DIETARY EFFECTS ON THE LEVELS OF SERUM CHOLESTEROL AND SERUM TOTAL LIPIDS IN THE GROWING CHICK

by

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ABSTRACT

An investigation was carried out into the effects of some dietary factors on the serum cholesterol and serum total lipid levels in both normal and hyperthyroid chicks. Dietary factors investigated were the effects of two levels of protein, two levels of fat, and two levels of vitamins. Basal diets were fed at either a 20% or 26% protein level and when dietary fat was investigated the dextrose of the basal diet was substituted for a hydrogenated vegetable oil to make up 12% of the diet. The vitamin supplement in the high vitamin diets fed consisted of additional amounts of the following B complex vitamins: choline chloride, calcium panthothenate, folacin, niacin and riboflavin.

Chicks were rendered hyperthyroid by feeding diets containing 0.02% iodinated casein. Hypothyroidism was induced by the feeding of 0.1% thiouracil.

Normal chicks showed lower levels of serum cholesterol and serum total lipids when they were fed basal diets consisting of 26% protein level than when fed basal diets consisting of a 20% protein level.

Normal chicks fed high fat diets showed higher levels of serum cholesterol and serum total lipids than normal chicks fed low fat diets.

Normal chicks fed diets low in the B complex vitamins showed higher levels of serum cholesterol than normal chicks fed diets high in the B complex vitamins.

The growth rate of chicks rendered hyperthyroid varied. In many instances hyperthyroid chicks grew at a significantly faster rate than normal chicks while in some instances no differences were noted. In some cases chicks rendered hyperthyroid showed depressed growth rates. No clear explanation could be given for such an effect on the growth rate of hyperthryoid chicks. It seems reasonable, however, to suspect that seasonal changes may affect thyroid activity and consequently the growth rate of the chicks in question.

No differences were noted between the serum cholesterol and serum lipid levels from hyperthryoid chicks fed the basal diets containing 20% and 26% protein. Hyperthyroid chicks, however, showed lower levels of serum cholesterol than normal chicks when the diet fed was low in the B complex vitamins, calcium panthothenate, choline chloride, folacin, niacin and riboflavin.

The effect on the serum cholesterol and serum total lipids when the chicks were rendered hyperthyroid varied.

The results suggest some interaction between thyroid state and diet on the serum cholesterol and serum total lipids.

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INTRODUCTION

There exists extensive literature on the effects of diet on the serum cholesterol level of various animal species. It is an established fact that administration of fat in the diet affects serum cholesterol level. In general, unsaturation of the fat has been associated with lowering of the serum cholesterol values. Also large quantities of saturated fats in the animals' diet have been associated with high levels of cholesterol in its blood serum. Recent investigations have demonstrated an increase in the serum cholesterol levels of chicks when corn oil (an unsaturated oil) has been included in the chicks' diet. In view of these findings it could no longer be generally said that unsaturated fats are associated with low levels of serum cholesterol in all animal species. It appears as has been suggested by some investigators that some factor, other than the degree of saturation of a fat, is responsible for influencing the serum cholesterol level of some animal species.

It has also been established that an adequate protein level in an animal's diet is an important factor in maintaining normal levels of serum cholesterol in the animal. By increasing the protein level of the diet, a decrease in serum cholesterol level can be produced.

The function of the thyroid gland has been shown to infleunce lipid metabolism in animal species. The general trend appears to be that lower serum cholesterol levels are associated with hyperthyroidism whereas hypothyroidism is associated with elevated serum cholesterol levels.

In general, the influence of vitamins on the serum cholesterol level of chicks is uncertain as there appears to be very little work done in this field. A number of vitamin deficiency states are associated with inanition and it is difficult to differentiate between a direct effect of vitamin deficiency or an indirect effect due to inanition.

In view of the above findings on the effect of diet and thyroid activity, on serum cholesterol levels in certain animal species, it seemed of interest to study the effects of certain dietary factors on the serum cholesterol levels of normal and hyperthyroid chicks.

LITERATURE REVIEW

There is a large volume of evidence suggesting that diet has an effect on serum lipid levels. The concept that there may exist a relationship between lipids and atherosclerosis has been entertained for over fifty years, ever since rabbit arterial lesions were produced by feeding cholesterol. Although this association of lipid metabolism and atherosclerosis appears to be well established, it is still not certain whether the association is one of cause and effect. It should be pointed out that many factors affect body lipid metabolism aside from diet. Age, sex, heredity, stress and other variables have been shown to affect lipid metabolism.

Diets rich in fat but not necessarily in cholesterol have been thought to be associated in man with high levels of plasma cholesterol. Keys and his associates (1952) have been the chief proponent of this concept, although recently Keys et al (1957) presented data to show that the type as well as the quantity of fat may play a role in determining the levels of cholesterol in the serum of blood. Prior to this, animal and vegetable fats were regarded as interchangeable in so far as their effect on the serum cholesterol was concerned. With further nutritional studies some scientists have come to associate higher levels of serum cholesterol with the intake of animal or saturated fatty acids, and the lack of unsaturated fatty acids. Among them are Ahrens et al (1957), Malmros and Wigand (1957).

In man, Beveridge et al (1955) have shown strong evidence indicating that intake of large quantities of vegetable fat or unsaturated oils in the diet reduces the plasma cholesterol significantly, and this fall can be overcome or reversed by the simultaneous feeding of similar amounts of animal fats or hydrogenated vegetable oils.

In experiments using mature cockerels, Kokatnur et al (1958) have concluded that serum cholesterol values were increased more rapidly in chickens which have been fed fat than those fed an essentially fat-free diet. As early as 1912 Stuckey carried out certain experiments from which he was able to discover that the portion of the animals diet responsible for the appearance of lesions in its blood vessels was the lipid portion and not the protein portion. From his experiments he noted that the egg yolk fat was responsible for the lesions produced in the animals' blood vessels.

Newburg and Clarkson (1922) however, were of a different opinion, for they were able to produce atherosclerosis in rabbits by feeding diets rich in protein. They achieved this condition in rabbits by feeding protein levels of 27% and 36%.

Meeker and Kesten (1940) produced hypercholesteremia in rabbits by feeding these animals high protein diets. In dogs, hypercholesteremia was produced by Li, Hough, Monahan and Freeman (1943) when they fed dogs a high fat cholesterol diet deficient in protein. An increase in serum cholesterol of 172% to 225% was achieved. When they fed a diet containing fat and cholesterol with adequate amounts of protein, serum cholesterol concentration, although increased, was not increased to the extent it would have been if the protein supplied was inadequate. Increases in serum cholesterol level of dogs when fed

(a) High fat diets with added cholesterol; and

(b) High fat diets, deficient in protein, with or without cholesterol

were again achieved by Li and Freeman (1946).

Moyer, Kritchevsky, Logan and Cox (1956) reported a progressive drop in serum cholesterol levels of rats by increasing the protein content of the diet. They used casein as their principal protein of the diet and fed it at four levels in addition to 0.5% choline chloride. In another experiment Isolated soya protein was used at levels of 10%, 40%, and 60% and with all their diets containing 25% lard, 5% H.M.W. salt mix, 1% cholic acid and 2% cholesterol. Fillios et al (1956) reported a similar drop in serum cholesterol concentration in rats fed a high protein level. They found that when the diets of 10%, 20%, and 60% casein, and 20% alpha protein were fed to rats, there was a significant drop in serum cholesterol concentration of these rats after being fed a 20% casein ration for a period of fifty-six days. The group of rats fed 60% casein diets displayed cholesteremic responses which were significantly lower than any of the three other groups. These workers concluded from their experiments that the higher the protein level in the diet, the lower the blood cholesterol level of the animals fed these diets.

Jones and Huffman (1956) demonstrated in rats that reduction or elevation of dietary casein beyond a modest range (12-18%) will lead to ultimate elevation of serum cholesterol and phospholipid. They obtained unusually high levels of serum cholesterol in old rats fed 40% casein.

In mature White Leghorn cockerels Kokatnur, Rand, Kummerow and Scott (1958) reported a drop in serum cholesterol level with an increase in protein level. These birds (12-18 weeks old) were fed diets containing 7.5%, 15%, or 30% protein and 0.1% or 15% corn oil throughout an experimental period of twenty-one days. The largest increase in serum cholesterol was noted in birds which had consumed the least amount of protein. There seemed to be no apparent relationship between the serum cholesterol levels and differences in feed intake or differences in the percentage of calories supplied by dietary fat. The serum cholesterol levels of hyper-cholestermic cockerels dropped rapidly during the three-week experimental period. This decrease did not reach the normal value in this period of time unless the protein level of the diet was high. In New Hampshire four-week old chicks, Nishida, Takenaka and Kummerow (1958) also demonstrated a decrease in serum cholesterol level when the protein level of the diet fed was high. These workers fed protein levels of 15%, 20%, and 30% to chicks, and concluded that a high protein level in combination with heated oil was successful in lowering the serum cholesterol level of the chicks.

Fillios et al (1954) suggested as a result of further experimental work with monkeys, that methionine in the protein of the diet is an important factor in the regulation of cholestermia in these monkeys. They were not able to confirm this state in rats, for although hypercholesteremia was noted when rats were fed a protein diet deficient in the sulphur amino acid methionine, they were able to prevent an increased hypercholestermia, only partially, by the supplementation of dl- methionine. In young chicks, when hypercholesteremia resulted from suboptimal protein intakes Johnson et al (1958) demonstrated that by supplementing the deficient protein with amino acids such that more protein could be made available to the chicks, protein deficiency was alleviated and normal levels of plasma cholesterol were observed. Recently, March and Biely (1959) reported lower levels of serum cholesterol in five-week old White Leghorn cockerels fed a 26% protein diet than similar chicks fed a 20% protein diet.

Inanition is involved in a number of vitamin deficiency states and for this reason it is often difficult to differentiate between a direct effect of vitamin deficiency and an indirect effect resulting from inanition.

The hypercholesteremia resulting from inanition or starvation in various laboratory animals is probably due to a depression in cholesterol synthesis. Any rise in blood cholesterol level may reflect increased mobilization of fat reserves.

Rinehart and Greenberg (1949) noted an elevated plasma cholesterol level in pyridoxine deficient monkeys. In a separate experiment when the diet was changed to one of high fat, both the control and the pyridoxine deficient monkeys showed increased levels of serum cholesterol with 1% added cholesterol in their diets. As a result these workers suggested that the high fat diet may have increased absorption of the cholesterol. Rinehart and Greenberg working with monkeys and Muschett and Emerson (1956) working with dogs demonstrated arteriosclerosis caused by pyridoxine deficiency but did not however measure the cholesterol concentration in these animals. Witten and Hollman (1952) studying the inter-relationship between pyridoxine and essential fatty acids in correcting the syndrome produced by pyridoxine deficiency, found that pyridoxine and linoleate corrected all aspects of the deficiency syndrome.

In the case of vitamin A a number of workers have demonstrated hypolipemia with high vitamin A intake. While Smith (1934) found an increase in blood fatty acids and cholesterol in deficient rats. Sure et al (1933) and Green et al (1955) found no significant differences in blood fatty acids, cholesterol and phospholipids in vitamin A deficient albino rats.

Van bruggen et al (1948) reported an increased blood cholesterol level with a high intake of vitamin A in humans. Similar results were reported by Ralli and Waterhouse (1933) in vitamin deficient dogs. Weitzel et al (1956) gave oral doses of vitamin A to old artherosclerotic hens, and reported only slight changes in the serum cholesterol of these hens. Wood (1960) reported that both the alcohol and acetate form of vitamin A when added to the chicks diet, significantly lowered the serum cholesterol concentration of cholesterol fed White Leghorn chicks.

Altschul et al (1955) working with humans, and Parsons et al (1956, 1957) working with rabbits, reported decreased blood cholesterol levels with oral administrations of nicotinic acid.

With regard to the effect of the B-complex group of vitamins on the level of serum cholesterol in chickens, Stamler et al (1958) and Pick et al (1957) reported the possible existence of an inter-relationship between the vitamins, fats, and cholesterol. The serum cholesterol levels of mature cockerels fed high protein, vitamin B deficient diets were not affected, but when cockerels were fed vitamin B deficient diets, low in protein, intensified hypercholesteremia was produced.

Hsu and Chow (1957) reported higher levels of serum cholesterol in eight New Hampshire chicks fed high fat, vitamin B_{12} deficient diets, than chicks fed high fat diets supplemented with vitamin B_{12}.

MATERIALS AND METHODS

Throughout the experiments, the same experimental conditions were maintained. The chicks were started on experimental diets when they were one day old. They were wing-banded and inoculated intraocularly against Newcastle Disease. All chicks were distributed at random into groups. They were housed in electrically heated Jamesway battery brooders. They were fed and watered ad libitum.

Two basal diets, the composition of which is shown in Table I were formulated to contain 20% and 26% protein respectively. It will be noted that dextrose makes up 12% of each basal diet. When fat was added, it was substituted for the dextrose. The source of thyroactive protein used in the experiments was iodinated casein.

Blood samples, taken from the wing vein of the chick were allowed to stand overnight in a refrigerator at 10^{o}C. The blood samples were then centrifugated, for approximately five minutes at 2500 revolutions per minute, in order to facilitate the separation of the serum. The serum was analysed for total cholesterol by the method of Zlatkis, Zak, and Boyle (1953) and for total lipids by the method of Huerga et al (1953).

The justification for using the method of Zlatkis, Zak, and Boyle for serum cholesterol determination was based on the simplicity and ease with which this method could be carried out in the laboratory, and because it is less time consuming. It must be realized, however, that this method has its limitations (Rhodes, 1959). In this study, the knowledge of the relative change in the levels of serum cholesterol is of greater importance than the knowledge of the absolute amounts.

Consequently, it is worthwhile to sacrifice a little accuracy in the absolute serum cholesterol determination for want of a simple method.

The method consists of adding a fixed volume of concentrated sulphuric acid, glacial acetic acid, and ferric chloride solution to 0.1 ml. of serum in 3.0 mls. of glacial acetic acid. Full development of the color reaction, which is purple, requires approximately one minute. The resultant color obeys Beer's Law and remains stable over a period of several hours.

METHOD

Reagents

I. Standard cholesterol solution (1 mg. per milliter)

II. Ferric chloride solution. Dissolve 10 mg. of ferric chloride, reagent grade, in 100 ml. of 100% glacial acetic acid.

III. Color Reagent. Dilute 2.0 mls. of the ferric chloride solution to 200 ml. with C.P. concentrated sulphuric acid.

PROCEDURE

(a) Determination of Total Serum Cholesterol.

Pipette 0.1 ml. of serum into a dry, clean 30 ml. test tube containing 3 ml. of glacial acetic acid. Shake test tube to disperse precipitate. Add 2.0 ml. of the color reagent, by carefully allowing it to flow down the side of the tube, thus producing two layers. Mix thoroughly, by striking the bottom of the tube sharply while holding it at the top between the thumb and forefinger, to effect mixing and even heat distribution. Measure the abosrbancy of the solution after it has cooled to room temperature, and determine the serum cholesterol concentration from the standard curve described below.

The absorbancy of the sample is measured against a blank prepared by mixing together 3.0 ml. of glacial acetic acid, 0.1 ml. of distilled water, and 2 ml. of ferric chloride color reagent. The absorbancy peak is at 560 mu.

(b) Standard Curve.

Pipette 0.1, 0.2, 0.3, 0.4, and 0.5 ml. aliquots of the standard cholesterol solution into clean, dry 30 ml. test tubes. Dilute each aliquot to 3.0 mls. with glacial acetic acid. Add 0.1 ml. of distilled water to each tube. Add carefully, 2.0 ml. of ferric chloride color reagent to each tube. Mix the solution thoroughly as explained above. When the solutions are cooled to room temperature, measure their absorbancies at 560 mu in a spectrophotometer. The standard curve as shown in (Fig.1), is obtained by plotting absorbancy against cholesterol concentration.

THE ESTIMATION OF SERUM TOTAL LIPIDS

The method presented here, for the determination of serum total lipids, has the advantage of being accurate for the purposes required. It is less time consuming, and requires no special equipment. This method is based on the turbidity, of the extracted lipids, in 4% sulphuric acid solution.

Reagents

I. Bloor's mixture (1 vol. ethyl ether: 3 vols of ethyl alcohol).

II. P Dioxan.

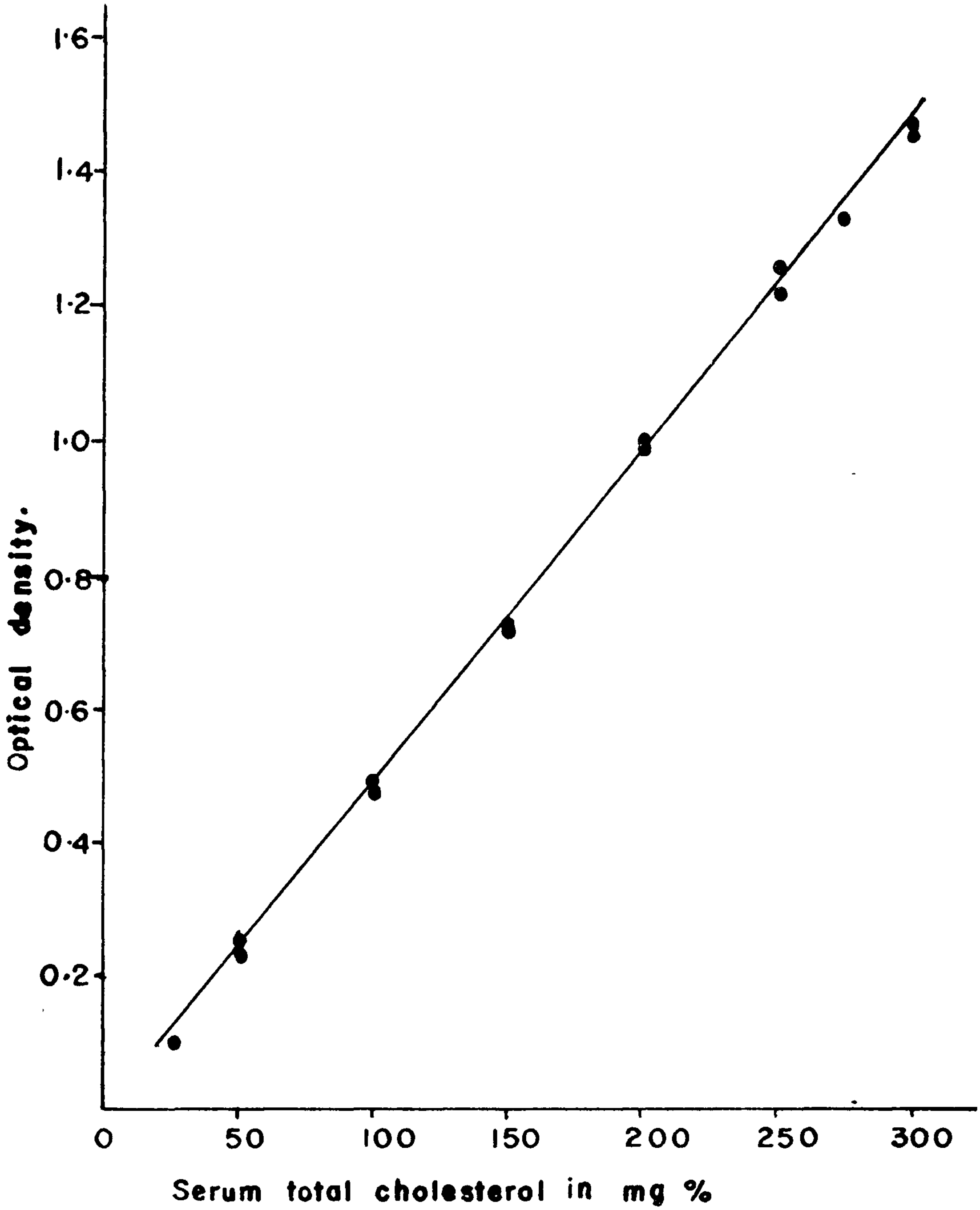
III. 4% sulphuric acid.

METHOD

Into a test tube graduated to 10 mls. transfer 9.5 mls. of Bloor's mixture. Add dropwise 0.5 ml. serum and place the tube in a water bath at 50 - 60°C for at least half-an-hour. After cooling to room termperature, make up the tube to the 10 ml. mark with Bloor's mixture. Centrifugate the tube to obtain a clear supernatant. Evaporate to dryness 1 cc aliquot of the supernatant. Cool the tube, and add 1.5 mls. p Dioxan to it. Emulsify the lipids dissolved in the p Dioxan with 5 mls. of 4% sulphuric acid. Allow the tube to stand for thirty minutes at room temperature before reading in a colorimeter at a wavelength of 650 mu or red filter, using water as a blank. The turbidity formed is stable for 20 - 60 minutes after addition of the sulphuric acid.

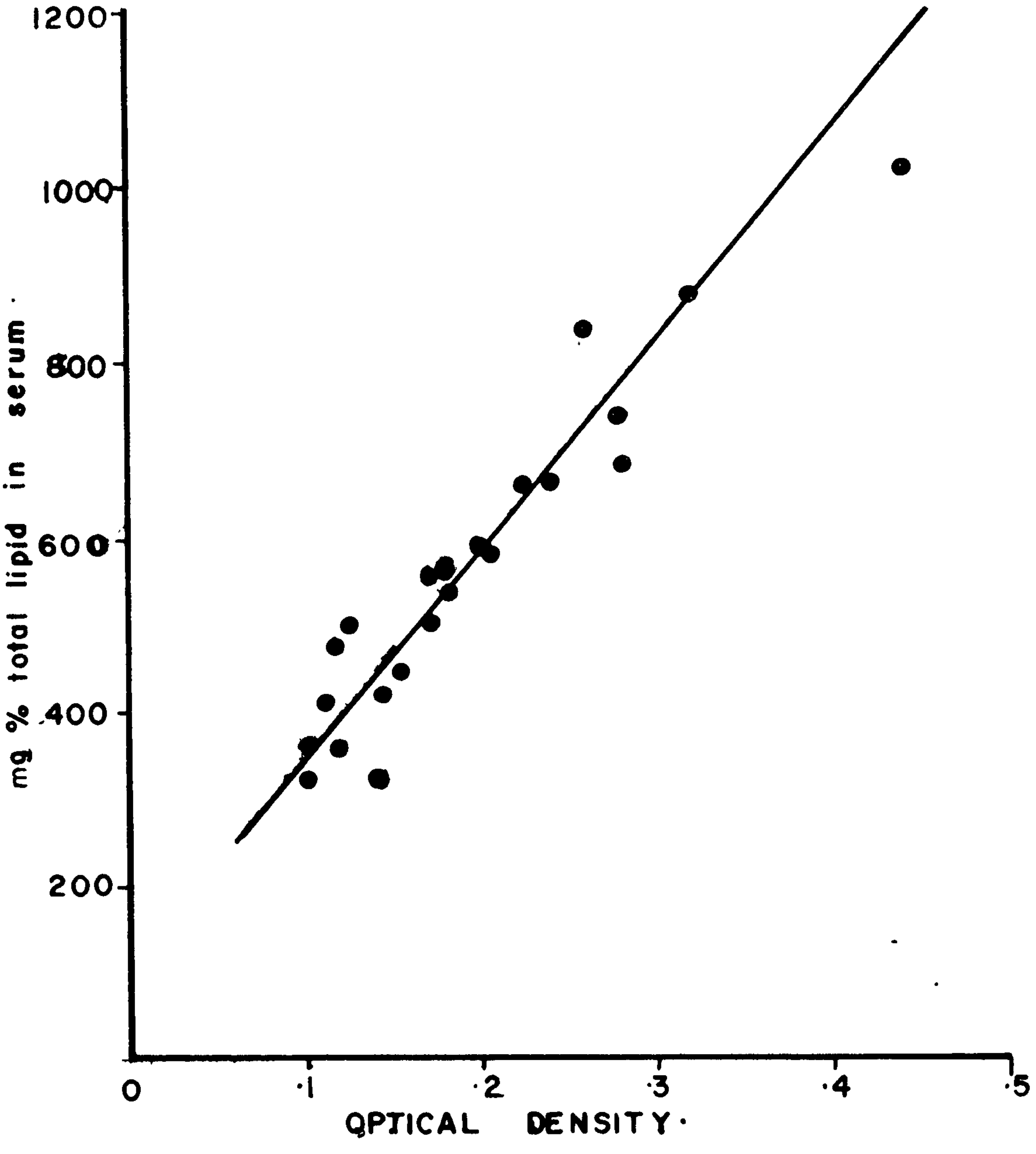
STANDARD CURVE

The standard curve is made by determining the concentration of total lipids in various serums gravimetrically. Extract 5 mls. of serum with 95 mls. of Bloor's mixture. After incubation for half-an-hour in a water bath at 50 - 60°C cool to room temperature, the volume is adjusted to 100 mls. with Bloor's mixture. This is now filtered and 80 mls. is taken and evaporated to dryness in a water bath. The impure liquid residue is taken up in four portions of 7 ml. each of petroleum ether and filtered. After evaporation of the solvent, the purified lipids are weighed to a constant weight and the amount of lipids in milligrams per 100 mls. of serum is calculated. From the same alcolol ether extract, 1 ml. aliquots are also taken for determination by the turbidimetric method. The optical readings obtained by the turbidimetric method is then plotted against the concentration of total lipids, as determined gravimetrically. The standard curve as shown in (Fig 2.) could now be drawn.



Standard curve for estimation of total cholesterol.

Figure 1.



<u>Standard curve for estimation of mg % lipid content in serum</u>·

Figure 2.

EXPERIMENT 1

Experiment 1 was designed to test the effects of

(i) two levels of protein;

(ii) two levels of fat;

(iii) two levels of B complex vitamins

and the interaction of these factors, on the serum cholesterol levels, serum total lipid levels, and growth of chicks.

The effects of the above factors was tested both on normal chicks and on chicks in which hyperthyroidism was induced. Hyperthyroidism was induced in chicks by feeding them diets containing 0.02% iodinated casein. The two levels of protein were 20% and 26% as shown in Table 1. High fat diets contained 12% hydrogenated vegetable oil which was substituted for the dextrose in the basal diets as shown in Table 1.

To make up diets with a high vitamin level, the basal diet as shown in Table 1 was further supplemented with 42.9 grams of choline chloride, 0.63 grams of calcium panthothenate, 0.375 grams of folacin, 0.2 grams of riboflavin and 1.8 grams of niacin per one hundred pounds of diet.

Twelve groups of New Hampshire cockerels were used in this experiment. Each group, consisting of twenty-two chicks, was fed an experimental diet as described in Table 1A. The chicks were weighed individually at seven day intervals up to the age of twenty-eight days. Blood samples were taken from six chicks selected at random in each group, when the chicks were twenty-eight, thirty-five, and forty-two days old.

Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953), and serum total lipids by the method of Huerga et al (1953).

GROWTH

As seen from the results in Table 1B chicks fed the higher protein level grew at a faster rate than chicks fed the lower protein level. Both the normal and hyperthyroid chicks grew at a faster rate, when they were fed a 26% protein diet than when they were fed a 20% protein diet.

Chicks fed diet 3 (low protein, high fat) grew at a slower rate than chicks fed diet 1 (low protein, low fat). High fat diets, low in protein, did not produce any increase in the growth rate of chicks fed these diets over those fed diets low in fat and low in protein.

Chicks fed diet 7 (high fat, high protein) displayed faster growth rates than chicks fed diet 5 (low fat, high protein). This illustrates that the amount of energy supplied in the basal diet at the low protein level was adequate, but at the higher protein level the chick required a greater amount of energy.

In all cases, either normal or hyperthyroid chicks grew at a faster rate when fed high protein diets supplemented with additional B complex vitamins than when they were fed diets with a low B complex vitamin supplement. The addition of fat in the basal diet apparently aggravated the vitamin deficiency induced by that diet on the basis of the growth response of the chicks. Chicks fed basal diets deficient in the B vomplex vitamins showed increased growth rates when the B complex vitamin supplement was fed. Inclusion of 12% fat in the diet depressed growth in chicks fed a vitamin deficient diet. Hyperthyroid chicks fed diets 1 (low protein, low fat, high vitamin), 3 (low protein, high fat, high vitamin), 7 (high protein, high fat, high vitamin), 9 (high protein, low fat, low vitamin) or II (high protein, high fat, low vitamin) grew at a slower rate than normal chicks fed similar diets. There were no differences between normal and hyperthyroid chicks fed diet 5 (high protein, low fat, high vitamin).

SERUM CHOLESTEROL

No differences were obtained between the serum cholesterol levels of normal and hyperthyroid chicks fed the basal diet (diet 1) (low protein, low fat) and normal or hyperthyroid chicks fed diet 5 (high protein, low fat). Hyperthyroid chicks fed diet 1 showed lower levels of serum cholesterol than hyperthyroid chicks fed diet 5. There were no differences between the serum cholesterol levels of normal chicks fed diet 3 (high fat, low protein) and normal chicks fed diet 7 (high fat, high protein). Hyperthyroid chicks fed diet 3 show higher levels of serum cholesterol than hyperthyroid chicks fed diet 7. On the basis of this experiment it appears that hyperthyroid chicks fed low protein, high fat diets tend to have higher levels of serum cholesterol than hyperthyroid chicks fed high protein, high fat diets. When high fat and low fat diets were fed to normal chicks, the chicks receiving high fat diets always had higher serum cholesterol levels than chicks receiving low fat diets.

The serum cholesterol levels of chicks fed basal diets high in B complex vitamins were lower than chicks fed basal diets low in the B complex vitamins. There are indications in the literature to suggest that chicks fed diets high in B complex vitamins had lower serum cholesterol levels than chicks fed diets low in the B complex vitamins (Hsu and Chow, 1957). This decrease in serum cholesterol level in chicks fed diets high in B complex vitamins was not achieved except in the case where chicks were fed basal diets. In some cases hyperthyroid chicks fed high vitamin B complex diets had lower serum cholesterol levels than normal chicks fed similar diets. This decrease in serum cholesterol level was not as great as that reported in the literature.

SERUM TOTAL LIPIDS

Generally, chicks fed the 26% protein diet showed lower serum total lipid levels than chicks fed the 20% protein diet. Only in one instance both normal and hyperthyroid twenty-eight day old chicks showed higher levels of serum cholesterol when fed a 26% protein diet than chicks of the same age and fed a 20% protein diet. High protein diets fed to hyperthyroid chicks were inconsistent in its effect on the serum total lipids of the chicks. While twenty-eight day old hyperthyroid chicks fed diet 5 produced higher serum total lipids than hyperthyroid chicks fed diet I, the reverse effect was obtained in chicks thirty-five days old.

Normal chicks fed high fat diets showed higher levels of serum total lipids than normal chicks fed low fat diets.

Hyperthyroid twenty-eight day old chicks fed diet 3 gave higher levels of serum total lipids than normal chicks of the same age and fed the same diet. In thirty-five day old hyperthyroid chicks the reverse effect was observed.

Hyperthyroid chicks fed diet 7 showed lower levels of serum total lipids than normal chicks fed the same diet at both sets of determinations. Generally, normal chicks fed high protein diets supplemented with additional B complex vitamins had lower levels of serum total lipids than normal chicks fed high protein diets of low B complex vitamin content.

TABLE 1

Composition of Basal Diets

Ingredients	26% Protein in Diet lb/100 lbs.	20% Protein in Diet lb/100 lbs.
Dextrose	12.0	12.0
Ground Wheat	22.0	30.0
Ground Yellow Corn	23.0	28.75
Soya Bean Oil Meal (44% Protein)	28.0	20.0
Fish Meal (70% Protein)	12.0	6.0
Limestone	1.0	1.0
Bone Meal	1.5	1.75
Iodized Salt	0.5	0.50
Vitamin A	2000 iu/lb.	2000 iu/lb.
Vitamin D	120 icu/lb.	120 icu/lb.
Manganeese Sulphate	6.0 gms.	6.0 gms.
Menadione	0.027 gms.	0.027 gms.
Nicarbazine (25%)	22.5 gms.	22.5 gms.

TABLE 1A

DIET	DESCRIPTION OF DIET
I N	+ Basal
I H	Diet 1 + 0.02% iodinated casein.
3 N	Diet 1 + 12% hydrogenated vegetable oil.
3 H	Diet 1 + 12% hydrogenated vegetable oil + 0.02% iodinated casein.
5 N	⊕ Basal
5 H	Diet 5 + 0.02% iodinated casein.
7 N	Diet 5 + 12% hydrogenated vegetable oil.
7 H	Diet 5 + 12% hydrogenated vegetable oil + 0.02% iodinated casein.
9 N	o Basal
9 H	Diet 9 + 0.02% iodinated casein.
II N	Diet 9 + 12% hydrogenated vegetable oil.
II H	Diet 9 + 12% hydrogenated vegetable oil. + 0.02% iodinated casein.

+ 20% Protein diet further supplemented with B complex vitamins.

⊕ 26% Protein diet further supplemented with B complex vitamins.

o 26% Protein diet without any further B complex vitamin supplement

N = Normal chicks.

H = Hyperthyroid chicks.

TABLE 1B

Body Weights, Serum Cholesterol Level and Total Lipid Level of Chicks in Experiment 1

Age in Days	*Average Weight (gms)	+Serum Cholesterol mgs/loo c.c.		+Serum Total Lipids mgs/100 c.c.	
	28	28	35	28	35
DIET					
1 N	332	166	162	665	833
1 H	288	157	170	714	759
3 N	319	178	177	824	859
3 H	242	190	188	868	812
5 N	360	165	165	687	697
5 H	365	166	172	756	703
7 N	389	179	184	739	717
7 H	356	166	171	700	611
9 N	342	174	182	710	625
9 H	327	161	170	690	598
11 N	317	175	194	755	756
11 H	299	144	186	609	649

*Average of 20 Chicks.

+Average of 6 Determinations.

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 1 TABLE 1C

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.023	.0369	18.0 * *
B	1	.002	.0023	1.1
C	1	.166	.1664	81.0 * *
AB	1	.012	.0124	6.0 *
BC	1	.015	.0149	7.2 * *
AC	1	.006	.0057	2.8
ABC	1	.000	.0001	.0
Total Treatment	7	.239	.0341	16.6 * *
Error	120	.247	.0021	
Total	127	.485		

A - Iodinated Casein
B - Fat
C - Protein

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.004	.0039	1.7
B	1	.000	.0000	.0
C	1	.050	.0497	21.2 * *
AB	1	.004	.0039	1.6
BC	1	.029	.0290	12.4 * *
AC	1	.003	.0029	1.2
ABC	1	.003	.0034	1.5
Total Treatment	7	.093	.0132	5.7 * *
Error	120	.281	.0023	
Total	127	.374		

A = Iodinated Casein
B = Fat
C = Vitamin

EXPERIMENT 2

Experiment 2 was designed to study the effects of

(i) two levels of fat;

(ii) two levels of vitamins

and the interactions of these factors on the serum total cholesterol level, serum total lipid level and growth of both the normal chick and the induced hyperthyroid chick.

As described in the previous experiment, hyperthyroidism was induced in the chick by the administration of 0.02% iodinated casein in the diet.

The high fat diet was formulated by substituting hydrogenated vegetable oil for dextrose in the 26% protein basal diet shown in Table 1. To obtain diets with a high vitamin level, the 26% protein basal diet shown in Table 1 was further supplemented with the following B vitamins; 42.9 grams of choline chloride, 0.63 grams of calcium panthothenate, 0.395 grams of folacin, 0.2 grams of riboflavin, and 1.8 grams of niacin per 100 pounds of diet.

Eight groups of White Leghorn mixed male and female chicks were used in this experiment. Each group consisting of approximately twenty-two chicks was fed an experimental diet as described in Table 2. Chicks were weighed individually at seven-day intervals up to a period of thirty-five days. Blood samples were taken from six chicks selected at random in each group when the chicks were twenty-eight, thirty-five and thirty-eight days old.

Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953), and serum total lipids by the method of Huerga et al (1953).

GROWTH

The effect of fat on the growth rate of chicks was inconsistent. Normal twenty-eight day old chicks fed diet 3 (high fat, high vitamin) grew at a slower rate than normal chicks of the same age and fed diet 1 (low fat, high vitamin). The growth rate of thirty-five day old normal chicks fed diet 3 was similar to the growth rate of normal chicks fed diet 1.

The growth rate of hyperthyroid chicks was also inconsistent. Hyperthyroidism resulted in an increased growth rate response in twenty-eight day old chicks fed diet 1 over normal chicks of the same age and fed a similar diet, while no effect was obtained from hyperthyroidism in thirty-five day old chicks fed the same diet. In both instances hyperthyroidism in chicks fed diet 3 resulted in increased growth rate response over normal chicks fed a similar diet.

Generally, chicks when fed diets low in the B complex vitamins grew at a slower rate than chicks fed diets high in B complex vitamins. High fat diets supplemented with additional B complex vitamins retarded the growth rate of twenty-eight day old chicks fed these diets. Apparently the inclusion of fat in these diets increased the requirement for B complex vitamins. Chicks fed diet 7 (high fat, low vitamin) grew at a slower rate than chicks fed diet 5 (low fat, low vitamins). Hyperthyroid chicks fed diets low in B complex vitamins did not differ in growth rate from normal chicks fed similar diets.

SERUM CHOLESTEROL

The effect of high fat diet on the serum cholesterol of the normal chick fed these diets was inconsistent. Twenty-eight day old normal chicks fed diet 1 (low fat, high vitamin) showed higher serum cholesterol levels than normal chicks of the same age fed diet 3 (high fat, high vitamin). There were no differences in the serum cholesterol levels of thirty-five day old normal chicks fed diet 1 and normal chicks of the same age fed diet 3. Similarly no differences in the serum cholesterol levels of thirty-eight day old normal chicks fed diet 1 and normal chicks of equal ages fed diet 3, were obtained.

The effect of induced hyperthyroidism in chicks fed diets 1 and 3, was inconsistent. Thirty-five and thirty-eight day old hyperthyroid chicks had higher levels of serum cholesterol than normal chicks when they were fed diet 1. Twenty-eight day old hyperthyroid chicks fed diet 1 showed lower levels of serum cholesterol than normal chicks of the same age and fed similar diets. Twenty-eight and thirty-five day old hyperthyroid chicks fed diet 3 showed higher levels of serum cholesterol than normal chicks of the same age fed similar diets. Under similar conditions the reverse effect on the serum cholesterol level of hyperthyroid chicks was obtained. When the level of B complex vitamins was low, the inclusion of fat in the diet was not consistent in its effect on the serum cholesterol level of chicks fed these diets. Twenty-eight day old normal chicks fed diet 7 (high fat, low vitamin) showed lower serum cholesterol levels than normal chicks of the same age and fed diet 5. Hyperthyroid chicks fed diet 7 showed lower levels of serum cholesterol than normal chicks fed similar diets. Normal chicks fed diets low in B complex vitamins generally showed higher levels of serum cholesterol than normal chicks fed diets high in the B complex vitamins.

SERUM TOTAL LIPIDS

Normal chicks fed high fat diets had higher serum total lipid levels than normal chicks fed low fat diets. Hyperthyroid chicks fed diet 1 (low fat, high vitamin) had higher levels of serum total lipids than normal chicks fed a similar diet. The serum total lipid level, however, of hyperthyroid twenty-eight and thirty-five day old chicks fed diet 3 (high fat, high vitamin) was higher than normal chicks of the same age and fed a similar diet. Thirty-eight day old hyperthyroid chicks had lower levels of serum total lipids than normal chicks equal in age and fed a diet high in the B complex vitamins (diet 1). The effect of feeding diets low in the B complex vitamins to hyperthyroid chicks on their serum total lipids, was inconsistent.

TABLE 2

DIET	DESCRIPTION OF DIET
I N	† Basal
I H	Diet I + 0.02% iodinated casein.
3 N	Diet I + 12% hydrogenated vegetable oil.
3 H	Diet I + 12% hydrogenated vegetable oil + 0.02% iodinated casein.
5 N	o Basal
5 H	Diet 5 + 0.02% iodinated casein.
7 N	Diet 5 + 12% hydrogenated vegetable oil.
7 H	Diet 5 + 12% hydrogenated vegetable oil. + 0.02% iodinated casein.

† 26% Protein supplemented with additional B complex vitamins.

o 26% Protein diet low in B complex vitamins.

N = Normal chicks.

H = Hyperthyroid chicks.

TABLE 2A

Body Weights, Serum Cholesterol Level and Serum Total Lipid Level of Chicks in Experiment 2

Age in Days	Average Weight in Grams.		Serum Cholesterol mgs/100 c.c.			Serum Total Lipids mgs/100 c.c.		
	28	35	28	35	38	28	35	38
DIET								
1 N	324	414	253	216	200	676	600	630
1 H	335	415	249	248	222	713	620	634
3 N	309	418	197	222	198	696	623	771
3 H	319	435	230	235	171	784	647	629
5 N	221	308	262	233	181	672	591	658
5 H	227	309	233	203	190	647	600	664
7 N	202	285	229	233	198	647	673	705
7 H	204	287	194	193	170	698	495	622

Average of 22 Chicks.

Average of 6 Determinations.

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 2 TABLE 2B

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.024	.0242	5.2 *
B	1	.003	.0029	.6
C	1	.389	.3886	83.9 * *
AB	1	.000	.0000	.0
BC	1	.034	.0338	7.3 * *
AC	1	.014	.0141	3.0
ABC	1	.001	.0008	.2
Total Treatment	7	.464	.0664	14.3 * *
Error	136	.630	.0046	
Total	143	1.094		

B = Fat
C = Vitamin

EXPERIMENT 3

Experiment 3 was designed to study the effect of

(i) two levels of fat;

(ii) two levels of B complex vitamins

and the interaction of these factors on the serum cholesterol level, the serum total lipid level, and growth of the chick. The effects of the above factors were studies on both the normal and the induced hyperthyroid chick.

Hyperthyroidism was induced in chicks by feeding them diets containing 0.02% iodinated casein.

High fat diets were formulated by substituting 12% hydrogenated vegetable oil for dextrose in the 26% protein basal diet shown in Table 1. To obtain diets with a high B complex vitamin level the 26% protein basal diet shown in Table 1 was further supplemented with the following B vitamins; 42.9 grams of choline chloide, 0.63 grams of calcium panthothenate, 0.395 grams of folacin, 0.2 grams of riboflavin, and 1.8 grams of niacin per 100 pounds of diet.

Eight groups of White Leghorn cockerels were used in this experiment. Each group consisting of approximately twenty chicks was fed an experimental diet, the description of which is given in Table 3. All chicks were weighed individually at seven-day intervals throughout an experimental period of thirty-five days. Blood samples were taken from six chicks selected at random in each group when the chicks were twenty-eight, thirty-five and forty-two days old.

The blood samples were analysed for serum cholesterol by the method of Zlatkis, Zak and Boyle (1953) and for serum total lipids by the method of Huerga et al (1953).

GROWTH

Chicks fed high fat, high B complex vitamin diets showed higher growth rates than chicks fed low fat, high B complex vitamin diets. The inclusion of fat in diets low in the B complex vitamins resulted in a depression of growth rate in chicks fed these diets. The addition of the fat in the basal diet apparently aggravated the vitamin deficiency induced by that diet on the basis of the growth response of the chicks. Chicks fed diets low in B complex vitamins grew at a slower rate than chicks fed high vitamin B complex diets.

The effect of induced hyperthyroidism in chicks fed high fat, high vitamin B complex diets was inconsistent. Twenty-eight day old hyperthyroid chicks showed an increased growth response when fed diet 3 (high fat, high vitamin) than normal chicks of the same age and fed a similar diet. The thirty-five day old hyperthyroid chick, however, showed a decrease in growth response as compared to the normal chick when fed diet 3.

Hyperthyroid chicks fed low vitamin B complex diets showed slower growth rates than normal chicks fed similar diets.

SERUM CHOLESTEROL

The inclusion of fat in the high B vitamin diets resulted in higher serum cholesterol levels of chicks fed these diets than of chicks fed low fat, high B complex vitamin diets. The high fat diets low in vitamin B complex had very little effect on the serum cholesterol levels of chicks fed these diets as compared to chicks fed low fat, low vitamin B complex diets.

The effect of the diet on the serum cholesterol levels of induced hyperthyroid chicks was very inconsistent. Thirty-five day old hyperthyroid chicks fed diet 1 (low fat, high vitamin) showed higher levels of serum cholesterol than normal chicks of the same age and fed the same diet. No differences were obtained in the serum cholesterol level between either twenty-eight day old or forty-two day old normal chicks and hyperthyroid chicks fed diet 1. No differences could be obtained in the serum cholesterol level between normal and hyperthyroid chicks fed diets low in the B complex vitamins.

SERUM TOTAL LIPIDS

Chicks fed diets high in fat generally showed higher serum total lipid levels than chicks fed diets low in fat. Induced hyperthyroid chicks fed high fat high B complex vitamin diets had higher serum total lipid levels than the hyperthyroid chicks fed low fat high B complex vitamin diets. The effect of feeding vitamin high diets to hyperthyroid chicks was inconsistent. Twenty-eight day old hyperthyroid chicks fed either diets 1 (low fat, high vitamin), 5 (low fat, low vitamin), or 7 (high fat, low vitamin) showed lower serum total lipid levels than normal chicks of the same age and fed the same diets. Thirty-five day old hyperthyroid chicks fed diets 1 had higher levels of serum total lipids than normal chicks similar in age and fed the same diet. Diet 3 (high fat, high vitamin) fed to thirty-five day old hyperthyroid chicks showed lower levels of serum total lipids, while diet 3 fed to forty-two day old hyperthyroid chicks showed higher serum total lipid levels than normal chicks of similar ages and fed similar diets. Chicks fed basal diets low in B complex vitamins generally showed higher levels of serum total lipids than chicks fed basal diets high in B complex vitamins.

TABLE 3

DIET	DESCRIPTION OF DIET
1 N	⸹ Basal
1 H	Diet I + 0.02% iodinated casein.
3 N	Diet I + 12% hydrogenated vegetable oil.
3 H	Diet I + 12% hydrogenated vegetable oil + 0.02% iodinated casein.
5 N	o Basal
5 H	Diet 5 + 0.02% iodinated casein.
7 N	Diet 5 + 12% hydrogenated vegetable oil.
7 H	Diet 5 + 12% hydrogenated vegetable oil + 0.02% iodinated casein.

⸹ = 26% Protein supplemented with additional B complex vitamins.

o = 26% Protein diet low in B complex vitamins.

N = Normal chicks.

H = Hyperthyroid chicks.

TABLE 3A

Body Weights, Serum Cholesterol Level and Serum Total Lipid Level of Chicks in Experiment 3

	*Average Weight in Grams		+Serum Cholesterol Level in mgs/100 c.c.			+Serum Total Lipid Level in mgs/100 c.c.		
Age in Days	28	35	28	35	42	28	35	42
DIET								
1 N	314	447	175	152	189	758	595	595
1 H	312	449	182	171	184	689	775	582
3 N	325	474	171	181	191	768	887	599
3 H	335	465	174	165	181	778	788	611
5 N	262	388	201	189	180	775	809	650
5 H	232	333	181	186	173	573	708	600
7 N	258	355	191	201	174	724	895	692
7 H	219	302	204	199	182	677	854	638

* Average of 20 chicks.

+ Average of 6 determinations.

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 3 TABLE 3B

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.001	.0013	.4
B	1	.001	.0012	.4
C	1	.603	.6033	178.3 **
AB	1	.000	.0002	.1
BC	1	.012	.0118	3.5
AC	1	.001	.0007	.2
ABC	1	.001	.0009	.3
Total Treatment	7	.619	.0885	26.1
Error	152	.514	.0034	
Total	159	1.134		

A = Iodinated Casein
B = Fat
C = Vitamin

EXPERIMENT 4

Experiment 4 was designed to study the effects of

(i) two levels of fat;

(ii) three levels of B complex vitamins

on the serum cholesterol level, serum total lipid level and growth of the chick. The above factors were studied in the normal chick, induced hyperthyroid chick, and the induced hypothyroid chick. Hyperthyroidism was induced in chicks by feeding them diets containing 0.02% iodinated casein. Hypothyroidism was induced by feeding chicks diets containing 0.1% thiouracil. To formulate diets of a high fat level, 12% hydrogenated vegetable oil was substituted for the dextrose of the 26% protein basal diet as shown in Table 1.

Diets containing three levels of B complex vitamins were made up as follows:-

(i) Level one - consisted of the 26% protein basal diet as shown in Table 1. This diet was not supplemented with additional B complex vitamins.

(ii) Level two - consisted of the 26% protein basal diet as shown in Table 1, and to this diet 90 grams of choline chloride and 0.375 grams of folacin per 100 pounds of feed was added.

(iii) Level three - consisted of the 26% protein basal diet as shown in Table 1 and to this diet a further B complex vitamin supplementation was made by the addition of the following - 90 grams of choline chloride, 0.375 grams of folacin, 0.02 grams of riboflavin, 0.63 grams of calcium panthothenate and 1.8 grams of niacin per 100 pounds of feed.

Eighteen groups of New Hampshire pullets were used in this experiment. Each group consisting of approximately thirty chicks was fed an experimental diet as shown in Table 4. The chicks were weighed individually at seven day intervals throughout an experimental period of forty-nine days, while blood samples were taken when the chicks were thirty-two, thirty-nine and forty-nine days old. Blood samples were taken from ten chicks selected at random in each lot.

Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953) while serum total lipids were determined by the method of Huerga et al (1953).

GROWTH

Chicks, thirty-five days old, fed high fat diets high in the B complex vitamins grew at a faster rate than chicks fed low fat diets low in the B complex vitamins. Normal, hyperthyroid and hypothyroid chicks fed diet 16 (high fat, high B complex vitamin) grew at a faster rate than normal chicks fed diet 13 (low fat, high vitamin). Hyperthyroid chicks grew at a faster rate than normal chicks fed diet 16, but no differences could be obtained between the growth rates of normal and hypothyroid chicks fed diet 16. Both hyperthyroid and hypothyroid chicks, however, gave increased growth responses over normal chicks when they were all fed diet 13.

When high fat diets supplemented with the B complex vitamins choline chloride and folic acid were fed to chicks, an improvement of growth rate over chicks fed low fat diets, supplemented with choline chloride and folic acid was seen only in hyperthyroid chicks. Both normal and hypothyroid chicks were not able to utilize the high fat diets to any advantage in improving growth rate over chicks fed diets low in fat. Apparently as noted in previous experiments, the inclusion of fat in the diet aggravated the vitamin deficiency induced by that diet, on the basis of its growth response. Similar observations were noted when chicks were fed high fat diets very low in the B complex vitamins. Either normal, hyperthyroid or hypothyroid chicks when fed diet 4 (high fat, high B complex vitamin) showed a lower growth rate response than normal, hyperthyroid and hypothyroid chicks fed diet 1 (low fat, vitamin level 1).

The effect of increasing the vitamin level to hyperthyroid chicks, on the basis of their growth response, was inconsistent. Hyperthyroid chicks fed diets 1, 10 (high fat, B complex vitamin, level 2) 13 (low fat, high B complex vitamin, level 3) and 16 (high fat, high B complex vitamin, level 3) showed higher growth rates than normal chicks fed similar diets.

Hypothyroid chicks, however, showed increased growth rate responses over normal chicks, when fed diets supplemented with the two additional B complex vitamins, choline chloride and folacin, or when the diets were further supplemented with the B complex vitamins calcium panthothenate, folacin, choline chloride, niacin and riboflavin. When basal diets were not supplemented with additional B complex vitamins there was little effect on the growth rates between normal and hypothyroid chicks fed these diets.

SERUM CHOLESTEROL

Normal chicks fed a basal diet high in fat, low in the B complex vitamins showed higher levels of serum cholesterol than chicks fed a basal diet low in fat and low in the B complex vitamins. There was very little change, however, in the levels of serum cholesterol of normal chicks fed high fat diets further supplemented with the B complex vitamins choline chloride and folacin alone, or high fat diets further supplemented with calcium panthothenate, choline chloride, folacin, riboflavin and niacin, and normal chicks fed low fat diets and with similar vitamin B complex content.

Apparently the increase in serum cholesterol level, which ordinarily occurs when the level of fat in the diet is high, would tend to be reduced with the intake of the B complex vitamins. The effect of the diet modification on the serum cholesterol level of hyperthyroid chicks was inconsistent as shown in Table 4A. In some instances high levels of serum cholesterol were noted in hyperthyroid chicks than in normal chicks fed a similar diet. In other instances smaller lower levels in the serum cholesterol were noted in hyperthyroid chicks than in normal chicks fed similar diets.

While thirty-two and thirty-nine day old hypothyroid chicks fed a basal diet low in the B complex vitamins showed higher levels of serum cholesterol than normal chicks of the same age and fed the same diet. Thirty-two and thirty-nine day old hypothyroid chicks fed diet 13 (low fat, high vitamin) showed lower levels of serum cholesterol than normal chicks of the same ages and fed similar diets. A similar observation was noted in thirty-two day old hypothyroid and normal chicks fed diet 16 (high fat, high vitamin). In other instances hypothyroid chicks showed lower levels or similar levels of serum cholesterol than normal chicks when they were fed similar diets.

SERUM TOTAL LIPIDS

Normal chicks fed diets high in fat showed higher levels of serum total lipids than chicks fed diets low in fat.

Thirty-nine day old hyperthyroid chicks fed high fat diets low in B complex vitamins showed a higher level of serum total lipids than hyperthyroid chicks fed low fat diets low in B complex vitamins. Hyperthyroid chicks fed high fat diets high in B complex vitamins showed higher levels of serum lipids than hyperthyroid chicks fed low fat diets high in B complex vitamins.

Thirty-two day old hyperthyroid chicks fed diet 7 (low fat, vitamin level 2) showed higher levels of serum total lipids than hyperthyroid chicks fed diet 10 (high fat, vitamin level 2) whereas thirty-nine day old hyperthyroid chicks showed a lower level of serum total lipids when fed diet 7 than hyperthyroid chicks fed diet 10. The comparison of the serum total lipids of the normal and the hyperthyroid chick shows a great deal of inconsistency in the effect of the diet. Whereas a lower serum total lipid level was noted in thirty-two day old hyperthyroid chicks fed diet 7 than the normal chick fed a similar diet, the reverse effect was noted in thirty-nine day old hyperthyroid and normal chicks fed diet 7.

In all instances hypothyroid chicks fed low fat diets, regardless of the level of B complex vitamins, showed higher levels of serum total lipids than normal chicks fed similar diets. Hypothyroid chicks fed diets high in fat and high in B complex vitamins also showed higher levels of serum total lipids than normal chicks fed similar diets. Thirty-two day old hypothyroid chicks fed diet 4 (high fat, vitamin level 1) or 10 (high fat, vitamin level 2) showed lower levels of serum total lipids than normal chicks fed similar diets, whereas thirty-nine day old hypothyroid chicks fed diet 4 or 10 showed higher levels of serum total lipids than normal chicks fed the same diet.

All thirty-two day old chicks fed diets high in the B complex vitamins showed lower serum total lipid levels than thirty-two day old chicks fed diets low in the B vitamins. Apparently the increase in serum lipid level, which ordinarily occurs when the level of fat in the diet is increased, would appear to be lessened with the intake of the B complex vitamins.

TABLE 4

DIET		DESCRIPTION OF DIET
1 N	†	Basal
1 HPR		1 + 0.02% iodinated casein
1 HPO		1 + 0.1% thiourasil
4 N		1 + 12% hydrogenated vegetable oil
4 HPR		1 + 12% hydrogenated vetetable oil + 0.02% iodinated casein
4 HPO		1 + 12% hydrogenated vegetable oil + 0.1% thiouracil
7 N	X	Basal
7 HPR		7 + 0.02% iodinated casein
7 HPO		7 + 0.1% thiouracil
10 N		7 + 12% hydrogenated vegetable oil
10 HPR		7 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
10 HPO		7 + 12% hydrogenated vegetable oil + 0.1% thiouracil
13 N	S	Basal
13 HPR		13 + 0.02% iodinated casein
13 HPO		13 + 0.1% thuouracil
16 N		13 + 12% hydrogenated vegetable oil
16 HPR		13 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
16 HPO		13 + 12% hydrogenated vegetable oil + 0.1% thiouracil

† + 26% protein basal diet with no further supplementation with B complex vitamins

X 26% protein basal diet + choline chloride + folacin

S 26% protein basal diet + choline chloride + folacin calcium panthothenate + niacin + riboflavin

N = Normal; HPR = Hyperthyroid; HPO = Hypothyroid

TABLE 4A

Body Weight, Serum Cholesterol Level and Total Lipid Levels of Chicks in Experiment 4

Age in Days	*Average Weight in Grams		+Serum Cholesterol mgm./100 c.c.			+Total Lipid Level mgm./100 c.c.	
	28	35	32	39	49	32	39
DIET							
1 N	286	459	161	177	165	627	558
1 HPR	305	467	178	184	158	627	616
1 HPO	282	438	197	203	165	772	664
4 N	282	449	187	182	179	752	630
4 HPR	264	428	164	173	163	623	565
4 HPO	275	436	182	193	165	703	706
7 N	304	460	186	186	197	616	645
7 HPR	275	426	185	193	200	534	686
7 HPO	306	473	177	169	187	651	712
10 N	278	437	183	170	199	721	658
10 HPR	302	481	162	183	194	519	718
10 HPO	305	467	179	178	189	626	763

TABLE 4A (Cont'd)

Age in Days	*Average Weight in Grams		+Serum Cholesterol mgm./100 c.c.			+Total Lipid Level mgm./100 c.c.	
DIET	28	35	32	39	49	32	39
13 N	311	419	177	171	193	434	570
13 HPR	322	481	172	182	190	498	605
13 HPO	310	469	194	185	185	588	675
16 N	319	478	174	187	188	582	670
16 HPR	323	502	167	178	190	530	712
16 HPO	318	485	201	183	188	591	806

\+ Average of 10 determinations per group

* Average of thirty chicks per group

EXPERIMENT 5

Experiment 5 was designed to study the effects of

(i) two levels of fat;

(ii) three levels of B complex vitamins.

on the interaction of these factors on the serum cholesterol levels, and growth of the chick. The above factors were studied in both the normal and the induced hyperthyroid chick. Hyperthyroidism was induced in chicks by feeding them diets containing 0.02% iodinated casein.

The diet with a high fat level was formulated by substituting 12% hydrogenated vegetable oil for the dextrose in the 26% protein basal diet shown in Table 1. Diets of different B complex vitamin levels were formulated as follows:

Vitamin level 1 - consisted of the 26% protein basal diet as shown in Table 1. This diet was not supplemented with additional B complex vitamins.

Vitamin level 2 - consisted of the 26% protein basal diet as shown in Table 1. In addition this diet was further supplemented with 90 grams of choline chloride, and 0.375 grams of folacin per 100 pounds of feed.

Vitamin level 3 - consisted of the 26% protein basal diet as shown in Table 1 and this diet was further supplemented with 90 grams of choline chloride, 0.375 grams of folacin, 0.63 grams of calcium panthothenate, 1.8 grams of niacin and 0.02 grams of riboflavin.

Twelve groups of New Hampshire pullets were used in this experiment. Each group consisting of approximately twenty-five chicks was fed an experimental diet as shown in Table 5. The chicks were weighed individually at seven day intervals up to a period of thirty-five days. Blood samples taken from ten chicks selected at random in each lot were analysed for serum cholesterol by the method of Zlatkis, Zak and Boyle (1953).

GROWTH

Chicks fed high fat high vitamin B complex diets grew at a faster rate than chicks fed low fat diets high in vitamins (vitamin level 3) as seen in Table 5A, both normal and hyperthyroid chicks fed diet 1 (high fat, high vitamin) grew at a faster rate than normal or hyperthyroid chicks fed diet 9 (low fat, high vitamin). The growth rate of normal twenty-eight day old chicks fed basal diets high in fat and low in B complex vitamins (vitamin level 1 or vitamin level 2) was lower than normal chicks twenty-eight days old fed low fat, low in B complex vitamins. As seen in previous experiments, the inclusion of fat in the basal diet apparently aggravated the vitamin deficiency induced by that diet on the basis of the growth response of the chicks. The growth rate of normal chicks thirty-five days old was not depressed by the inclusion of a high fat level in their low B complex vitamin basal diets. Hyperthyroid chicks, fed low fat diets generally showed an increase in growth rate over normal chicks fed similar diets. The growth rate of hyperthyroid chicks fed high fat diets low in B complex vitamins was generally lower than the growth rate of normal chicks fed high fat diets low in B.complex vitamins. It seems that the inclusion of a high level of fat in the low B complex vitamin diets of hyperthyroid chicks, tend to aggravate the already existing vitamin B complex deficiency to a greater degree than in normal chicks, on the basis of their growth response. Generally chicks fed diets high in B complex vitamins grew at a faster rate than chicks fed diets low in B complex vitamins.

Chicks fed basal diets supplemented with the vitamins choline chloride and folacin alone (vitamin level 2) grew at a slower growth rate than chicks fed diets low in B complex vitamins (vitamin level 1).

On the basis of this particular experiment it might appear that an inter-relationship exists among the B vitamins. It appears as if the presence of the two B complex vitamins, choline chloride and folacin alone in the diet, when fed to the chick may have aggravated the already existing deficiency of B complex vitamins. This probably accounts for the slower growth rates of chicks fed diets supplemented with choline chloride and folacin alone than chicks fed diets without any additional supplementation with the B complex vitamins (i.e., vitamin level 1).

SERUM CHOLESTEROL

Chicks fed basal diets high in B complex vitamins and high in fat showed higher levels of serum cholesterol than chicks fed basal diets high in B complex vitamins and low in fat. The serum cholesterol level of thirty day old chicks fed diet 7 (high fat, low vitamin-level 2) was lower than that of thirty day old chicks fed diet 5 (low fat, low vitamin-level 2). In other instances there appeared to be no differences between the serum cholesterol levels of chicks fed high fat, low B complex vitamin level diets (vitamin levels 1 or 2) than the serum cholesterol level of chicks fed low fat, low B complex vitamin diets.

The serum cholesterol levels of hyperthyroid chicks were lower than those of normal chicks in some instances while in other instances the serum cholesterol level was higher in hyperthyroid chicks than that of normal chicks although fed the same diets. Twenty-five day old hyperthyroid chicks fed diet 1 (low fat, low vitamin-level 1) was lower than normal chicks fed the same diet whereas the serum cholesterol level of hyperthyroid chicks fed diet 9 (low fat, high vitamin-level 3) was higher than normal chicks fed the same diet.

TABLE 5

DIET		DESCRIPTION OF DIET
1 N	†	Basal
1 H		1 + 0.02% iodinated casein
3 N		1 + 12% hydrogenated vegetable oil
3 H		1 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
5 N	X	Basal
5 H		5 + 0.02% iodinated casein
7 N		5 + 12% hydrogenated vegetable oil
7 H		5 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
9 N	S	Basal
9 H		9 + 0.02% iodinated casein
11 N		9 + 12% hydrogenated vegetable oil
11 H		9 + 12% hydrogenated vegetable oil + 0.02% iodinated casein

+ 26% protein basal diet - no additional B vitamins

X 26% protein basal diet + choline chloride + folacin

S 26% protein basal diet + choline chloride + folacin + riboflavin + niacin + calcium panthothenate.

N = Normal

H = Hyperthyroid

TABLE 5A

Body Weights, and Serum Cholesterol Levels of Chicks in Experiment 5

	+Average Weight in Grams		*Serum Cholesterol mgm./100 c.c.		
Age in Days	28	35	25	30	38
DIET					
1 N	298	407	218	206	214
1 H	299	425	203	210	212
3 N	289	426	217	211	210
3 H	272	394	214	213	211
5 N	274	360	228	233	229
5 H	298	430	227	228	218
7 N	252	377	226	204	226
7 H	246	366	219	209	214
9 N	306	443	227	209	216
9 H	358	496	216	222	219
11 N	363	500	236	218	225
11 H	377	527	219	229	211

+ Average of twenty-five chicks per group

* Average of 10 determinations per group

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 5 TABLE 5B

Source of Variation	df	Sum of Squares	Mean Squares	F
A	2	.492	.2458	51.1 * *
B	1	.002	.0017	.3
C	1	.030	.0296	6.1 *
AB	2	.054	.0270	5.6 * *
BC	1	.035	.0345	7.2 * *
AC	2	.024	.0118	2.5
ABC	2	.003	.0013	.3
Total Treatment	11	.638	.0580	12.1 * *
Error	240	1.154	.0048	
Total	251	1.792		

A = Vitamin
B = Fat
C = Iodinated Casein

EXPERIMENT 6

Experiment 6 was designed to study the effect of

(i) two levels of fat;

(ii) two levels of B complex vitamins

and the interaction of these factors on the serum cholesterol level, serum total lipid level and growth of both the normal and the hyperthyroid chick.

Hyperthyroidism was induced in the chick by feeding them diets containing 0.02% iodinated casein.

In previous experiments the effect of various diets on the serum cholesterol and serum total lipid level of hyperthyroid chicks were inconsistent. It was suspected that hyperthyroidism was not always induced in the chick when diets containing 0.02% iodinated casein was fed. As a result an additional diet was formulated to contain twice the level of iodinated casein as was previously used.

In this experiment the above factors were studied in chicks rendered hyperthyroid by feeding them diets containing levels of 0.02% and 0.04% iodinated casein.

Diets containing a high level of fat were formulated by substituting 12% hydrogenated vegetable oil for dextrose in the 26% protein basal diets as shown in Table 1. Diets containing a high vitamin B complex level were formulated by the addition of the following B complex vitamins to the 26% protein basal diet shown in Table 1; 90 grams of choline chloride, 0.375 grams of folacin, 0.02 grams of riboflavin, 1.8 grams of niacin, and 0.63 grams of calcium panthothenate per 100 pounds of diet.

Twelve groups of New Hampshire cockerels were used in Experiment 6. Each group consisting of approximately twenty-five chicks was fed an experimental diet as described in Table 6. The chicks were weighed at seven day intervals up to an experimental period of thirty-one days. Blood samples were taken from ten chicks selected at random in each lot. Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953) while serum total lipids was estimated by the method of Huerga et al (1953).

RESULTS

GROWTH

Chicks fed basal diets high in fat, high in B complex vitamins grew at a faster rate than chicks fed basal diets low in fat, and high in B complex vitamins. No differences were observed in the growth rate of chicks fed basal diets low in the B complex vitamins, and at either the high or low fat level. Hyperthyroid chicks fed high fat diets showed higher growth rates than hyperthyroid chicks fed low fat diets, provided the chicks were high in the B complex vitamins.

Hyperthyroid chicks (level 0.04%) fed diet 7 (low fat, low B complex vitamins) or diet 10 (high fat, low B complex vitamins) showed lower growth rates than normal chicks fed similar diets.

The effect of the diet on the growth rate on chicks rendered hyperthyroid, when either level of thyroactive protein was fed, was inconsistent. Whilst chicks rendered hyperthyroid by administration of the higher level of thyroactive protein showed higher growth rates than chicks rendered hyperthyroid from a lower level of thyroactive protein when they were fed diets 1 (low fat, high B complex vitamins) or 4 (high fat, high B complex vitamins), similar effects could not be observed in hyperthyroid chicks fed low vitamin diets.

Hyperthyroid twenty-eight day old chicks (level 0.02%) grew at a faster rate than hyperthyroid chicks (level 0.04%) of the same age and fed the same diet (diet 7). In twenty-eight day old hyperthyroid chicks the reverse effect was noted in the growth rate of these chicks fed diet 7.

Chicks fed diets with a high B complex vitamin level grew at a faster rate than chicks fed diets of a low vitamin B complex level. Chicks fed high fat diets low in the B complex vitamins grew at a slower rate than chicks fed diets low in fat and low in the B complex vitamins. Apparently as previously noted the inclusion of fat in the low B complex vitamin diets aggravated the vitamin deficiency already existing on the basis of the growth response of the chicks fed these diets.

SERUM CHOLESTEROL

Chicks fed high fat diets generally showed higher levels of serum cholesterol than chicks fed low fat diets. Chicks rendered hyperthyroid from the administration of thyroactive protein showed higher levels of serum cholesterol than normal chicks when they were fed diet 1 (low fat, high vitamin). Twenty-eight day old hyperthyroid chicks (at both levels) fed diet 4 (high fat, high vitamin) showed higher levels of serum cholesterol than normal chicks of the same age and fed the same diet. No differences were observed in thirty-one day old hyperthyroid chicks fed diet 4. The effect of chicks rendered hyperthyroid when either level of thyroactive protein was fed, was not consistent. Chicks fed diets low in B complex vitamins generally showed higher levels of serum cholesterol than chicks fed diets high in the B complex vitamins.

SERUM TOTAL LIPIDS

Chicks fed diets high in the B complex vitamins and high in fat showed higher levels of serum total lipids than chicks fed diets low in fat and high in B complex vitamins. Twenty-five day old chicks fed basal diet 7 (low fat, low vitamin) showed higher levels of serum total lipids than t twenty-five day old chicks fed diet 10 (high fat, low vitamins). The reverse effect on the serum total lipids was noted in thirty-one day old normal chicks fed similar diets. Hyperthyroid chicks fed diets low in fat and high in B complex vitamins showed higher levels of serum total lipids than normal chicks fed similar diets. The effect of feeding diets 4 (high fat, high vitamin) 7 (low fat, low vitamin) and 10 (high fat, low vitamin) to hyperthyroid chicks was inconsistent. Chicks fed a basal diet low in B complex vitamins showed higher levels of serum total lipids than chicks fed a basal diet high in B complex vitamins.

TABLE 6

DIET	DESCRIPTION OF DIET
1 N	† Basal
1 Hx	1 + 0.02% iodinated casein
1 Hxx	1 + 0.04% iodinated casein
4 N	1 + 12% hydrogenated vegetable oil
4 Hx	1 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
4 Hxx	1 + 12% hydrogenated vegetable oil + 0.04% iodinated casein
7 N	o Basal
7 Hx	7 + 0.02% iodinated casein
7 Hxx	7 + 0.04% iodinated casein
10 N	7 + 12% hydrogenated vegetable oil
10 Hx	7 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
10 Hxx	7 + 12% hydrogenated vegetable oil + 0.04% iodinated casein

† 26% protein basal diet as shown in Table 1 supplemented with additional B complex vitamins.

o 26% protein basal diet as shown in Table 1.

N = Normal chicks

Hx = Chicks rendered hyperthyroid by feeding them diets containing 0.02% iodinated casein

Hxx = Chicks rendered hyperthyroid by feeding them diets containing 0.04% iodinated casein

RESULTS

TABLE 6A

Body Weights, Serum Cholesterol Levels and Total Lipid Levels of Chicks in Experiment 6

	*Average Weight in Grams		+Serum Cholesterol mgm./100 c.c.		+Total Lipids mgm./100 c.c.	
Age in Days	21	28	25	31	25	31
DIET						
1 N	189	309	144	157	657	553
1 Hx	205	316	157	189	755	579
1 Hxx	219	344	152	205	710	730
4 N	216	347	156	202	814	819
4 Hx	223	332	186	194	848	715
4 Hxx	231	371	176	216	815	696
7 N	173	285	183	203	735	589
7 Hx	166	268	175	181	712	551
7 Hxx	152	287	186	202	784	639
10 N	167	284	213	212	720	682
10 Hx	179	312	208	224	781	533
10 Hxx	160	267	187	209	754	632

\+ Average of 10 determinations per group

* Average of twenty-five chicks per group

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 6 TABLE 6B

Source of Variation	df	Sum of Squares	Mean Squares	F
A	2	.008	.0041	1.7
B	1	.016	.0163	6.9 * *
C	1	.247	.2467	103.6 * *
AB	2	.000	.0001	.1
BC	1	.006	.0058	2.4
AC	2	.024	.0120	5.1 * *
ABC	2	.002	.0012	.5
Total Treatment	11	.304	.0276	11.6 * *
Error	240	.571	.0024	
Total	251	.875		

A = Iodinated Casein
B - Fat
C - Vitamin

EXPERIMENT 7

Experiment 7 was designed to study the effect of

(i) two levels of fat;

(ii) two levels of riboflavin

and the interaction of these factors on the serum cholesterol, serum total lipids, and growth of the chick. The effect of the above factors were studied both in the normal chick and in the induced hyperthyroid chick. Hyperthyroidism was induced in chicks by feeding them diets containing 0.02% iodinated casein.

Diets containing a high level of fat were formulated by substituting 12% hydrogenated vegetable oil for the dextrose in the 26% protein basal diet shown in Table 1. All diets were supplemented with the following B complex vitamins:

90 grams of choline chloride
0.375 grams of folacin
0.8 grams of niacin
0.63 grams of calcium panthothenate
0.02 grams of riboflavin per 100 pounds of diet

Diets containing a low level of riboflavin were formulated by the addition of the above B vitamins with the exception of riboflavin to the 26% protein basal diet as shown in Table 1. It will be noted that diets of both vitamin levels differ only in respect to the amount of additional riboflavin.

Eight groups of New Hampshire cockerels were used in this experiment. Each group consisting of approximately twenty-five chicks was fed an experimental diet as described in Table 7C. The chicks were weighed individually at seven day intervals throughout an experimental period of twenty-eight days. Blood samples were taken from ten chicks selected at random from each group when the chicks were twenty-one and twenty-eight days old. Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953) and serum total lipids by the method of Huerga et al (1953).

RESULTS

GROWTH

Chicks fed diets high in fat and high in riboflavin only grew at a faster rate than chicks fed low fat diets high in riboflavin. There were no differences in the growth rate of twenty-one day old chicks fed high fat basal diets, low in riboflavin and chicks fed low fat basal diets low in riboflavin. Twenty-eight day old normal chicks fed diet 3 (high fat, low riboflavin) grew at a slower rate than normal chicks fed diet 1 (low fat, low riboflavin). This observation agrees with those indicated in the literature, which state that the inclusion of fat in the diet of experimental animals results in an increased need for dietary riboflavin by those animals. (Harrill et al, 1959).

Hyperthyroid chicks fed the high fat diet supplemented with riboflavin grew at a faster rate than normal chicks fed a similar diet. Hyperthyroid chicks fed diet 3 grew at a faster rate than normal chicks fed the same diet and also grew faster than hyperthyroid chicks fed diet 1.

SERUM CHOLESTEROL

Normal chicks fed the basal diet high in fat and high in riboflavin showed higher levels of serum cholesterol than chicks fed a basal diet low in fat and high in riboflavin. Chicks fed a basal diet high in fat and low in riboflavin showed higher levels of serum cholesterol than chicks fed a basal diet low in fat and low in riboflavin.

Hyperthyroid chicks fed diet 1 (low fat, low riboflavin), 3 (high fat, low riboflavin), 5 (low fat, high riboflavin) or 7 (high fat, high riboflavin) showed slightly lower levels of serum cholesterol than normal chicks fed similar diets. There were no differences in the serum cholesterol levels of chicks fed diets low in riboflavin and those fed diets high in riboflavin.

SERUM TOTAL LIPIDS

Chicks fed high fat diets either high or low in riboflavin showed higher levels of serum total lipids than chicks fed diets low in fat. Hyperthyroid chicks fed low fat diets showed lower levels of serum total lipids than normal chicks fed similar diets. In one instance, however, there were no differences between the serum total lipid levels of hyperthyroid and normal chicks fed a low fat diet, viz. hyperthyroid chicks fed diet 1 (low fat, low riboflavin). Chicks fed diets low in riboflavin showed lower levels of serum total lipids than chicks fed diets high in riboflavin. At this stage it may be of interest to mention that although chicks were fed diets high in riboflavin, they still showed symptoms typical of chicks suffering from a riboflavin deficiency.

TABLE 7

Summary of Results in Experiment 7

	Average Weight in Grams		Serum Cholesterol Level mgm./100 c.c.		Total Lipid Level mgm./loo c.c.	
Age in Days	21	28	21	28	21	28
DIET						
1 N	198	322	206	185	377	270
1 H	195	314	195	176	301	270
3 N	197	310	211	208	386	329
3 H	217	343	203	201	405	366
5 N	207	340	192	181	419	321
5 H	214	348	188	174	396	303
7 N	227	366	210	202	490	382
7 H	255	406	191	191	486	389

TABLE 7A

Serum Cholesterol Level from Individual Chicks in mgm./100 c.c. in Experiment 7

DIET	21 day old chicks										Average
1 N	208	200	245	245	196	206	208	194	196	158	206
1 H	188	241	184	228	180	210	184	194	184	160	195
3 N	225	200	194	228	307	180	200	200	203	173	211
3 H	164	178	203	160	164	203	265	231	249	215	203
5 N	186	191	180	184	203	241	203	173	184	170	192
5 H	200	164	184	208	196	192	170	182	172	210	188
7 N	220	200	192	215	208	231	203	208	196	228	210
7 H	172	213	173	192	173	194	184	212	208	184	191
	28 day old chicks										
1 N	163	197	184	180	166	173	213	191	188	196	188
1 H	166	180	168	186	153	166	203	172	194	172	176
3 N	180	194	203	206	245	192	200	220	228	212	208
3 H	192	170	241	184	184	220	180	215	227	194	201
5 N	175	145	180	182	172	194	168	184	197	215	181
5 H	149	173	191	173	170	237	192	180	138	138	174
7 N	186	203	203	186	194	208	228	191	212	208	202
7 H	203	170	196	197	191	178	158	194	227	191	191

TABLE 7 B

Total Lipid Level of Individual Chicks in mgm./100 c.c. Serum in Experiment 7

DIET											Average
	21 day old chicks										
1 N	380	580	330	370	320	320	345	460	320	345	377
1 H	310	380	300	300	370	360	300	270	210	210	301
3 N	345	485	270	510	-	345	330	370	310	285	386
3 H	405	345	345	345	370	395	485	415	485	462	405
5 N	460	320	345	370	510	510	498	415	380	380	419
5 H	395	345	370	415	498	370	330	395	370	476	396
7 N	560	510	462	580	440	580	440	415	450	462	490
7 H	450	535	395	535	485	550	462	485	510	450	486
	28 day old chicks										
1 N	235	310	225	285	225	270	320	320	310	200	270
1 H	270	225	235	320	270	265	200	246	345	320	270
3 N	360	246	360	310	405	345	320	302	360	285	329
3 H	330	300	370	370	370	320	415	395	300	485	366
5 N	320	345	345	300	260	360	380	300	285	310	321
5 H	320	320	330	330	260	310	380	260	270	246	303
7 N	440	285	330	450	476	285	395	450	360	345	382
7 H	330	345	380	300	450	380	370	345	485	450	389

TABLE 7C

DIET			DESCRIPTION OF DIET
1	N	†	Basal
1	H		1 + 0.02% iodinated casein
3	N		1 + 12% hydrogenated vegetable oil
3	H		1 + 12% hydrogenated vegetable oil + 0.02% iodinated casein
5	N	o	Basal
5	H		5 + 0.02% iodinated casein
7	N		5 + 12% hydrogenated vegetable oil
7	H		5 + 12% hydrogenated vegetable oil + 0.02% iodinated casein

† 26% protein basal diet as shown in Table 1 and supplemented with B complex vitamins with the exception of riboflavin.

o 26% protein basal diet as shown in Table 1 and supplemented with B complex vitamins including riboflavin.

N = Normal chick

H = Hyperthyroid chick

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 7 TABLE 7D

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.012	.0117	5.7 *
B	1	.023	.0229	11.2 * *
C	1	.069	.0691	33.7 * *
AB	1	.015	.0145	7.1 *
BC	1	.008	.0081	3.9 *
AC	1	.000	.0004	.2
ABC	1	.000	.0000	.0
Total Treatment	7	.127	.0181	8.8
Error	128	.262	.0021	
Total	135	.389		

A = Iodine
B = Fat
C = Riboflavin

EXPERIMENT 8

Experiment 8 was designed to study the effects of

(i) two levels of protein;

(ii) two levels of fat

and the interaction of these factors on the serum cholesterol levels, the serum total lipid levels and growth of the chick. The effects of the above factors were studied both in the normal and the hyperthyroid chick. Hyperthyroidism was induced in the chick by feeding them diets containing 0.02% iodinated casein. The two levels of protein used in this experiment were 20% and 26% as shown in Table 1. Diets with a high level of fat were formulated by substituting 12% lard for dextrose in the 20% and 26% protein basal diets as shown in Table 1.

From the previous experiments carried out it was noted that there is a great variation in the serum cholesterol and serum total lipid levels of the chick at different intervals. It was therefore decided to study the effects of the above factors on the serum cholesterol and serum total lipid levels of the chick from an earlier age (five days old).

Eight groups of White Leghorn cockerels were used in this experiment. Each group consisting of approximately twenty-five chicks was fed an experimental diet, the description of which is shown in Table 8. The chicks were weighed at approximately seven day intervals and blood samples were taken periodically from ten chicks selected at random in each group. Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle (1953) while serum total lipids were determined by the method of Huerga et al (1953).

RESULTS

GROWTH

As seen from the results in Table 8B, chicks fed a 26% protein diet grew at a faster rate than chicks fed a 20% protein diet. Only in one instance the reverse effect was noted viz. chicks fed diet 5 (high protein, low fat) grew at a slower rate than chicks fed diet 1 (low protein, low fat). This effect was noted in chicks twenty-eight days old.

Hyperthyroid chicks fed diets 1, 3 (low protein, high fat) or diet 7 (high protein, high fat) grew at a slower rate than normal chicks fed a similar diet. Apparently hyperthyroid chicks gave an increased gorwth rate response only when the protein to energy ratio of the diet was high (see Table 8B).

Chicks fed 26% protein diets high in fat grew at a faster rate than chicks fed 26% protein diets low in fat. Normal chicks fed 20% protein diets high in fat grew at a slower rate than normal chicks fed 20% protein diets low in fat. Hyperthyroid chicks fed 20% protein diets high in fat grew at a slower rate than hyperthyroid chicks fed 20% protein diets low in fat.

SERUM CHOLESTEROL

Generally chicks fed 26% protein diets showed lower levels of serum cholesterol than chicks fed 20% protein diets. In one instance, thirty-three day old normal chicks fed diet 5 (high protein, low fat) showed higher levels of serum cholesterol than thirty-three day old normal chicks fed diet 5 showed very little difference in their serum cholesterol levels, from the twenty-six day old normal chicks fed diet 1.

Hyperthyroid chicks fed diet 5 showed slightly lower levels of serum cholesterol than hyperthyroid chicks fed diet 1. The serum cholesterol levels of hyperthyroid chicks fed diet 7 (high protein, high fat) were much lower than those of hyperthyroid chicks fed diet 3 (low protein, high fat). The effect of induced hyperthyroidism on the serum cholesterol levels of the chicks fed diets 1 or 3 was inconsistent. As seen in Table 8A hyperthyroid chicks fed diets 1 or 3 showed higher levels of serum cholesterol in some instances than normal chicks fed similar diets, whilst in other instances the reverse effect was noted. Hyperthyroid chicks fed diets 5 or 7 generally showed lower levels of serum cholesterol than normal chicks fed similar diets. Forty-nine day old hyperthyroid chicks fed diet 7 showed higher levels of serum cholesterol than normal forty-nine day old chicks fed the same diet. Chicks fed high fat diets generally showed higher levels of serum cholesterol than chicks fed low fat diets.

TOTAL LIPIDS

Except for thirty-three day old normal chicks, the total lipid levels of normal chicks recorded at all other ages, were lower in those chicks fed a 26% protein diet than those fed a 20% protein diet. Thirty-three day old normal chicks fed 26% protein diets showed higher levels of serum total lipids than normal chicks of the same age and fed the 20% protein diet. Normal chicks fed diet 3 (low protein, high fat) generally showed higher levels of serum total lipids than normal chicks fed diet 7 (high protein, high fat). Twenty-six and thirty-three day old normal chicks fed diet 3 showed lower levels of serum total lipids than normal chicks of similar ages and fed diet 7.

Hyperthyroid chicks fed diets 5 (high protein, low fat) or 7 generally showed lower levels of serum total lipids than normal chicks fed diets 5 or 7. The effect of hyperthyroidism on the serum total lipids of chicks fed diets 1 or 3 was inconsistent. Hyperthyroid chicks fed diet 1 generally showed lower levels of serum total lipids than normal chicks fed similar diets, while forty day old hyperthyroid chicks showed slightly higher serum total lipid levels than normal chicks of a similar age. There were no differences between the serum total lipid levels of twenty-six day old hyperthyroid chicks and normal chicks fed diet 1. Hyperthyroid chicks fed diet 7 generally showed lower levels of serum total lipids than normal chicks fed the same diet. Forty-six day old hyperthyroid chicks, however, showed higher levels of serum total lipids than forty-six day old normal chicks when they were fed diet 7.

Normal chicks fed high fat, high protein diets generally showed higher serum total lipid levels than normal chicks fed low fat, high protein diets. Five day old and twenty-six day old normal chicks fed diet 3 showed lower levels of serum total lipids than normal chicks of similar ages and fed diet 1.

TABLE 8

DIET		DESCRIPTION OF DIET
1 N	†	Basal
1 H		1 + 0.02% iodinated casein
3 N		1 + 12% lard
3 H		1 + 12% lard + 0.02% iodinated casein
5 N	o	Basal
5 H		5 + 0.02% iodinated casein
7 N		5 + 12% lard
7 H		5 + 12% lard + 0.02% iodinated casein

† 20% protein basal diet as shown in Table 8.

o 26% protein basal diet as shown in Table 8.

N = Normal chicks.

H = Hyperthyroid chicks.

TABLE 8A

Body Weights, Serum Cholesterol Level and Total Lipid Level of Chicks in Experiment 8

DIET \ Age in Days	5	12	19	26	33	40	46
	*Total Cholesterol - 20% Protein						
1 N	227	210	200	175	175	179	182
1 H	255	202	182	172	184	193	184
3 N	278	206	238	199	216	202	197
3 H	252	220	233	205	196	197	195
	26% Protein						
5 N	169	163	189	182	197	183	157
5 H	170	135	180	181	188	172	178
7 N	205	169	216	204	201	190	179
7 H	198	158	210	186	189	180	166
	*Total Lipids - 20% Protein						
1 N	473	304	326	340	324	291	294
1 H	429	291	297	341	270	301	285
3 N	421	315	415	264	339	285	378
3 H	402	301	380	330	306	291	408
	26% Protein						
5 N	279	239	333	318	350	246	291
5 H	275	197	306	276	336	264	302
7 N	370	247	380	348	401	236	314
7 H	346	227	337	307	352	221	348

TABLE 8B

DIET	Age in Days	+Average Weights in Grams - 20% Protein						
		5	12	19	26	33	40	46
1	N	72	126	180	272	369	488	614
1	H	68	118	168	260	375	461	612
3	N	69	122	167	245	338	432	550
3	H	66	109	150	220	308	409	518
					26% Protein			
5	N	74	128	185	258	374	493	613
5	H	77	133	186	282	395	524	647
7	N	77	142	211	310	439	580	708
7	H	77	131	200	296	430	559	692

* Average of 10 determinations per group

\+ Average of twenty-five chicks per group

ANALYSIS OF VARIANCE (GROWTH)

EXPERIMENT 8 TABLE 8C

Source of Variation	df	Sum of Squares	Mean Squares	F
A	1	.007	.0073	.9
B	1	.007	.0075	.9
C	1	.233	.2330	28.8 **
AB	1	.037	.0371	4.6 *
BC	1	.132	.1325	16.4 **
AC	1	.000	.0002	.0
ABC	1	.015	.0152	1.9
Total Treatment	7	.433	.0618	7.6 **
Error	152	1.229	.0081	
Total	159	1.662		

A = Iodine Level
B = Fat Level
C = Protein Level

Diagram VIII(a)

Diagram to Show the Growth Rate of Chicks in Experiment 8

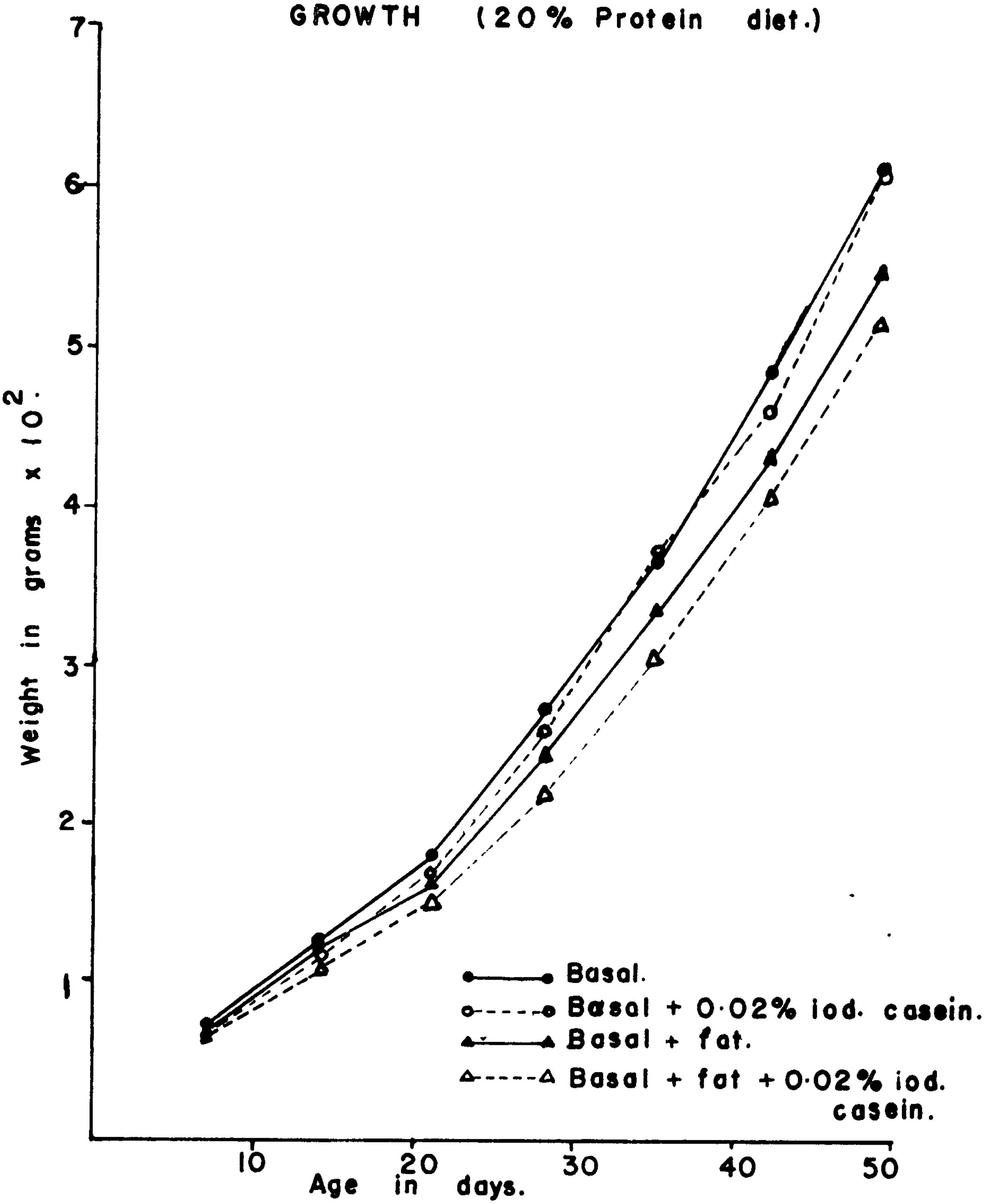


Diagram VIII(b)

Diagram to Show the Growth Rate of Chicks in Experiment 8

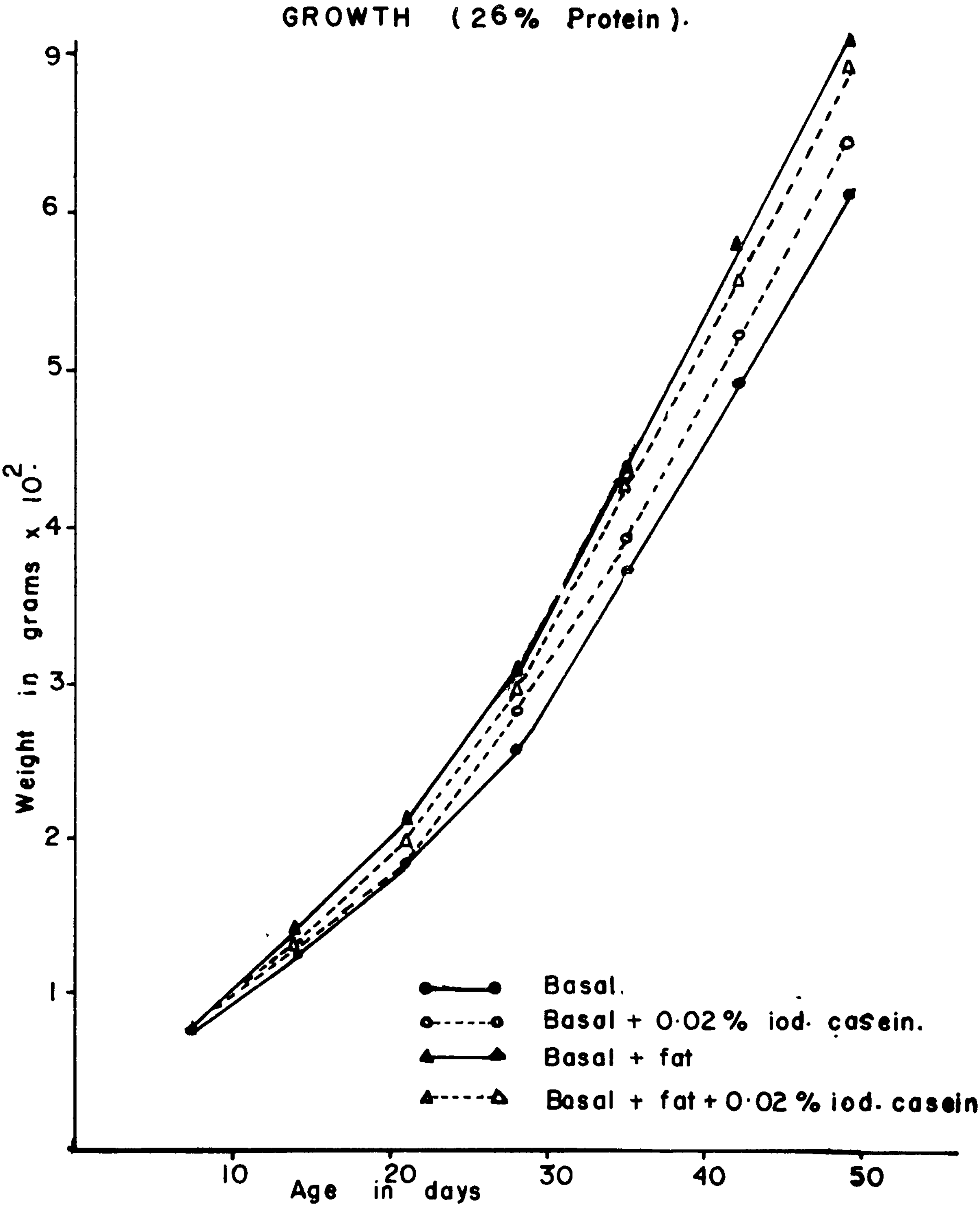


Diagram VIII(c)

Diagram to Show the Serum Cholesterol Level of Chicks in Experiment 8

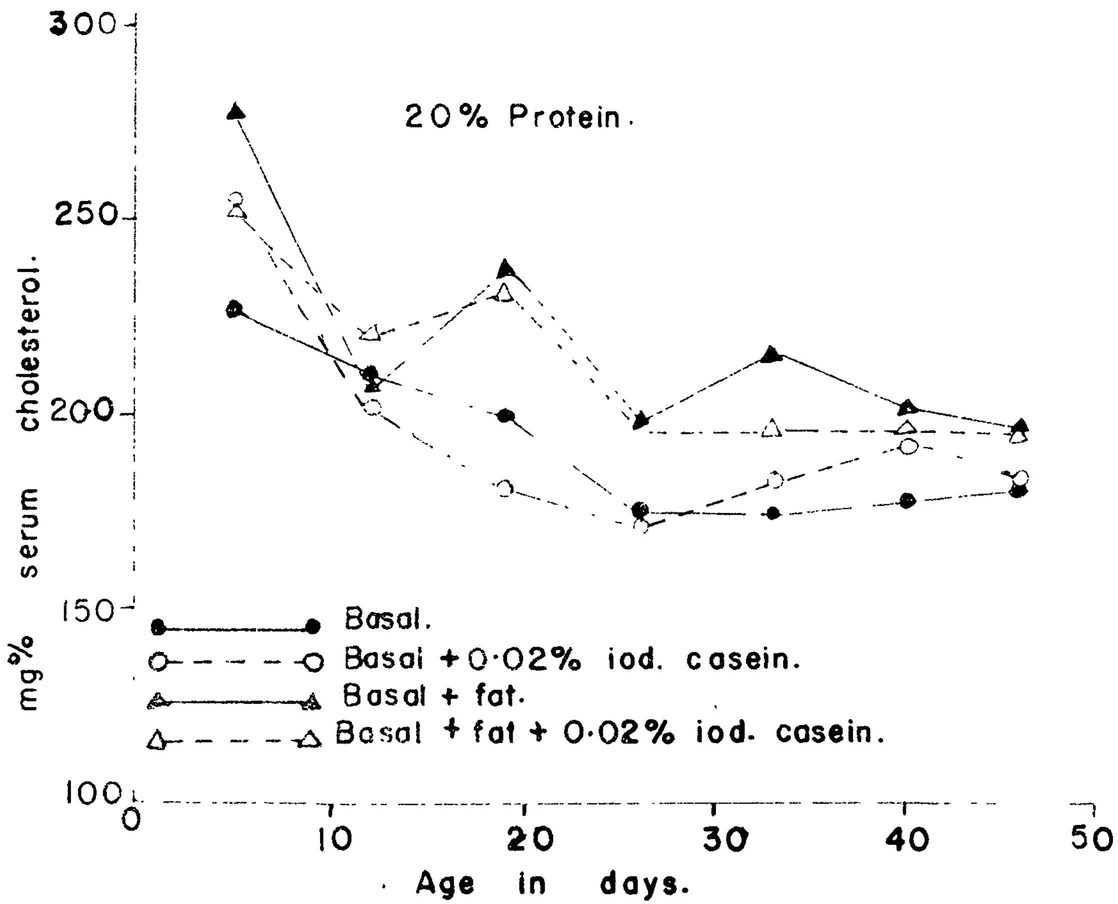


Diagram VIII(d)

Diagram to Show the Serum Cholesterol Level of Chicks in Experiment 8

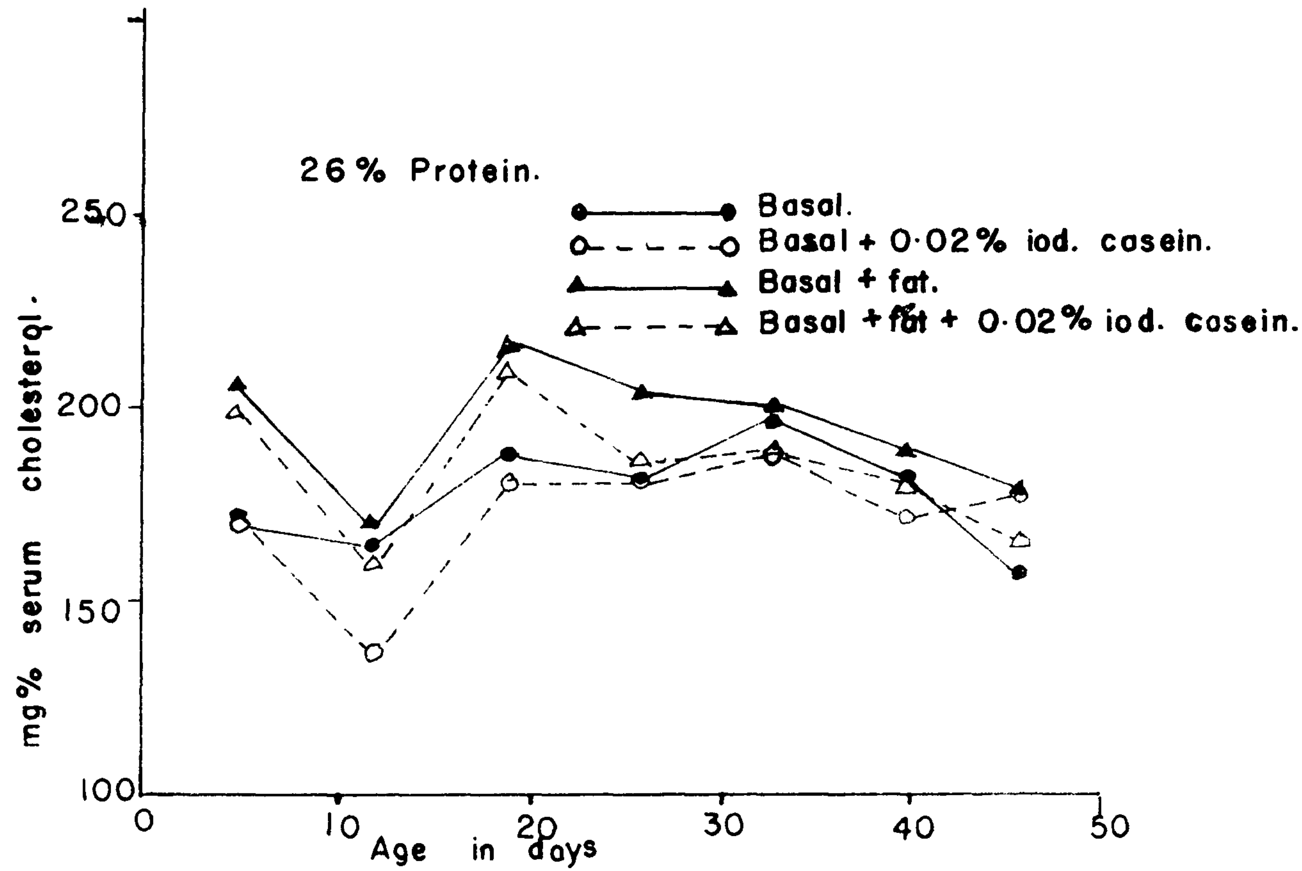


Diagram VIII(e)

Diagram to Show the Serum Total Lipid Level of Chicks in Experiment 8

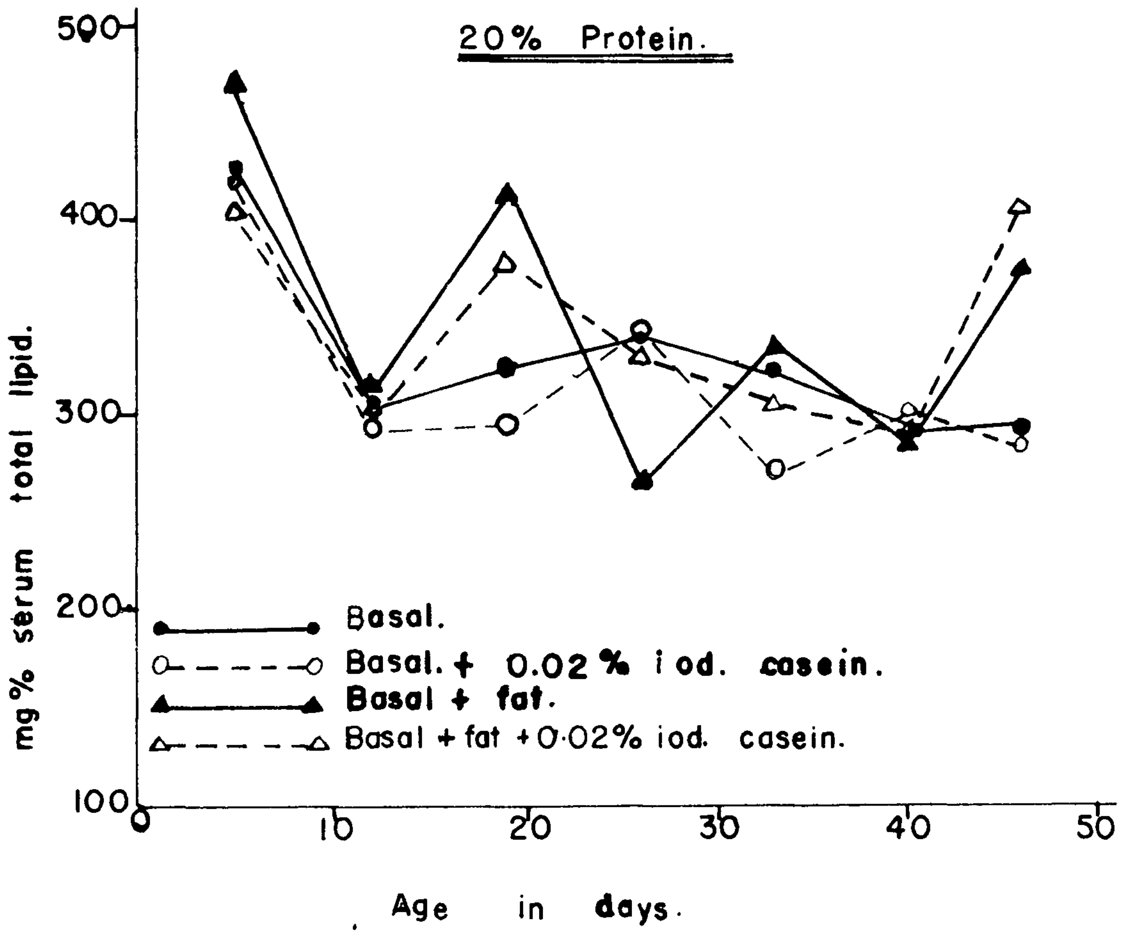
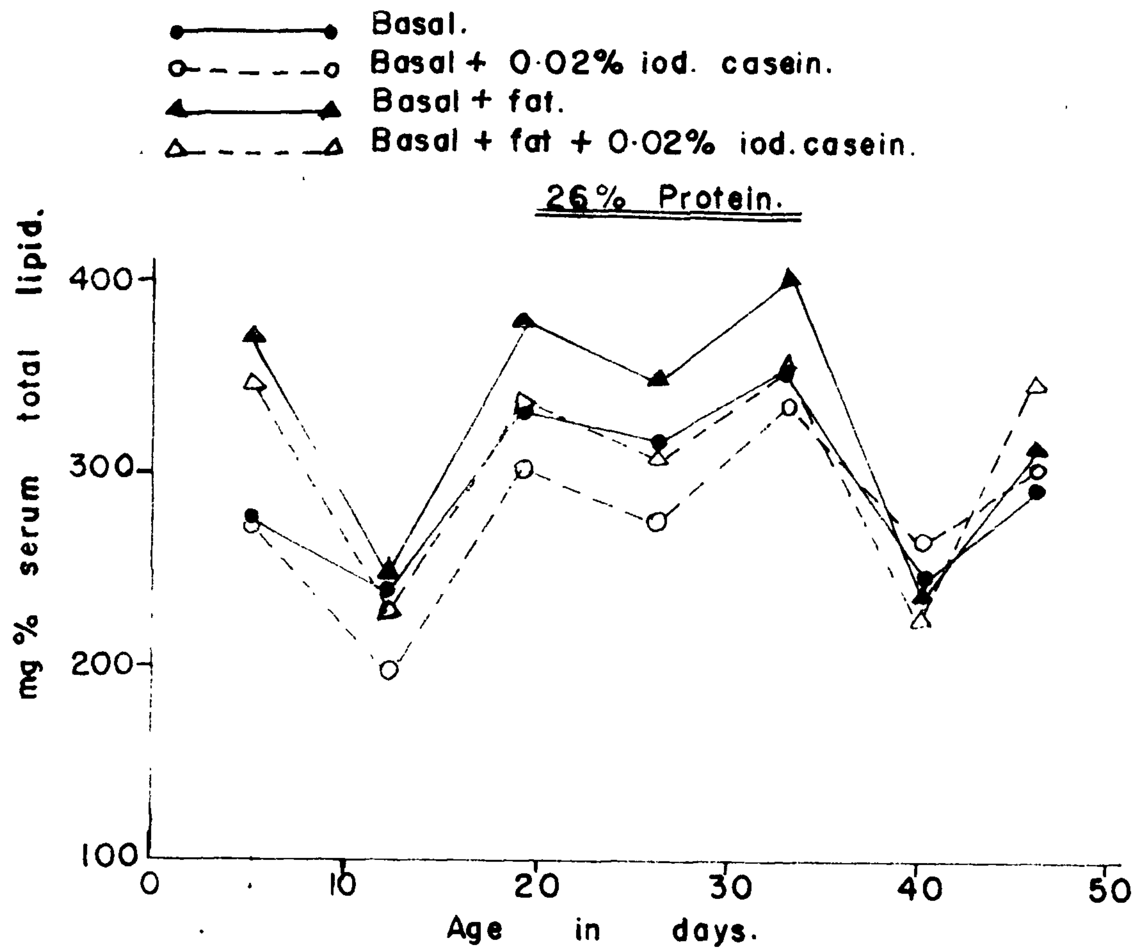


Diagram VIII(f)

Diagram to Show the Serum Total Lipid Level of Chicks in Experiment 8



GENERAL DISCUSSION

GROWTH

The results of experiments 1 and 8 indicate that

(i) Normal chicks fed a 26% protein diet grew at a significantly faster rate than normal chicks fed a 20% protein diet.

(ii) When 12% fat was included in the diets of normal chicks, either in the form of hydrogenated vegetable oil or lard, normal chicks fed the fat diet containing a 26% protein level grew at a significantly faster rate than normal chicks fed a 20% protein diet.

(iii) Induced hyperthyroidism increased the growth rate of chicks fed a 26% protein diet, low in fat content, as compared to normal chicks fed a similar diet.

(iv) Administration of the thyroactive protein to chicks fed a 20% protein diet at either fat level depressed the growth of these chicks.

(v) The administration of thyroactive protein retarded the growth of chicks fed a 26% protein diet, high in fat content, as compared to normal chicks fed a similar diet.

The above findings agree with those reported in the literature.

Biely and March (1954) reported that the effect of fat supplements in chick diets depends in part upon the protein content of the diets. The addition of fat to low protein diets depresses growth and feed efficiency whereas the addition of fat to high protein diets has been shown to stimulate growth and to improve efficiency of feed utilization. Barnes, Primrose and Burr (1944) showed that a lower protein content in the diet fed to rats was associated with a lower fat digestibility.

The effect on the growth rate of chicks rendered hyperthyroid was inconsistent. Whereas a significant increase in the growth rate of chicks fed thyroactive protein was obtained in experiments 2, 5, and 7, and a highly significant increase in growth rate of hyperthyroid chicks fed a 26% protein diet in experiment 1, no effect was obtained in the growth rate of hyperthyroid chicks from experiments 3, 6, and 8. In experiment 1 growth rate was retarded in hyperthyroid chicks fed a 20% protein diet. Data is available to indicate that requirements for a number of nutrients are markedly increased in the hyperthyroid animal. Drill et al (1943) have shown that the requirements for the three B complex vitamins, thiamin, pyridoxine, and panthothenic acid are increased during experimental hyperthyroidism of the rat. Martin (1952) has demonstrated an increase requirement for folic acid, while Nichol et al (1949) have demonstrated an increased requirement for vitamin B_{12} following the administration of large doses of thyroactive substances in the rat. If the administration of thyroactive substances in the chick has similar physiological effects as have been shown to occur in the rat, then it is likely that the effect of feeding thyroactive protein has increased the requirements of the chick for certain vitamins or essential growth factors.

It is possible that a decrease in the growth rate of chicks fed a 20% protein basal diet together with the administration of thyroactive protein, over normal chicks fed similar diets, can be explained by a possible vitamin deficiency which may have resulted from inducing hyperthyroidism in the young chick.

Experiments 1, 2, 3, 5, 6, and 7 show that when chicks were fed diets containing additional amounts of the B complex vitamins, calcium panthothenate, folacin, niacin, riboflavin and choline chloride, significant increases in growth rate were obtained from chicks fed these diets as compared to chicks fed diets low in the above B complex vitamins.

With regard to the effect of the quantity of fat in the diet, on the growth rates of chicks, experiments 1 and 8 show a significant increase in the growth rates of chicks fed high fat diets, provided the protein content of the diet was adequate. In experiments 3, 4, 5, 6, and 7, the protein content of the diets used was 26%. Although increases in growth rate of all chicks were obtained when 12% fat was included in their diets, the increased growth rates shown by chicks from experiments 1, 6, 7, and 8 were significant.

It is apparent that the energy content of the diets, containing 12% hydrogenated vegetable oil as in experiment 1, or 12% lard as in experiment 8, was adequate when the protein level of the diet was 20%. The energy content of the high fat diets was not adequate when the protein level was 26%. This may explain the increase in growth obtained in young chicks fed a high fat, high protein diet over those fed a high fat, low protein diet. This finding is supported by x those of Dam et al (1959) and Baldini and Rosenberg (1957) in chicks. Dam et al obtained highly significant growth increases with chicks, when the hydrogenated vegetable oil or lard content of the diet was increased from 2-5% to 10%. The protein content of the diet was adjusted to take care of the increased energy content.

SERUM TOTAL CHOLESTEROL

By increasing the protein level of a diet, a decrease in serum cholesterol concentration was obtained by Moyer et al (1956) in rats, Kokatnur et al (1958) in mature cockerels, Nishida et al (1958), Johnson et al (1958), and March et al (1959) in the young chick.

The results presented in experiment 8 confirm the findings of the above workers. When basal diets of 20% and 26% protein level respectively were fed to young chicks, lower serum cholesterol and lipid levels were obtained from chicks fed the 26% protein diet than from those fed the 20% protein diet. Diagrams IX(a) and IX(b) compare the serum cholesterol level obtained in chicks fed both a 20% and a 26% protein basal diet.

In experiment 1, no differences in the serum cholesterol level of both normal chicks and chicks rendered hyperthyroid could be obtained from feeding either a 20% or 26% protein basal diet. In experiment 1 and 8, however, chicks rendered hyperthyroid and fed a high fat diet containing a protein level of 20%, showed higher serum cholesterol levels than those chicks rendered hyperthyroid and fed a high fat diet consisting of a 26% protein level.

Much work has been done on the effect of experimentally produced changes in the thyroid status. An increase in thyroid activity has been found to decrease the serum cholesterol and serum total lipid level in some experimental animals. The data obtained in the various experiments and illustrated in diagram X(a) and X(b) show that there is a great amount of variability in the serum cholesterol level, of chicks fed 0.02% iodinated casein.

As seen in diagram X(a) the levels of serum total cholesterol and serum total lipids of induced hyperthyroid chicks are compared. In experiments 2, 3, and 6 hyperthyroid chicks showed somewhat larger levels of serum cholesterol than normal chicks while, in experiments 4 and 5 no differences could be seen. However, in experiments 7 and 8 serum cholesterol levels were lower in hyperthyroid chicks than in normal chicks. An increase in cholesterol level due to a hyperthyroid state is in contradiction with the work reported in the literature. Bertheil et al (1947) have demonstrated a decrease in serum cholesterol level in the hyperthyroid rat while Smith (1935) was the only worker to find the opposite effect in rabbits. It seemed reasonable to suspect that the level of thyroactive protein fed may not have been effective in producing a hyperthyroid state in the chicks at all times. This possibility was investigated further and in experiment 5 the effect of feeding twice the level of thyroactive protein (0.04% iodinated casein) was determined. The effect of the increased level of thyroactive protein on the serum cholesterol concentration was still inconsistent. While in some instances a high cholesterol concentration was obtained, on other occasions a decreased level was produced. These observations would tend to rule out the possibility that the level of 0.02%

iodinated casein was ineffective in producing a hyperthyroid state in young chicks. 0.02% iodinated casein may have had a seasonal effect in producing increased thyroid activity in the young chick. In order to confirm this postulate further experimental work should be done which would involve long-term experiments.

Using the rat as the experimental animal Rosenman et al (1952) have shown that the rate of synthesis of cholesterol has increased in hyperthyroidism, and conversely there is a lower synthesis rate in hypothyroidism. Byers (1958) has stated that these changes in the rate of synthesis are opposite in direction to the blood cholesterol concentration changes. The plasma cholesterol level has been shown to fall in the hyperthyroid state of the rat and rise in the hypothyroid state. Byers (1958) suggested that the rate of excretion of plasma cholesterol during hyperthyroidism is increased while during hypothyroidism the rate of cholesterol excretion is decreased. The lower levels of serum cholesterol obtained in hyperthyroid chicks than in normal chicks as seen in experiments 7 and 8 confirms the suggestions of Byers (1958). In experiments 7 and 8, it is possible that the rate of excretion of cholesterol is increased in those chicks rendered hyperthyroid. The above reasoning, however, does not explain why higher levels of serum cholesterol were obtained in hyperthyroid chicks from experiments 2, 3, and 6.

The effect of hyperthyroidism on serum total lipids followed almost the same pattern as that for serum total cholesterol. Diagram XI(c) shows this pattern and the same explanation which held for serum cholesterol can hold for the effect of thyroactive protein on the total lipid levels. In experiment 4 while no differences were obtained between the levels of cholesterol in the hyperthyroid chick and the normal chick, the lipid levels of the hyperthyroid chick was much higher than those of the normal chick.

Effects of Fat

Animal experiments have consistently demonstrated during the past half century that dietary cholesterol is one of the most important factors which influence the serum cholesterol concentration. We are unaware of any mammalian or avian species systematically studied which under certain circumstances does not respond to dietary cholesterol with increased serum cholesterol levels. This is an established fact. In man Keys and his associates have presented data to show that the quality as well as the quantity of fat may play a role in determining the levels of cholesterol in the serum of the blood. With further nutritional studies Ahrens et al (1957), Malmros and Wigand (1957) have come to associate higher levels of serum cholesterol with the intake of animal or saturated fatty acids.

Using mature cockerels, Kokatnur et al (1958) have concluded that serum cholesterol values were increased in chickens which have been fed fat over those fed an essentially fat free diet. In young chicks March and Biely (1959) have shown that diets supplemented with saturated fat increase serum cholesterol level to a greater degree than those supplemented with unsaturated fat. The saturated fat used in experiments 1, 2, 3, 4, 5, and 6 was hydrogenated vegetable oil. In experiment 8 lard was the saturated fat used (see diagram XIII(a) and XIII(b).

The effects on the level of serum cholesterol of chicks fed saturated fat, as seen from experiments carried out, confirm those obtained by other workers. The results as shown in diagram XIII(a) illustrates this. Experiments 1, 3, 4, 5, 6, 7, and 8 show increases in serum cholesterol level of chicks fed high fat basal diets over those fed basal diets without supplemental fat. In experiment 2 a lower level of serum cholesterol was obtained from normal chicks fed high fat basal diets than from normal chicks fed low fat basal diets. When hyperthyroidism was induced, chicks from experiment 6 were the only ones to show a reasonably high level of serum cholesterol, when they were fed high fat diets (see diagram XII(c)).

Effects of Vitamins

Diagram XII(d) summarizes the effect on the serum cholesterol levels when low B complex vitamin diets were fed to chicks. In experiments 4 and 5 small decreases in serum cholesterol concentration were obtained when B complex vitamin low diets were fed. Apparently there is no evidence in the literature to show the effects obtained on the serum cholesterol and lipid level of chicks when their diets were deficient or low in the B complex vitamins, choline chloride, folic acid, nicotinic acid, riboflavin, and calcium panthothenate, as a group. Herman (1952) showed that by oral administration of nicotinic acid alone, the serum cholesterol concentration of rabbits was reduced. The effect of deficiency of any one particular vitamin is likely to influence the serum cholesterol concentration and total lipid concentration in one direction, but this influence may not be maintained when other vitamins are deficient as well.

Although a vitamin inter-relationship has been known to exist for several years now, there is still very little information as to this relationship, and this is true especially among the B complex vitamins. It is difficult to say whether the dietary level of one B complex vitamin effects the growth of animals receiving inadequate amounts of a second B vitamin. Gyorgy et al (1934), Chick et al (1935) and Harris (1935) by experimental production of deficiency diseases in rats, found that a deficiency of riboflavin and pyridoxine did not exhibit florid dermatitus, characteristic of pyridoxide deficiency, unless adequate riboflavin was added to the diet. Lepkovsky et al (1936) also showed that symptoms of acute pyridoxine deficiency did not appear in the rat unless adequate panthotenic acid was present in the diet. As a result it is difficult to pin-point the effect of a specific vitamin.

When inanition occurs in animals an increase in serum cholesterol and serum lipid concentration results (Cook, 1958).

Whether this increase in cholesterol and lipids concentration is a direct effect of inanition or whether it is indirectly related to a vitamin inadequacy, is difficult to determine. Chicks used in these experiments were not depleted for any particular vitamin prior to the experimental period and there is a possibility that the carryover of vitamins from the egg to the newly hatched chick, is adequate to maintain normal growth and development for a short period of time.

There may also exist the case where the carryover of some vitamins is so low that the amount supplemented may not be adequate to prevent an effect of vitamin deficiency. In experiment 7 this is probably what happened, for symptoms of riboflavin deficiency (curled toe paralysis) were noted not only in the chicks of riboflavin low diets but also in chicks fed the control diets.

Of the seven experiments carried out where low B complex vitamin diets were fed, five experiments showed increased serum cholesterol concentration which may be due to the effects of low levels of the B complex vitamins, choline chloride, folic acid, calcium panthothenate, nicotinic acid, and riboflavin. When the diets were supplemented with choline chloride and folic acid only, serum cholesterol level was still increased. In experiments 4 and 5 a small increase in serum cholesterol was obtained.

Effect of Thiouracil

The opposing effect of thiouracil and thyroxine were first used by Dempsey and Astwood (1943) to estimate the rate of thyroid secretion in rats and by Mixner et al (1944) for the assay of thyroid activity in chicks. These workers found that thiouracil decreased the metabolic rate of animals treated. Diagrams XV(a), XV(b) and X(c) compare the results obtained by induding hypothyroidism in young chicks. Both the serum cholesterol and serum total lipids are higher in induced hypothyroid chicks than normal chicks. These results confirm those already found by Fleischmann et al (1945) in the thiouracil treated chicks, and those of Roseman et al (1952) in the thiouracil treated rat. In experiment 4 in most instances it was found that by producing a hypothyroid state in the young chick the serum cholesterol and serum total lipid levels were elevated.

When hypothyroidism was induced in chicks fed high fat diets, no differences were noted in the cholesterol levels of these chicks and those of normal chicks fed diets low in the B complex vitamins, calcium panthothenate, niacin, folic acid, riboflavin and choline chloride or in the B complex vitamins choline chloride and riboflavin only.

Hyperthyroid chicks fed basal diets low in B complex vitamins showed lower serum cholesterol levels than normal chicks fed basal diets low in B complex vitamins. This result was achieved in experiments 1, 2, 3, 5, 6, and 7. Experiment 4 showed higher levels of serum cholesterol in hyperthyroid chicks than in normal chicks, fed basal diets low in vitamin B complex (see diagram XIV(a)).

From the results obtained it seems reasonable to say that in most cases when hyperthyroidism is induced in the young chick there is a tendency for these chicks to have lower serum cholesterol levels than normal chicks, provided the basal diets fed were low in the B complex vitamins, calcium panthothenate, nicotinic acid, riboflavin, folacin and choline chloride.

Experiments 1, 4, 6, and 7 showed higher levels of serum cholesterol when chicks were fed basal diets high in fat and low in the B complex vitamins than when they were fed basal diets low in fat and low in B complex vitamins, (diagram XIV(b)). No effect was obtained in experiments 3 and 5. While in experiment 2 a lower level of serum cholesterol was obtained by feeding chicks low vitamin B complex basal diets, high in fat, than feeding chicks basal diets low in B complex vitamins and low in fat content.

In almost all cases, however, hyperthyroid chicks fed high fat low vitamin B complex diets showed lower levels of serum cholesterol than normal chicks fed similar diets, (diagram XIV(c)).

Diagram IX

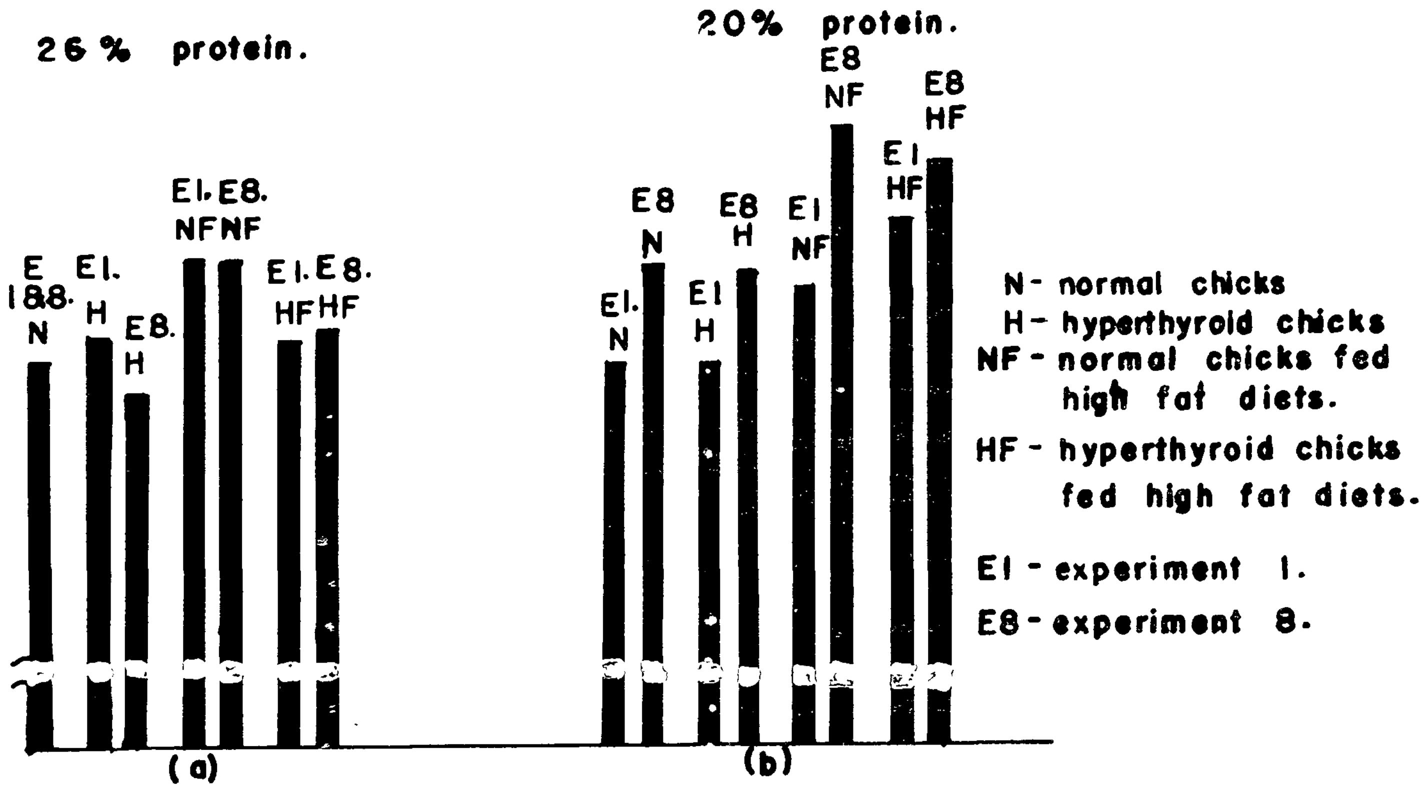
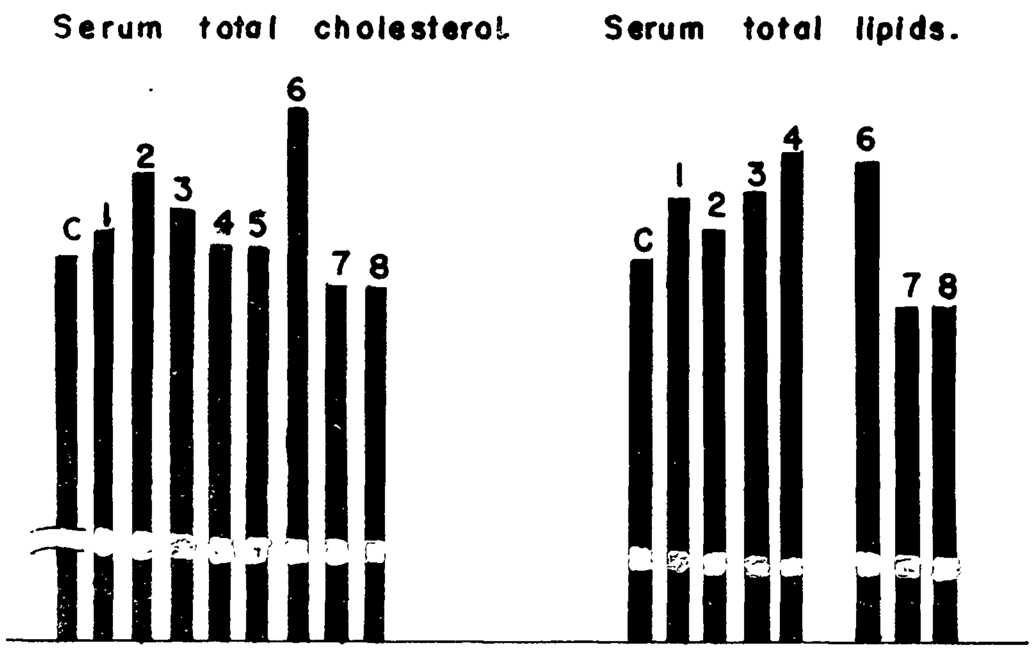


Diagram to show the effect of the protein level on the serum cholesterol concentration of chicks in experiments 1 & 8.

Diagram X

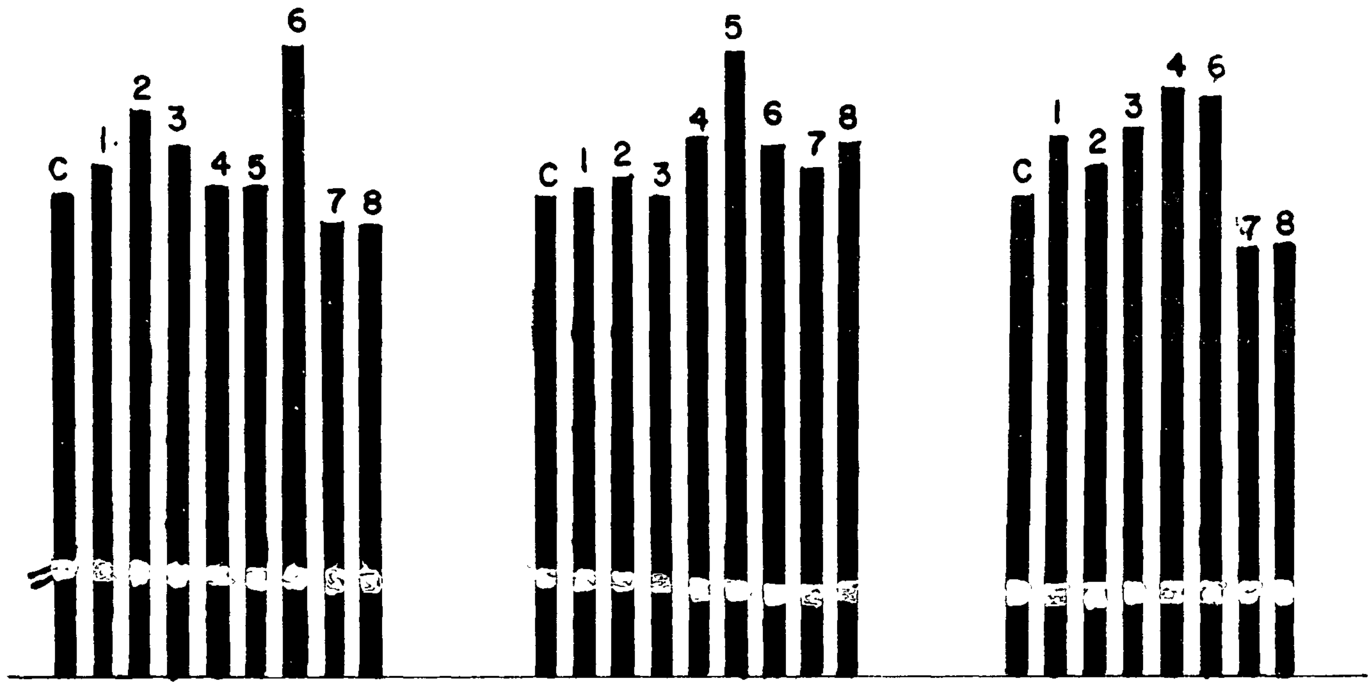


Serum total cholesterol & total lipid in induced hyparthyroid chicks.

C = control.
1-8 = experiments.

Diagram XI

Diagram to Show Effect of 0.02% Iodinated Casein on Growth, Serum Cholesterol and Serum Total Lipid Levels in Chicks from Experiments 1-8



A-Effect of 0·02% iod. casein on serum cholesterol.

B-Effect of 0·02% iod. casein on growth.

C-Effect of 0·02% iod. casein on total lipids.

Diagram XII

Diagram to Show Effects of High Fat Diets, 0.02% Iodinated Casein and Vitamin Deficiency on Chicks in Experiments 1-8

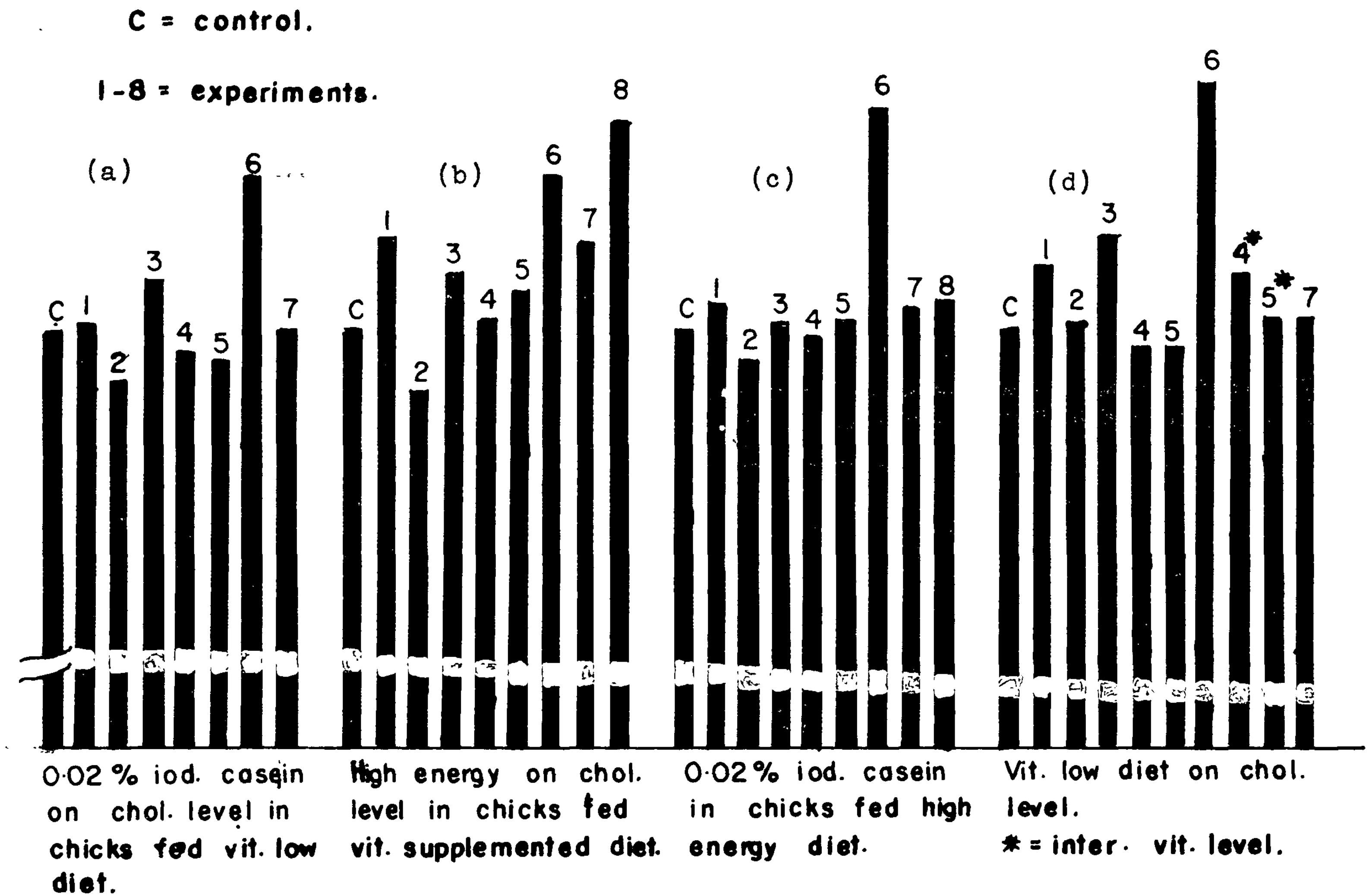
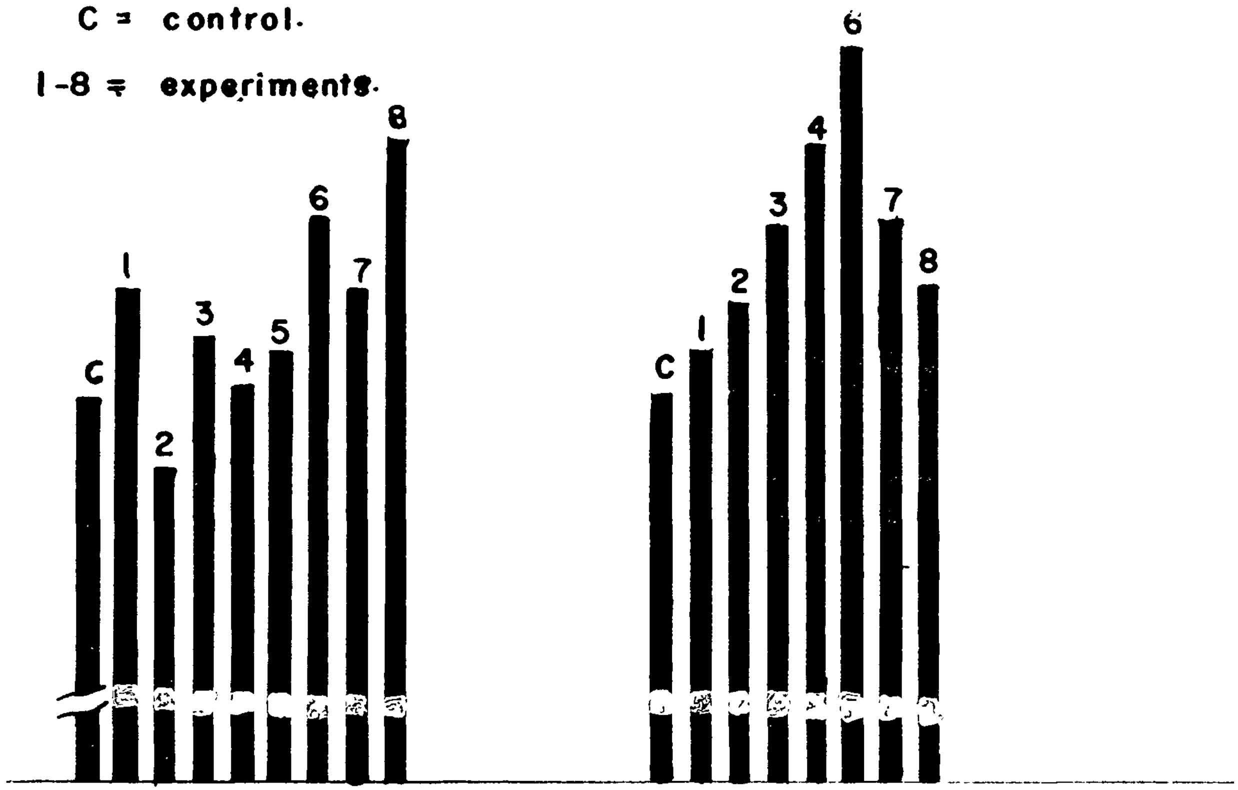


Diagram XIII

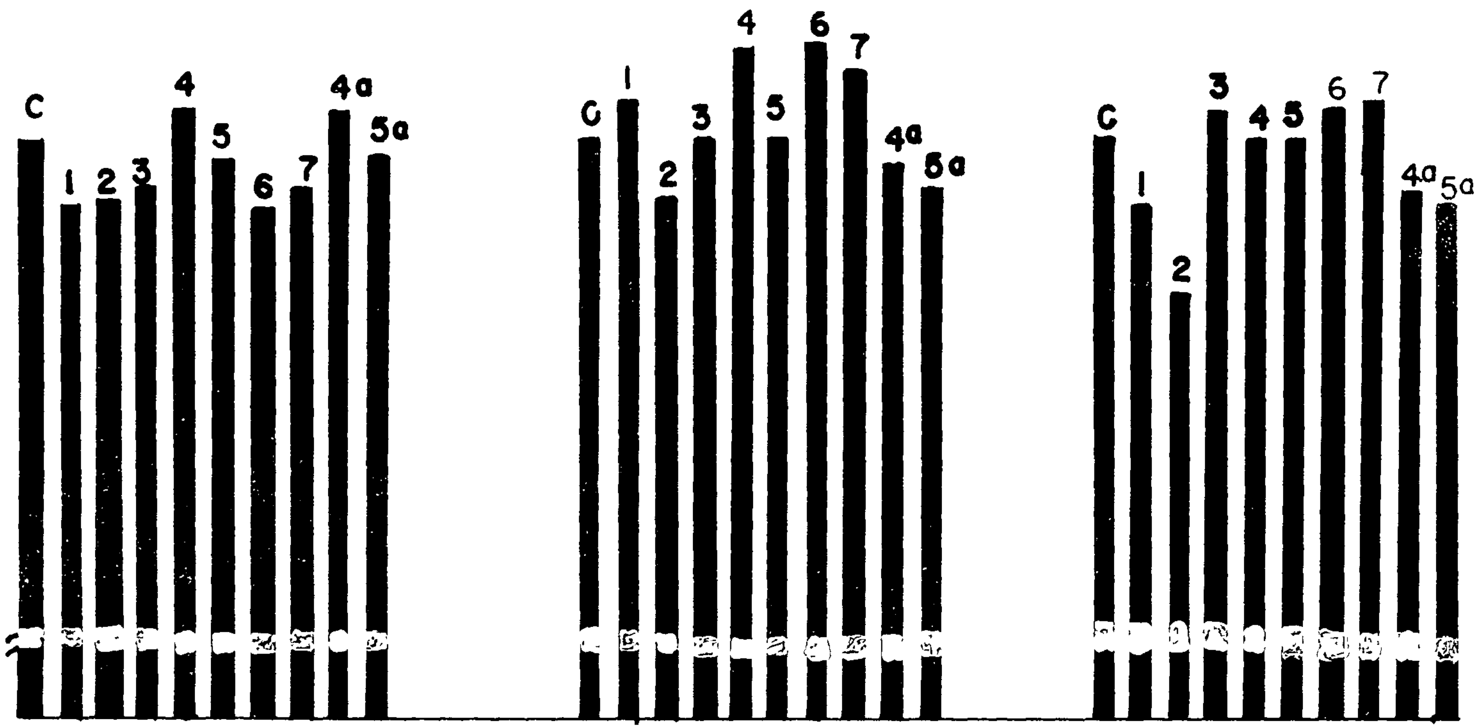


A- cholesterol. B- lipid.

High fat diet on the cholesterol level and lipid level of normal chicks.

Diagram XIV

Serum cholesterol level of chicks fed vit. low diet.



A. effect of 0·02% iod. casein.

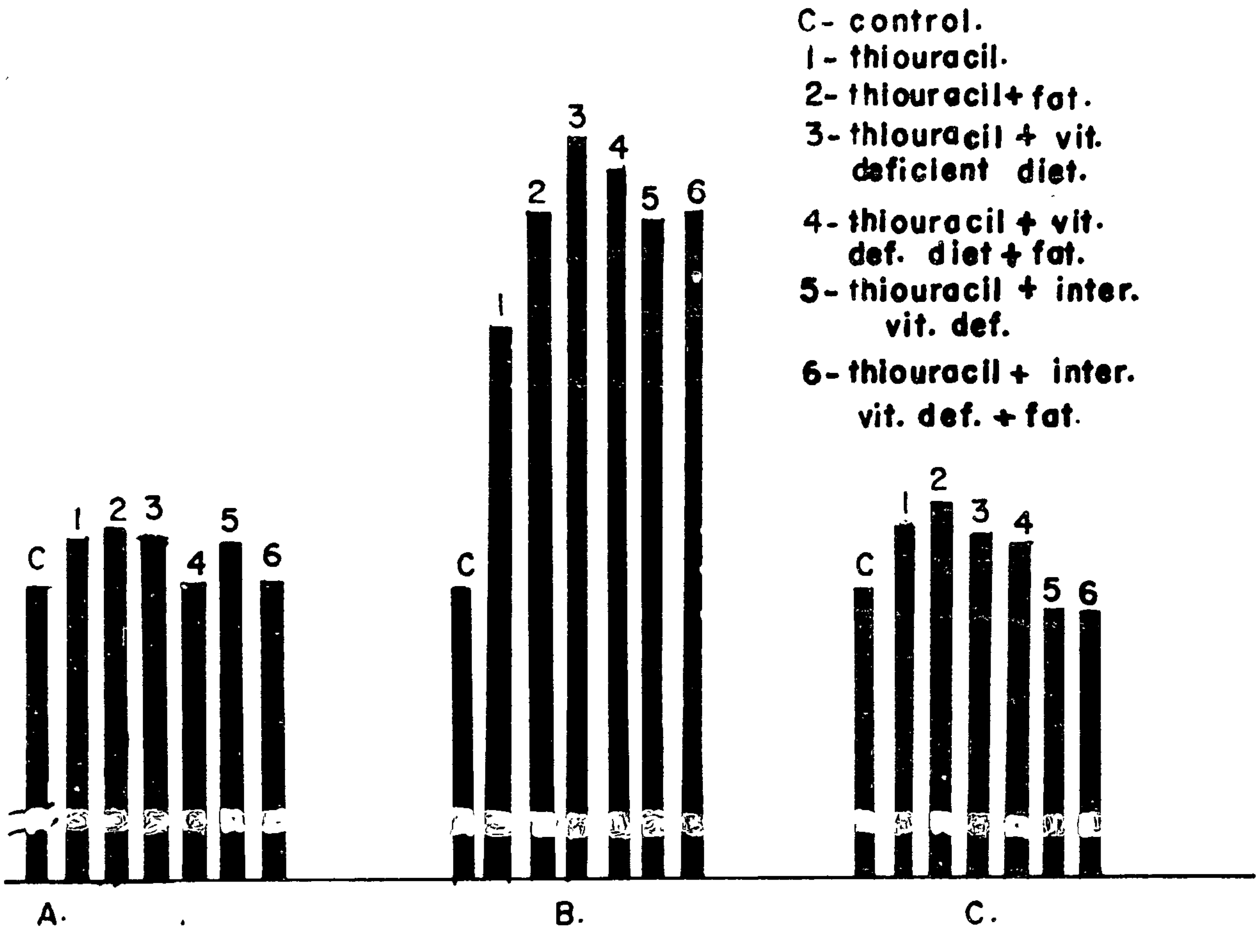
B. effect of high fat diet.

C. effect of 0·02% iod. casein high fat diet.

C = control.
1-7 = experiments.

Diagram XV

Diagram to Show the Effect of Thiouracil on Serum Cholesterol and Serum Total Lipid Levels and Growth of Chicks in Experiment 4



Effect of thiouracil on A - cholesterol.
B - total lipids.
C - growth.

GENERAL CONCLUSIONS

A series of experiments was conducted to observe the effect of certain dietary factors on the serum cholesterol and serum total lipid levels in the growing chick.

Among the factors tested were dietary protein, dietary fat and a group of B complex vitamins consisting of choline chloride, calcium panthothenate, niacin, folacin and riboflavin.

The above dietary factors were tested in several experiments on both the hyperthyroid and the normal chick. In one experiment the hypothyroid chick was used.

Hyperthyroidism was induced in the chicks by feeding them diets containing 0.02% iodinated casein, whereas hypothyroidism was induced by feeding chicks diets containing 0.1% thiouracil.

The growth rate of chicks rendered hyperthyroid varied. In many instances hyperthyroid chicks grew at a significantly faster rate than normal chicks. In other instances however no difference was noted between the growth rate of hyperthyroid and normal chicks, whereas on some occasions when hyperthyroidism was induced in chicks, the growth of these chicks was depressed.

When a 26% protein basal diet was fed to normal chicks, the serum cholesterol observed in these chicks was lower than those fed a 20% protein basal diet.

Hyperthyroid chicks fed a high fat diet containing 26%

protein showed lower serum cholesterol and serum lipid levels than hyperthyroid chicks fed a high fat diet containing a protein level of 20%.

Normal chicks fed a high level of fat showed higher levels of serum cholesterol and total lipids than normal chicks fed a low level of fat.

Normal chicks fed diets low in the B complex vitamins (choline chloride, folacin, calcium panthothenate, niacin, and riboflavin) showed higher levels of serum cholesterol than chicks fed diets high in the above B complex vitamins.

Chicks rendered hypothyroid showed higher levels of serum cholesterol and total lipids than normal chicks.

BIBLIOGRAPHY

Ahrens, E.H. Jr., Blankenhorn, D.H. and Tsalta, T.T. - "Effect on human serum lipids of substituting plant for animal fat in the diet". Proc. Soc. Exp. Biol. Med., 86:872-878, 1954.

Ahrens, E.H. Jr., Hirsch, J., Insull, W. Jr., Tsaltas, T.T., Blowstrand, R. and Peterson, M.L. - "The Influence of dietary fats on serum lipid levels in man". Lancet, i:943-953, 1957.

Altschul, R., Hoffer, A. and Stephen, J.D. - "Influence of nicotinic acid on serum cholesterol in man". Archiv. of Biochem. and Biophysics, 54:558, 1955.

Baldini, J., Rosenberg, H.R. - "The effect of calorie source in a chick diet on growth, feed utilization and body composition". Poultry Science, 36, 432-434, 1957.

Barnes, R.H., Primrose, M.F., and Burr, G.O. -"The influence of the protein content of the diet upon fat digestibility". J. Nutrition, 27:179-184, 1944.

Betheil, J.J., Wiebelhaus, V.D., and Lardy, H.A. - "A nutritional factor which alleviates the toxicity of ingested thyroid substance". J. Nutrition, 34:431-441, 1947.

Beveridge, J.M.R., Connell, W.F., Mayer, G.A., Firstbrook, J.B., and Dewolfe, M.S. - "The effects of certain vegetable and animal fats on the plasma lipids of humans". J. Nutrition, 56:311-320, 1955.

Biely, J., and March, B.-"Fat studies in poultry". Poultry Science, 33:1220-1227, 1954.

Biely, J., and March, B. - "Fat studies in poultry". Poultry Science, 36:1236-1240, 1957.

Brontee-Stewart, B., Antonis, A., Eales, L. and Brock, J.F. "Effects of feeding different fats on serum cholesterol level". Lancet, i:521-526, 1956.

Byers, S.O. - "The mechanism for changes in blood cholesterol in deranged thyroid states". Amer. Jour. of Clin. Nut., 6:642, 1958.

Chick, H., Copping, A.M., and Edgar, G.E. - "The Water Soluble B Vitamin. (iv) The components of vitamin B12". Biochem. J., 29:722-734, 1935.

Combs, G.F., and Romoser, G.L. - "A new approach to poultry feed formulation". Maryland Agr. Exp. Stat. Misc. Pub., 226: 1955.

Cook, R.P. - Cholesterol. University of St. Andrews, New York Academic Press, 1958. p.288.

Dam, Richàrd, Leach, R.M. Jr., Nelson, T.S., Norris, L.C., and Hill, F.W. - "Studies on the effect of quantity and type of fat on chick growth". J.of Nut., 68:615-631, 1959.

Dempsey, E.W., and Astwood, E.B. - "Determination of the rate of thyroid hormone secretion at various environmental temperatures". Endocrinol, 32:509, 1943.

Donaldson, W.E., Combs, G.F., Romoser, G.L., and Supplee, W.G. - "Body composition, energy intake, feed efficiency, growth rate and feather condition of growing chickens as influenced by calorie protein ratio of the ration". Poultry Science, 34:1190, 1955.

Drill, V.A., and Overman, R. - "Increased requirements of panthothenic acid and vitamin B6 during experimental hyperthyroidism". Am. J. Physiol., 135: 474-477, 1941.

Fillios, L.C., and Mann, G.V. - "Influence of sulphur-amino acid deficiency on cholesterol matabolism". Metabolism, 3:16-26, 1954.

Fillios, L.C., Mann, G.V., and Stare, F.J. - "Diets low in protein favour hypercholesteremia in rats". J. Exp. Med., 104:539-551, 1956.

Fleischmann, W., and Fried, I.A. - "Studies on the mechanism of the hyperolesterolemia and hypercolcemia induced by estrogen in immature chicks". , 35:406, 1945.

Green, B., Lowe, J.S., and Morton, R.A. - "The effect of vitamin A deficiency on the cholesterol level of the plasma and liver of the rat". Biochem. J., 61:447-453, 1955.

Greenberg, L.D., and Rinehart, J.D. - "Plasma cholesterol levels of cholesterol fed control and pyridoxine deficient monkeys". Proc. Soc. Exp. Biol. Med., 76:580-583, 1951.

Gyorgy, P. - "Vitamin B12 and the pellegra-like dermatities in rats". Nature, 133:498, 1934.

Hardinge, M.G., and Stare, F.J. - "Dietary and Serum levels of cholesterol". Am. J. Clin. Nut., 2:83, 1954.

Harrill, Inez, Kylen, A.M., Weis, A., and Dyar, E. "Relation of dietary fat and supplementary riboflavin to tissue levels of cholesterol, riboflavin and total lipids in the rat". J. Nutrition, 69:356-364, 1959.

Harris, L.J. - "Flavin and the pellegra-preventing factor as separate constituents of complex B2". Biochem. J., 29:776-781, 1935.

Herman, George - "Methionine decholesterolization in old hens". University of Texas Bulletin, 1952.

Hildreath, E.A., Mellinkoff, S.M., Blair, G.W., and Hildreath, D.M. - "The effect of vegetable fat ingestion on human serum cholesterol concentration". Circ., 3:641-646, 1951.

Hsu, J.M., and Chow, B.F. - "Vitamin B12 deficiency and hypercholesterolemia". Fed. Proc., 16:63, 1957.

Huerga, J. de la, Charlotte, Y., and Popper, H. "Estimation of total serum lipids by a turbidimetric method". Am. J. Clin. Path., 23:1163-1167, 1953.

Irwin, M.R., Reineke, E.P., and Turner, C.W. - "Effect of feeding thyroactive iodocasein on growth, feathering, and weight of glands of young chicks". Poultry Science, 22:374-380, 1943.

Johnson, D. Jr., Leveille, G.A., and Fisher, H. - "Influence of amino acid deficiencies and protein level on the plasma cholesterol of the chick". J. Nutrition, 66:367-376, 1958.

Jones, R.J., and Huffman, S. - "Chronic effect of dietary protein on hypercholestermia in the rat". Soc. for Exp. Biol. Med., 93:519, 1956.

Keys, A., Anderson, J.T., Fidanza, F., Keys, M.H., and Swalen, B. - "Effects of diet on blood lipids in man, particularly cholesterol and lipoprotein". Clin. Chem., 1:34-52, 1955.

Keys, A., Anderson, J.T., and Grande, F. - "Serum Cholesterol Response to Natural and Hydrogenated Fats". Circ., 16:480 (Abstr.)., 1957.

Kinsell, L.W., Patridge, J., Boling, L., Margen, S., and Michaels, G.D. - "Dietary modification of serum cholesterol and phospholipid levels". J. Clin. Endocrinal and Metabolism, 12:909-913, 1953.

Kokatnur, M., Rand, R.T., Kummerow, F.A., and Scott, H.M. "Effect of dietary protein and fat on changes of serum cholesterol in mature birds". J. Nutrition, 64:177-183, 1958.

Lepkovsky, S., Dukes, T.H., and Krause, M.E. - "The multiple nature of the third factor of the vitamin B complex". J.B.C., 115:557-565, 1936.

Leong, K.C., Sunde, M.L., Bird, H.R., and Elvehjem, C.A. "Effect of energy: protein ratio and growth rate, efficiency, feathering and fat deposition in chickens". Poultry Science, 34:1206, 1955.

Li, T., and Freeman, S. - "Effect of protein deficiency and cholesterol feeding on the liver of dogs". Am. J. Physiol., 145:646-659, 1946.

Li, T., and Freeman, S. - "Experimental lipemia and hypercholestermia by protein depletion and by cholesterol feeding in dogs". Am. J. Physiol., 145:660-665, 1946.

Malmros, H., and Wigand, G. - "Treatment of Hypercholesteremia". Minn. Med. In symposium on arterosclerosis. U. Minn., 38:864, 1955.

Malmros, H., and Wigand, G. - "The effect on serum cholesterol of diets containing different fats". Lancet, 2:1-7, 1957.

Mann, G.V., Andrus, S.B., McNally, A., and Stare, F.J. "Experimental atherosclerosis in Cebus monkeys". J. Exp. Med., 98:195-217, 1953.

March, B.E., and Biely, J. - "The effect of dietary fat level on thyroid activity in the growing chick". Poultry Science, 36:1270-1277, 1957.

March, B.E., and Biely, J. - "Dietary modification of serum cholesterol in the chick". J. Nutrition, 62:105-110, 1959.

Martin, G.T. - "Increased folic acid requirements resulting from thyroxin infections". Am. Digest Dis., 14:341, 1952.

Matterson, L.D., Potter, L.M., Stinson, L.D., and Singsen, E.P. - "Studies on the effect of varying protein and energy levels in poultry rations on growth and feed efficiency". Poultry Science, 34:1210, 1955.

Meeker, B.R., and Kesten, H.D., - "The effect of high protein diet on experimental atherosclerosis of rabbits". Archiv. of Path., 31:147-162, 1940.

Mixner, J.B., Reineke, E.P., and Turner, C.W., - "Effect of Thiouracil and Thiourea on the thyroid gland of the chick". Endocrinol, 34:168, 1944.

Moyer, A.W., Kritchevsky, D., Logan, J.B., and Cox, H.R. "Dietary protein and serum cholesterol in rats". Proc. Soc. Exp. Biol. Med., 92:736-737, 1956.

Muschett, C.W., and Emerson, G.A. - "Arteriosclerosis in pyridoxine deficient monkeys and dogs". Fed. Proc. 15:526, 1956.

Newburgh, M.D., and Clarkson, Sarah - "Production of artheriosclerosis in rabbits by diets rich in animal protein". J.A.M.A., 79:1106-1108, 1922.

Nichol, C.A., Dietrich, L.S., Cravens, W.W., and Elvehjem, C.A. - "Activity of vitamin B12 in the growth of chicks". Proc. Soc. Exp. Biol. Med., 70:40, 1949.

Ibid., - "Observations on folic acid deficiency in the chick in the presence of vitamin B12". J. Nutrition, 39:289-297, 1949.

Nishida, T., Takenaka, F., and Kummerow, F.A. - "Effect of dietary protein and heated fat on serum cholesterol and beta-lipoprotein levels, and on the incidence of experimental atherosclerosis in chicks". Circ. Res., 6:194-201, 1958.

Page, I.H., Lewis, L.A., and Gilbert, J.B. - "Plasma lipids and proteins and their relationship to coronary disease among Navajo Indians". Circ., 13:675, 1956.

Parsons, W.B., Achor, R.W.P., Berge, K.G., McKenzie, B.F., and Barker, N.W. - "Changes in concentration of blood lipids following prolonged administration of large doses of nicotinic acid to persons with hyper-cholestermia". Proc. Staff Meetings of the Mayo Clinic, 31:377, 1956.

Parsons, W.B., and Flinn, J.H. - "Reduction in elevated blood cholesterol levels by large doses of nicotinic acid". J. Am. Med. Assoc., 165:234-238, 1957.

Pick, R., Stamler, J., and Katz, L.M. - "Effects of high protein high vitamin supplements on cholesterolemia and atherogenisis in cholesterol fed cockerels".

Fed. Proc., 16:101, 1957.

Ralli, E., and Waterhouse, A. - "Blood cholesterol in dogs, on an A deficient diet". Proc. Soc. Exp. Biol. Med., 30:519-523, 1933.

Rinehart, J. D., and Greenberg, L.D. - "Arteriosclerotic lesions in pyridoxine deficient monkeys". Am. J. Path., 25:481-485, 1949.

Rinehart, J.D., and Greenberg, L.D. - "Vitamin B6 deficience in Rhesus monkey". Am. J. Clin. Nutrition, 4:318, 1956.

Rhodes, D.N. - "Interference by polyunsaturated fatty acids in the determination of cholesterol". Biochem. J., 71:26, 1959.

Robblee, A.R., Nichol, C.A., Cravens, W.W., Elvehjem, C.A., and Halpin, J.G. - "Relation between induced hyperthyroidism and an unidentified chick growth factor". Proc. Soc. Exp. Biol. Med., 67:400-404, 1948.

Rosenman, R.H., Byers, S.O., and Friedman, M. - "The mechanismresponsible for the altered blood cholesterol in deranged thyroid states". J. of Clin. Endocrinol, 12:1287, 1952.

Scott, H.M., Matterson, L.D., and Singsen, E.P. - "Nutritional factors influencing growth and efficiency of feed utilization. The effect of the source of the carbohydrate". Poultry Science, 26:554, 1957.

Smith, M.E. - "The effect of vitamin A deficiency on the concentration of the blood lipids of the albino rat". J. Nutrition, 8:675-687, 1934.

Sperry, W.M., and Webb, M. - "A revision of the Schoeinheimer-Sperry Method for cholesterol determination". J. Biol. Chem., 187:97, 1950.

Stamler, J., Pick, R., and Katz, L. - "Effects of vitamin deficiency on cholestermia and atherogenisis in cockerels on highfat, high cholesterol diets". Circ., 18:511, 1958.

Stuckey, N.W. - "Ueber die Ueranderungen der Kaninchen aorata bei der Futterung mit uerschiedenen Fettsorten. Zentralb F. Allg. Path., 23:910-911, 1912.

Sure, B., Kik, M.C., and Church, A.E. - "Effects of vitamin A deficiency on concentration of blood lipids of the albino rat". Proc. Soc. Exp. Biol. Med., 30:620-621, 1933.

Van Bruggen, J.T., and Staumfjord, J.V. - "High vitamin A intake and blood levels of cholesterol, phospholipids, carotene and vitamins C, A, and E". J. Lab. Clin. Med., 33:67, 1948.

Wheeler, R.S., Hoffman, E., and Graham, C.L. - "The value of thyroprotein in starting, growing and laying rations". Poultry Science, 27:103-111, 1948.

Wittan, P.W., and Holman, R.T. - "Polyethenoid fatty acid metabolism. (vi) Effect of pyridoxine on essential fatty acid conversion". Archiv. Biochem. Biophysics, 41:266-273, 1952.

Wood, J.D. - "The effect of vitamin A and lingcod liver oil components on the serum cholesterol levels in chicks". Can. J. Biochem. and Physiol, 39:19, 1960.

Zlatkis, A., Zak, B., and Boyle, A.J. - "A new method for the direct determination of serum cholesterol". J. Lab. Clin. Med., 41:486-491, 1953.