Trials in the production of fish crackers

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SUMMARY

The technique of fish cracker production has been tested using species of fish caught in Ghana. Storage trials have shown that the product can be kept for over 1 year. Laboratory organoleptic and consumer tests have shown that the product is widely acceptable. With the fairly high protein content of the meal, fish crackers are promising as nutritious snacks.

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RÉSUMÉ

OKRAKU-OFFEI, GLADYS: *Essais de production de biscuits au poisson.* La technique de production de biscuits (crackers) au poisson a été essayée avec des espèces de poisson prises au Ghana. Des essais de conservation ont montré que ce produit peut être gardé plus qu'une année. Des essais organoleptiques de laboratoire et des experts ont prouvé que ce produit est largement acceptable. Avec leur haute teneur en protéines, les biscuits au poisson semblent prometteurs pour de légers repas très nourrissants.

Introduction

Fish is a highly perishable food and much attention has consequently been focused on its preservation, especially by the conventional methods such as smoking, drying, salting and fermentation. New methods have, however, been developed in which the fish is processed into a completely different form of food. Van Veen (1953) reported on a preparation called *krupuk* widely used in South-east Asia. Van Veen (1965) later indicated that this preparation was also popular in Indonesia. Fish flakes (fish crackers) prepared from cooked and deodorized fish was reported by Venugopalan & Govindan (1967).

Non-fatty fish has been recommended for the processing of fish crackers (Meyboom, 1970; personal communication from B. Meyboom, Institute for Fishery Products, TNO, Ijmuiden, the Netherlands). The objective of this work was to apply the methods of fish cracker preparation to non-fatty fish species generally caught in Ghana and to introduce this as an additional use of fish in Ghana.

Materials and methods

Species of fish used

Four species of non-fatty fish, namely, cassava fish (*Cynoscion senegalla*), grouper (*Epinephelus* sp.), red snapper (*Lutjanus fulgen*) and grey snapper (*Lutjanus agennes*) were selected and used for this study. They were bought fresh from Tema beach and fillets made from them. The fillets were minced in a Hobart mincer.

Chemical analysis of fish

The proximate composition of the flesh of these species of fish was determined by the following analytical methods:

Moisture was determined on 5-g well-mixed sample in a ventilated drying oven at 105 ± 1°C to a constant weight.

Protein. Nitrogen was determined on 2-g mixed sample by macro-Kjeldahl method and percentage protein calculated as N × 6·25.

Fat was extracted from 5-g sample by Soxhlet extraction method for about 8 h.

Ash. 5-g minced sample in a silica crucible was

heated on a burner in a fume cupboard for some time to remove excess water and later ashed in electric furnace at 550°C.

Calcium was determined by the standard method as described by A.O.A.C. (1970).

Phosphorus was determined according to the method as described by Fogg & Wilkinson (1958).

Iron was determined by the method described by Food Research Institute (1968).

Recipes for fish cracker preparation

Three recipes were tried (Table 1). Recipe 1 was that recommended by Meyboom (1970; personal communication). Recipes 2 and 3 were modifications of Recipe 1.

Cassava starch in Recipes 1 and 3 was pure dried starch prepared from fresh cassava in the laboratory. Gelatinized starch (tapioca) was bought from the market and ground into powder. This tapioca powder was used in Recipe 2 in place of raw cassava starch.

Fish cracker preparation

The ingredients for each recipe were weighed in the specified quantities and mixed well in a Hobart mixer. The specified volume of water was added a little at a time until a homogeneous mass, which would not break easily, was obtained.

TABLE 1

Recipes for Fish Cracker Preparation

Recipe 1

500 g minced fish fillet
1000 g cassaca starch
50 g table salt
50 g sugar
1000 cm^3 water
(ratio of fish to starch 1 : 2)

Recipe 2

500 g minced fish fillet
1000 g tapioca powder
50 g table salt
50 g sugar
1000 cm^3 water
(ratio of fish to tapioca 1 : 2)

Recipe 3

1000 g minced fish fillet
1000 g cassava starch
50 g table salt
50 g sugar
1000 cm^3 water
(ratio of fish to starch 1 : 1)

From this mass, 200–g rolls were made, and a square piece of soft calico (0·1 m^2) wrapped around each roll. These were suspended by a string in hot water at 75°C for 1·5 h after which they were steamed at 100°C for 4 h.

The cooked rolls were left at a temperature of −20°C overnight to freeze. The wrappers were removed the next day; the rolls were immediately cut up into thin slices, dried at 60°C for 2 h, allowed to cool and packed in sealed polyethylene bags.

Chemical and organoleptic tests

The dried fish crackers were analysed for moisture and protein contents.

Samples of the dried fish crackers were fried in very hot (200°C) salad oil for 2–3 s. A panel of six tasters evaluated the quality of the product. Good fish cracker should on frying swell up or puff up to three or four times their original dried size and must be light and crispy. These were the main criteria used in evaluating all the fried fish cracker samples.

Storage trials

To assess the keeping quality of fish cracker the following experiments were performed:

(a) samples of dried fish crackers in sealed polyethylene bags were kept at room temperature (28–30°C) and about 80% relative humidity for 1 year, from March 1972 to March 1973;
(b) samples of fried fish cracker in sealed polyethylene bags were kept at room temperature (28–30°C) and about 80% relative humidity;
(c) samples of fried fish crackers in sealed polyethylene bags were kept in a refrigerator at 5°C.

All the samples were examined daily.

Results and discussion

The proximate composition of the flesh of the four fish species used in making the fish crackers is shown in Table 2. The fat content of the species of fish was low.

Chemical analyses of the dried fish crackers showed that the moisture and protein contents were in the ranges 8–10% and 12–16% respectively.

Taste panel scores for fish crackers prepared

TABLE 2

Proximate Composition of the Flesh of the Four Types of Fish Used in Making Fish Crackers

Content	*Cassava fish* (Cynoscion senegalla)	*Grouper* (Epinephelus *sp.*)	*Red snapper* (Lutjanus fulgen)	*Grey snapper* (Lutjanus agennes)
Moisture %	78·6	78·4	79·9	76·4
Protein (N × 6·25) %	18·4	19·2	18·5	14·4
Fat %	2·8	1·0	1·5	1·5
Ash %	1·0	1·4	1·0	1·1
Calcium (mg/100 g)	1·2	41·0	26·0	53·0
Phosphorus (mg/100 g)	200	188	73	92
Iron (mg/100 g)	1·2	1·2	1·4	1·4

TABLE 3

Average Comparative Scores for Fried Fish Crackers Prepared with Three Recipes

Recipe	*Selling capacity*				*Crispness*				*Flavour*			
	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
1	2	2	2	2	3	2·5	3	3	2	2	2	2
2	1	1	1·5	1	1	1	1	1	2	1	2	2
3	4	4	4	4	4	4	4	4	3	3	3	4

F_1 = Cassava fish cracker; F_2 = Grouper cracker;
F_3 = Grey snapper cracker; F_4 = Red snapper cracker.
Subjective scores: 4 = Excellent, 3 = Good, 2 = Fair, 1 = Poor.

from the four species of fish were very similar (Table 3). This indicates that all four types of fish are suitable for making fish crackers.

Although the mixed mass should normally be wet but not sticky, the mass prepared with Recipe 1 was found to be too sticky. The swelling capacity of the dried crackers was also not up to standard and the product had a starchy flavour. As a result of this, Recipe 2 was tried in which pregelatinized starch was used. The swelling capacity of the flakes prepared with Recipe 2 was negligible. Recipe 3 which had a reduced amount of starch was the best.

Taste panel assessment of the dried fish cracker samples stored for 1 year at room temperature of 28–30°C and about 80% relative humidity showed that they were still light and crispy. There had been no change in taste. Fried samples kept at room temperature of 28–30°C remained crispy and in good taste and flavour for 12 days after which they became flat and slightly rancid. The fried refrigerated samples remained in good condition for 4 weeks.

Conclusion

Fish crackers can be successfully processed from cassava fish, grouper, red snapper and grey snapper. The storage trials show that fish in the form of dried fish crackers can be stored for at least 1 year. The protein content of the product, though lower than that of fish, was found to be higher than that of most cereal products in use in Ghana, which have a protein range of 1–12%.

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REFERENCES

A.O.A.C. (1970) *Official methods of analysis*, 11th ed., pp. 294–310. Washington, D.C.: Association of Official Analytical Chemists.

Fogg, D. N. & **Wilkinson, N. T.** (1958) The colorimetric determination of phosphorus. *Analyst, Lond.* **83,** 406–414.

Food Research Institute (1968) *Food analysis sheet* No. **3** (Mimeo.). Accra: the Institute.

Van Veen, A. G. (1953) Fish preservation in South-east Asia. *Adv. Fd Res.* **4,** 221–227.

Van Veen, A. G. (1965) Fermented and dried sea food products in South-east Asia. In *Fish as food,* vol. **3,** pp. 244–245. New York: Academic Press.

Venegopalan, V. & **Govindan, T. K.** (1967) Utilization of trash fish—Preparation of fish flake. *Fish. Tech.* **4**(1), 35–43.

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RETINOL IN CHOLESTROL BIOSYNTHESIS: EFFECT ON LIPID METABOLISM

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Introduction

Josephs[1] reported hyperlipemia with high intake of vitamin A as well as with carotenoids. Van Bruggen and Struamfjord[2], observed that long-term administration of large doses of vitamin A to patients raised their plasma cholesterol and phospholipid levels. Pallotta and Krause[3] observed a rise in serum phospholipid levels with administration of vitamin A. Misra[4] 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] Misra[9] 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. Kordylas[11] 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 devided 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 IUper 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 I U 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 reported[11].

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) mixture[13] 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 Sauberlich[14]. Lipid phophorus for plasma was determined by the method of Zilversmit and Davies[15]. To obtain phospholipid values the lipid phosphorus results were multiplied by 25 (1:25) being the ratio of phophorus in the phosphotide contained in an individual lipid[16]. Plasma total and free cholesterol were determined using the method by Searcy and Bergquist[13]. 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 Eden[18]. 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 bath[20] 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 reported[11]. Methods used for tissue vitamin A determination was as reported[11].

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 Practique 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 IUper 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 published[11]. Total Vitamin A, Vitamin A ester and alcohol levels of plasma, liver and kidneys are as previously reported[11].

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 tissues[21]. 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 lipoproteins[22]. According to Steinberg[22], 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 1*a*: *The effects of feeding vitamin A or Carotenoids (Palm Oil) on the mean values ± SD of Plasma Lipids of Chickens.*

Groups	*I* *Oil*	*II* *Oil + Oit. A*	*III* *Palm Oil*	*IV* *No Oil*
mg/100 CC	M ± SD	M ± SD	M ± SD	M
60 *days on diet*				
	(3) 1/			
Total lipids...	2 183.0 ± 170.2	2 156.0 ± 318.4	1 969.0 ± 159.9	—
Phospholipids	196.3 ± 19.2	168.3 ± 32.1	206.9 ± 48.4	—
Total cholesterol	99.0 ± 6.2	118.7 ± 32.2	127.5 ± 36.4	—
Free cholesterol	14.7 ± 5.0	19.5 ± 5.7	21.0 ± 12.3	—
Cholesterol ester 2/	84.3 —	99.2 —	106.5 —	—
Glycerides 3/	1 887.7 —	1 869.0 —	1 634.6 —	—
120 *days on diet*				
			(3)	
Total lipids...	2 225.0 ± 177.9	1 900.0 ± 54.0*	1 808.0 ± 101.0**	—
Phospholipids	203.8 ± 36.5	179.0 ± 41.4	199.4 ± 21.7	—
Total cholesterol	150.0 ± 42.7	97.0 ± 11.7	97.0 ± 11.5*	—
Free cholesterol	33.5 ± 9.3	19.0 ± 3.3	18.0 ± 2.2	—
Cholesterol ester 2/	117.2 —	78.0 —	79.0 —	—
Glycerides 3/	1 870.5 —	1 624.0 —	1 511.6 —	—
170 *days on diet*				
			(3)	(3)
Total lipids...	2 094.0 ± 235.8	1 838.0 ± 101.0*	1 858.0 ± 87.8	1 817.0
Phospholipids	186.1 ± 6.0	147.2 ± 9.8	225.1 ± 1.6	206.5
Total cholesterol	78.0 ± 4.2	68.0 ± 5.0*	72.0 ± 11.1	75.0
Free cholesterol	16.0 ± 4.7	18.0 ± 6.1	27.0 ± 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 1*b*: *The effects of Feeding Vitamin A or Carotenoids (Palm Oil) on the mean values ± SD of Liver Lipids of chickens.*

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

Groups	*I* *Oil*	*II* *Oil + Vit. A*	*III* *Palm Oil*	*IV* *No Oil*
mg / gm	M ± SD	M ± SD	M ± SD	M
60 *days on diet*	(3) 1/			
Total lipids...	59.5 ± 7.5	64.8 ± 11.4	74.7 ± 7.6	—
Phospholipids	— —	— —	— —	—
Total cholesterol	5.4 ± 0.5	6.6 ± 1.8	4.3 ± 1.7	—
Free cholesterol	3.0 ± 0.6	3.0 ± 0.9	2.9 ± 1.6	—
Cholesterol ester 2/	2.4 —	3.6 —	1.4 —	—
Glycerides 3/	— —	— —	— —	—
120 *days on diet*			(3)	
Total lipids...	64.9 ± 8.5	72.5 ± 12.6	77.7 ± 19.6	—
Phospholipids	— —	— —	— —	—
Total cholesterol	4.6 ± 1.0	6.9 ± 1.3*	5.3 ± 1.5	—
Free cholesterol	3.0 ± 1.0	3.2 ± 0.8	3.0 ± 0.5	—
Cholesterol ester 2/	1.6 —	3.7 —	2.3 —	—
Glycerides 3/	— —	— —	— —	—
170 *days on diet*			(3)	(3)
Total lipids...	61.1 ± 14.4	78.4 ± 12.8	59.6 ± 2.8	46.1
Phospholipids	19.7 ± 1.4	21.4 ± 1.1	19.7 ± 1.5	25.1
Total cholesterol	4.3 ± 0.7	11.7 ± 1.5**	4.3 ± 0.5	5.0
Free cholesterol	3.1 ± 0.9	4.0 ± 0.9	3.6 ± 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 elsewhere[11]. Table 1*b*,1*c*, 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.

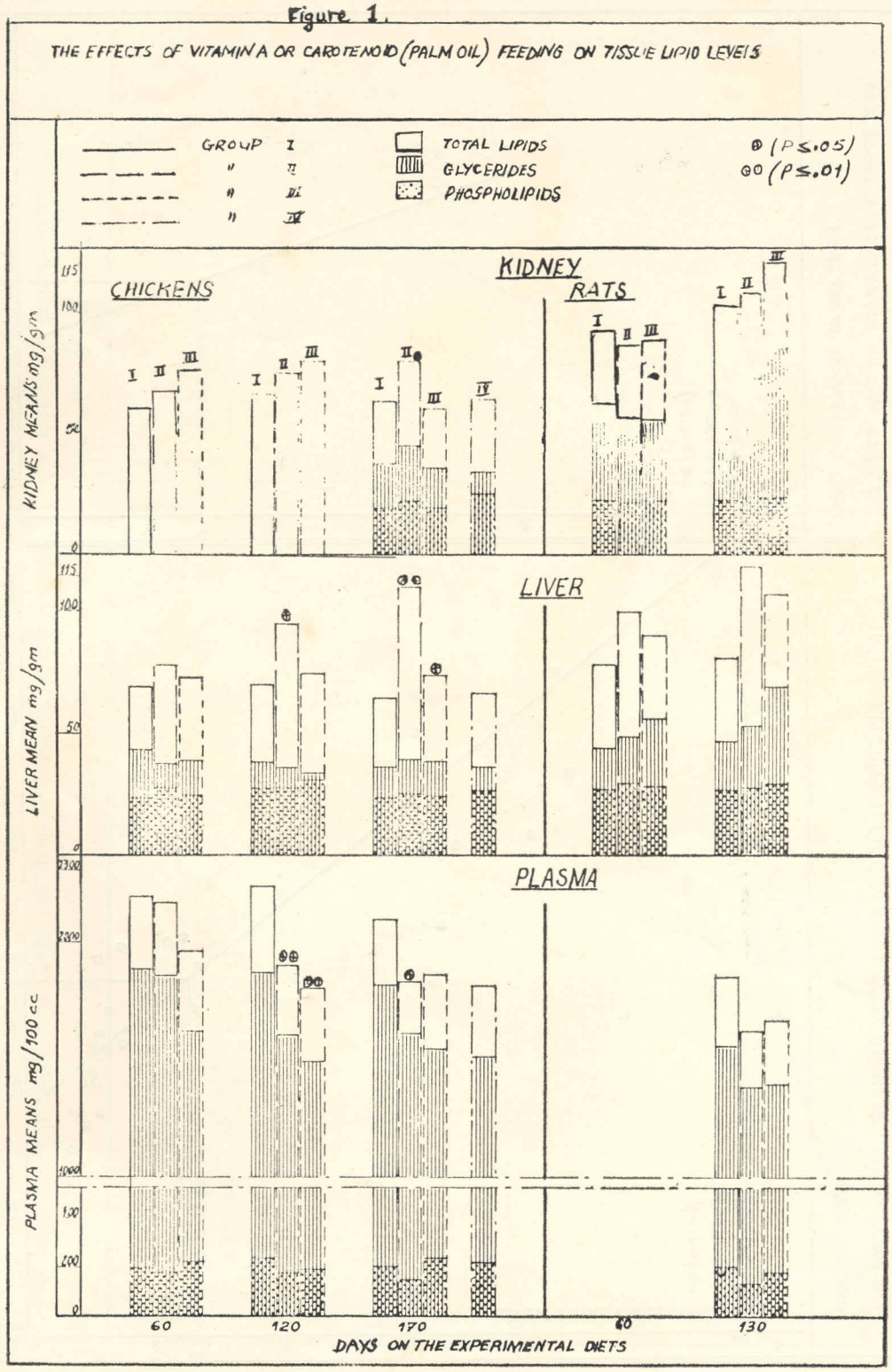
their tissue cholesterol levels remained unchanged. This evidence can therefore be used to support the suggestion that vitamin A administration could cause an increase in fatty acid oxidation in order to provide necessary acetate procursors for cholesterol biosynthesis which has been suggested to be one of the main reactions of vitamin A[11]. Singh *et al*[10], also provided evidence to show that administration of vitamin A causes mobilization of fatty acids from deport 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 1*b*, 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
GROUP I
" II
" III
" IV
TOTAL LIPIDS
GLYCERIDES
PHOSPHOLIPIDS
⊕ (P≤.05)
⊕⊕ (P≤.01)
KIDNEY
CHICKENS
RATS
LIVER
PLASMA
KIDNEY MEANS mg/gm
LIVER MEAN mg/gm
PLASMA MEANS mg/100 cc
115
100
50
0
2300
2000
1000
150
100
60
120
170
60
130
DAYS ON THE EXPERIMENTAL DIETS

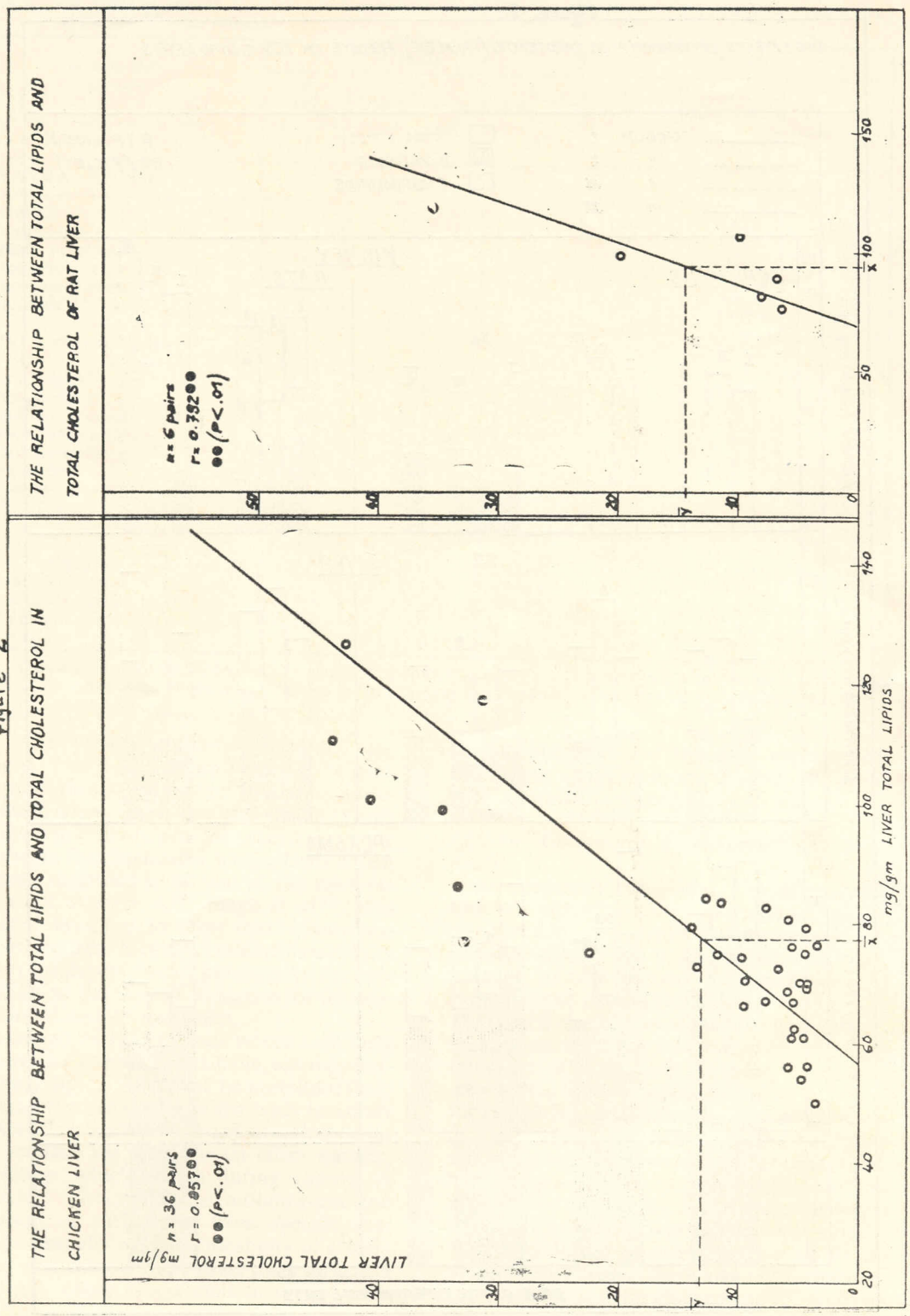

THE RELATIONSHIP BETWEEN TOTAL LIPIDS AND TOTAL CHOLESTEROL OF RAT LIVER
n = 6 pairs
r = 0.7320 ●●
●● (P<.01)
THE RELATIONSHIP BETWEEN TOTAL LIPIDS AND TOTAL CHOLESTEROL IN CHICKEN LIVER
n = 36 pairs
r = 0.8578 ●●
●● (P<.01)
LIVER TOTAL CHOLESTEROL mg/gm
mg/gm LIVER TOTAL LIPIDS

Figure 2

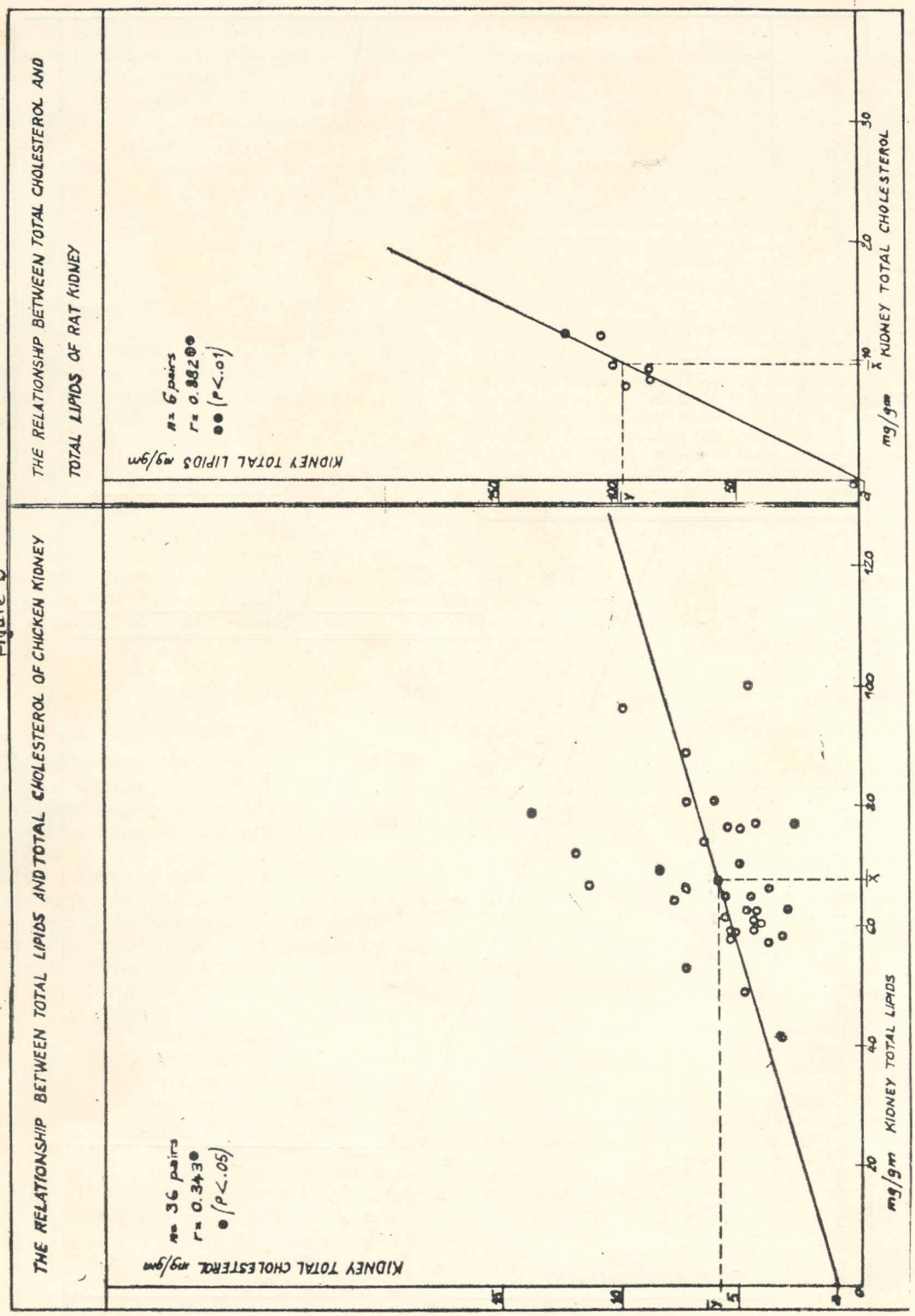


Figure 3
THE RELATIONSHIP BETWEEN TOTAL LIPIDS AND TOTAL CHOLESTEROL OF CHICKEN KIDNEY
KIDNEY TOTAL CHOLESTEROL mg/gm
n= 36 pairs
r= 0.343●
●(P<.05)
mg/gm KIDNEY TOTAL LIPIDS
THE RELATIONSHIP BETWEEN TOTAL CHOLESTEROL AND TOTAL LIPIDS OF RAT KIDNEY
KIDNEY TOTAL LIPIDS mg/gm
n= 6 pairs
r= 0.882●●
●●(P<.01)
mg/gm KIDNEY TOTAL CHOLESTEROL

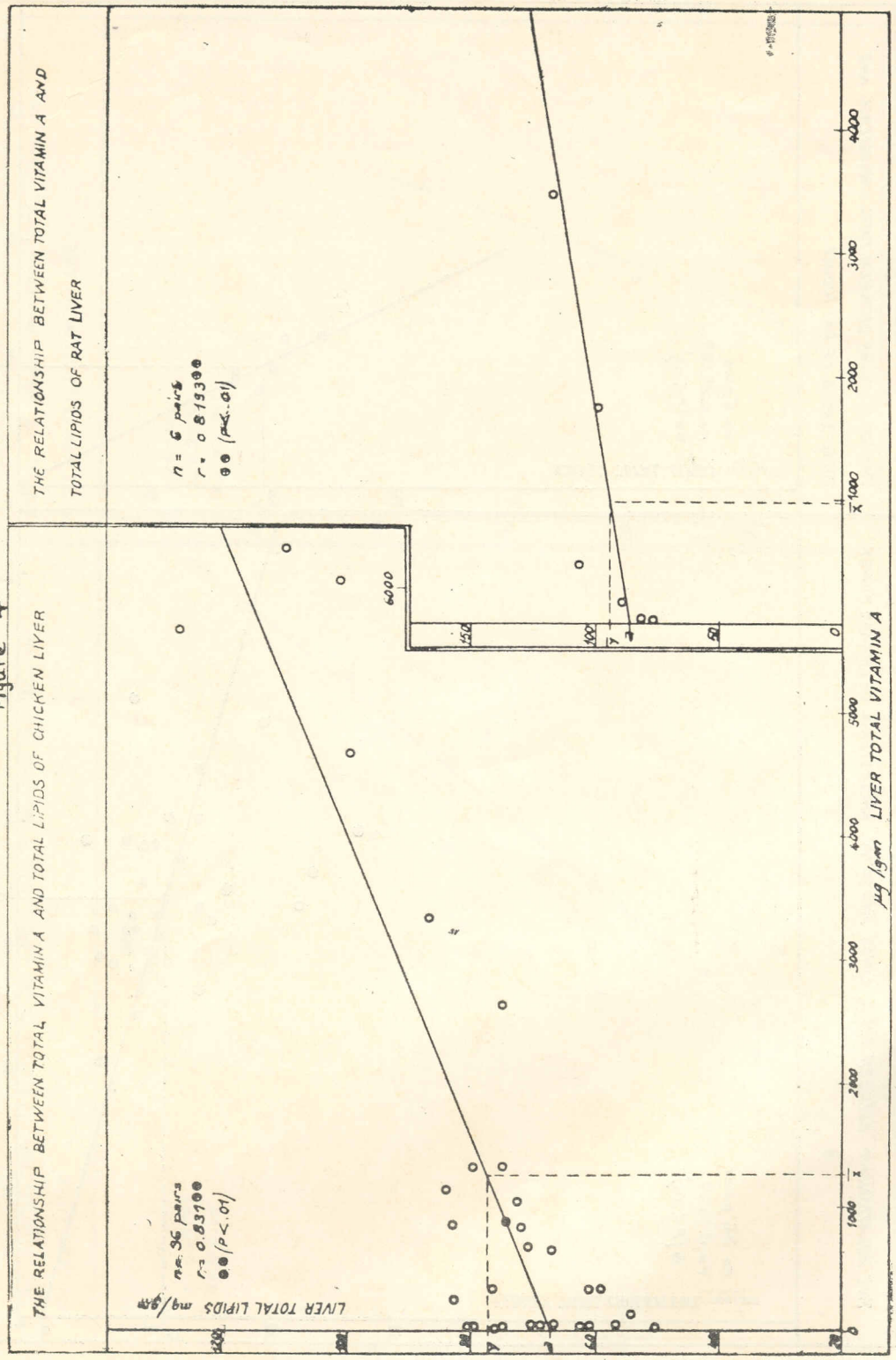

THE RELATIONSHIP BETWEEN TOTAL VITAMIN A AND TOTAL LIPIDS OF CHICKEN LIVER
THE RELATIONSHIP BETWEEN TOTAL VITAMIN A AND
TOTAL LIPIDS OF RAT LIVER
n = 6 pairs
r = 0.819300
●● (p<.01)
n= 36 pairs
r = 0.83700
●● (p<.01)
LIVER TOTAL LIPIDS mg/gm
μg/gm LIVER TOTAL VITAMIN A

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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References

[1]Joseph, H.W. Hypervitaminosis A and Carotenaemia. *Amer. J. Dis. Child.* **67**: 33–43 (1944).

[2]Van Bruggen, J.T. and Strauamfjord, J. V. and Ore, A. High vitamin A intake and blood levels of cholesterol phospholipids, carotene, and vitamins C, A and E. *J. Lab. and Clin. Med.* **22** : 67–74 (1948)

[3]Pallotta, M.A. and Krause, R. F. Effect of vitamin A on certain blood constituents of geriatric subjects. *Amer. J. Clin. Rutri.* **13** : 201–208 (1963).

[4]Misra, U.K. Effect of hypervitaminosis A on rat plasma lipids: Rat plasma phospholipids in hyper vitaminosis A. *Naturwissen schaften* **52**: 188, 306–307 (1965).

[5]Domagk, G. and Von Dobeneck, P. Uber. Histologische Befunele bei der Uberdosierung mit Vitamin A—Konzentrat. *Virchow's Arch physiol Path. Anat.* **290** : 385–395 (1933). Cited by moore, T. Vitamin A Elsevier Publishing Loo V.Y. (1957).

[6]Ray, A. and Sadhu, D.P. Carbohydrate metabolism in hypervitaminosis A *Nature* **184** *suppl.* 17 : 1323 (1959).

[7]Squibb, R.L. Vitamin Atoxicity and its effect on liver nucleic acids, proteins and lipids in the chick: *Poultry Sci.* **42** : 1332–1335 (1963).

[8]Misra, U.K. Lipid metabolism in hypervitaminosis A.*Nature* **209** : 910 (1966).

[9]Misra, U.K. Liver Lipids of rats administered excessive amounts of retinol. *Can. J. Bioch.* **46**: 697–701 (1968).

[10]Singh, V.N., Singh, M. and Venkitasubramanian, T. A. Early effects of feeding excess vitamin A: mechanism of fatty liver production in rats. *J. Lipid. Res.* 10 : 395–401 (1969).

[11]Kordylas, J.M. Retinol in cholesterol biosynthesis: *Ghana Med. J.* **11** : 71–82 (1972).

[12]Glick, D. Methods of Biochemical Analyses Vol. 2: 83–111: Interscience Publishers, N.Y. (1955).

[13]Searcy, R.L. and Bergquist, L.M. A new colour reaction for the quantitation of serum cholesterol. *Clin. Chim. Act.* **5** : 192–199 (1960).

[14]Leveille, G.A. and Sauberlich, H.E. Lipid changes in plasma alpha lipoproteins, liver and aorta of chicks fed different fats. *Proc. Soc. Exp. Biol. Med.* 1:12 300–303 (1963).

[15]Zilversmit D.B. and Davis, A.K. Micro determination of plasma phospholipids by trichloroacetic acid precipitation. **4**. *Lab. & Clin. Med.* **35** : 155–160 (1950).

[16]Marinetti, G.V. Chromatographic separation identification and analysis of phosphatides. *J. Lipid Res.* **3** : 1–20 (1962).

[17]Glick, D. Methods of Biochemical Analyses—Determination of vitamin A. Interscience Publichers, N.Y. 4 : 43–98. (1957).

18Eden, E. A micro method for separating free and esterified vitamin A *Bioch. J.* **46** : 259–261 (1950).

19Nishida, T., Ueno, A.'and Kummerow, F. A. Effect of dietary protein on the metabolism of sodium acetate 1–C14 in chicks. *J. Rut.* **71** : 379–386 (1960).

20Youngburg, G.E. and Youngburg, M.V. Phosphorus metabolism; system of blood phosphorus analysis. *J. Lab. Clin. Med.* **16** : 158–166. (1930).

21Fredrickson, D.A. Levy, R.I. and Lees, R. S. Fat Transport in lipoproteins—An integrated approach to mechanisms and disorders. *New Eng. J. Med.* **276** : 34–44 (1967).

22Steinberg, D. The control of Lipid Metabolism. J. K· Grant, Editor, Academic Press, Inc. London (1963)