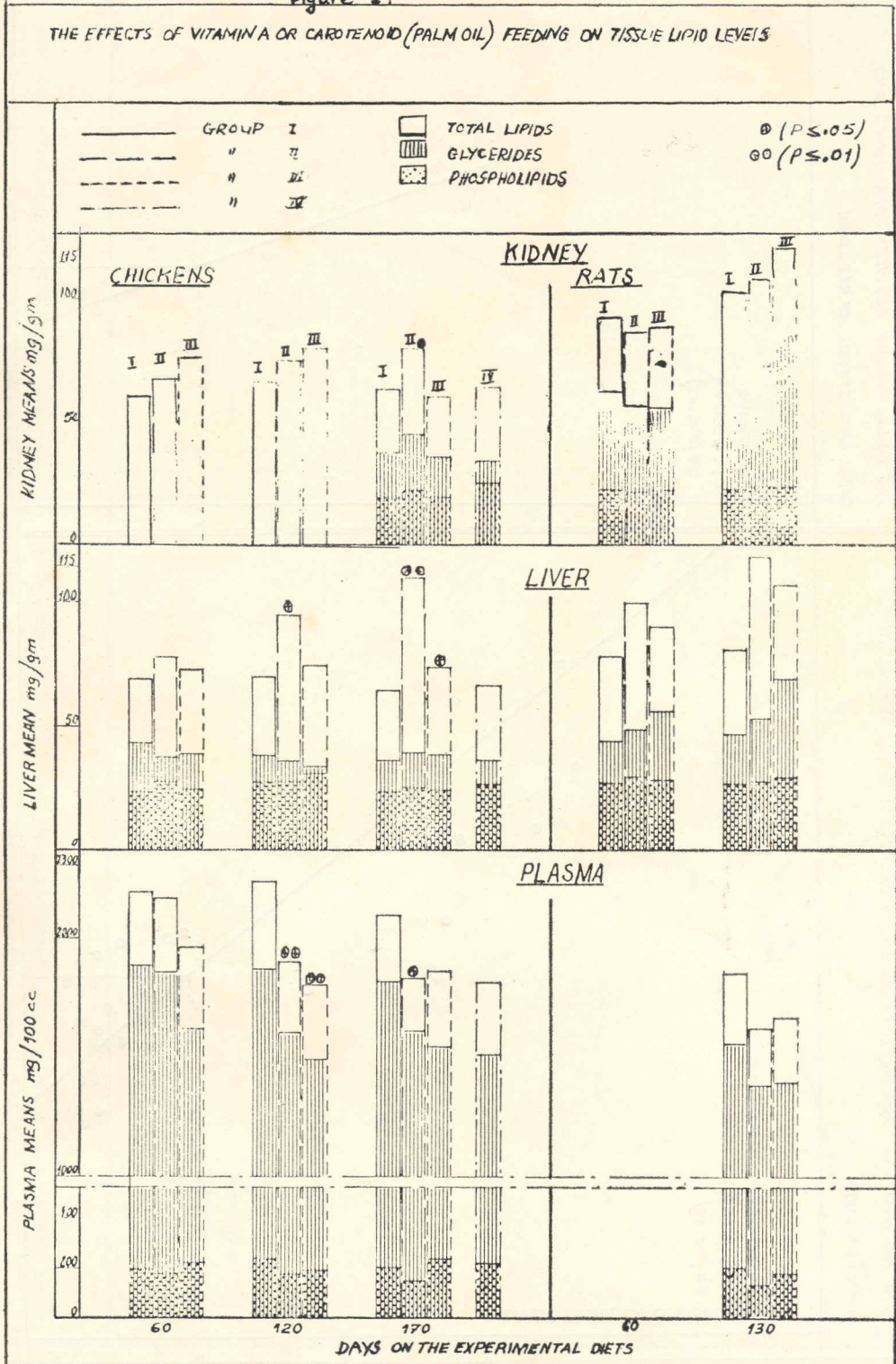


Figure 2

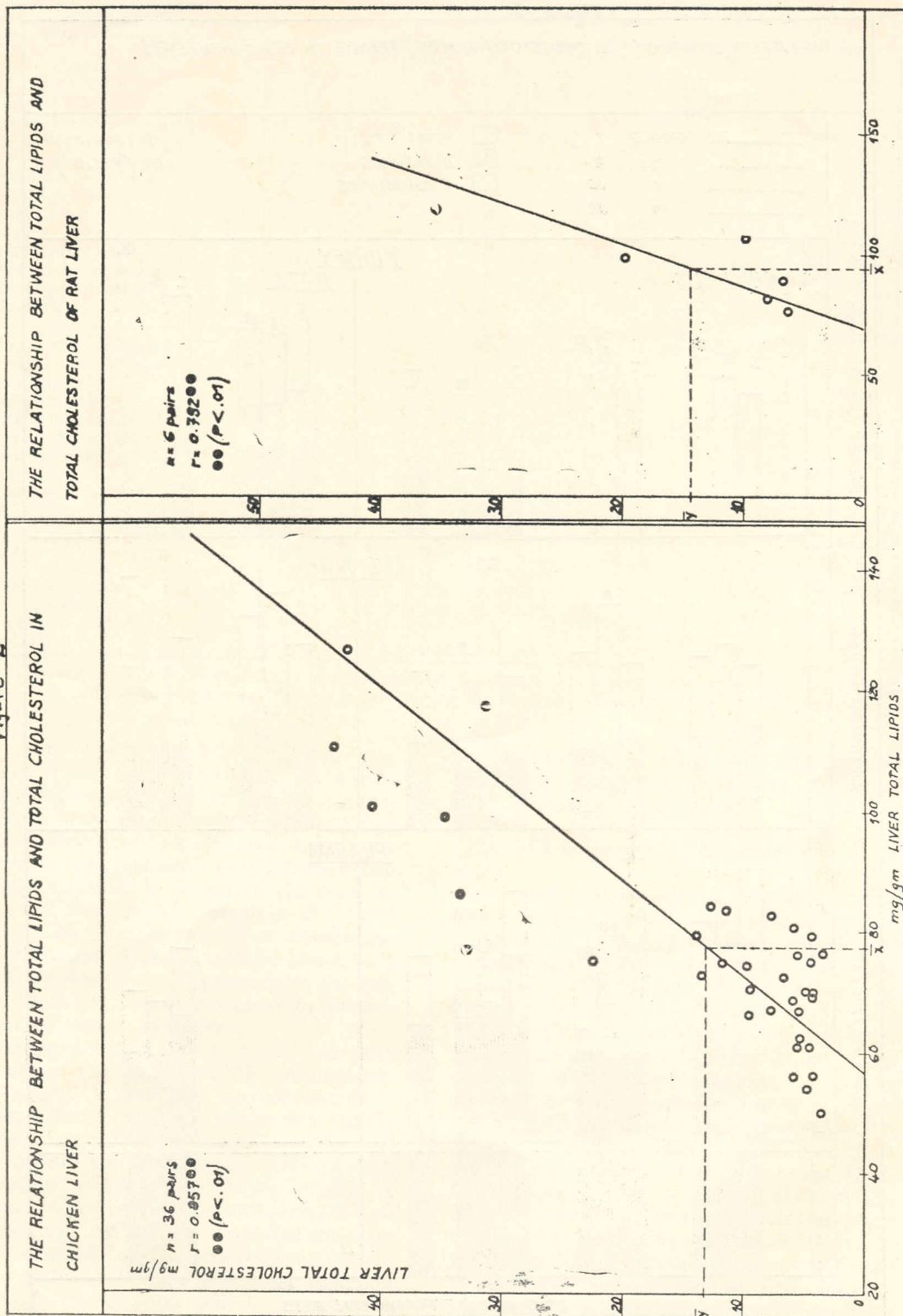


Figure 3

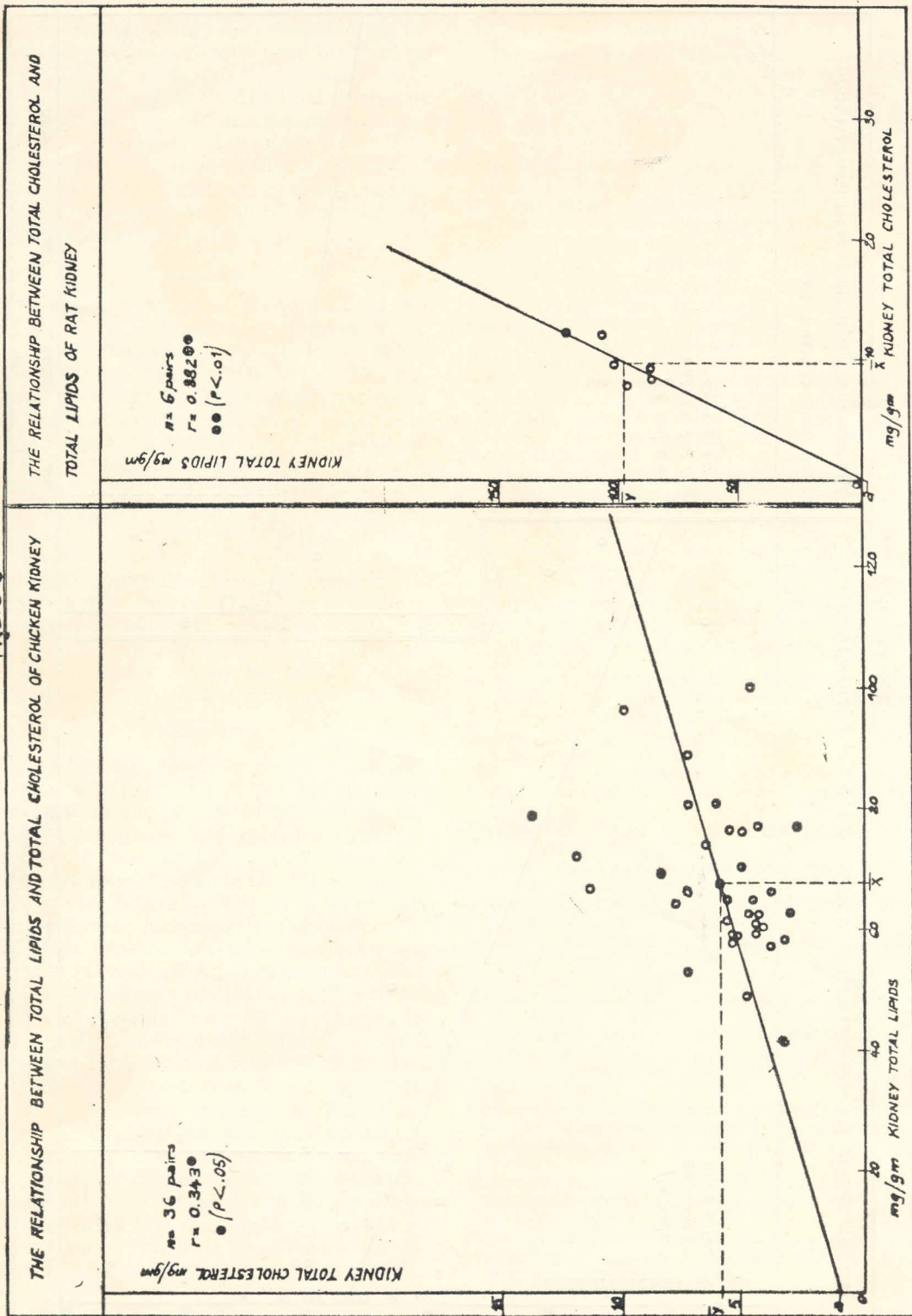
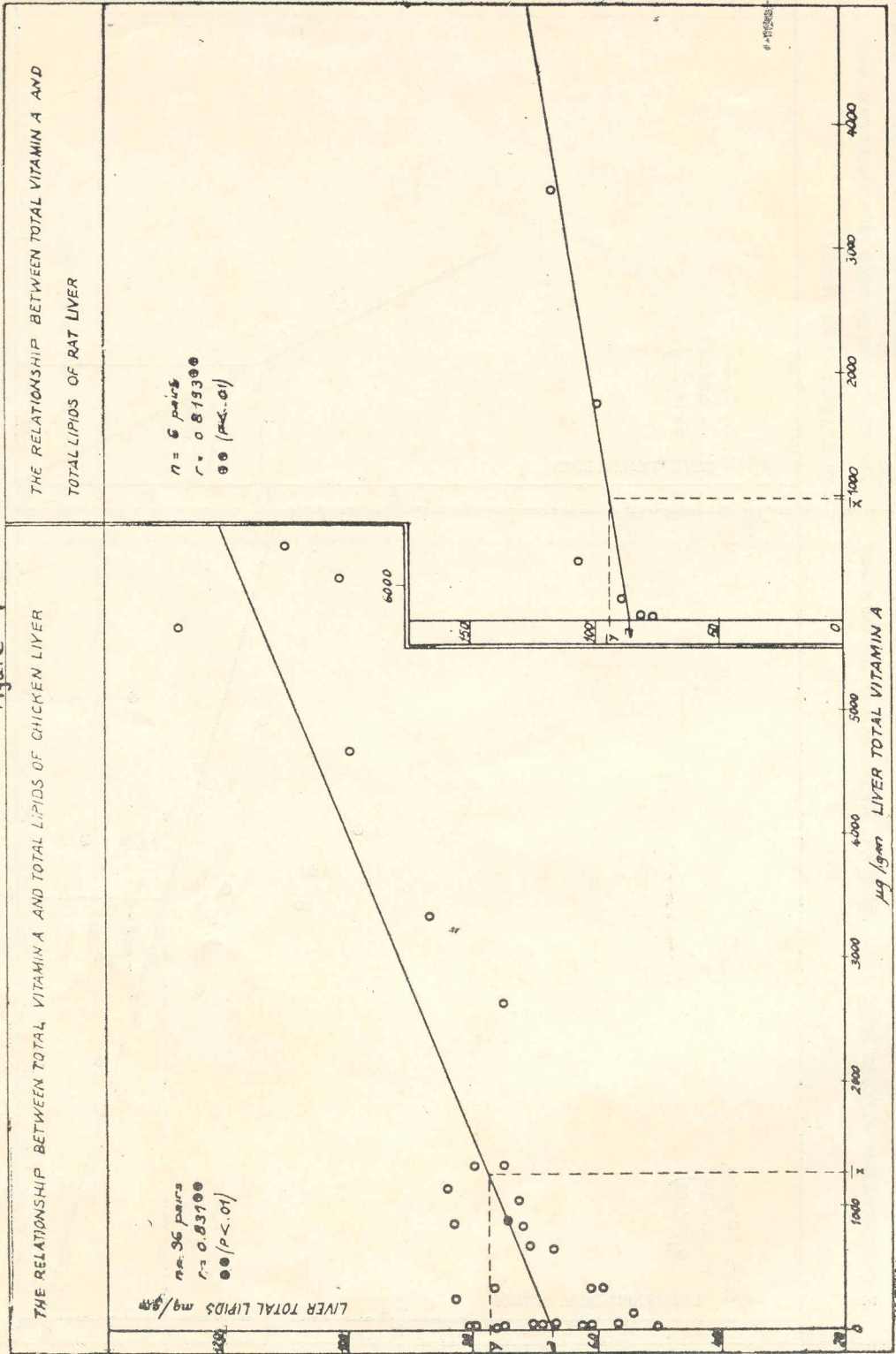


Figure 4



(Fig. 2). The rat kidney also showed a highly significant positive correlation and the chicken kidney showed a less striking but also significant correlation (Fig. 3). The lipid levels can also be seen in Fig. 4 to be significantly correlated to the vitamin A levels in the livers. The tissue lipid accumulation can be said therefore to be dependent on cholesterol accumulation, which in turn can be seen to be dependent on vitamin A intake and storage.

Conclusion

The evidence presented above leads to the conclusion that, Vitamin A administration triggers off a series of metabolic reactions. The major one being the necessity for cholesterol biosynthesis. The suggestion is made that, this necessity for cholesterol biosynthesis brings about an increase in fatty acid oxidation for the production of acetate precursors for the acetate/ vitamin A/ cholesterol/ reaction. The increase in fatty acid oxidation is also suggested to prompt up mobilization of fatty acids either from depot tissue or from dietary fat, when easily available, as brought to light in this study. One may therefore conclude that metabolic effects of vitamin A can be traced through a series of reactions all of which can be said to be linked with its involvement with cholesterol biosynthesis.

Summary

Physiologically tolerable doses of carotenoids in oil, (palm oil) or the feeding of its equivalent in pre-formed vitamin A to experimental animals, had provided sufficient evidence to lead to the proposal that, the administration of vitamin A triggers off a series of metabolic reactions all of which can be traced to the biosynthesis of cholesterol in which the vitamin has been previously reported to be involved. An increase in fatty acid oxidation which showed up as lack of increase in plasma glyceride levels in the vitamin A fed animals was suggested. The livers and kidneys of these animals revealed high levels of lipids. Cholesterol was found to be responsible for these increases in the tissue lipids. Highly significant positive correlations were found to exist between the cholesterol and the lipid levels and also between lipid and vitamin A levels in these tissues. It was therefore concluded that the effects of vitamin A are mediated through its involvement in cholesterol biosynthesis.

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