1965

Effects of B Vitamin Deficiencies on Energy Metabolism of the Chick

William Clarence Lockhart

EFFECTS OF B VITAMIN DEFICIENCIES ON ENERGY METABOLISM OF THE CHICK

BY

WILLIAM CLARENCE LOCKHART

A thesis submitted
in partial fulfillment of the requirements for
the degree Doctor of Philosophy, Major in
Animal Science, Department of Poultry
Science, South Dakota
State University

June 1965

EFFECTS OF B VITAMIN DEFICIENCIES ON
ENERGY METABOLISM OF THE CHICK
Abstract

WILLIAM CLARENCE LOCKHART

Under the supervision of Professor C. W. Carlson

Experiments were conducted over a four year period to study the effects of pyridoxine, thiamine, riboflavin, pantothenic acid and niacin nutrition as related to deutectomy of the day-old chick, liver vitamin storage, efficiency of metabolizable energy (ME) utilization and oxygen consumption. Broiler-type chicks used in the several feeding trials were housed in a 20' X 30' insulated battery brooding room. Temperature was maintained at approximately 80°F. and fluorescent lights provided continuous lighting. The chicks were contained in conventional battery brooders during the vitamin depletion period and were put in shifting-type metabolism pens during the experimental periods at which time droppings were collected and growth data obtained.

One casein-cerelose type diet was used for all depletion studies. Chromic oxide was used as a dietary indicator to determine caloric utilization quantitatively. A Parr adiabatic calorimeter was used to determine heats of combustion of diet and feces. Oxygen consumption data were obtained by use of a Minute Oxygen Uptake Spirometer and carbon dioxide was determined by gravimetric procedure.

Vitamin depletion studies were conducted with each B vitamin under normal (yolk intact) and deutectomized (yolk removed) conditions.

The body weight reduction of the chicks due to deutectomy was not regained by the chicks for any vitamin during experimental period, the longest period being 32 days. The removal of the yolk had little if any effect on vitamin depletion time or severity of deficiency signs.

A comparison of growth and liver storage at various vitamin dosage levels showed different repletion patterns. Thiamine and pyridoxine did not tend to accumulate in the liver until dosage levels supporting maximum growth were attained. For riboflavin, pantothenic acid and niacin, liver storage of these vitamins started at the low dosage levels but each followed a different repletion pattern.

The efficiency with which ME was utilized by the five vitamins was determined at 11 vitamin dosage levels. The efficiency of ME utilization was significantly depressed by various planes of B vitamin nutrition. The mcg. dosage per 100 gm. of body weight at and below which the various vitamins depressed ME utilization were: pyridoxine, 49.0; thiamine, 27.0; riboflavin, 33.0; pantothenic acid, 85.0; and niacin, 90.0. With the exception of thiamine the range of decrease in efficiency of ME utilization was less than three percent of maximum efficiency. The range of ME decline from maximum utilization for thiamine approximated nine percent.

Oxygen consumption of chicks receiving all experimental vitamins was shown in two differently designed trials to be significantly

higher than chicks deprived of any one of the experimental vitamins. Even with 4-5 days of repletion, the highest dosage level did not support oxygen consumption equal to normal chicks. Although there was an upward trend in oxygen consumption with increased vitamin levels, thiamine deficiency seemed to give the only clear evidence of a reduction due to a vitamin *per se*.

EFFECTS OF B VITAMIN DEFICIENCIES ON ENERGY METABOLISM OF THE CHICK

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Poultry Department Date

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to:

Dr. C. Wendell Carlson and Professor William Kohlmeyer for their suggestions, assistance and constructive criticisms in the preparation of this dissertation.

Dr. W. L. Tucker for statistical advisement.

Dr. Reece L. Bryant, Dr. William E. Dinusson, Mr. Donald W. Bolin and Mr. Clayton N. Haugse of North Dakota State University, Fargo, North Dakota for their helpful suggestions in outlining the project and their assistance in the analysis of data.

His wife, Vera, for her encouragement and her helpfulness in final preparation of this manuscript.

Shari Ness and Patty Busch for stenographic assistance.

The North Dakota Agricultural Experiment Station for allowing the use of experimental data collected at that station.

WCL

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
PROCEDURE AND MATERIALS	25
Experimental Animals	25
Housing and Brooding Units	25
Experimental Period	27
Dietary Indicator	28
Calorimetry	29
Basal Metabolism Rate	30
Liver Vitamin Assay	35
Experimental Diet Development	36
Adjustment of Data	38
Growth	40
Statistics	40
RESULTS AND DISCUSSION	42
Deutectomy and Vitamin Depletion	42
Pyridoxine	43
Thiamine	44
Riboflavin	46
Pantothenic acid	46
Niacin	49

Page

The Effects of B Vitamin Deficiencies on the Efficiency of Metabolizable Energy Utilization 53

Pyridoxine. 56

Thiamine. 57

Riboflavin. 59

Pantothenic acid. 61

Niacin. 64

Variation Between Vitamins 67

B Vitamin Nutrition and Basal Metabolism 68

Diurnal Variation 69

Expression of Basal Metabolism Rate 70

Metabolic Body Size 73

B Vitamin Nutrition and Oxygen Utilization 75

Growth and Vitamin Storage 81

Pyridoxine and Thiamine 82

Riboflavin, Pantothenic acid and Niacin 82

SUMMARY AND CONCLUSIONS 89

LITERATURE CITED. 92

APPENDIX I. 101

APPENDIX II 106

LIST OF TABLES

Table		Page
1.	Composition of experimental diet	37
2.	The effects of time of day and vitamin deficiency on oxygen consumption.	71
3.	Correlation coefficients for body weights and O_2 consumption at various vitamin intake levels	75
4.	Effects of pyridoxine, riboflavin, pantothenic acid, thiamine, niacin and intake levels of oxygen consumption	77

LIST OF FIGURES

Figure		Page
1.	Schematic of basal metabolism unit.	33
2.	Effects of deutectomy, pyridoxine depletion and repletion on chick growth	45
3.	Effects of deutectomy, thiamine depletion and repletion on chick growth	45
4.	Effects of deutectomy, riboflavin depletion and repletion on chick growth	47
5.	Effects of deutectomy, pantothenic acid depletion and repletion on chick growth	48
6.	Effects of deutectomy, niacin depletion and repletion on chick growth	50
7.	Effects of method of niacin supplementation on chick growth.	50
8.	Effect of pyridoxine on the efficiency of ME utilization	58
9.	Effect of thiamine on the efficiency of ME utilization	58
10.	Effect of riboflavin on the efficiency of ME utilization	65
11.	Effect of pantothenic acid on the efficiency of ME utilization.	65
12.	Effect of niacin on the efficiency of ME utilization	65
13.	Effects of pyridoxine intake on body weight gains and liver pyridoxine of chicks from 11 to 17 days of age	83
14.	Effects of thiamine intake on body weight gains and liver thiamine of chicks from 9 to 15 days of age	83

Figure Page

15. Effects of riboflavin intake on body weight gains and liver riboflavin of chicks from 14 to 20 days of age 84

16. Effects of pantothenic acid intake on body weight gains and liver pantothenic acid of chicks from 14 to 20 days of age 85

17. Effects of niacin intake on body weight gains and liver niacin of chicks from 14 to 20 days of age. . . 86

LIST OF APPENDICES TABLES

Table		Page
	APPENDIX I. ADJUSTED METABOLIZABLE CALORIC ADJUSTED WEIGHT GAIN AND LIVER VITAMIN CONTENT DATA.	101
1.	Thiamine	101
2.	Pyridoxine.	102
3.	Riboflavin.	103
4.	Pantothenic acid.	104
5.	Niacin.	105
	APPENDIX II. THE ANALYSIS OF VARIANCE OF THE EXPERIMENTAL DATA	106
1.	Metabolizable energy values	106
2.	Vitamins and vitamin intake levels as influencing oxygen consumption.	107
3.	Vitamins and time of day as influencing oxygen consumption	107

INTRODUCTION

Approximately two decades ago Robertson et al. (1948) reported that high-energy diets supported faster growth of young chicks with an improvement in feeding efficiency. This seemed to open an era in poultry nutrition in which much work was conducted in order to establish the quantitative relationships between dietary energy levels and nutrients. Donaldson et al. (1956) published data showing a relationship between the caloric and protein (C:P) content of the diet.

With the current emphasis on the energy content of the diet, a knowledge of those factors which interfere with the most efficient utilization of energy is important. Nutritional imbalance was shown by Sure (1941) to decrease the net energy or productive energy content of the diet. Brody (1945) stated that the efficiency of biologic transformation is dependent on the precise timing and completeness of the oxidative reactions. If the oxidation process stops short of the final oxidation products, H_2O and CO_2, as for example when fat oxidation stops with the aceto-acetic acid stage, or carbohydrate oxidation with the pyruvic acid, lactic acid or alcohol stage, the energetic efficiency of food utilization is reduced by this much. In addition untoward effects are developed with corresponding pathologic conditions.

The B complex vitamins are also associated with growth, efficiency of production and general health of monogastric animals.

Several of the vitamins are associated with coenzymes which hold key positions in the metabolism of energy in the intermediary processes. A deficiency of one or more of these vitamins may well interfere with the physiological processes of energy metabolism.

Various nutritional levels of thiamine, riboflavin, pyridoxine, pantothenic acid and niacin were investigated. The objectives of this study were to determine:

1. If and to what degree each vitamin deficiency depressed the efficiency of metabolizable energy utilizable by the chicks.
2. The relationship between liver vitamin storage and growth.
3. The effects of vitamin deficiency upon the basal metabolism rate.
4. The effect of deutectomy on B vitamin depletion time.

LITERATURE REVIEW

General

The discovery and characterization of vitamins have an interesting history and were covered by the reviews of Robinson (1951) and Hogan (1957). It is not the purpose of the present review to cover the historical accounts in detail but a brief resume is here presented.

According to Hogan (1957) the medical profession in the latter part of the nineteenth century was attempting to cure all disease on the premise of Pasteur and Koch that many diseases were caused by pathogenic microorganisms. According to Lusk (1928), Eijkman and associates showed that a substance in rice polishings cured human beriberi and avian polyneuritis. The curative substance which was found to be water soluble was called the antiberiberi or antipolyneuritic factor and later was named thiamine. According to Eddy (1949), Funk in about 1912 proposed the first so-called "Vitamin Hypothesis of Deficiency Diseases."

McCollum and Davis (1913) showed that egg yolk and butter fat contained a fat soluble substance necessary for rat growth. McCollum and Kennedy (1916) suggested that the word vitamin be replaced by referring to the substances as "fat-soluble A" and "water-soluble B." Although the proposal was not fully accepted, the fat and water soluble vitamin groups were therewith established.

With the passing of time, other vitamin-like substances were discovered and characterized mainly on the basis of clinical deficiency signs and isolation techniques. During the 1930's, most of the B vitamins, with the exception of vitamin B_{12} and folic acid, were chemically characterized by degradation and synthesis methods.

Enzymatic Role

The vitamins of interest in the present study are a part of one or more coenzymes. The activity of the apoenzymes with which these are associated cover many different types of biological reactions. The activities of the various coenzymes listed in this review will deal with those important in energy metabolism.

Thiamine

Peters (1936) showed that normal pigeon brain tissue had a higher oxygen uptake than brain tissue from the avitaminous bird. The addition of thiamine to the tissue solutions showed that this vitamin restored oxygen consumption to normal in avitaminous tissue.

Lohmann and Schuster reported, as described by Rosenberg (1945), that the yeast enzyme responsible for anaerobic fermentation of pyruvic acid to acetaldehyde and CO_2 was dependent upon thiamine pyrophosphate (TPP). Lipschitz *et al.* (1938) reported that adenosine triphosphate converted thiamine into cocarboxylase. Weil-Malherbe (1939) showed that neither thiamine nor thiamine monophosphate *per se* functioned as a coenzyme of carboxylase. This

work confirmed the earlier report of lipschitz and associates that thiamine could be converted into cocarboxylase by the action of adenosine triphosphate.

Long and Peters (1939) reported that an enzyme system of yeast was active in decarboxylation of the alpha-oxo-carboxylic acids, and keto-glutaric, keto-valeric, keto-butyric acid and others. Stern (1940) listed 9 different biological reactions in which the metabolism of pyruvic acid alone had been found to occur in various animal tissues.

Two enzymatic oxidative decarboxylation reactions occur in the Krebs cycle of mammalian metabolism (Wagner and Folkers, 1964). One of these is the condensation of "active acetyl" with oxaloacetate with the formation of citric acid. A second important reaction of the cycle is the oxidative decarboxylation of alpha-ketoglutaric acid with the formation of succinyl coenzyme A.

In the hexosemonophosphate shunt the enzyme transketolase is responsible for the cleavage of ribulose-5-phosphate. Here TPP functions as a coenzyme with the formation of "aldehyde-thiamine pyrophosphate" as an intermediate.

Riboflavin

According to Robinson (1951) Warburg and Christian in 1931 showed that a substance called the "yellow enzyme" was an essential link in carbohydrate metabolism, and Kuhn and Rudy in 1936 identified

the coenzyme as riboflavin mononucleotide (FMN). A second coenzyme containing riboflavin was discovered about 1937. According to Horwitt (1954), Warburg and Christian in 1938 reported the structural components. Schrecker and Kornberg (1950) and Christie et al. (1954) contributed to the structural identity of the coenzyme which was called flavin adenine dinucleotide (FAD).

Snell (1953) reviewed the activity of riboflavin in coenzyme systems. It was pointed out that either FMN or FAD served as components of enzyme systems then known to depend upon riboflavin for activity. That report was substantiated by West and Todd (1961) and Wagner and Folkers (1964).

FMN and FAD serve as coenzymes to several enzyme systems known to function directly in the main stream of energy metabolism. West and Todd (1961) state that the flavoprotein enzymes operate in electron transport systems. These authors showed diagramatically, under "biological oxidation and reduction" two important places in the citric acid cycle where flavoproteins act as acceptors and donors for hydrogen obtained directly and indirectly from the substrate, succinate and diphosphopyridine nucleotide, respectively. This cycle is responsible for approximately 90 percent of the chemical energy, adenosine triphosphate (ATP), formed in oxidative processes according to Harrow and Maxur (1962).

Some of the specific enzyme systems with which FMN is associated are well known. Blanchard et al. (1945, 1946) reported

that l-amino acid oxidase was active in enzyme systems that oxidize l-alpha-amino acids and l-alpha-hydroxy acids to alpha-keto acids. Several if not all of the alpha-keto acids may be further oxidized to yield energy. Haas et al. (1940) showed that FMN was associated with an apoenzyme that reduced cytochrome c. West and Todd (1961) indicated that in animal and plant tissues the coenzyme for succinic dehydrogenase was riboflavin phosphate.

Flavin-adenine dinucleotide is the coenzyme of several enzyme systems. There was some indication among various literature sources that FMN or FAD might serve as the same coenzyme in some systems. Conn and Stumpf (1963) indicated that FAD was the coenzyme of NAD^+-cytochrome c reductase, aldehyde oxidase, succinic dehydrogenase, lipoyl dehydrogenase and others. Wagner and Folkers (1964) stated that this group of flavoproteins participates in biochemical oxidation by receiving hydrogen from nicotinamide-adenine dinucleotides and transferring it to the cytochrome system. Conn and Stumpf (1963) reported that $FADH_2$ was produced in the degradation of carbohydrates, fatty acids and amino acids. It is in these systems that high energy phosphate bonds are formed. For each hydrogen that was transported by FAD to the cytochrome system, one molecule of ATP was formed at the FAD reduction-oxidation level.

Niacin

Niacin is a part of two coenzymes which are very active in cellular respiration. Neilands and Stumpf (1958) reported that,

while von Euler's laboratory of Stockholm was working with coenzyme I, Warburg and associates were working on a similar factor, coenzyme II, that was necessary for the oxidation of glucose-6-phosphate to gluconic acid-6-phosphate. Hundley (1954) reported that in 1935 both groups isolated nicotinamide from the coenzymes.

A coenzyme III was suggested by Singer and Kearney (1953) as an essential cofactor for the enzyme system necessary for the oxidation of cysteine sulfinic acid to cysteic acid. Since West and Todd (1961) do not show that a coenzyme containing nicotinamide is essential for that reaction, the existence of such a coenzyme is doubtful.

The names of the two coenzymes were changed as more was learned of their structural formulae. Coenzyme I became "diphosphopyridine nucleotide" (DPN) and coenzyme II became "triphosphopyridine nucleotide" (TPN) (West and Todd, 1961 and Wagner and Folkers, 1964). Dixon (1960) proposed that coenzymes I and II be called nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP). The last two names of each coenzyme are currently used in identification.

The role of both NAD and NADP is that of hydrogen transfer. Wagner and Folkers (1964) state, "the nicotinamide moiety of the coenzyme operates in these systems by reversibly alternating between an oxidized quarternary pyridinium ion and a reduced tertiary amine."

These coenzymes operate in many metabolic systems and are especially important in glycolysis and in the citric acid cycle.

In the oxidation of carbohydrates, fatty acids and alpha-keto acids, the coenzymes NAD and NADP act not only as hydrogen carriers but participate in a chain of events that causes chemical energy to be trapped. If the hydrogen is passed to the flavoprotein-cytochrome system the energy is trapped by the formation of high-energy bonds, (ATP). Wagner and Folkers (1964) give several enzyme systems in which NAD or NADP are of considerable importance in energy metabolism. NAD functions in dehydrogenase systems that converts lactic acid to pyruvic acid; 1,3-diphosphoglyceric acid to 3-phosphoglyceraldehyde; l-alpha-glycerophosphate to dihydroxyacetone phosphate and l-glutamic acid to iminoglutaric acid. NADP-containing dehydrogenases include those that convert glucose to gluconic acid, pyruvic acid to malic acid, alpha-ketoglutaric acid to isocitric acid and 6-phosphogluconic acid to glucose-6-phosphate.

Pantothenic Acid

Jukes (1939) and Woolley *et al.* (1939) showed that the filtrate factor, pantothenic acid, was an effective cure for chick dermatitis or pellagra. The biochemical role of pantothenic acid did not become evident until after Lipmann (1945) discovered coenzyme A. Lipmann *et al.* (1947) reported that pantothenic acid was a part of the coenzyme A (CoA) molecule.

Lipmann (1945) showed that the newly discovered coenzyme was necessary for the acetylation of sulfanilamide by pigeon liver tissue. Other metabolic acylation systems were soon discovered. By 1953 evidence for the existance of metabolic reactions of acetyl CoA was sufficient for Novelli (1953) to present a schematic outline of these activities. Primary sources of acetyl groups were shown to be carbohydrates, fatty acids and amino acids. Acetyl groups were shown to feed into the citric acid cycle for energy production.

Wagner and Folkers (1964) described the metabolic role of CoA as a shunt between acyl donors such as pyruvate and acetoacetate and acyl acceptors such as oxaloacetate, hydroxyamine and others. West and Todd (1961) Harrow and Mazur (1962) and others showed that pyruvate, a principal acyl donor, was one of the key metabolic products in carbohydrate metabolism. With the aid of cocarboxylase, NAD and CoA-SH, a two carbon fraction of pyruvic acid reacted with oxaloacetate to form citric acid, the initial six carbon compound of the citric acid cycle.

At another point in the citric acid cycle CoA is very important in formation of succinyl CoA. According to Conn and Stumpf (1963) this reaction proceeds in much the same way as when acyl CoA is formed from pyruvic acid. However, in this reaction a thioester high-energy bond was formed and eventually the reaction of ADP and guanosine triphosphate gives rise to a molecule of ATP. In several reactions involving CoA a dehydrogenase enzyme also removes hydrogen.

This hydrogen, if transported through the electron transport system, will also stimulate the formation of chemical energy in the form of ATP.

The beta-oxidation scheme first presented by Knoop in 1905 and described by West and Todd (1961) requires CoA as an essential component in fatty acid degradation. In this process, as previously pointed out by Wagner and Folkers (1964), CoA acts as a shunt mechanism for getting acetate into the citric acid cycle.

Pyridoxine

Pyridoxine-5-phosphate is active as a coenzyme in decarboxylation, transamination and deamination reactions. Feldman and Gunsalus (1950) reported that alpha-ketoglutaric acid may undergo transamination with aspartic acid, alanine, leucine, norleucine, tryptophan, tyrosine, phenylalanine and methionine. Some of the resulting keto acids would no doubt be used for energy production.

Nitrogen retention data presented by Bolin and Lockhart (1960), showed that between 40 and 50 percent of the ingested nitrogen was retained in the birds body. This indicates that of the 70 to 80 percent of the total protein that was digested, about 30 percent was used for energy by oxidation of the deaminated amino acids. West and Todd (1961) show oxidative pathways whereby this may be accomplished.

Pyridoxine is usually not thought of as being important to carbohydrate metabolism and energy production. West and Todd (1961)

point out the importance of the reversible reaction between alpha-ketoglutaric acid and glutamic acid in which the protein and carbohydrate metabolism systems are tied together. Pyridoxal-5-phosphate acts as the coenzyme in this reaction and shows an indirect relationship between pyridoxine and energy metabolism. That pyridoxal-5-phosphate was directly associated with carbohydrate metabolism as a coenzyme was shown by Cori and Illingworth (1957). Muscle "phosphorylase a" and "phosphorylase b" were reported to contain 4 and 2 moles of pyridoxal-5-phosphate, respectively, per mole of enzyme. The removal of pyridoxal-5-phosphate inactivated the enzyme but the activity could be restored by incubating the enzyme with pyridoxal-5-phosphate for about 30 minutes.

Phosphorylase was reported by Conn and Stumpf (1963) to be widely distributed in nature. The enzyme catalyzes the phosphorylitic cleavage or synthesis of the alpha-1-4-glucosidic linkage at the nonreducing end of the starch or glycogen chain. When glycogen is broken down in the muscle, phosphorolase is responsible for the formation of alpha-D-glucose-1-phosphate, the first step in the glycolytic process.

Metabolizable Energy and Nutrient Imbalance

Metabolizable energy (ME) was defined by Titus (1956) as the digestible energy of a diet or feedstuff less the total energy of the resulting urine and of any combustible gases that were formed from the

ingested material as it passed through the animal. The production of gases by the simple stomached animal is usually minor and is neglected in direct calorimetry techniques.

The literature review which preceded this section pointed out the importance of the vitamins in nutrition and further showed that the enzyme systems in which each played a part were often associated with energy metabolism. The present section deals more specifically with the effects of intermediary nutrient deficiencies or imbalances on the efficiency of metabolizable energy utilization.

The first work reported on imbalance and ME efficiency dealt with mineral deficiencies. Mitchell and Carman (1926) working with chickens reported that the metabolizable energy derived from a given weight of corn was not lowered by the sodium and chlorine deficiency of such a ration. Riddell and associates, according to Brody (1945), showed declines in utilization of metabolizable energy when phosphate was a limiting factor. However, it is questionable as to whether this interpretation was directed at efficiency with which the ME was utilized. Kleiber et al. (1936) reported that a phosphorus deficiency in beef cattle nutrition had no effect on the digestibility and metabolizability of food energy. Black et al. (1937) showed that an iron and copper deficient diet yielded slightly more ME per unit of diet than a supplemented diet. It was interesting to note that eight out of ten pair-feeding trials showed a decrease in ME efficiency when the diet was supplemented with iron and copper.

Sibbald et al. (1961) indicated that calcium in the presence of Aureomycin reduced the amount of ME obtained from a unit of feed. In the same experiment three different phosphorus sources at various levels were studied. One source supported greater ME efficiency than the other two sources. This increase in efficiency amounted to about 1.2 percent. The phosphorus levels were reported to influence ME efficiency but these data were difficult to interpret. Although there were several significant treatment differences reported, none of these seemed large enough to be of any practical importance.

Black and Bratzler (1952) reported that rats fed diets with and without streptomycin, but otherwise similar, metabolized 66 and 67 percent of the total calories, respectively. Knoebel and Black (1952) similarly reported on the addition of vitamin B_{12} and antibiotics to the diet of rats. The ME efficiency of utilization of the supplemented and unsupplemented diets was 88 and 89 percent, respectively. Neither of these trials showed any dramatic changes in ME efficiency due to vitamin and/or antibiotic additions.

In the area of amino acid supplementation, Swift et al. (1934) reported that a dietary cystine deficiency impaired growth but did not affect the efficiency with which ME was utilized. Baldini (1961) reported that a methionine deficient diet had more metabolizable energy per pound than the supplemented diet. Sibbald et al. (1962)

reported that methionine additions to a diet deficient in methionine depressed ME values in only a few treatments. Several interactions were shown between methionine and riboflavin, niacin and vitamin B_{12}. However, these were too complex for a definite interrelationship pattern to be established.

McClure et al. (1934) were apparently the first workers to investigate the effect of B vitamin deficiency on the efficiency of energy metabolism. The apparent prominent role of B vitamins in intermediary metabolism of carbohydrate and energy prompted these investigators to determine the effects of B vitamin deficiency on the efficiency of ME utilization and other criteria. It was shown that a vitamin B supplement added to a vitamin B deficient diet fed to rats had no effect on improving the efficiency of ME utilization. In contrast, Voris (1937) showed that the efficiency of ME utilization decreased by about 12 percent when rats were thiamine deficient.

More recently Sibbald et al. (1961) reported data in which four levels of pantothenic acid, 5.6, 6.8, 14.8 and 28.8 mg. per lb. of diet, were fed to chicks. The three lower levels had no effect upon energy metabolism whereas the highest level depressed ME utilization. Sibbald et al. (1962) reported on the effects of riboflavin, niacin, vitamin B_{12} and methionine as related to the efficiency of ME utilization. This work showed several first order interactions and a third order interaction involving the four

supplements. The authors stated "the interactions in the ME data are too complex to allow definite conclusions to be drawn from the data available," but concluded that ME values may be influenced by levels of riboflavin, niacin, vitamin B_{12} and methionine present in the ration.

Tissue Vitamin Levels

Vitamins of the B complex groups usually function at the cellular level in most tissues. Some of the various enzyme systems in which these have a part were previously cited. This part of the literature review will be confined primarily to those tissues or organs that contain relatively high levels of B vitamins. Data for aves and other species are included.

Niacin

Rosenberg (1945) reported that nicotinamide occurs mainly as bound nicotinamide in the liver, kidney and muscle while free nicotinic acid or its amide has been found only in the liver. Dann (1937) indicated that niacin occurs in all living cells in small amounts with a greater amount in liver tissue.

Childs *et al.* (1952) reported that two levels of dietary niacin, 2.8 and 5.8 mg. per kg. of feed, were each fed to groups of chicks for 3, 5, 6 and 8 weeks. At the 3, 5 and 6-week intervals the chicks were placed on niacin-free diets and continued to 8 weeks of age at which time liver samples were taken from all groups for

niacin content determination. The results of the assay for chicks deprived of niacin for 5, 3 and 2 weeks showed no consistent trend in liver storage. These workers concluded that liver storage of niacin was not an adequate criteria for measuring the amounts of niacin required to support rapid growth.

Pantothenic Acid

Waisman et al. (1939) indicated that pantothenic acid is widely distributed in animal tissues and most abundant in liver and kidney tissues. Rosenberg (1945) stated that the liver and kidneys are able to store this vitamin to some extent.

Bauernfeind et al. (1942) reported on the pantothenic acid requirement of the White Leghorn chick. Diets containing 300, 415, 472, 531, 588 and 648 mcg. of pantothenic acid per 100 gms. of diet supported liver storage of the vitamin in amounts of 12.0, 16.7, 27.7, 34.3, 32.0 and 38.0 mcg. per gm. of fresh tissue respectively. A second experiment showed a similar storage pattern with the exception that at the higher supplementation levels the vitamin storage was less.

Olsen and Kaplan (1948) reported on the amounts of CoA in the liver of normal and deficient ducks. Starting with an approximate value of 65 mcg. of the vitamin per gm. of fresh tissue for the normal duck, the vitamin content decreased to about 36 mcg. when the ducks were fed a pantothenic acid deficient diet for 5 days.

A sharp drop in the vitamin level also occurred in heart tissue under the same feeding regime.

Riboflavin

Kuhn and associates, according to Rosenberg (1945), reported that relatively large quantities of riboflavin were found in liver and kidney tissues. An intake of large amounts of this vitamin did not increase the liver content to any appreciable extent. Animals dying of riboflavin deficiency contained about one-third of the normal level in liver tissue.

According to Brody (1945), Ochoa and Rossiter reported that the amount of FAD was closely correlated with riboflavin tissue levels. A decrease of riboflavin caused a similar response in FAD. Burch and Combs (1956) reported that under riboflavin deficient conditions FMN and FAD are lost from the liver of the rat very rapidly.

Wase (1956) measured the relative riboflavin turnover rate with riboflavin-C14. In normal rats the half-life of riboflavin was found to be 6.52 $\pm$ .5 days.

Thiamine

Westenbrink stated, as described by Rosenberg (1945), that thiamine was not stored to any great extent but relatively high concentrations are found in the liver, kidneys, heart, muscle and brain. The amount is enough to maintain life for only a few days

and a daily intake was reported to be necessary. Jansen (1954) confirmed the report of Westenbrink with regard to liver and kidney thiamine levels and indicated that the muscle thiamine level was proportional to the work performed.

De Caro and associates as described by Novelli (1957) examined the liver, muscle and brain tissues of mice for both free thiamine and TPP. Most of the thiamine existed as TPP.

Warnick et al. (1956) reported that the thiamine content of the heart, liver and kidneys did not vary with the diet. These workers suggested that unknown factors associated with source of dietary thiamine may influence the utilization and deposition of this vitamin.

Pyridoxine

Rosenberg (1945) reported that pyridoxine intake in excess of that needed was destroyed by the organism and that no storage of this vitamin in the organism had been observed.

Rabinowitz and Snell (1948) indicated that normal chicken heart and liver contained 13.8 and 63.3 mcg. of pyridoxine per gram of fresh tissue, respectively.

Cheslock (1958) reported on two experiments with rats. The liver pyridoxine content in experiment A did not change when doses of 2.5 to 15.0 mcg. were administered to each rat daily. In experiment B there was a consistent increase in liver storage of

pyridoxine at daily vitamin intake levels of 5 to 20 mcg. When food intake increased, the vitamin B_6 content of the liver decreased when the vitamin intake was held constant.

Basal-Metabolism and Vitamin Deficiency

There is a paucity of literature concerned with the effects of vitamin B complex deficiencies on the basal-metabolism rate (BMR) of poultry. Some of the review here presented is for other species. Even though specie differences may exist one would expect the BMR of each to react quite similarly if the vitamin involved is part of a necessary coenzyme system.

Thiamine

According to Kleiber (1945), Rnomino in 1916 seemed to be the first to observe that a vitamin B deficiency resulted in a decreased rate of oxygen consumption. Kleiber (1945) also reviewed the work of Abderhaden who in 1920 and 1921 reported that polyneuritic pigeons had a reduced respiratory exchange. Feeding yeast or an alcohol extract of yeast to polyneuritic pigeons raised their metabolic rate to normal.

Anderson and Kulp (1922) reported that the basal metabolic rate of vitamin B deficient hens was reduced by about two-thirds that of normal.

Gulick (1924) working with rats concluded that the decrease in metabolic rate of vitamin B deficient animals could be explained on the decreased intake of food energy alone. Voris (1937) using a paired-feeding technique and an ad libitum fed control, showed that decreased feed intake reduced the metabolic rate and that the thiamine deficient rat had a metabolic rate about 5 percent lower than the pair-fed control.

Williams and associates in 1942, as reviewed by Kleiber (1945) reported that a thiamine deficiency in man reduced the BMR by 10 to 33 percent of normal.

Riboflavin

According to Kleiber (1945), Wahl reported that the basal metabolic rate of guinea pigs was not affected by a riboflavin deficiency per se. Kleiber and Jukes (1942) using an experimental technique similar to Voris (1937) showed that the BMR of two-week old chicks was reduced when normal dietary intake was restricted. Paired-feeding of chicks on the normal and riboflavin deficient diet showed that riboflavin deficiency per se had no effect on the BMR. Horwitt et al. (1949) reported that humans maintained on a daily riboflavin intake of .55 mg. per day, about one-third of the daily requirement according to Bogert (1949), showed no abnormal BMR.

Pyridoxine

Beaton et al. (1953) reported that the BMR was not different from the pair-fed control but was lower than the ad libitum-fed

control. Orsini and associates according to Beaton et al. (1953) reported that the BMR of the pyridoxine deficient rat was lower than the ad libitum-fed control.

Basal metabolism studies with other B complex vitamins are quite scarce and probably do not exist for poultry. Kleiber (1945) reviewed basal metabolism studies in which specific nutrients were involved. That review gave no basal metabolism data for niacin, pantothenic acid or pyridoxine. A lack of information in these areas could well be due to the development of in vitro technique. Although, this method only attempts to simulate what is going on in vivo, it has been very useful in studying tissue metabolism rate by measuring oxygen uptake. The usefulness of in vitro technique in studying biological systems was discussed by West and Todd (1961).

Calorimetry Methods

Direct and indirect calorimetry methods have been used in studying the basal metabolic rate of animals. Brody (1945) and Kleiber (1961) discussed the various methods and instrumentation. The direct method measured the heat given off by the animal while the indirect method measured the oxygen consumed and carbon dioxide produced from which the heat production was estimated.

About 1895 Rubner, according to Brody (1945), demonstrated that heat measured by direct calorimetry was in good agreement with the heat computed from indirect calorimetry. According to Lusk

(1928), Zuntz and Schumberg in 1901 presented a table of thermal equivalents of oxygen and carbon dioxide for respiratory quotients (R.Q.) ranging from .707 to 1.0. These values were derived for animals that excrete waste nitrogen mainly in the form of urea.

Since the bird excretes most of the waste nitrogen in the form of uric acid it becomes questionable as to whether the thermal equivalents of Zuntz and Schumberg are applicable to indirect calorimetry of aves. Because of this problem Mellen and Hill (1953) and (1955) reported basal-metabolism data in terms of oxygen consumption rather than heat production.

King (1957) thoroughly reviewed the theory of indirect calorimetry as applied to birds. One of the main criticisms of indirect calorimetry was that R.Q.'s below .7 often occur in avian studies whereas a R.Q. of .707 with mammals should occur only when fat is the sole source of metabolic energy. According to King (1957), Benedict and Lee in 1937 predicted that careful attention to techniques would eliminate R.Q.'s below .707. Despite this prediction these workers as well as Smith and Riddle (1944), Mellen and Hill (1955) and others continued to obtain R.Q. values below .707.

As reviewed by King (1957), Henry and associates in 1934 hypothesized that R.Q. values below .71 in fowl were a result of protein catabolism. By feeding egg albumen to a fasting fowl having an initial R.Q. of .685 it was observed that the R.Q. fell to .65-.66 during the next 210 minutes which was considered the period in

which most of the absorbed protein was undergoing oxidation. These workers showed that from the stoichiometric point of view alanine and some other amino acids when oxidized to uric acid, water and carbon dioxide gave R.Q.'s below .71 while similar oxidation to urea, water and carbon dioxide gave R.Q.'s well above .71. Mellen and Hill (1955) suggested that the R.Q. of protein catabolism for aves was lower than that for mammals.

King (1957) concluded that judgment of the validity of a large portion of the caloric data accumulated through the study of aves by indirect calorimetry must be suspended until such time that the datum of Zuntz and Schumberg is found applicable or rejected for BMR studies of aves.

PROCEDURE AND MATERIALS

Experimental Animals

Broiler-type chicks were the experimental animals used in all trials. Day-old chicks were purchased from a commercial hatchery and were of common ancestry. Nothing was known about the nutritional status of the hatchery flock or flocks that produced the hatching eggs. It was assumed that the breeder hens were receiving a diet having proper protein and carbohydrate balance with adequate trace mineral and vitamin fortification to support the well-being of the developing embryo and hatched chick. Average initial chick weight varied slightly among the groups purchased during the course of the several trials. This, however, had no apparent effect upon the experimental responses.

Housing and Brooding Units

Housing of the brooding units was in a 20' X 30' insulated battery brooding room. Fluorescent lights were used in providing evenly distributed light to all pens. Room temperature was maintained at approximately 80°F, but during July and August the temperature was often higher than 80°F. A hot-air type furnace with a duct system provided good distribution of heat throughout the room. A fresh-air duct leading to the furnace, as well as a ventilation exhaust fan, provided adequate air change.

Conventional battery brooders and shifting-type metabolism pens were used in the course of the experimental work. The conventional pens were electrically heated, had raised wire floors and provided the chicks with feed and water *ad libitum*. This type of pen was used for brooding the chicks used in diet development, in vitamin-depletion-time studies and for holding chicks being conditioned (depleted of the vitamin in question) prior to an experimental period. During the experimental period the shifting-type pens were used to contain the chicks.

The shifting-pens mechanism was developed at the North Dakota Agricultural Experiment Station for metabolizable energy evaluation studies (Lockhart *et al.*, 1963). The apparatus had an over-all length of approximately 15 feet consisting of two units of 6 pens per unit (back to back) which were mounted on separate tracks and shifted by a single fulcrum attached to a motor drive and time clock unit. This unit shifted the pens from a feeding area to a droppings collection area and vice versa. The chicks were allowed to eat for 15 minutes and then were shifted over to the droppings collection area for a period of 30 minutes. This timing schedule was found to result in procuring more than 50 percent of the droppings voided in the collection area. Water was available to the chicks at all times. Each pen was 16 inches wide, 18 inches deep and 12 inches high, with a 200-watt thermostatically controlled electric heating unit suspended from the top at the rear. The walls and partitions for each pen, including the floor, were made of 3/4 inch hardware cloth.

The rear third area of each pen was covered on the sides, top and back with fireproof canvas forming an open front canopy over each heating unit. Air-duct systems that blew air across the droppings collection trays were mounted between each unit of pens. The forced air movement maintained dry conditions in the collection trays at all times, and helped to reduce the enzymatic loss of ammonia and carbon dioxide.

Experimental Period

After the chicks were conditioned for the experimental period they were divided into pen-groups of 6 to 8 chicks per group, depending on the trial. The pen-groups within a trial were weight-equalized so that differences of 10 grams between pen initial weights rarely existed. The chicks were then placed in the shifting-pens for an adjustment period of approximately 72 hours prior to the start of the droppings collection period. Collection periods usually lasted from 72 to 96 hours. Growth, feed consumption and other data were obtained during the course of the experimental period.

At the end of the droppings collection period the droppings were cleaned of down and other feather-like substances by passing a current of air over the collection trays and by the removal of larger feathers with a forceps. By use of a broad, thin-blade putty knife, the feces, including uric acid deposits, were removed from the trays and placed in 1000 ml. beakers. Some of the uric acid

deposits stuck tightly to the collection trays and were not easily removed under dry conditions. A small amount of water was sprinkled on the tray to facilitate the removal of these deposits. As soon as the collection was completed the beakers containing the droppings were placed in a convection-type drying oven for a 2-4 hour period with the temperature at 65°C. After this period the beakers and contents were removed from the oven and allowed to recover moisture to an air-dry basis. This was usually about 7 percent. The droppings samples were then ground through a small Wiley mill having a .040 inch size screen, sealed in mason jars and stored for heats of combustion and chemical analysis.

Dietary Indicator

Throughout this study chromic oxide (Cr_2O_3) was used as the indicator material. According to Maynard and Loosli (1962) this substance was first suggested in 1918 by Edin, a Swedish scientist, to fill such a role. In preparing the chromic oxide for experimental use it was first mixed with water and celluflour in a Waring Blender. The mixture was dried in a convection-type drying oven and then pulverized. The celluflour-chromic oxide mixture was added to each diet in sufficient amounts to bring the chromic oxide level to about .20 percent. Analysis of the feed and feces for chromic oxide was according to the procedure of Bolin and Lockhart (1960). A summary of the procedure is as follows:

A five to ten gram sample in an aluminum dish was ashed overnight in an electrically controlled muffle furnace set at a low dull red heat, approximately 600°C. The ash was transferred to a 100 ml. Kjeldahl flask to which was added 15 ml. of the oxidizing reagent. This was composed of 5 grams of sodium molybdate dissolved in one liter of H_2O to which one liter of perchloric acid, $HClO_4$, (70-72%) was added. The Kjeldahl flasks and contents were put into a metal holder and placed on a slow cooking hotplate. Oxidation of the sample with perchloric acid completely changed the chromium oxide (Cr_2O_3) to chromium trioxide (CrO_3), a reddish-yellow soluble compound. Upon complete oxidation the sample was then made up to definite volume with distilled water.

Calorimetry

Heats of combustion were determined for the feed and feces sample by use of a Parr Adiabatic Calorimeter. The instrument was equipped with Beckmann Differential thermometers which were calibrated to the hundredth of a degree and estimated to the thousandth of a degree. Duplicate determinations were made on one gram quantities of each sample. If the heat values received from the two determinations were different by more than 20 calories, additional determinations were made. The calculation of a caloric value was made by multiplying the temperature rise in Centigrade degrees per 1000 ml. of water (flask calibration at 25°C.) times a correction factor for heat retained by the bomb and bomb-bucket. This was 2430

for the instrument used. Other corrections made were (1) heat associated with nitric acid production in the combustion process, (2) heat produced by the burning of ignition wire, and (3) the variation in the thermometer bore.

Using determined chromic oxide values and heats of combustion values for a feed sample and corresponding feces sample, a classical metabolizable calorie value (CMC) was obtained according to the formula:

$$\text{Feed caloric value} - \frac{\text{Feed } Cr_2O_3 \text{ value X Feces caloric value}}{\text{Feces } Cr_2O_3 \text{ value}} = \text{CMC}$$

In order to fully evaluate a unit of feed for its ability to furnish heat, a correction must be made for the amount of dietary nitrogen retained in the body of the experimental animals during the experimental period. A correction factor of 8.21 kilocalories per gram of nitrogen retained was calculated and subtracted from each CMC value, thus yielding nitrogen corrected metabolizable caloric energy values (MC). The feed and feces samples were analyzed for nitrogen according to the Kjeldahl Process, boric acid modification, as discussed by Triebold (1946).

Basal Metabolism Rate

The chicks used in determining the fasting metabolism rate were of different groups than those used in the metabolizable energy (ME) studies. Conditioning of the chicks for the metabolic rate

studies followed a similar depletion time pattern for each vitamin as was established for the ME studies.

The experimental period started with the chicks being administered various levels of the vitamin in question for approximately 48 hours before taking of the first oxygen consumption measurement (O^1). At 5:00 p.m. the day previous to that on which O values were taken, all feed was removed from the chicks with water remaining available at all times. The first O determination of a daily series started at about 7:00 a.m. Two or three chicks were used in each determination. The chicks were allowed a 30-minute adjustment period inside the metabolism chamber with the metabolism system being purged simultaneously. At the end of the adjustment and purge period an experimental period of approximately 60 minutes was used in taking oxygen consumption and carbon dioxide production data.

After the determination of O^1 data the chicks continued to receive the daily vitamin intake level as before. They were fed for a 72-hour period, fasted as before and O^2 data determined. The chicks used in O^1 and O^2 at a given vitamin intake level were not necessarily the same chicks, but were representative of the group at that time. Metabolism chamber temperature was maintained at approximately 93°F. According to Barott and Pringle (1946), chicks of the weight used in these studies should have been at or near thermal neutrality at this temperature.

Body heat production data were obtained by using indirect calorimetry techniques. A Minute Oxygen Uptake Spirometer was

employed to measure the amount of oxygen consumed. The oxygen pressure within the metabolism unit was approximately equal to that of the atmosphere. Pressure was maintained by a water transducer unit. The transducer was connected to a two-way electric switch which controlled the motor-drive that propelled the piston within the spirometer oxygen cylinder. Oxygen from a commercial source having a purity in excess of 99.5 percent was used in all respiration trials.

Oxygen consumption data were corrected to standard conditions. Atmospheric temperature measurements were taken near the spirometer oxygen cylinder. The oxygen volume was corrected to zero degrees Centigrade as follows:

$$O_2 \text{ Vol.} \times \frac{273°A}{273 + \underset{(C°)}{\text{Atm. Temp.}}} = O_2 \text{ Vol. (temp. corr.)}$$

Atmospheric pressure measurements were obtained at 12:00 a.m. daily. The data were corrected to an atmospheric pressure of 760 mm Hg as follows:

$$\frac{O_2 \text{ Vol. (temp. corr.)} \times \text{Atm. Press. (mm Hg)}}{760 \text{ mm Hg}} = O_2 \text{ Vol. (std. cond.)}$$

The metabolism chamber (a four quart size pressure cooker) was adapted with 4 gas flow orifices. A top view, Figure 1, gives a diagrammatic description of the unit. Orifice A was connected to

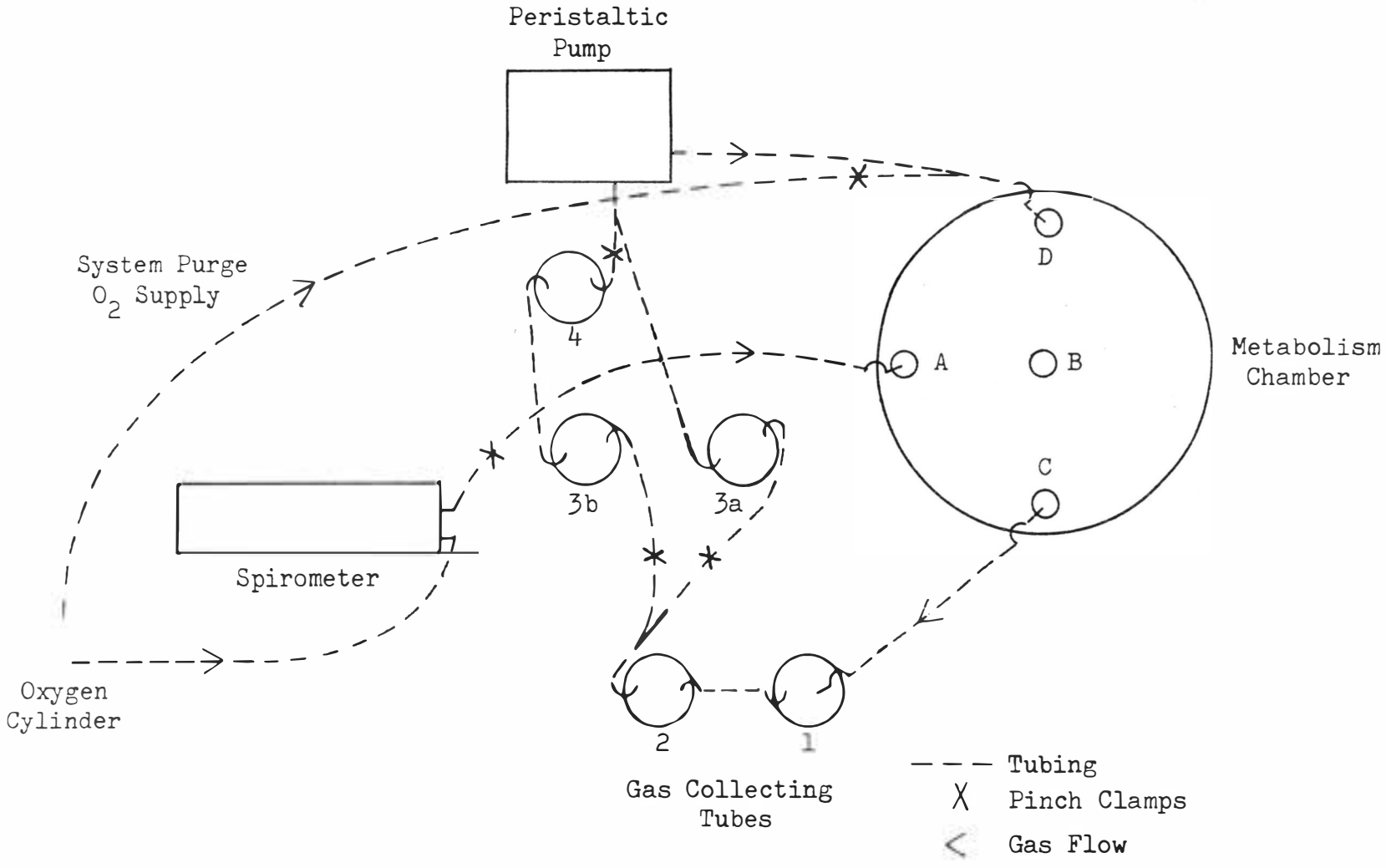

Fig. 1. Schematic of basal metabolism unit.

the spirometer for oxygen intake. Orifice B was used to facilitate gas release when purging the system. Orifices C and D were used as an outlet and inlet to complete a gas flow circuit through a series of water and carbon dioxide collecting tubes. The first collecting tubes 1 and 2 were used to remove respiratory water from the system during the period that chicks were in the metabolism unit. A third tube, 3a, was continuous with tubes 1 and 2 and was used to remove all carbon dioxide from the circulating gases during the system purge period. Collecting tube 3b, an alternate to 3a, was used to collect the carbon dioxide from the system during the experimental period. Tube 4 was continuous with tube 3b and was used to absorb any water being lost from 3b in the reaction of carbon dioxide and sodium hydroxide. Absorber materials were Drierite ($CaSO_4$) for water and Ascarite (NaOH dried on asbestos) for carbon dioxide. A peristaltic pump was used to circulate the gases through the system. The metabolism chamber, collecting tubes, peristaltic pump and spirometer were connected together by the use of rubber tubing. The flow of gas was controlled by pinch clamps. The minute-gas volume turnover rate was set by a flow meter to approximate the volume of the metabolism chamber.

The amount of carbon dioxide produced during the experimental period was obtained by taking an initial weight of tubes 3b and 4 containing absorber materials and by reweighing the tubes at the end. The weight gain multiplied by the conversion factor .509 gave

the volume of CO_2 produced. This volume of CO_2 was divided by the volume of O_2 consumed to give the respiratory quotient (RQ).

Liver Vitamin Assay

At the end of each experimental period involving metabolizable energy, approximately 6 chicks were killed to determine the liver vitamin content. The chicks were frozen at about -6°F. until such time that the liver samples could be taken. Before taking the liver samples the frozen chicks were allowed to thaw overnight under refrigerated conditions. The livers were removed and refrozen on a pooled basis for each vitamin level. When the foregoing process was completed the livers were quartered and samples made up for analysis. The liver samples were packed with dry-ice and shipped to the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin, for analysis. References to the chemical or microbiological method used were as follows:

Pyridoxine:

Atkins, Schultz, Williams and Frey, Ind. and Eng. Chem., Anal. Ed., 15 141 (1943) (S. Carlsbergensis).

Thiamine:

A. O. A. C., 655 (1960) 9th Ed.

Riboflavin:

A. O. A. C., 669 (1960) 9th Ed.

Pantothenic acid:

A. O. A. C., 668 (1960) 9th Ed.

Niacin:

A. O. A. C., 667 (1960) 9th Ed.

Experimental Diet Development

Of great concern at the beginning of this study was the development of a diet that would support good growth when well fortified with vitamins and trace minerals. A purified diet, Table 1 was formulated after similar diets used by Sunde (1955) and West *et al.* (1952).

The purified diet was prepared with and without the addition of 6.66 mg. of pyridoxine•HCl per kilogram of diet. In trial 1 each diet was fed to one pen of chicks. The diet with the pyridoxine addition supported good growth whereas chicks on the unsupplemented diet made very poor growth. On the 11th day of the trial one chick raced aimlessly about the pen with its head held in a downward position; convulsions occurred and death came during the day. From the 11th to the 15th day several chicks showed similar symptoms. On the 15th day of the trial and each day thereafter, each deficient chick was injected with 5 mcg. of pyridoxine•HCl dissolved in 1/20 cc. of distilled water. The injected chicks showed improved growth from the 15th to the 22nd day.

The first trial served as a pilot for examining the purified diet for deficiency symptoms and growth response. Trial 2 was planned to: (1) determine the repeatability of deficiency symptoms

Table 1. Composition of experimental diet

Ingredient	gm./kg. of diet
Cerelose	534.3
Casein (purified)	129.7
Gelatin	90.3
Corn oil	39.4
Cellulose (Alphacel)	98.5
Indicator premix	
Chromic Oxide (Cr_2O_3), 20%	12.6
Cellulose (Alphacel), 80%	
Minerals (premix)	64.4
Vitamins (premix)	30.9
Methionine hydroxy analogue - Ca	1.2

Vitamin	amt./kg. of diet	
Vitamin A	13320.00	U.S.P.U.
Vitamin D_3	1665.00	I.C.U.
Vitamin E	22.20	U.S.P.U.
Menadione • $NaHSO_3$	2.22	mg.
Biotin	.44	mg.
Folic Acid	3.33	mg.
Inositol	111.10	mg.
P- Aminobenzoic Acid	8.88	mg.
Choline Xanthate	2220.00	mg.
Vitamin B_{12}	13.30	mcg.
Riboflavin	5.55	mg.*
Thiamine • HCl	6.66	mg.*
Niacin	55.50	mg.*
Pantothenic Acid	22.20	mg.*
Pyridoxine • HCl	6.66	mg.*

Mineral	amt./kg. of diet	
$CaCO_3$	20.0	gm.
$CaHPO_4$	15.0	gm.
K_2HPO_4	8.6	gm.
NaCl (iodized)	4.1	gm.
Na_2HPO_4	7.3	gm.
$MgSO_4 \cdot 7 H_2O$	5.0	gm.
Fe_2O_3	5.7	mg.
$MnSO_4 \cdot 4 H_2O$	1.06	gm.
$ZnSO_4$	80.0	mg.
$CuSO_4 \cdot 5 H_2O$	19.9	mg.
Cobalt Acetate	1.0	mg.

* Each vitamin was omitted from the diet when that particular vitamin deficiency was being studied.

as previously shown, and (2) observe the growth response to vitamin intake at 4 different levels if (1) above were satisfactory. Vitamin injection levels of 1.25, 2.50, 5.00 and 10.00 micrograms of pyridoxine•HCl per chick per day were arbitrarily selected.

The pyridoxine deficiency symptoms observed in trial 1 were likewise observed in trial 2. Of the chicks originally started on the basal diet, 10 percent died of these symptoms by the 11th day. The remaining chicks were divided into four pens. The chicks in a given pen were administered pyridoxine at one of the levels previously stated. The growth data supported the observation of trial 1. In general the responses were proportional to the level of the vitamin administered.

At this point the diet was accepted as the experimental diet for the pyridoxine studies and was later found to be useful in studying deficiency symptoms of thiamine, riboflavin, pantothenic acid and niacin.

Adjustment of Data

The metabolism pens used in conducting the various experiments were not of sufficient number to accommodate the testing of all vitamin treatment levels simultaneously. The array of treatments were divided into a low vitamin dosage series and a high dosage series. In order that the data of the low and high dosage series might be integrated, the greater dosage levels of the low series and the lesser dosage levels of the high series were the same

treatment levels. A general arrangement of the data is presented here to give clarity in describing the integration process as follows:

	Vitamin Dosage Level										
Trials	1	2	3	4	5	6	7	8	9	10	11
1	X_1	-	-	-	X_1	X_1					
	X_2	-	-	-	X_2	X_2					
2	X_1	-	-	-	X_1	X_1					
	X_2	-	-	-	X_2	X_2					
3						X_1	X_1	-	-	-	X_1
						X_2	X_2	-	-	-	X_2
4						X_1	X_1	-	-	-	X_1
						X_2	X_2	-	-	-	X_2
						$\overline{X}t_1\text{-}t_4$					

The "X" represents a determined ME value. The subscript of the X, i.e. X_1 and X_2, identifies data that were obtained from a unit of six metabolism pens which were previously discussed. In this example the four groups of data of trials 1 through 4 are integrated by use of the ME values under dosage level number 6 (L6). A percentage multiplier for each unit of data was calculated by dividing the mean $\overline{X}t_1\text{-}t_4$ by each of the X values (8 in all) shown under L6. The product of each multiplied by 100 gives the

percentage multiplier by which the respective X values of a unit were adjusted.

Gross caloric, ME values and weight-gain data were adjusted by similar processes as outlined above. In some cases the process varied slightly from that shown here but the principle involved was the same.

Growth

Growth data were obtained to show evidence of chick response to graded levels of vitamin intake and to obtain the optimum growth response (growth plateau) for use as a reference point. This point was used in determining the relative degree to which vitamin deprivation affected other criteria for which experimental data were obtained. There were instances when the growth plateau was not used as the reference point and these will be cited when discussing the data involved.

Statistics

The analysis of variance was calculated as outlined by Steel and Torrie (1960). The procedure of Dunnett as described by Steel and Torrie (1960) was used in comparing means with a control. This method was modified to test group means with unequal numbers of replications by the procedure of Kramer (1956). Integrating of

correlation coefficient values was accomplished by the Z transformation procedure as described by Snedecor (1946).

RESULTS AND DISCUSSION

Deutectomy and Vitamin Depletion

The egg yolk within the abdomen of the newly hatched chick was considered to be a likely source of the B vitamins investigated in these studies. The first work conducted with each vitamin was to determine if the vitamin in question was present in sufficient quantities to prolong the time required to produce deficiency signs. Day-old chicks contain approximately 5.5 grams of yolk material within the yolk sac, Romanoff and Romanoff (1949). This material is a source of nutrients for the first 5 to 7 days of life after hatching. Romanoff (1949) reported that the amounts of thiamine, riboflavin and pantothenic acid within the egg were not changed after 21 days of incubation.

Sloan et al. (1934) reported that the removal of the yolk from newly hatched chicks reduced by about 2 to 3 weeks the time required to show deficiency signs for vitamins A, D_3 and E. The effects of deutectomy on depleting the chick of B vitamins were not given.

The yolk removal procedure used in the study presently reported was similar to that of Sloan (1936), with some modification. He made an incision on the "ventral surface of the abdomen nearly parallel to the long axis of the body." However, it was found that an incision of about one-fourth to three-eighths of an

inch in length running parallel to the long axis of the body just below and to the left of the vent helped to prevent the extrusion of the small intestine through the incision during and subsequent to the operation. Forceps were used to remove the yolk and the umbilicus was severed by an electric cauterizing instrument originally designed for caponization. The incision was sutured with cotton thread and covered with a red colored no-pick paste. Mortality due to the operation was very small. The chicks showed good activity within 12 hours.

Growth response data and other pertinent factors concerning each experiment are discussed below. Each figure shows growth data that are representative of the responses obtained.

Pyridoxine

Figure 2 shows the growth response of the deutectomized and "normal" (yolk intact) chicks when fed a pyridoxine deficient diet (.63 mg./kg.). The removal of the yolk reduced the average chick weight approximately 7.5 grams. This initial difference in weight remained about the same to the 11th day at which time the trial was terminated. Pyridoxine body stores were insufficient to support growth by the ninth day of the trial. Daily injections of a third group of the "normal" chicks with 10 mcg. of pyridoxine·HCl per chick showed that this vitamin was the primary growth limiting factor.

The removal of yolk nutrients from the chicks did not facilitate a faster pyridoxine depletion rate. The growth response data of Figure 2 support this conclusion.

Epileptic-like convulsions were associated with most of the mortality. The convulsions per se may not have been responsible for all of the deaths. Young chicks fed a pyridoxine deficient diet tend to accumulate a considerable amount of crop fluid which was described as a pendulous crop by Miller (1963). Klosterman (1960) suggested that the excessive amount of fluid in the crop may flow into the pharynx during the convulsions and bring about death by anoxia.

Thiamine

The removal of the yolk reduced the average weight of the deutectomized chicks by approximately 4.5 grams. The amount of thiamine in the egg when laid is not great, about 50 micrograms, according to Siedler (1963). This amount supported growth to about 3 days of age when the chicks were fed a thiamine deficient diet, (.34 mg./kg.). If thiamine was not made available to the chicks, weight losses ensued until death, see figure 3.

In two different trials "normal" chicks fed the thiamine deficient diet were all dead by the end of the ninth day. The deutectomized chicks of one trial were all dead by the 10th day and the last chick of another trial died on the 11th day. The daily injection of a group of deutectomized and "normal" chicks with

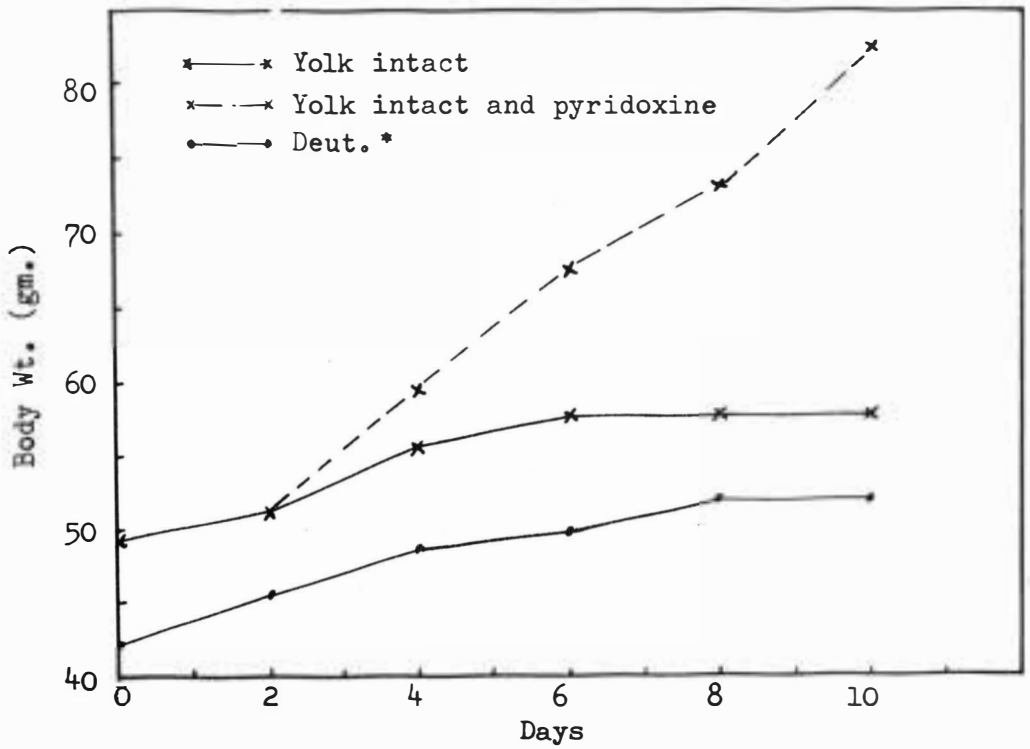

Fig. 2. Effects of deutectomy, pyridoxine depletion and repletion on chick growth

*Deutectomized

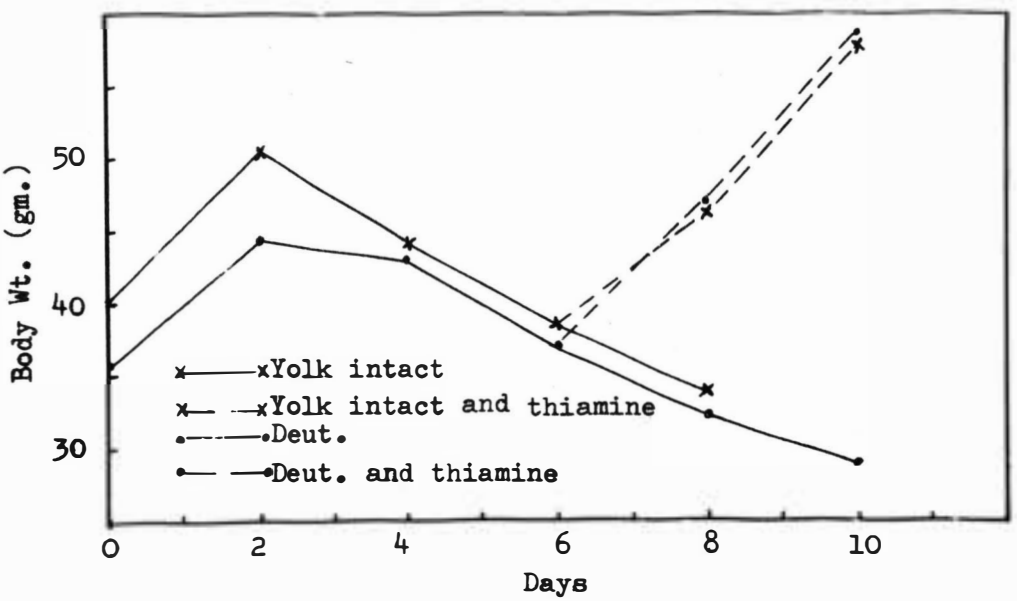

Fig. 3. Effects of deutectomy, thiamine depletion and repletion on chick growth

10 mcg. of thiamine per chick reduced the mortality and greatly improved the rate of growth.

Deutectomy had little if any effect upon the thiamine depletion time. After the chicks began to lose weight the difference in chick size due to yolk removal was greatly reduced.

Riboflavin

The growth data for deutectomized and "normal" chicks on a riboflavin deficient diet (.28 mg./kg.) are shown in Figure 4. The deutectomized chicks had an initial weight of about 4 gm. less than the "normal" chicks. Both groups of chicks were quickly depleted of riboflavin stores as evidenced by the greatly reduced growth rate after the first 4 days.

The difference in average chick weight of the duetectomized and "normal" chicks increased with age. At 24 days of age the deutectomized chicks were approximately 19 percent smaller than the "normal" chicks. With similar groups that were given riboflavin injections after 10 days of depletion, the differences were less, but the deutectomized chicks still remained smaller at 24 days of age.

Pantothenic Acid

The growth data for deutectomized and "normal" chicks on a pantothenic acid deficient diet (2.32 mg./kg.) are shown in Figure 5. The deutectomized chicks weighed approximately 3 grams less than the "normal" chicks at the beginning of the experiment.

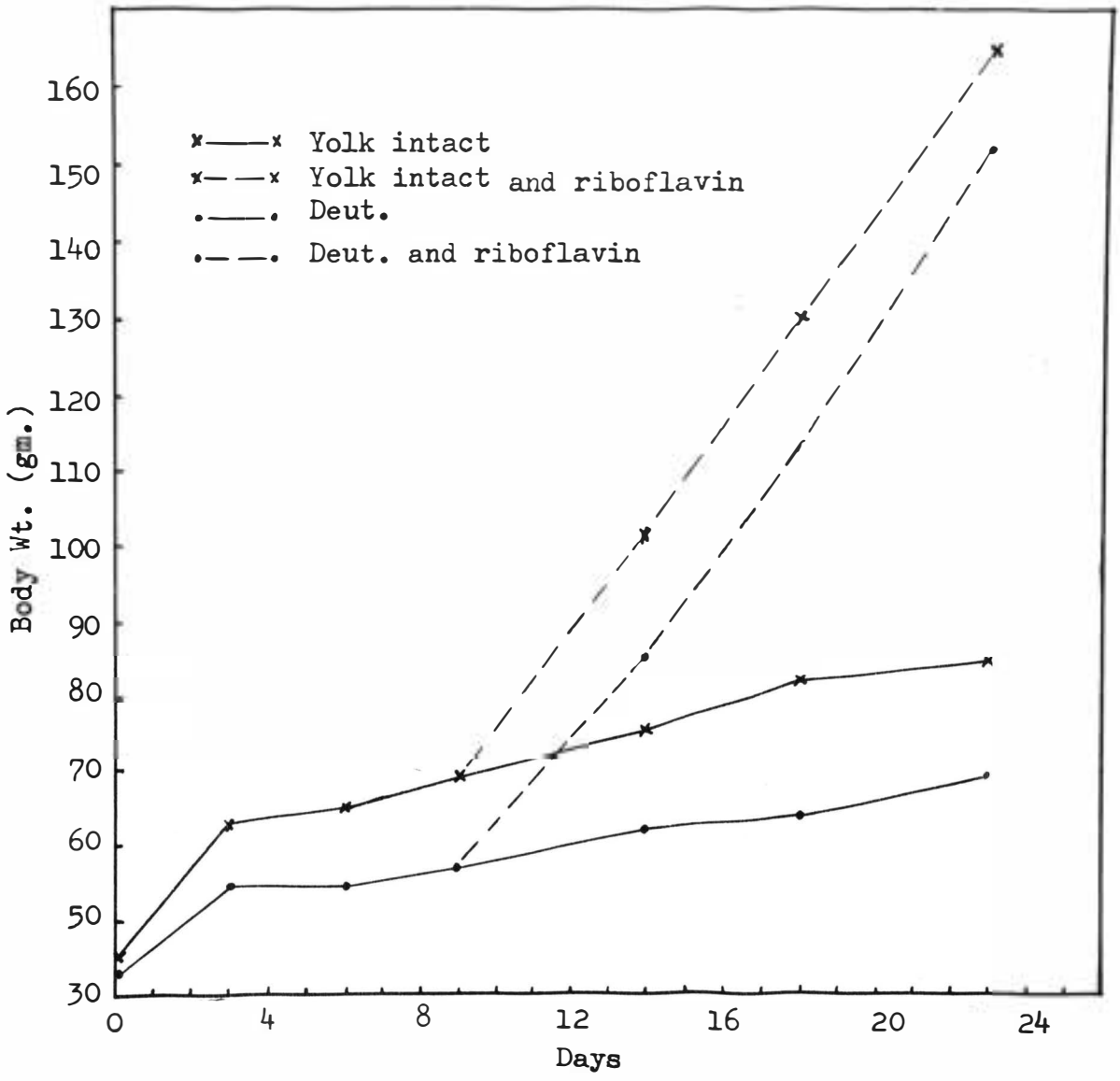

Fig. 4. Effects of deutectomy, riboflavin depletion and repletion on chick growth

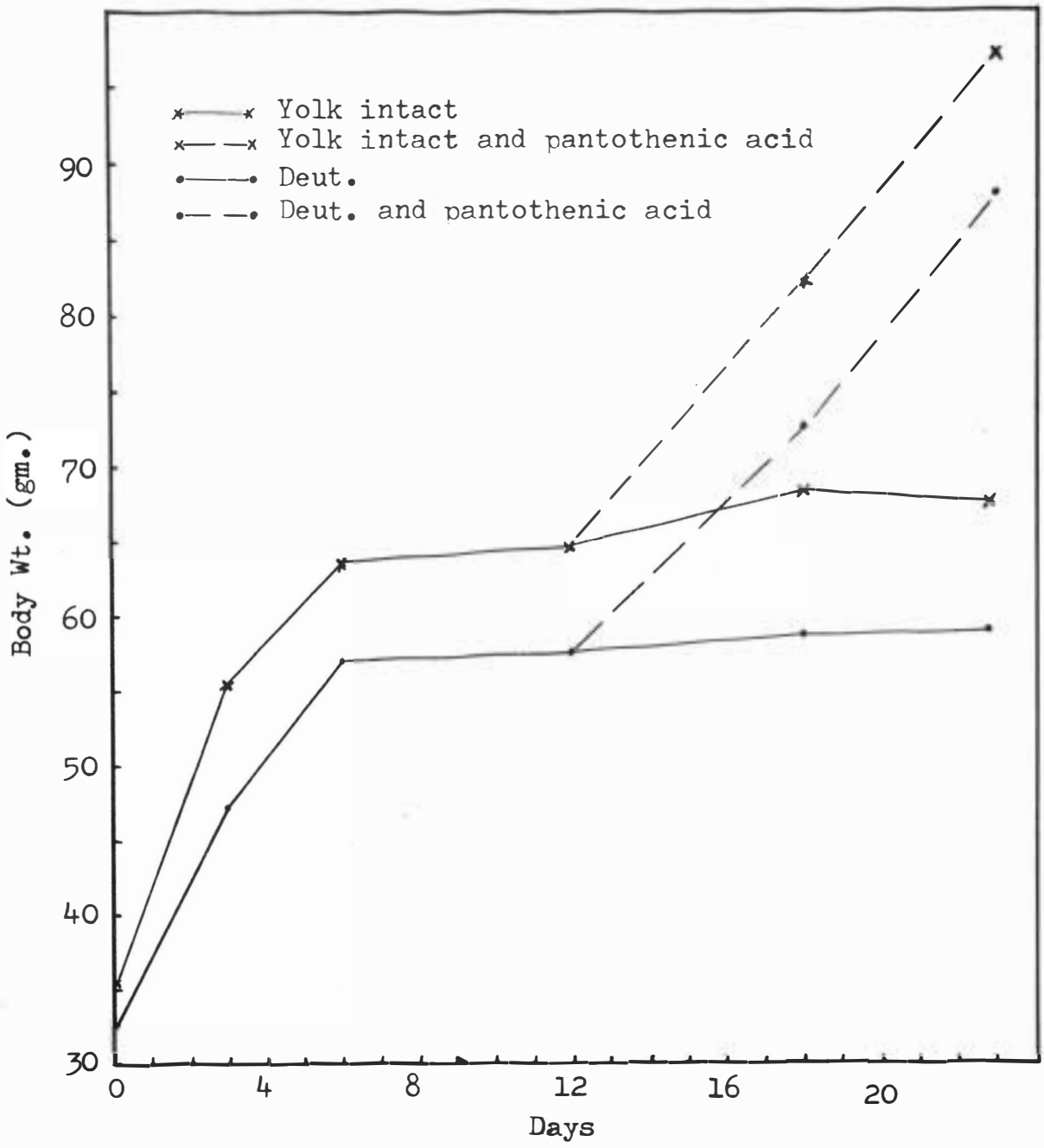

Fig. 5. Effects of deutectomy, pantothenic acid depletion and repletion on chick growth

Here also, the weight advantage of the "normal" chicks increased with age. At 24 days of age the deutectomized chicks not receiving supplemental pantothenic acid weighed approximately 15 percent less than the "normal" chicks. When a group of "normal" and a group of deutectomized chicks were injected with 40 mcg. of pantothenic acid per chick per day there were rapid improvements in growth. The average chick weight difference due to deutectomy remained about the same numerically.

Deutectomy was without effect in shortening the period of time required for depletion signs to appear. Growth plateaus for each group appeared on the sixth or seventh day of the experiment.

Niacin

Procedure similar to that established previously with the other vitamins was used for the initial niacin experiments. Two replicate trials, of which Figure 6 is representative, again showed that deutectomy was without effect in bringing about depletion signs faster than for the "normal" chicks. The difference in growth response followed a pattern similar to that established for the vitamins previously studied.

In both trials, the injection of each chick with 80 mcg. of niacin per day did not give a growth pattern like that established for the other vitamins. The data given in Figure 6 show that the chicks responded negatively to supplemental niacin. It appeared that the chicks could have been incorrectly identified. This was

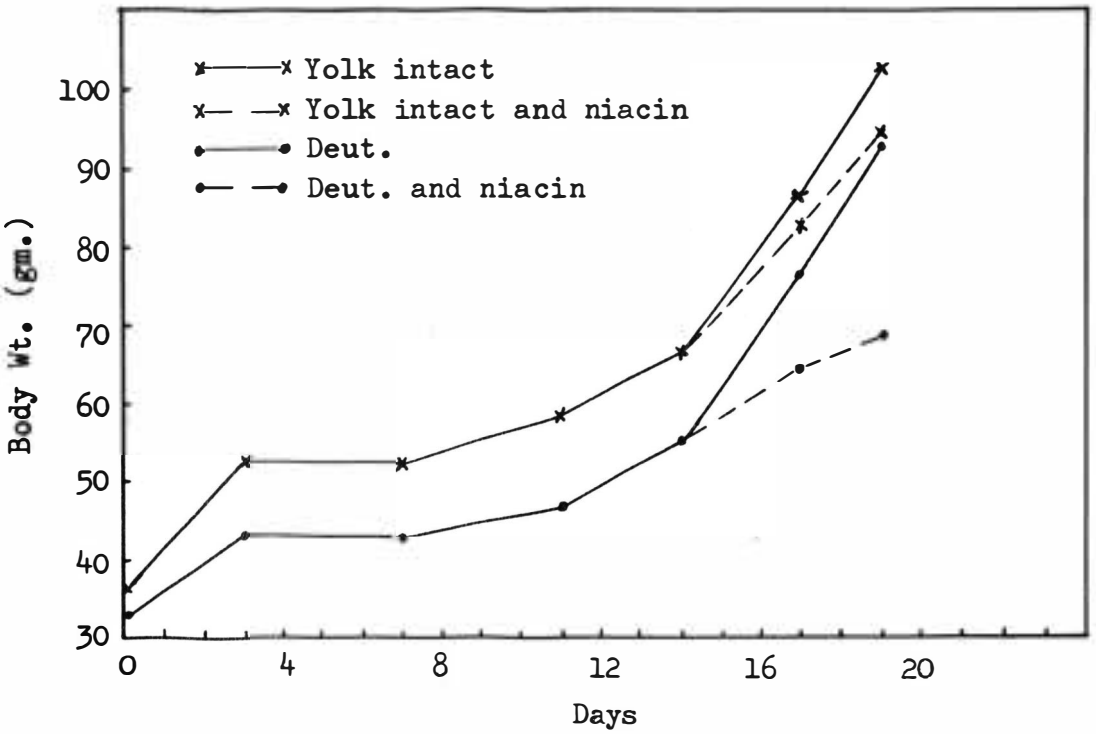

Fig. 6. Effects of deutectomy, niacin depletion and repletion on chick growth

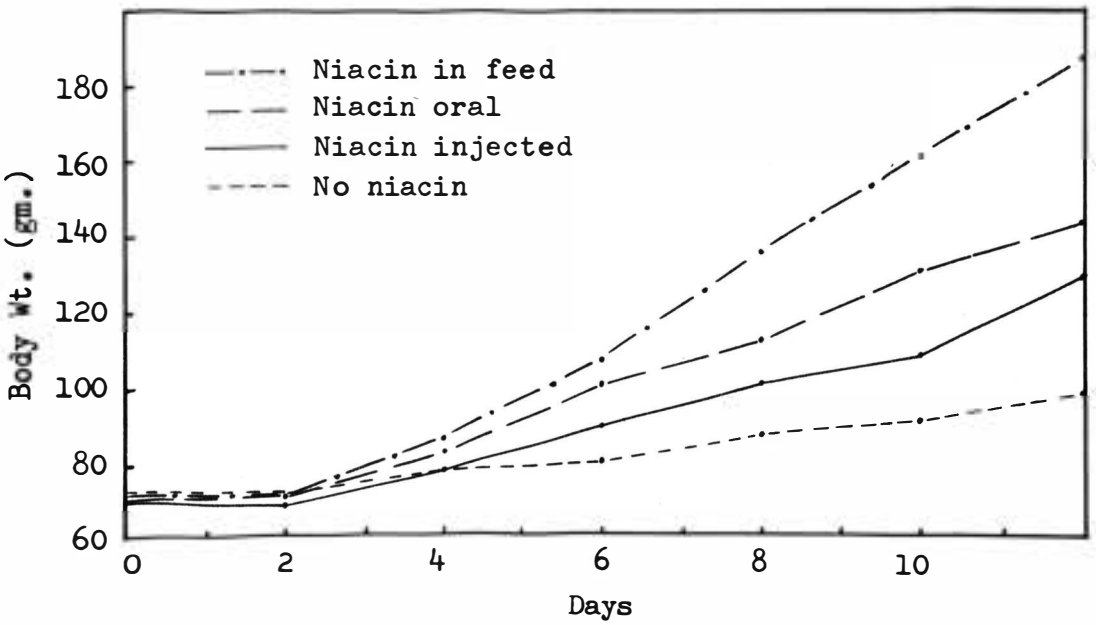

Fig. 7. Effects of method of niacin supplementation on chick growth

not the case, however, because the results were similar to the irregular growth response of the trial previously run. Experiments were then designed to study the effectiveness of intramuscular injections as compared to other methods of administration.

The first trial conducted in this study consisted of 4 groups of niacin-depleted chicks. Each group was given one of the following treatments: (1) control, (2) 35.2 mg. of niacin per kg. of diet, (3) 400 mcg. injections of niacin daily per bird, (4) as (3) but given orally. The results of this trial showed that the oral administration of the vitamin promoted better growth than the injected amount. To verify or repudiate the results obtained, a second trial was conducted, but with three pen replications for each treatment. The results of this trial are shown in Figure 7. At the end of a 13-day experimental period the results corroborated those obtained in the first trial. The orally administered niacin was approximately 50 percent more effective in promoting growth than the intramuscularly injected niacin. The oral method was then used in all subsequent work involving niacin.

Romanoff and Romanoff (1949) indicated that a preponderance of pyridoxine, thiamine, and pantothenic acid were found in the egg yolk while the amounts of riboflavin and niacin are divided between the yolk and albumen. The deutectomy and depletion data herewith reported showed that the yolk material removed from the chick had little, if any, pyridoxine, thiamine, riboflavin, pantothenic acid and niacin when measured by the time required to obtain a growth

plateau. These findings were rather surprising in consideration of the effects of incubation on the initial levels of riboflavin, thiamine and pantothenic acid reported by Romanoff (1949). Dann and Handler (1941) reported that niacin was synthesized in the egg during the incubation period. Snell and Quarles (1941) reported the initial levels of pantothenic acid, riboflavin and niacin at approximately 12.0, 2.0 and 0.87 mcg./gm., respectively for the nonincubated egg. These authors stated that the biosynthesis of niacin was from 10 to 20-fold during the incubation period. Although these workers did not indicate that riboflavin increased during the course of incubation, the data showed an increase of about 75 percent. More recent workers, Fischer et al. (1958) and Sharzynski et al. (1958) with vitamin B_{12} and Swendseid et al. (1958) with folic acid, support the earlier reports of vitamin biosynthesis in the incubating egg. The responses here with deutectomized chicks indicated that the vitamins were selectively moved from the yolk into the developing chick prior to the end of the incubation period. Romanoff (1949), while not reporting on the removal of vitamins from the yolk, indicated that there are increases and decreases of certain metabolic products within the yolk during the incubation period.

The average amount of yolk removed from the chicks by deutectomy differed in the various experiments. The difference was probably due to two factors, (1) chick weight when hatched and (2)

the time lapse between hatching and deutectomy. The data for the various vitamin trials indicated that the average weight loss was about 4 grams. The loss ranged from 3 grams for the pantothenic acid experiment to 7.5 grams for the pyridoxine experiment. Regardless of the weight loss due to deutectomy, the average chick weight difference between the deutectomized and "normal" was about 7 grams at 4 days of age. This effect was consistent among the several vitamins studied.

Sloan (1936) showed that the deutectomy weight-depression effect existed until the chicks were past 10 weeks of age. The longest period of observation for any vitamin of the present study was 32 days. The deutectomy growth-depressing effect was still very evident at the end of this period. In view of the fact that deutectomy greatly depresses chick weight gains to 4 or 5 days of age and maintains this depression for a long period of time thereafter, it is possible that this was due to more than yolk-fat and protein loss. Administering of any one of the vitamins studied gave little or no effect in correcting the deutectomy growth retardation.

The Effects of B Vitamin Deficiencies on the Efficiency of Metabolizable Energy Utilization

At the time this study was begun, little or no information of the type herewith reported was known to exist for poultry obtained

under vitamin deficient conditions. Knoebel and Black (1952) working with swine showed that the feeding of a diet containing a vitamin B_{12}-antibiotic supplement significantly decreased the metabolizable calories derived from the diet. The major part of this decrease was due to digestion rather than to intermediary processes. Sibbald et al. (1961) reported on the efficiency of ME utilization of pantothenic acid deficient chicks. Sibbald et al. (1962) reported similar data for riboflavin, vitamin B_{12} and their interrelationships. These findings will be discussed in more detail where applicable to the data of the present study.

The reports of Heuser et al. (1938), Bauernfeind et al. (1942), Briggs et al. (1942), Arnold and Elvehjem (1938), Childs et al. (1952) and many others showed that B vitamins were important in promoting chick growth. The feeding of pyridoxine, thiamine, riboflavin, pantothenic acid or niacin at graded levels below that amount required for maximum growth, not only depressed growth but in general decreased the efficiency of food utilization. That this decrease in efficiency may be the result of more than a simple decrease in weight gain was demonstrated by Sure (1941).

Using a paired-feeding technique, Sure (1941) reported that rats fed a riboflavin deficient diet gained 6.1 grams in 125 days. Litter mates receiving the same amount of diet but supplemented with 20 mcg. of riboflavin daily, gained 61.3 grams in a similar period of time. This work showed the importance of riboflavin in improving body weight gain and, presumably, a gain of net energy. In reference

to this work, Brody (1945) stated that a difference in food utilization resulted in part from differences in completeness of food oxidation in which several flavoproteins are known to participate.

Baldini (1961) reported that supplemental methionine added to a chick growing diet depressed the efficiency of ME utilization. In the review of literature Baldini (1961) construed information presented by Brody (1945) to mean "the metabolizable energy value of a feed ingredient is not decreased by a nutrient deficiency while productive energy is." This statement seems to be an interpretation of several fragments of information rather than a specific statement. That the last part of this quotation concerning productive energy is true there is little doubt. The report of Sure (1941) supports this conclusion. The first part of the quotation concerning metabolizable energy, if interpreted in true context, is subject to some doubt. Experimental evidence concerned with the physiological processes of digestion, absorption and intermediary metabolism suggests that a change in the efficiency of ME utilization may occur. The data of Baldini (1961) suggested that nutritional factors may alter the efficiency with which ME was used. The data of Sibbald et al. (1961) (1962) and the data herewith reported clearly show that some nutritional deficiencies, differing in kind and degree, are capable of decreasing the efficiency of ME utilization.

Pyridoxine

Metabolizable calorie values were determined for the basal diet, Table 1, over a pyridoxine intake range of 1.52 to 59.4 mcg. per 100 gm. of body weight. Adjusted caloric data are shown in Appendix I, Table 2. The mean caloric values are plotted in Figure 8.

These data show that there was a gradual decrease in the efficiency of ME utilization as the vitamin intake decreased from 49.0 to 1.52 mcg. The range of the decrease was from about 2995 MC to a low of about 2930 MC. Calculated LSD values showed that all MC values at and below the 9.0 mcg. level were significantly different ($P = .05$) from the MC value at which optimum growth response was obtained. With the minimum level of supplementary pyridoxine necessary to maintain chick life, the efficiency of ME utilization was reduced by only 2.1 percent below that obtained at a level of pyridoxine (37.0 mcg.) supporting optimum growth.

Information that might explain the decrease in efficiency of ME utilization is limited. Absorption of fat and carbohydrate, Carter and Phizackerely (1951), was not impaired under pyridoxine deficient conditions in the rat. The work of Jacobs (1962) indicated that a pyridoxine deficiency in the rat impaired the absorption of methionine. Guggenheim and Diamant (1957) reported that glucose utilization was increased in the muscle tissue of the pyridoxine-deficient rat but found no deviation from normal processes of carbohydrate metabolism.

The work of Chubb (1959) showed that significant amounts of free amino acids accumulate in chicken urine under pathological stress conditions. This may account in part for the slight depression of ME utilization efficiency as here reported.

Thiamine

Metabolizable caloric values were determined at daily thiamine intake levels ranging from 2.17 to 53.56 mcg. per 100 gm. of body weight. Adjusted caloric values are shown in Appendix I, Table 1. The mean values are plotted in Figure 9.

These data show that there was a decrease in efficiency of ME utilization from a daily vitamin intake of 27.0 to 2.17 mcg. with MC values of 2978 to 2795, respectively. The decrease in efficiency was greatly accelerated, relatively speaking, at a daily intake of 5 mcg. and below. The calculated LSD values showed that all MC values at and below a thiamine intake of 11.0 mcg. per day were significantly different ($P = .05$) from the MC value at which the optimum growth response was obtained. Maximum thiamine deprivation reduced the efficiency with which the ME was utilized by 9.38 percent. This is similar to the decreased efficiency of ME utilization reported for thiamine deficient rats by Voris (1937).

The physiological disturbance most likely associated with this decrease in energetic efficiency was a cellular decrease in the coenzyme, cocarboxylase. Peters (1936) showed that a thiamine deficiency in the diet resulted in a deficiency of cocarboxylase

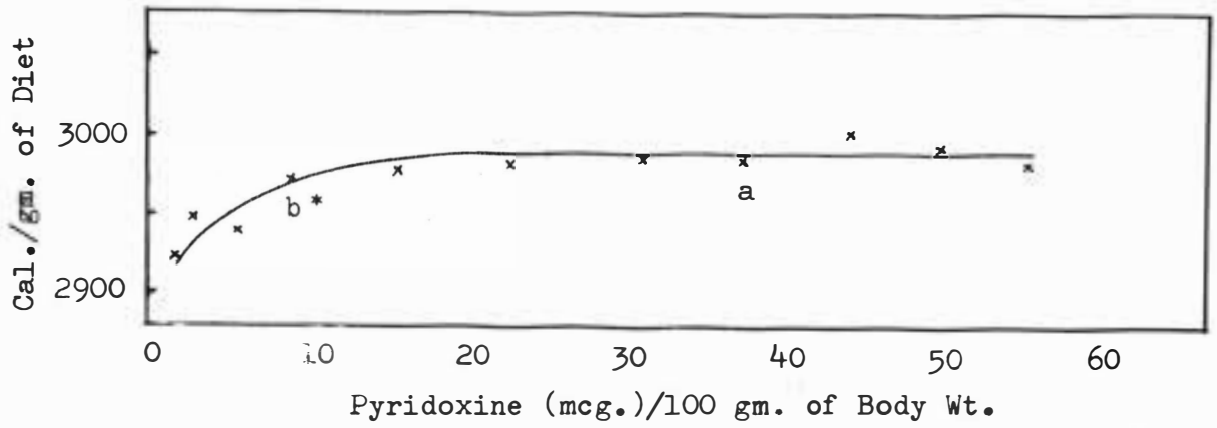

Fig. 8. Effect of pyridoxine on the efficiency of ME utilization

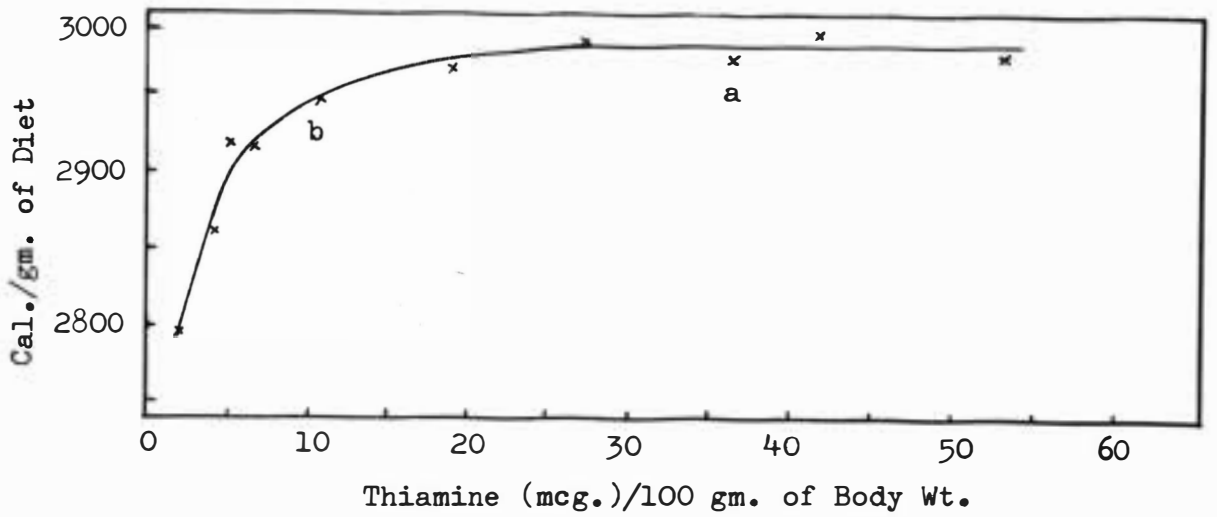

Fig. 9. Effect of thiamine on the efficiency of ME utilization

*Caloric values at b and below are significantly different from the value at a (P = .05).

which with the enzyme decarboxylase catalyzes the decarboxylation of pyruvic acid. According to Brody (1945), Peters in 1937 reported that under thiamine deficiency conditions the anaerobic oxidation product, pyruvic acid, accumulated in the blood. Geiger and Rosenberg in 1933 and Lehman in 1935 as described by Williams (1939) reported pyruvic acid in the urine of thiamine deficient infants and rats respectively. Mitchell (1956) similarly reported that in thiamine deficiency pyruvic acid tends to accumulate in the blood and is excreted in the urine. This would in part tend to account for the decrease in ME efficiency.

Optimum growth response was obtained at a daily vitamin intake level of 36.5 mcg. per 100 gm. of body weight. The reduction in energetic efficiency started near the 19.0 mcg. intake level. Neglecting the small amount of thiamine contributed by the feed, this indicated that the thiamine needed per day to prevent a decrease in energetic efficiency was approximately 60 percent of that required to support optimum growth. The observed vitamin intake level at which clinical deficiency signs appear was usually below 10 mcg. These data indicated that as the result of the change in some physiological process the decrease in energetic efficiency occurred before deficiency signs appeared.

Riboflavin

Metabolizable calorie values were determined at daily riboflavin intake levels ranging from 1.47 to 64.3 mcg. per 100 gm. of

body weight. Adjusted caloric values are shown in Appendix I, Table 3. The adjusted mean values are plotted in Figure 10.

These data indicated that there was a decrease in the efficiency of ME utilization from a daily riboflavin intake level of approximately 32.6 to 1.47 mcg. with MC values of 2810 to 2750 respectively. The decrease in efficiency of ME utilization was approximately linear between intake levels of about 25.0 to 5.0 mcg. The ME values at 1.47, 2.84 and 5.12 mcg. levels were about 2750 calories, approximating a horizontal plane. Calculated LSD values showed that ME values at and below the 18.0 mcg. level were significantly different from the MC value at which optimum growth was attained. The ME efficiency at which optimum growth response was attained was reduced by 2.15 percent at the lowest vitamin intake level.

Sibbald et al. (1962) reported that riboflavin was a factor that affected the efficiency of ME utilization. The basal diet used in that study was of the corn-soya type. The addition of riboflavin to this diet increased the amount of ME derived regardless of the combination of other supplements (niacin, vitamin B_{12} and methionine) with which it may have been simultaneously fed. The effect of riboflavin in this work increased the caloric efficiency by 2.4 percent in contrast to 2.15 percent for the data herewith reported. Sibbald et al. (1962) also reported a slight interaction between riboflavin and niacin effects. The addition of riboflavin to the diet did not

affect the niacin response but in the absence of riboflavin, niacin seemed to have a greater influence. Several other interactions, one of them being a third order, were apparent. These, although statistically significant, were of a relatively small magnitude.

There is little, if any, evidence as to how a riboflavin deficiency might cause such a change. Mann et al. (1952) working with the Cebus monkey reported that the plasma FAD level decreased when dietary riboflavin was severely restricted. A moderate restriction of dietary riboflavin in rats and man failed to cause a decline in plasma FAD levels. Horwitt et al. (1949) reported that there were no apparent changes in the carbohydrate metabolism of man suffering from a riboflavin deficiency. In contrast to thiamine no mention was made in the literature cited of improperly metabolized products occurring in the urine. This may be a factor of digestion rather than one of intermediary metabolism. According to Cowgill (1939), Reder and Gallup in 1935 reported that rats deficient in thiamine and riboflavin exhibited slower digestion and absorption of carbohydrates than normal control animals. The addition of thiamine to the diet did not improve the condition but the addition of riboflavin to the diet was effective in correcting the abnormality.

Pantothenic Acid

Metabolizable calorie values were determined at daily pantothenic acid intake levels ranging from 4.9 to 112.4 mcg. per 100 gm.

of body weight. Adjusted caloric values are shown in Appendix I, Table 4. The adjusted caloric value means are plotted in Figure 11.

These data showed that there was a decrease in the efficiency of ME utilization from a daily vitamin intake of approximately 85 mcg. to the minimum intake of 4.9 mcg. The caloric decrease ranged from about 3000 MC to a low of approximately 2925 MC. The calculated LSD values showed that all MC values at and below a vitamin intake of 35.0 mcg. were significantly different (P = .05) from the MC value at the vitamin intake level of 112.4 mcg. In previous studies the ME value to which other values were statistically compared was that occurring at the vitamin intake level where growth plateaued. In this study no definite growth plateau was established. The 112.4 mcg. level was used as a reference point because it was the greatest vitamin intake level at which caloric data were obtained. The decrease in caloric efficiency at the 4.9 mcg. intake level was 2.3 percent less than at the 112.4 mcg. level.

Sibbald et al. (1961) reported on the effects of four dietary levels of pantothenic acid on the efficiency of ME utilization with two levels of Aureomycin and calcium. These data and the data herewith reported are not directly comparable because of the wide difference in pantothenic acid treatment levels. In the study herewith reported it was shown that low pantothenic acid intake levels did depress the efficiency of ME utilization. The maximum intake level approximated Sibbald's low dietary levels of 5.6 or 6.8 mg. per pound of diet. The ME values for that work was approximately the

same at pantothenic acid levels of 5.6, 6.8 and 14.8 mg. per lb. of diet but appeared to be depressed when the diet contained 28.8 mg. per lb. of diet.

Since pantothenic acid and thiamine are both associated with pyruvic acid metabolism one might expect similar ME assay patterns to exist for the two vitamins. That this was not the case is shown in Figures 9 and 11. The degree of decline in caloric efficiency from vitamin deficiencies was about three times greater for thiamine than that shown for pantothenic acid.

The lack of similarity in ME response as stated in the previous paragraph may have been due to several factors. One of these is the degree of dietary vitamin deprivation and subsequent depletion of the chicks. The growth curve, Figure 16, and clinical deficiency signs show that the chicks were well depleted but it is doubtful that the degree of depletion equaled that of the thiamine deficient chicks. Secondly, the removal of pyruvate from the tissues is apparently not so dependent on coenzyme A as it is on the coenzyme cocarboxylase. Pilgrim et al. (1942) pointed out that there are many pathways for the removal of pyruvate from the organism deficient in coenzyme A. Stern (1940) outlined several diphosphothiamine dependent enzyme systems, whereby pyruvic acid might be broken down in various tissues. This perhaps accounts for the fact that little if any comment is made in the literature about the excretion of pyruvic acid in the urine under pantothenic acid deficiency conditions.

Niacin

Metabolizable calorie values were determined at daily niacin intake levels ranging from 0.0 to 1289.0 mcg. per 100 mg. of body weight. Adjusted MC values are shown in Appendix I, Table 5. Mean values for the adjusted data are plotted in Figure 12.

These data showed that there was a decrease in the efficiency of ME utilization from a daily vitamin intake of approximately 900 mcg. to the minimum intake of 0.0 mcg. The decrease in ME efficiency ranged from about 2890 MC to approximately 2805 MC. The relative efficiency of ME utilization was decreased by 2.9 percent with maximum niacin deprivation.

ME values were changed over a wider range of niacin intake levels than were the growth data. For this reason the ME value at which optimum efficiency occurred, namely 2891 MC, was used as the reference value for making statistical comparisons. Calculated LSD values showed that all ME values at and below a niacin intake of 400 mcg. (2850 MC) were significantly different ($P = .05$) from the ME value of 2891 MC obtained at the 900 mcg. intake level.

Sibbald (1962) reported the results of a factorially designed experiment in which niacin, riboflavin, vitamin B_{12} and methionine were factors. Niacin was found to improve the efficiency of ME utilization in the absence of riboflavin supplement. This interaction was accentuated by a methionine deficiency. The data herewith reported were obtained under conditions in which the diets were

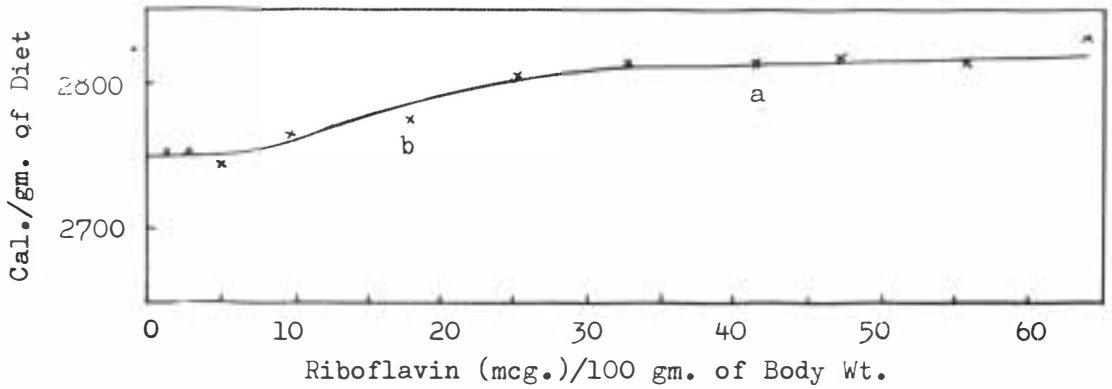

Fig. 10. Effect of riboflavin on the efficiency of ME utilization

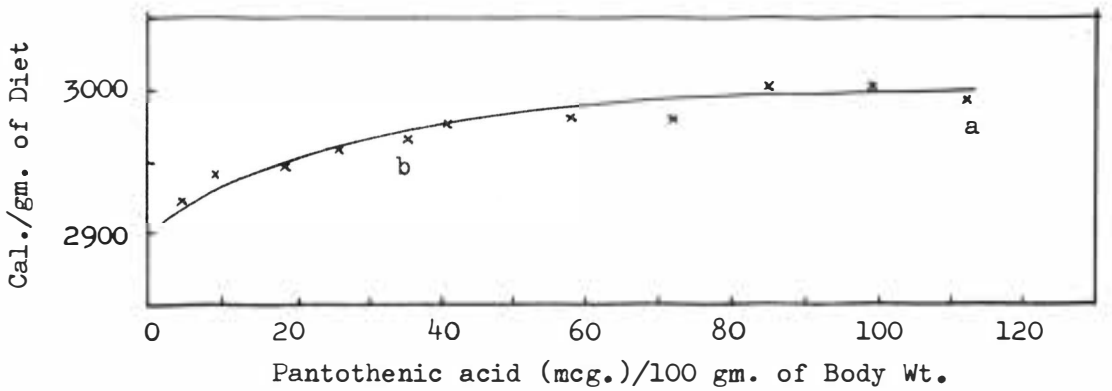

Fig. 11. Effect of pantothenic acid on the efficiency of ME utilization

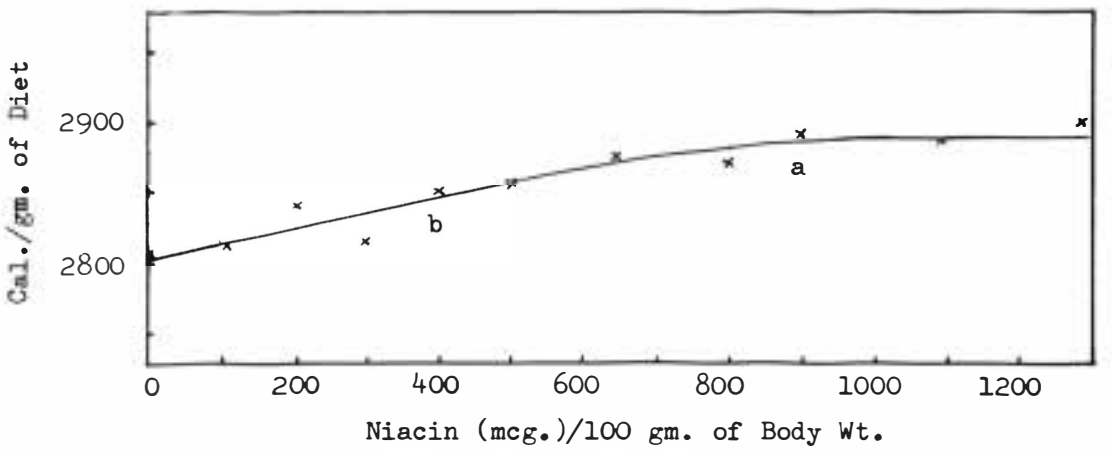

Fig. 12. Effect of niacin on the efficiency of ME utilization

supplemented with 5.5 mg. of riboflavin and 1270 mg. of methionine hydroxy analogue + calcium per kilogram of diet. In spite of these supplementations the addition of niacin to the niacin deficient diet improved the efficiency of ME utilization. This observation does not concur with the report of Sibbald et al. (1962).

There is a paucity of information that might explain the decrease in ME utilization efficiency. Spies and associates, according to Rosenberg (1945) reported that a niacin deficiency causes iron-containing porphyrins to undergo decomposition. As a result an increased amount of porphyrin compounds was excreted through the urine. The work of Sullivan as described by Rosenberg (1945) indicated that tryptophane catabolism under pellagrin conditions resulted in the excretion of indoxyl-ethyl-amin in the urine. Matsuoka and Yoshimatsu according to West and Todd (1961) reported that kynurenine, an intermediate in the tryptophane-niacin conversion scheme, was found in the urine of rats injected with tryptophane. Other products of this scheme have been found in urine according to Coon and Robinson (1958).

According to the National Research Council (1960) the chick diet should contain about .20 percent tryptophane. If the chicks were capable of converting all of the tryptophane to metabolites that were excreted in the urine the energy loss would be only a small fraction of the 2.9 percent loss here reported. It appears that the loss of porphyrin and/or tryptophane catabolic products

through the urine under niacin deficiency conditions could have only a minor effect on the decrease in ME efficiency.

Variation Between Vitamins

The information diagrammed in Figures 8, 9, 10, 11 and 12 shows that the diets used for testing the ME effects of thiamine, pyridoxine and pantothenic acid yielded about the same amount of ME when the vitamin-level effect was not restricting the efficiency of metabolism. The diets used for the studies with niacin and riboflavin yielded less ME under similar conditions.

These differences are here reported and briefly discussed. The ME values presented in this chapter are adjusted values. As previously discussed under Procedure and Materials, adjustments were made in combining experiments and for the difference in gross caloric contents of the experimental diets. The gross caloric content of the experimental diet was theoretically the same but a small amount of variation existed between mixes within and among vitamins. Elimination of the variation of gross ME values leaves a variation between vitamins that cannot be accounted for with the experimental data here presented. This variation may, in part, be due to a technician's error, or a difference in digestibility of feed ingredients or a vitamin deficiency effect. It is possible that an imposed deficiency could alter the nutrient absorption process or some other physiological process in early life of the animal that would

continue after the imposed deficiency is alleviated. This, however, is speculation and can be answered only with additional studies.

A summary of the results of the five trials indicates that an accentuated deficiency of B complex vitamins reduced the efficiency with which ME was utilized. The data from the several trials presented many MC values which were significantly different. The magnitude of decrease in energy utilization efficiency was within three percent of optimum efficiency for pyridoxine, riboflavin, pantothenic acid and niacin. Thiamine deficiency had the greatest effect on ME utilization. This was evidenced by a 9.3 percent reduction in caloric efficiency. In the interpretation of the percentage values here used to express magnitude of difference, one must keep in mind that the percentage values are not absolute because of adjusted data used in the calculations. The values do, however, give an estimate of ME change.

B Vitamin Nutrition and Basal Metabolism

The evaluation of basal metabolism data obtained under experimental conditions of this study is dependent upon several factors. For this reason experimental data determined to aid in the evaluation of the effect of B vitamin nutrition and BMR are presented previous to the presentation and discussion of the main effects.

Diurnal Variation

Several workers have reported that time of the day affects the rate of metabolism of chickens. Barott et al. (1938) and Barott and Pringle (1946) observed diurnal variation of oxygen consumption. The high level of consumption was at approximately 8:00 a.m. while the low level was at 8:00 p.m. Mellen (1958) reported that oxygen consumption by chicks decreased with increased fasting time and that a diurnal pattern similar to that reported by Barott et al. (1938) and Barott and Pringle (1946) was observed.

Brody (1945) discussed the diurnal effects of oxygen consumption from the point of view that light per se might be a factor. Barott and Pringle (1946) showed that chicks housed in dark quarters during the night had a decrease in oxygen consumption of about 18 percent from 8:00 a.m. to 8:00 p.m.

The chicks used in the work here reported were kept in the battery room and conventional-type pens heretofore described. Fluorescent lights were used to maintain constant lighting 24 hours per day.

Studies were conducted to determine if a diurnal effect existed under the conditions in which the basal-metabolism work was measured. This study included birds receiving the experimental diet with all experimental B-vitamins added and groups of birds fed diets deficient in each of the experimental B-vitamins. From each of these lots, basal metabolism was determined on 4 small

groups of 3 to 4 chicks per group. The small groups were weight-equalized in order to eliminate this variable. Basal-metabolism tests were conducted during the course of the day with 60 minute experimental periods starting near 8:30 and 11:00 a.m. and 1:30 and 4:00 p.m. The results are shown in Table 2.

This study was carried on for 14 days. On only two out of 14 days was there clear evidence of a diurnal effect continuing throughout the day. The average value for the 8:30 a.m. determinations was 22.10 microliters of oxygen consumed per minute per gram of tissue and the 4:00 p.m. value was 20.56 microliters. This decrease in oxygen consumption amounted to 7 percent which was statistically significant. The 11:00 a.m. value was smaller than that at 1:30 p.m. but both were smaller than the 8:30 a.m. value. Barott and Pringle (1946) showed about 17.5 percent decrease in oxygen consumption in a similar period for chicks of about the same age. Because of the general diurnal effect here obtained, the oxygen data shown in Table 4 were increased by one percent for each hour that an experimental period was started subsequent to 8:00 a.m. This increase was less than that reported by Barott and associates but seemed to be a reasonable correction for the data presented here.

Expression of Basal-Metabolism Rate

Basal metabolic rate is usually expressed as calories per unit of tissue for a given period of time. Brody (1945) expressed BMR as calories per 24 hours per kg. of body weight. When using the

Table 2. The effects of time of day and vitamin deficiency on oxygen uptake

Vitamin Deficiency	Days	Time a.m. 8:00	a.m. 11:00	p.m. 1:30	p.m. 4:00	Vitamin av.
		Mcl. of O_2 per gm. of tissue per minute				
None (control)	4	22.75	19.28	23.01	20.19	
	9	23.53	24.28	24.52	24.85	
	15	30.04	28.43	25.86	24.64	
	18	25.12	21.02	22.38	24.19	24.00 a*
Niacin		20.79	18.98	18.02	15.05	
		19.18	20.09	19.61	18.58	18.79 d
Thiamine		21.14	21.73	21.06	20.82	
		19.54	18.39	21.96	18.50	20.39 c
Pantothenic acid		21.25	22.11	19.56	20.41	
		18.46	19.05	17.89	17.84	19.57 cd
Pyridoxine		24.49		25.00	24.45	
		21.96	22.79	23.26	20.12	23.15 a
Riboflavin		20.50	21.17	21.17	19.48	
		20.65	20.88	22.66	18.68	21.44 b
	Av.	22.10 a*	21.40 a	21.85 a	20.56 b	

* Values having the same letters are not significantly different, P = .05.

indirect calorimetry technique it is possible to calculate the calories of heat produced by an animal with the oxygen caloric coefficients of Zuntz and Shumberg as described by Lusk (1928).

Since the data of Zuntz and Schumberg was for animals that produce urea instead of uric acid, the use of these values for calculating the heat produced by chickens was criticized by several research groups. In an effort to avoid this, Mellen and Hill (1953) expressed the metabolic rate of chickens in terms of oxygen consumption. Mellen (1958) and Siegel and Washburn (1964) expressed metabolism data on an oxygen consumption basis rather than a calculated caloric basis.

The data presented here are also expressed on an oxygen consumption basis although caloric estimates were made using the oxygen caloric coefficients of Zuntz and Schumberg. Correlation coefficients (r) were calculated with the measurements of caloric values (Y) dependent on oxygen values (X). The resulting r values were as follows:

Vitamins	r value
Thiamine	0.953
Pyridoxine	0.989
Riboflavin	0.976
Pantothenic acid	0.987
Niacin	0.968

The r values were all significant (P = .01) and showed that the correlations between measurements were highly significant. The

error inherent to these measurements may be due in part to CO_2 "washout" as described by Kleiber (1961).

From the data collected it was impossible to determine the true metabolic rate of the chickens. Both measurements were relative, since oxygen consumption per se could only estimate the true BMR and the calculated caloric values of the BMR were probably not precise because of the use of the Zuntz-Schumberg oxygen caloric coefficients. Theoretically the caloric values should be more representative of the heat produced because these values have been corrected by use of the various respiratory quotients which have different CO_2 and O_2 caloric coefficients. This, however, may not be true if error involved in CO_2 production greatly influences the RQ values and subsequent caloric calculations.

Metabolic Body Size

The metabolic size of the chicken does not reflect the use of energy according to the formula 70 (kg. wt.$^{.75}$) of Kleiber (1961) or to the formula 70.5 (kg. wt.$^{.73}$) of Brody (1945) during the first two weeks of life. These formulas estimate the amount of energy required per 24 hours. Barott et al. (1938) showed that the BMR of the chick increased with increase in body size to about 90 gms. during the first two weeks of life and then developed a metabolic rate of the type shown by the formulas of Kleiber (1961) and Brody (1945). A similar metabolic rate per age pattern was established in this study for standard control chicks during the

first two weeks of life. This was shown in Table 2 by data on non-deficient chicks obtained at 4, 9, 15 and 19 days of age.

One of the problems in analyzing the oxygen consumption data of the various vitamins was that the metabolic rate was not functioning according to the standard type formula 70. (kg. wt.$^{.75}$) because of their immature physiological age. What effect a particular vitamin deficiency would have on a chick's apparent physiological age to about 90 gms. weight was not known. Kleiber and Jukes (1942), working with a 50 to 70 gram chick deficient in riboflavin used the standard formula for estimating the heat produced. In order to get an estimate of the type of metabolic pattern during the course of the experiment, the oxygen consumption per gram of tissue was correlated to chick size at each vitamin intake level. This gave evidence as to whether the BMR was increasing or decreasing in O_2 consumption with increased body weight.

Correlation coefficient data are shown in Table 3. The several r values for each vitamin showed both positive and negative correlation values with the exception of niacin which had all positive r values. The several r values for each vitamin were converted into a single r value by use of the Z transformation method as described by Snedecor (1946). The combined r value of niacin was significant (P = .05). The combined r values for the other vitamins were small and not significant.

The niacin O_2 consumption data were adjusted for body weight using the formula: adj. $Y = Y-b\ (X-\bar{X})$. This formula was used with

Table 3. Correlation coefficients for body weight and O_2 consumption at various vitamin intake levels.

Vitamin levels	Vitamins				
	Pyridoxine	Thiamine	Riboflavin	Pantothenic acid	Niacin
	r	r	r	r	r
0	-.60	+.025	-.364	-.070	+.163
1	+.22	-.347	-.615	-.097	+.351
2	-.59	+.557	-.467	-.063	+.417
3	+.55	-.296	-.139	+.750	+.960
4	-.27	+.248	-.905	+.097	+.675
5	+.75	-.230	+.783	-.010	+.916
6	+.80	-.940	+.440	-.880	--
All	+.015	-.065	-.408	+.097	+.88*

* Significant P = .05.

the assumption that the change in O_2 consumption and body weight was a linear function.

B Vitamin Nutrition and Oxygen Utilization

A trial designed to give information on the diurnal effects of oxygen consumption also gave information on the oxygen consumption effects of vitamin deficient and normal (control) chicks. The diet fed the normal chicks was the same as that fed to the deficient

chicks with the exception that all test vitamins were present. Since vitamin levels were not a part of the trial, an attempt was made to keep the chicks at a low level of vitamin nutrition but not to the extent that the chicks died of a deficiency. Thiamine and pyridoxine deficient chicks were given daily doses of 5 mcg. to maintain relatively good activity during the course of the trial.

The oxygen consumption data of trial one are shown in Table 2. Statistical analysis data are shown in Appendix II, Table 2. These data show that the chicks deficient in riboflavin, thiamine, pantothenic acid and niacin consumed significantly less oxygen per unit of tissue than the control chicks. Oxygen consumption of the pyridoxine deficient chicks were significantly different than the oxygen consumption of the other vitamin deficient chicks. This was not true for the control chicks. These data in general suggest that the deficiency of B-vitamins here studied tended to lower the rate of metabolism of the chick.

A second group of oxygen consumption data are shown in Table 4. These data were determined at 6 and 7 of the lowest intake levels for the several vitamins studied. Four determinations at each vitamin intake level were designed for the first series of tests. A second series of determinations for the 5 lowest vitamin intake levels added two additional oxygen consumption estimates to each mean. Additional estimates were determined for several of the zero levels and in other cases where the validity of a given estimate was in question.

Table 4. Effects of pyridoxine, riboflavin, pantothenic acid thiamine, niacin and intake levels of oxygen consumption.

Vitamin Deficiency	Vitamin intake levels							Av.
None								25.08 a*
Pyridoxine:								
Daily (mcg.)	0	1.25	2.50	5.00	10.00	20.00	30.00	
Mcg./100 gm. t.	0	2.11	4.03	7.28	14.14	25.80	38.00	
Mcl. O_2/gm.	23.71 b*c	20.21 a	22.53 b	22.73 b	22.33 b	25.09 c	23.84 bc	22.92 b
Riboflavin:								
Daily (mcg.)	0	1.25	2.50	5.00	10.00	20.00	30.00	
Mcg./100 gm. t.	0	1.65	3.07	6.04	11.34	20.72	29.7	
Mcl. O_2/gm. t.	18.52 a	20.11 b	20.90 b	21.37 b	21.29 b	21.37 b	20.47 b	20.57 d
Pantothenic Acid:								
Daily (mcg.)	0	5	10	20	30	40	50	
Mcg./100 gm. t.	0	5.39	10.93	21.16	31.85	40.61	51.97	
Mcl. O_2/gm. t.	20.30 a	22.16 b	21.16 b	21.27 b	22.38 b	22.81 b	23.22 b	21.90 c
Thiamine:								
Daily (mcg.)	0	1.25	2.50	3.75	5.00	10.00	20.00	
Mcg./100 gm. t.	0	3.09	5.59	7.14	9.43	17.69	31.40	
Mcl. O_2/gm. t.	16.56 a	18.38 ab	19.52 b	22.13 c	21.57 c	22.17 c	22.31 c	20.38 d
Niacin:								
Daily (mcg.)	0	100	200	300	400	500		
Mcg./100 gm. t.	0	145.0	304.0	454.0	551.0	705.0		
Mcl. O_2/gm. t.	19.73 a	19.12 a	21.13 b	23.06 c	20.63 ab	22.28 cd		20.99 d

* Treatments with different letters are significantly different, P = .05.

** Tissue.

As previously mentioned, the original oxygen consumption data were corrected for diurnal variation to approximately 8:00 a.m. determinations. Since a regression trend was not established between body weight and O_2 consumption for thiamine, pyridoxine, pantothenic acid and riboflavin, a correction of O_2 consumption per unit of body weight was not made. A positive relationship was established for niacin and the oxygen consumption data were corrected. The control data which were a part of the diurnal experiment given in Table 2 were also used as the control for these data. These data were also corrected for diurnal effects.

The results of these oxygen consumption data are shown in Table 4. The statistical analysis is given in Appendix II, Table 3. The difference between oxygen consumption for the control birds and the oxygen consumption for the deficient birds followed a pattern similar to that established in the first trial. With these data, oxygen consumption per unit of tissue was not significantly different for thiamine, riboflavin or niacin but the data for these three were significantly different from that for pantothenic acid. The data for these four vitamins were significantly different from that for pyridoxine and the five vitamins gave significantly different results from the controls.

The effect of vitamin deprivation was to generally lower the metabolic rate per unit of tissue. In no case did the higher vitamin injection levels stimulate oxygen consumption equal to that of the control.

It is possible that a metabolic rate similar to that of the control birds would have been developed at the highest intake levels if the chicks had remained on vitamin repletion for a longer period of time. The nutritive plane of the diet as expressed by caloric intake cannot be discounted as a factor. Mellen et al. (1954) reported that nutritional regimes differing widely in caloric content produced significantly different basal metabolic rates for male chickens. As reviewed by Kleiber (1945), Williams and associates in 1942, working with humans, and Voris (1937), working with rats, indicated that a reduction of food intake reduced the BMR under thiamine deficient conditions. Kleiber and Jukes (1942), working with chicks, reported that riboflavin deficiency caused a decrease in the BMR by decreasing food intake. As reviewed by Kleiber (1945), Wahl in 1939 and Bersohn in 1943, working with guinea pigs and rats, respectively, reported that riboflavin deficiency per se did not effect BMR.

The effects of vitamin dosage levels on oxygen consumption for the several vitamins are shown in Table 3. Daily thiamine dosage levels of 0, 1.25, and 2.50 mcg. significantly depressed oxygen consumption in comparison to 3.75, 5.00, 10.00 and 20.00 mcg. levels. The latter dosage levels were not different statistically.

The oxygen consumption data of riboflavin and pantothenic acid show a slight upward trend with increased vitamin intake. The zero vitamin dosage levels significantly depressed oxygen

consumption below that consumed with vitamin dosage levels at or above 1.25 mcg.

The oxygen consumption data for niacin were quite variable. There appeared to be a trend of increased oxygen consumption as the level of niacin intake increased to the 300 mcg. dosage level. The relationships of oxygen consumption values at the 0 and 400 mcg. dosage levels to other levels cannot be determined without additional observations.

There seemed to be little evidence that pyridoxine *per se* had an effect on BMR. The data of Tables 2 and 3 show that chicks with this deficiency demonstrated the greatest level of oxygen consumption of the five vitamin effects studied. The zero dosage level supported an oxygen intake of 23.71 mcl. per gram of tissue per minute. A dosage level of 1.25 mcg. of the vitamin daily caused a decrease in oxygen consumption to 20.20 mcl. with an upward trend at greater pyridoxine dosage levels.

The greater oxygen consumption at the zero dosage level ranged from 20.09 to 26.06 mcl. The generally higher oxygen consumption as evidenced by the mean was believed to be due to the more active condition of the pyridoxine deficient chicks. Chicks deficient in this vitamin but demonstrating no nervous symptoms at the beginning of an experimental period were often found to be very excitable when removed from the metabolic chamber.

In summary, the data show that individual vitamin deprivation decreased the metabolic rate of chicks below those receiving all of the vitamins in the diet. Evidence of a vitamin effect per se was probably demonstrated for thiamine. The effects of the other vitamins per se were not clear. Aside from the general decrease in the BMR, which was not corrected by the short-term repletion treatment, the general effect of these vitamins on the BMR was very limited although growth was greatly depressed.

Growth and Vitamin Storage

The storage of B vitamins in the body of the animal seems to be rather limited. As previously cited in the literature, the existence of B vitamins or their metabolites in the urine suggest that these vitamins have a relatively fast turnover rate in the body. Rosenberg (1945) indicated that the thiamine nutritional status of an animal could be determined by the quantity of this substance excreted in the urine. Similar information was reported for riboflavin by Goldsmith (1949) and for niacin by Sarett and Goldsmith (1950).

Since the liver has been reported to be the major tissue for vitamin storage it was selected to determine the effects of vitamin repletion upon body stores.

Pyridoxine and Thiamine

These two vitamins are considered together because of similar chick repletion patterns. Liver samples were assayed from the repleted chicks grown on each treatment and from birds which died of the deficiency.

Liver stores of these vitamins are contrasted to growth data for pyridoxine, Figure 13 and thiamine, Figure 14. The assay data are quite variable for both vitamins but do give an estimate of the over-all response as shown by the Figures and in Appendix I, Tables 1 and 2.

Livers from chicks dying from the deficiencies of the basal diets contained about 3.26 and 1.47 mcg. per gm. of wet liver of pyridoxine and thiamine, respectively. The graph line fitted to each set of data showed that the vitamin content of the liver did not increase with increased daily vitamin intake until optimum growth response was attained. After this, the stores of pyridoxine and thiamine increased in a linear fashion with additional vitamin supplementation.

Riboflavin, Pantothenic Acid and Niacin

Liver samples from chicks grown on each basal and treatment diet were assayed for each of these vitamins. Liver stores of these vitamins are shown in Figures 15, 16 and 17 together with the respective growth curve. The numerical data are given in Appendix I, Tables 3, 4 and 5.

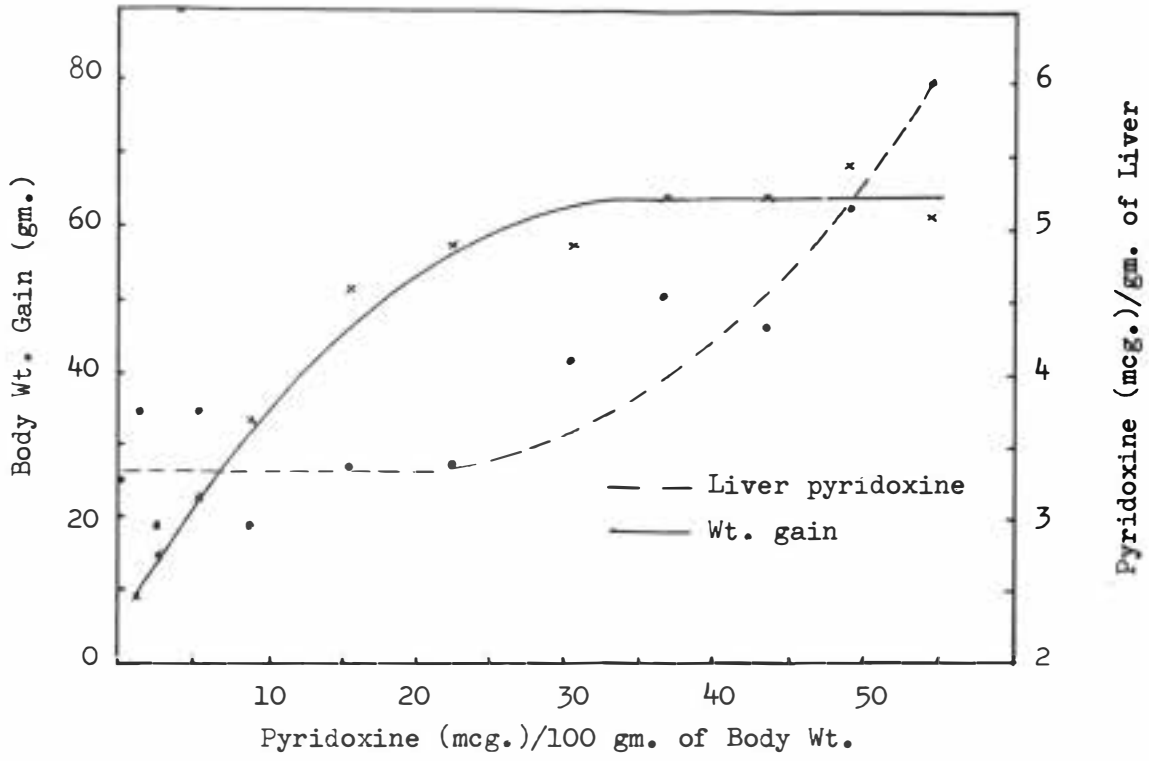

Fig. 13. Effects of pyridoxine intake on body weight gains and liver pyridoxine of chicks from 11 to 17 days of age.

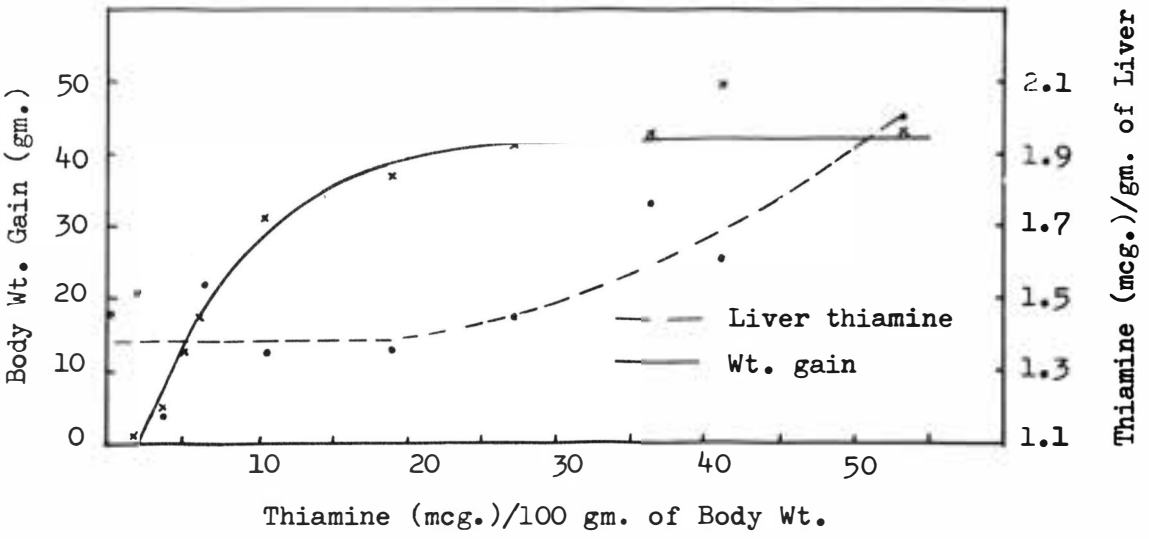

Fig. 14. Effects of thiamine intake on body weight gains and liver thiamine of chicks from 9 to 15 days of age.

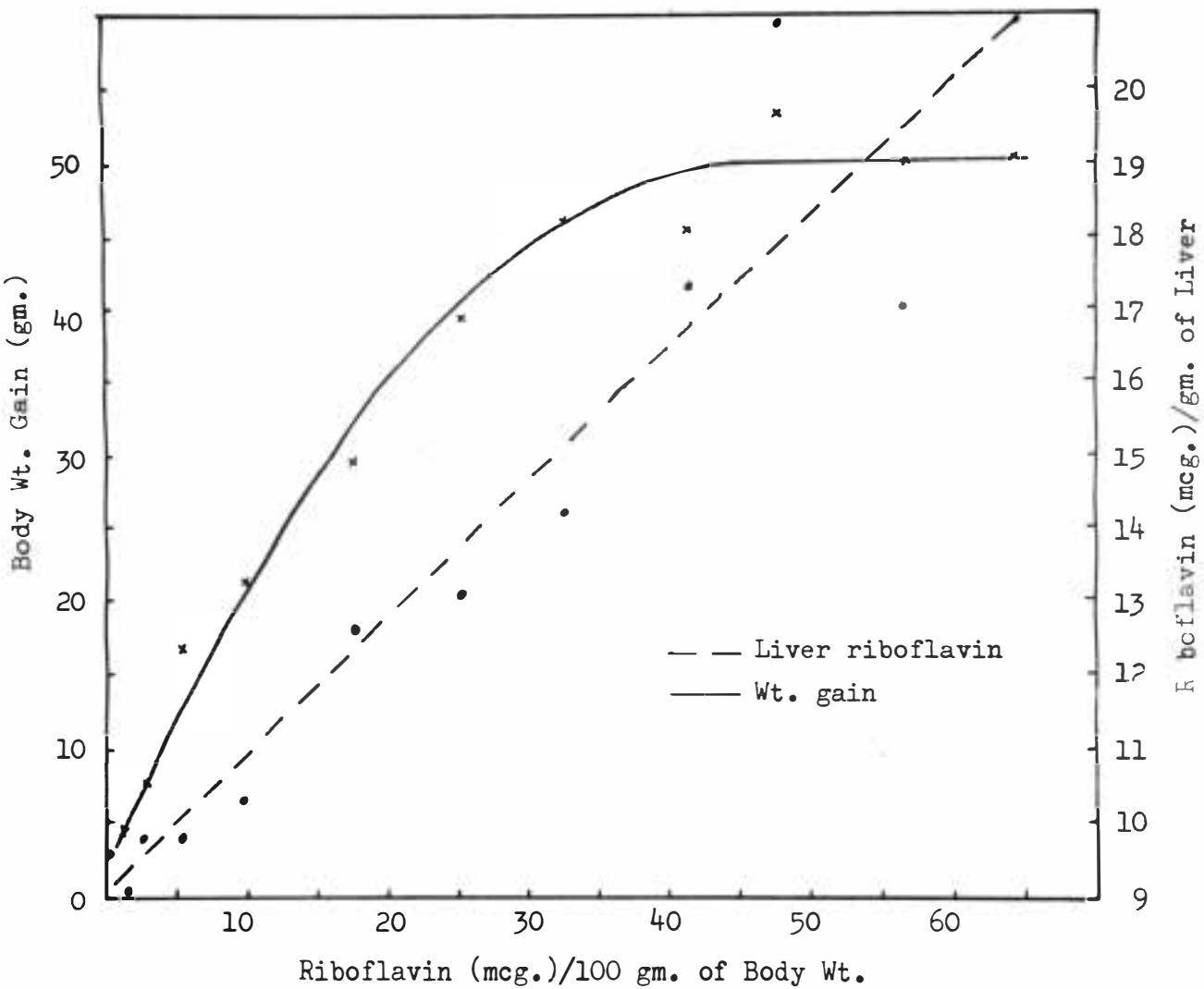

Fig. 15. Effects of riboflavin intake on body weight gains and liver riboflavin of chicks from 14 to 20 days of age.

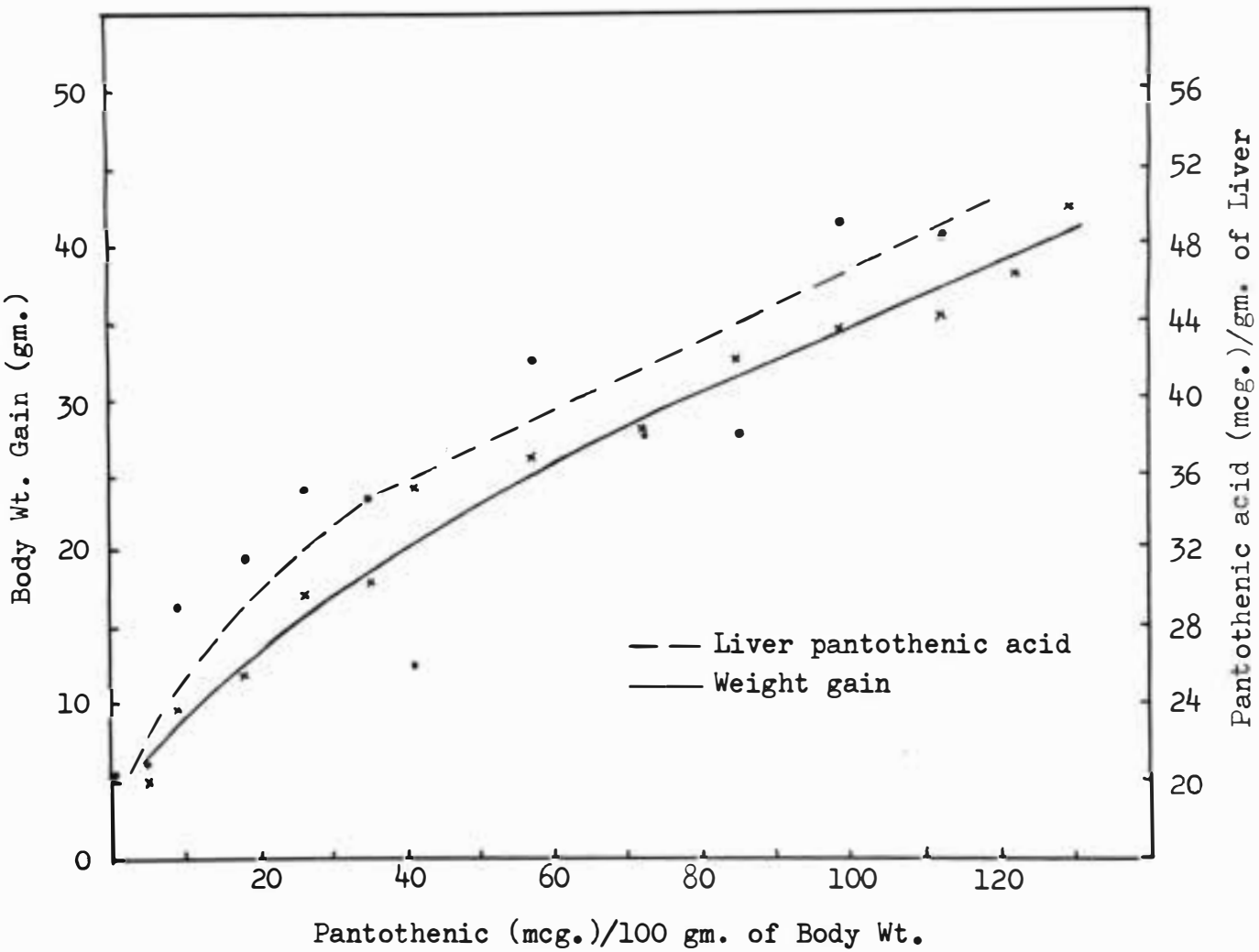

Fig. 16. Effects of pantothenic acid intake on body weight gains and liver pantothenic acid of chicks from 14 to 20 days of age.

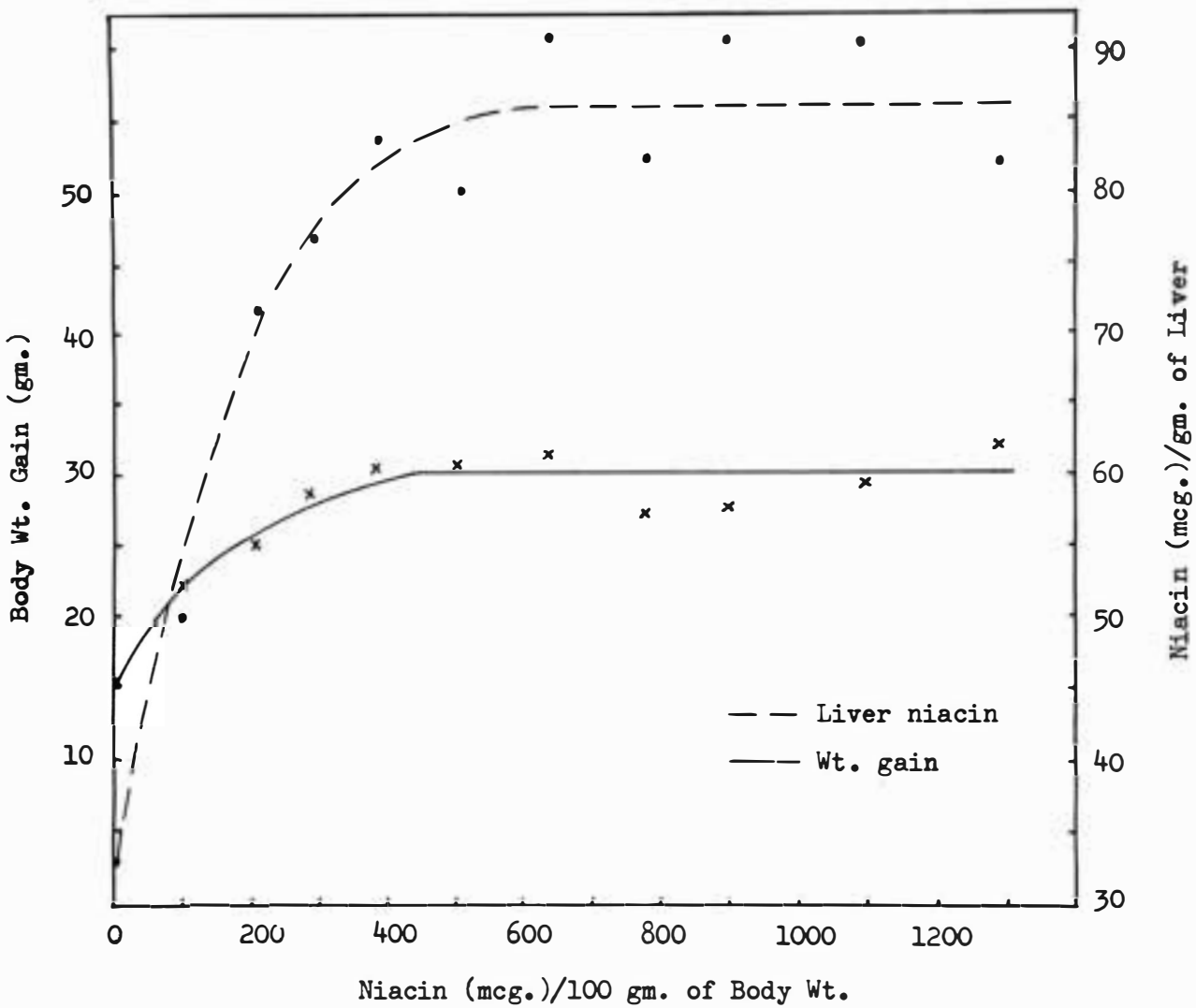

Fig. 17. Effects of niacin intake on body weight gains and liver niacin of chicks from 14 to 20 days of age.

In general, liver tissue levels of these vitamins began to increase at relatively lower levels of vitamin intake. At the lower levels the riboflavin content of the liver increased at a rate approximately one-half the rate of the improvement in growth. The storage of riboflavin followed a variable pattern for chicks given more than 40 mcg. per 100 gm. of body weight, the level supporting optimum growth. The plotted data indicated that the liver could store greater amounts of riboflavin than here shown.

The liver storage of pantothenic acid showed considerable variability with the several vitamin intake levels. A comparison of the growth and liver vitamin level curves indicates, however, that the general trend of effects were correlated within the range of this study.

In the niacin study, growth obtained with the basal diet was approximately fifty percent of maximum growth. A comparison of the growth and liver assay curves showed that they reached a maximum response simultaneously. These were obtained with 400 mcg. of niacin per 100 gm. of body weight. Maximum growth was obtained when the liver contained about 60 mcg. of niacin per gm. of wet liver.

These results further demonstrate that liver sample analysis might be used to obtain an expression of nutritional status. A serious disadvantage for the use of such a method for estimating the nutritional status of an animal for thiamine and pyridoxine is

that the vitamin levels in the liver remain practically unchanged from severe depletion to the minimum amount required to support maximum growth. The method does have merit in the case of riboflavin, pantothenic acid and niacin.

SUMMARY AND CONCLUSIONS

Depletion studies were conducted with several B-vitamin deficiencies under normal (yolk intact) and deutectomized (yolk removed) conditions. It was concluded that the absorbed yolk material contributed very little to the B-vitamin nutritional status of the day-old chick.

The efficiency of metabolizable energy (ME) utilization was determined at 11 dosage levels for each of five vitamins. Vitamin dosage levels (mcg./100 gm. body wt.) below which the ME utilization was decreased were: pyridoxine, 49.0; thiamine, 27.0; riboflavin, 33.0; pantothenic acid, 85.0 and niacin, 90.0.

A comparison of ME and growth data suggested that approximately 25 percent of the amounts of thiamine, pyridoxine and pantothenic acid required to support maximum growth were needed to support optimum ME efficiency. For riboflavin the amount required to support optimum efficiency was about 50 percent of the growth requirement. The niacin required to support optimum ME efficiency was in excess of that required for maximum growth.

With the exception of thiamine the range of decrease in efficiency of ME utilization was less than three percent of maximum efficiency. The range of ME decline from maximum utilization for thiamine was approximately nine percent.

Basal metabolism rate and several factors pertaining thereto were studied in a series of determinations. The BMR was

highest at 8:00 a.m. and decreased to the end of the experimental day, approximately 7:00 p.m. Therefore, oxygen consumption data were corrected on the basis of a one percent increase for each hour that an experimental period was started after 8:00 a.m.

It was shown that young normal chicks do not observe the classical BMR formula, 70.(kg. wt.$^{.75}$). The BMR showed a significant increase with age to 15 days of age. However, neither thiamine, pyridoxine, pantothenic acid nor riboflavin deficient-repleted chicks showed this increase, whereas, chicks of the niacin study did follow a metabolism pattern similar to control chicks.

Correlation coefficients were determined for oxygen consumption and calculated caloric data. Correlation coefficient values (r) were: thiamine, .953; pyridoxine, .989; riboflavin, .976; pantothenic acid, .987 and niacin, .968. These data indicate that oxygen consumption values can be used if caloric data per se are of little interest.

Deprivation of all five B-vitamins significantly lowered the metabolic rate. The BMR of pyridoxine repleted birds approached that of the control birds, but in no case did vitamin injections stimulate oxygen consumption equal to that of the controls.

Liver vitamin storage in chicks fed the basal diets was found to be approximately 1.5, 3.5, 9.0, 20.0 and 35.0 mcg. per gm. of wet liver for thiamine, pyridoxine, riboflavin, pantothenic acid and niacin deficiencies, respectively. With increasing levels of

supply, storage of thiamine and pyridoxine did not change appreciably until the amounts obtained were sufficient to support maximum growth. Riboflavin, pantothenic acid and niacin contents of the liver showed increases rather well correlated with increased growth rate.

LITERATURE CITED

Anderson, R. J. and W. L. Kulp, 1922. A study of the metabolism and respiratory exchange in poultry during vitamine starvation and polyneuritis. J. Biol. Chem. 131: 85-93.

Arnold, A. and C. A. Elvehjem, 1938. Studies on the vitamin B_1 requirements of growing chicks. J. Nutr. 15: 403-410.

Baldini, J. T., 1961. The effect of dietary deficiency on the energy metabolism of the chick. Poultry Sci. 40: 1177-1183.

Barott, H. G., J. C. Fritz, E. M. Pringle and H. W. Titus, 1938. Heat production and gaseous metabolism of young male chickens. J. Nutr. 15: 145-167.

Barott, H. G. and E. M. Pringle, 1946. Energy and gaseous metabolism of the chicken from hatch to maturity as affected by temperature. J. Nutr. 31: 35-50.

Bauernfeind, J. C., L. C. Norris and G. F. Heuser, 1942. The pantothenic acid requirement of chicks. Poultry Sci. 21: 142-146.

Beaton, J. R., J. L. Beare, G. H. Beaton, J. M. White and E. W. McHenry, 1953. The basal metabolic rate and the effects of thiouracil administration and of thyroidectomy on control and vitamin B_6-deficient rats. J. Nutr. 51: 599-608.

Black, A., O. J. Kahlenberg, J. W. Bratzler and E. B. Forbes, 1937. The utilization of energy producing nutriment and protein as affected by deficiency of iron and copper. J. Nutr. 14: 521-532.

Black, A. and J. W. Bratzler, 1952. The effects of a vitamin B_{12} supplement, vitamin B_{12} and streptomycin on the metabolism of the rat. J. Nutr. 47: 159-173.

Blanchard, M., D. E. Green, V. Nocito and S. Ratner, 1945. Isolation of l-amino acid oxidase. J. Biol. Chem. 161: 583-597.

Blanchard, M., D. E. Green, V. Nocito-Carroll and S. Ratner, 1946. L-hydroxy acid oxidase. J. Biol. Chem. 163: 137-144.

Bogert, L. J., 1949. Nutrition and Physical Fitness, 5th Ed., W. B. Saunders, Philadelphia.

Bolin, D. W. and W. Lockhart, 1960. The use of perchloric acid as a sole oxidizing reagent for the organic matter and chromium oxide. North Dakota Acad. Sci. 14: 102-104.

Briggs, G. M., Jr., R. C. Mills, D. M. Hegsted, C. A. Elvehjem and E. B. Hart, 1942. The vitamin B_6 requirement of the chick. Poultry Sci. 21: 379-383.

Brody, S., 1945. Bioenergetics and Growth. Reinhold, New York.

Burch, H. B. and A. M. Combs, 1956. Regeneration of flavin enzymes during recovery from riboflavin deficiency. Fed. Proc. 15: 227.

Carter, C. W. and P. J. R. Phizackerely, 1951. The influence of pyridoxine on fat metabolism in the rat. Biochem. J. 49: 227-232.

Cheslock, K. E., 1958. The influence of diet upon tissue concentration of vitamin B_6. J. Nutr. 65: 53-61.

Childs, G. R., C. W. Carrick and S. M. Hauge, 1952. The niacin requirement of young chickens. Poultry Sci. 31: 551-558.

Christie, S. M. H., G. W. Kenner and A. R. Todd, 1954. A synthesis of flavin-adenine dinucleotide. J. Chem. Soc. PT. 1: 46-52.

Chubb, L. G., 1959. Application of paper chromatography to avian pathology. 1. The free amino acids in the blood, urine and body tissues of the normal fowl. Poultry Sci. 38: 668-675.

Conn, E. E. and P. K. Stumpf, 1963. Outlines of Biochemistry. John Wiley and Sons, New York.

Coon, M. J. and W. G. Robinson, 1958. Amino acid metabolism. Ann. Rev. Biochem. 27: 561-612.

Cori, C. F. and B. Illingworth, 1957. The prosthetic group of phosphorylase. Proc. Natl. Acad. Sci. 43: 547-552.

Cowgill, G. R., 1939. The Vitamins (symposium) pp. 159-178, Am. Med. Assoc., Chicago.

Dann, W. J., 1937. Nicotinic acid and vitamin B_2. Science, 86: 616-617.

Dann, W. J. and P. Handler, 1941. Synthesis of nicotinic acid by the chick embryo. J. Biol. Chem. 140: 935-936.

Dixon, M., 1960. Nomenclature of the nicotinamide nucleotide coenzymes. Science, 132: 1548-1550.

Donaldson, W. E., G. F. Combs and G. L. Romoser, 1956. Studies on energy levels in poultry rations. 1. The effect of calorie-protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35: 1100-1105.

Eddy, W. H., 1949. Vitaminology, Williams and Wilkins, Baltimore.

Feldman, L. I. and I. C. Gunsalus, 1950. The occurrence of a wide variety of transaminases in bacteria. J. Biol. Chem. 187: 821-830.

Fischer, N. A., E. M. Benson and M. E. Swendseid, 1958. The distribution of vitamin B_{12} in the developing chick egg. Arch. Biochem. Biophys. 74: 458-464.

Goldsmith, G. A., 1949. Chemical measurements in relation to physical evidence of malnutrition. Fed. Proc. 8: 553-567.

Guggenheim, K. and E. J. Diamant, 1957. Carbohydrates metabolism in pyridoxine deficient rats. J. Biol. Chem. 224: 861-869.

Gulick, A., 1924. The basal metabolism of white rats in relation to the intake of vitamin B. Am. J. Physiol. 68: 131-132.

Haas, E., B. L. Horecker and T. R. Hogness, 1940. The enzymatic reduction of cytochrome C. J. Biol. Chem. 136: 747-774.

Harrow, B. and A. Mazur, 1962. Textbook of Biochemistry. 8th Ed. W. B. Saunders, Philadelphia.

Heuser, G. F., H. S. Wilgus and L. C. Norris, 1938. The quantitative vitamin-G requirement of chicks. Poultry Sci. 17: 105-108.

Hogan, A. G., 1957. Vitamins required by the chick. Missouri Agr. Exp. Sta. Res. Bul. No. 634.

Horwitt, M. K., O. W. Hills, C. C. Harvey, E. Liebert and D. L. Steinberg, 1949. Effects of dietary depletion of riboflavin. J. Nutr. 39: 357-373.

Horwitt, M. K., 1954. The Vitamins, Vol. III, pp. 379-394, Academic Press, New York.

Hundley, J. M., 1954. The Vitamins, Vol. II, pp. 452-587, Academic Press, New York.

Jacobs, F. A., 1962. Role of vitamin B_6 in intestinal absorption of amino acids *in situ*. J. Am. Med. Assoc. 179: 523-525.

Jansen, B. C. P., 1954. The Vitamins, Vol. III, pp. 425-472, Academic Press, New York.

Jukes, T. H., 1939. Pantothenic acid and the filtrate (chick anti-dermatitis) factor. J. Am. Chem. Soc. 61: 975-976.

King, J. R., 1957. Comments on the theory of indirect calorimetry as applied to birds. Northwest Science, 31: 155-169.

Kleiber, M., H. Goss and H. R. Guilbert, 1936. Phosphorus deficiency metabolism and food utilization in beef heifers. J. Nutr. 12: 121-153.

Kleiber, M. and T. H. Jukes, 1942. Metabolism and food utilization of riboflavin-deficient chicks. Proc. Soc. Exp. Biol. Med. 49: 34-37.

Kleiber, M., 1945. Dietary deficiencies and energy metabolism. Nutr. Abstr. Rev. 15: 207-222.

Kleiber, M., 1961. The Fire of Life, John Wiley and Sons, New York.

Klosterman, H., 1960. Personal communication.

Knoebel, L. K. and A. Black, 1952. The influence of vitamin B_{12} and antibiotics on protein and energy utilization in a low protein diet. J. Nutr. 48: 477-487.

Kramer, C. Y., 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics, 12: 307-310.

Lipmann, F., N. O. Kaplan, G. D. Novelli, L. C. Tuttle and B. M. Guirard, 1947. Coenzyme for acetylation, a pantothenic acid derivative. J. Biol. Chem. 167: 869-870.

Lipmann, F., 1945. Acetylation of sulfanilamide by liver homogenates and extracts. J. Biol. Chem. 160: 173-190.

Lipschitz, M. A., V. R. Potter and C. A. Elvehjem, 1938. The mechanism of the enzymatic synthesis of cocarboxylase. J. Biol. Chem. 124: 147-150.

Lockhart, W. C., R. L. Bryant and D. W. Bolin, 1963. A shifting type metabolism pen. North Dakota Farm Res. Bul. No. 1, Vol. 23: 16-19.

Long, C. and R. A. Peters, 1939. Pyruvate oxidation in brain. 5. Evidence derived from the metabolism of alpha-keto-butyric acid. Biochem. J. 33: 759-773.

Lusk, G., 1928. The Science of Nutrition, 4th Ed., W. B. Saunders, Philadelphia.

Mann, G. V., P. L. Watson, A. McNally and J. Goddard, 1952. Primate nutrition. 2. Riboflavin deficiency in the Cebus monkey and its diagnosis. J. Nutr. 47: 225-241.

Maynard, L. A. and J. K. Loosli, 1962. Animal Nutrition, 5th Ed., McGraw-Hill, New York.

McClure, F. J., L. Voris and E. B. Forbes, 1934. The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. 2. The effects of vitamin B deficiency. J. Nutr. 8: 295-308.

McCollum, E. V. and M. Davis, 1913. The necessity of certain lipins in the diet during growth. J. Biol. Chem. 15: 167-175.

McCollum, E. V. and C. Kennedy, 1916. The dietary factors operating in the production of polyneuritis. J. Biol. Chem. 24: 491-502.

Mellen, W. J. and F. W. Hill, 1953. Effects of thiouracil, thyroprotein, and estrogen upon the basal metabolism and thyroid size of growing chickens. Poultry Sci. 32: 994-1001.

Mellen, W. J. and F. W. Hill, 1955. Studies on the avian respiratory quotient. Poultry Sci. 34: 1085-1089.

Mellen, W. J., F. W. Hill and H. H. Dukes, 1954. Studies of the energy requirements of chickens. 2. Effect of dietary energy level on the basal metabolism of growing chickens. Poultry Sci. 33: 791-798.

Mellen, W. J., 1958. Duration of effect of thyroxine and thiouracil in young chickens. Poultry Sci. 37: 672-679.

Miller, B. F., 1963. Pendulous crop in pyridoxine deficient chicks. Poultry Sci. 42: 795-796.

Mitchell, H. H. and G. G. Carman, 1926. Does the addition of sodium chloride increase the value of a corn ration for growing animals? J. Biol. Chem. 68: 165-181.

Mitchell, P. H., 1956. A Textbook of General Physiology, 5th Ed., McGraw-Hill, New York.

National Research Council, 1960. Recommended nutrient allowances for domestic animals. Number 1. Recommended nutrient allowances for poultry. Pub. 827, Nat. Acad. Sci.

Neilands, J. B. and P. K. Stumpf, 1958. Outlines of Enzyme Chemistry, 2nd Ed., John Wiley and Sons, New York.

Novelli, G. D., 1953. Metabolic functions of pantothenic acid. Physiol. Rev. 33: 525-543.

Novelli, G. D., 1957. Water-soluble vitamins. 3. Pantothenic acid, thiamine, lipoic acid, riboflavin and inositol. Ann. Rev. Biochem. 26: 243-274.

Olson, R. E. and N. O. Kaplan, 1948. The effect of pantothenic acid deficiency upon the coenzyme A content and pyruvate utilization of rat and duck tissues. J. Biol. Chem. 175: 515-529.

Peters, R. A., 1936. The biochemical lesion in vitamin B_1 deficiency. Lancet, 230: 1161-1165.

Pilgrim, F. J., A. E. Axelrod and C. A. Elvehjem, 1942. The metabolism of pyruvate by liver from pantothenic acid- and biotin-deficient rats. J. Biol. Chem. 145: 237-240.

Rabinowitz, J. C. and E. E. Snell, 1948. The vitamin B_6 group. 14. Distribution of pyridoxal, pyridoxamine and pyridoxine in some natural products. J. Biol. Chem. 176: 1157-1167.

Robertson, E. I., R. F. Miller and G. F. Heuser, 1948. The relation of energy to fiber in chick rations. Poultry Sci. 27: 736-741.

Robinson, F. A., 1951. The Vitamin B Complex, John Wiley and Sons, New York.

Romanoff, A. L., 1949. Fertility and Hatchability of Chicken and Turkey Eggs, pp. 178-208. John Wiley and Sons, New York.

Romanoff, A. L. and A. J. Romanoff, 1949. The Avian Egg, John Wiley and Sons, New York.

Rosenberg, H. R., 1945. Chemistry and Physiology of the Vitamins, Interscience Publishers, New York.

Sarett, H. P. and G. A. Goldsmith, 1950. Metabolism of l- and dl-tryptophan in normal man and in pellagrins. J. Biol. Chem. 182: 679-690.

Schrecker, A. W. and A. Kornberg, 1950. Reversible enzymatic synthesis of flavin-adenine dinucleotide. J. Biol. Chem. 182: 795-803.

Sharzynski, B., Z. Zak, W. Ostrowski, M. K. Davies, M. E. Gregory and S. K. Kon, 1959. Vitamin B_{12} in the developing chick embryo. Brit. J. Nutr. 13: 72-78.

Sibbald, I. R., S. J. Slinger and G. C. Ashton, 1961. Factors affecting the metabolizable energy content of poultry feeds. 4. The influences of calcium, phosphorus, antibiotics and pantothenic acid. Poultry Sci. 40: 945-951.

Sibbald, I. R., S. J. Slinger and W. F. Pepper, 1962. Interrelationships between riboflavin, niacin, vitamin B_{12} and methionine measured in terms of chick weight gains and dietary metabolizable energy values. Poultry Sci. 41: 380-386.

Siedler, A. J., 1963. Nutritional contributions of the meat group to an adequate diet. Borden's Rev. Nutr. Res. 24: 29-41.

Siegel, P. B. and K. W. Washburn, 1964. Adjustment for body weight in the measurement of oxygen consumption. Poultry Sci. 43: 782-783.

Singer, T. P. and E. B. Kearney, 1953. Metabolic functions of coenzyme III in animal tissues. Fed. Proc. 12: 269.

Sloan, H. J., L. E. Card and F. B. Adamstone, 1934. The effect of vitamin deficient rations on normal and deutectomized chicks. Poultry Sci. 13: 304-305.

Sloan, H. J., 1936. The operative removal of the yolks from newly-hatched chicks. Poultry Sci. 15: 23-27.

Smith, G. C. and O. Riddle, 1944. Effects of fasting on the respiratory metabolism of normal and hypophysectomized young pigeons. Am. J. Physiol., 141: 303-311.

Snedecor, G. W., 1946. Statistical Methods, 4th Ed., Iowa State College Press, Ames.

Snell, E. E. and E. Quarles, 1941. The effect of incubation on the vitamin content of eggs. J. Nutr. 22: 483-489.

Snell, E. E., 1953. Metabolic significance of B-vitamins. Physiol. Rev. 33: 509-524.

Steel, R. G. D. and J. H. Torrie, 1960. Principles and Procedures of Statistics, McGraw-Hill, New York.

Stern, K. G., 1940. Biological oxidations and reductions. Ann. Rev. Biochem. 9: 1-42.

Sunde, M. L., 1955. The niacin requirement of chickens from 6 to 11 weeks. Poultry Sci. 34: 304-311.

Sure, B., 1941. Further observations on riboflavin as a food factor in economy of food utilization. J. Nutr. 22: 295-301.

Swendseid, M. E., R. Jackson and E. M. Benson, 1958. The formation of citrovorum factor in the developing chick egg and the effect of injecting folic acid antagonists. Arch. Biochem. Biophys. 78: 221-226.

Swift, R. W., O. J. Kahlenberg, L. Voris and E. B. Forbes, 1934. The utilization of energy producing nutriment and protein as affected by individual nutrient deficiencies. 1. The effects of cystine deficiency. J. Nutr. 8: 197-219.

Titus, H. W., 1956. Energy values of feedstuffs for poultry. Proc. Nutr. Council, pp. 10-14, Am. Feed Manuf. Assoc., Chicago.

Triebold, H. O., 1946. Quantitative Analysis With Applications to Agricultural and Food Products, D. Van Nostrand, New York.

Voris, L., 1937. Effect of vitamin B deficiency on heat production of the rat. J. Nutr. 14: 199-213.

Wagner, A. F. and K. Folkers, 1964. Vitamins and Coenzymes, John Wiley and Sons, New York.

Waisman, H. A., O. Mickelsen and C. A. Elvehjem, 1939. The distribution of the chick antidermatitis factor (pantothenic acid) in meats and meat products. J. Nutr. 18: 247-256.

Warnick, K. P., M. V. Zaehringer, S. V. Bring and E. Woods, 1956. Physiological availability of thiamine from potatoes and from brown rice. J. Nutr. 59: 121-133.

Wase, A. W., 1956. The biological half-life of riboflavin-C^{14} in the liver of normal rats and rats fed 2-acetylaminofluorene. Arch. Biochem. 61: 174-178.

Weil-Malherbe, H., 1939. The enzymic phosphorylation of vitamin B_1. Biochem. J. 33: 1997-2007.

West, J. W., C. W. Carrick, S. M. Hauge and E. T. Mertz, 1952. The tryptophan requirement of young chickens as influenced by niacin. Poultry Sci. 31: 479-487.

West, E. S. and W. R. Todd, 1961. Textbook of Biochemistry, 3rd Ed., Macmillan, New York.

Williams, R. R., 1939. The Vitamins (symposium), pp. 141-157, Am. Med. Assoc., Chicago.

Woolley, D. W., H. A. Waisman and C. A. Elvehjem, 1939. Nature and partial synthesis of the chick antidermatitis factor. J. Am. Chem. Soc. 61: 977-978.

APPENDICES

APPENDIX I. ADJUSTED METABOLIZABLE CALORIC, ADJUSTED WEIGHT GAIN AND LIVER VITAMIN CONTENT DATA

Table 1.--Thiamine

Daily Vitamin Intake mcg./100 gms. of body wt.										
0	2.17	3.91	5.00	6.29	10.56	19.38	27.22	36.75	41.80	53.56
Metabolizable Calories										
T_1*			2905	2929	2980	2983	2978			
			2920	2922	2940	2961	2978			
T_2	2804	2933		2905	2910	2959	2978			
		2850		2893	2944	2933	2978			
T_3	2785	2830		2903		2974	2978	2981		
	2795	2827						2992		
T_4					2952	2952	2978	2958	2987	2963
					2918	2979	2978	2940	2987	2955
Av.	2795	2860	2912	2910	2940	2963	2978	2968	2987	2959
Body Wt. Gains (gms.)										
T_1			10.4	17.2	29.0	45.0	40.8			
T_2	5.3	5.3		19.9	26.6	39.9	36.4			
T_3	-1.0	2.7		16.8	34.0	35.4	33.6			
	0.0	4.1					43.6			
T_4					29.1	36.8	45.8	45.4	49.1	42.7
					31.4	40.9	45.4	41.3	50.9	44.5
T_5	0.0	4.1	10.9	20.0	34.0	39.0				
	2.3	4.1	14.5	14.9	34.5	25.0				
Av.	1.3	4.1	11.9	17.8	31.2	37.4	40.9	43.3	50.0	43.6
Liver Vitamin Content (mcg./gm. - wet)										
1.47	1.47	1.51	1.18	1.54	1.34	1.34	1.44	1.77	1.60	2.06

*Trial

APPENDICES

APPENDIX I. CONTINUED

Table 2.--Pyridoxine

Daily Vitamin Intake mcg./100 gms. of body wt.											
0	1.52	2.72	5.13	8.98	15.18	22.84	30.69	36.96	43.54	49.19	54.4
Metabolizable Calories											
T_1		2952	2943	2975	2976						
T_2		2935	2931	2980	2989						
T_3	2906	2947	2959	2966	2981						
T_4				2989	2987	2978	3002	2987			
T_5				3018	2973	3011	3012	3017			
T_6	2927	2941		2972	2990						
	2947										
T_7						2990	2979	2993	3002	2990	2982
						2973	2978	2972	3010	2998	2990
T_8	2959	2973		2951	2991						
	2907	2968									
Av.	2929	2952	2944	2979	2984	2988	2993	2992	3006	2994	2986
Body Wt. Gains (gms.)											
T_1		11.9	17.7	30.9	55.2						
T_2		16.6	29.4	35.9	50.1						
T_3	10.6	14.2	26.3	28.5	57.5						
T_4				39.0	47.1	63.6	58.4	64.8			
T_5				34.2	51.8	54.5	58.1	65.8			
T_6	12.9	13.4		35.7	50.3						
T_7						51.2	63.5	66.5	68.1	66.5	61.5
						67.1	52.2	64.5	66.1		69.1
T_8	9.0	21.6		35.6	50.3						
	12.2	13.0									
T_9						59.1	58.6	55.5	64.5	73.6	57.6
						64.5	59.0	68.4	62.6	54.6	59.1
T_{10}	8.4	12.4	20.9	35.1	51.1	58.6					
	6.6	10.3	21.8	31.1	54.7	46.9					
Av.	9.9	14.2	23.2	34.0	52.0	58.2	58.3	64.2	65.3	64.9	61.8
Liver Vitamin Content (mcg./gm. - wet)											
3.26	3.67	2.92	3.67	2.96	3.37	3.40	4.10	4.57	4.35	5.12	6.00

APPENDICES

APPENDIX I. CONTINUED

Table 3.--Riboflavin

	Daily Vitamin Intake mcg./100 gms. of body wt.										
	0	1.47	2.84	5.12	9.95	17.85	25.35	32.70	41.50	47.20	55.80	64.30
	Metabolizable Calories											
T_1		2782	2711	2731	2775	2732	2800					
		2749	2790	2748	2781	2779	2801					
T_2		2760	2704	2765	2761	2810	2803					
		2702	2780	2708	2739	2765	2801					
T_3							2801	2807	2818	2835	2799	2850
							2803	2833	2856	2852	2887	2853
T_4							2803	2795	2751	2794	2773	2794
							2801	2804	2809	2775	2784	2819
Av.		2748	2746	2738	2764	2771	2802	2810	2808	2814	2811	2829
	Body Wt. Gains (gms.)											
T_1		6.1	6.1	15.6	17.7	20.5	40.7					
		3.6	11.4	16.5	16.5	38.6	38.1					
T_2		3.0	7.3	21.1	32.2	30.0	34.8					
		5.5	6.1	14.5	20.1	30.7	43.9					
T_3							40.2	42.9	51.7	51.7	51.8	59.7
							38.6	46.3	51.2	64.1	52.2	49.8
T_4							32.9	49.5	38.1	40.4	54.4	42.2
							46.0	45.8	42.2	58.5	42.3	50.2
Av.		4.5	7.7	16.9	21.6	29.9	39.4	46.1	45.8	53.7	50.2	50.5
	Liver Vitamin Content (mcg./gm. - wet)											
	9.58	9.05	9.80	9.80	10.3	12.6	13.0	14.2	17.3	21.4	17.0	20.9

APPENDICES

APPENDIX I. CONTINUED

Table 4.--Pantothenic Acid

	Daily Vitamin Intake mcg./100 gms. of body wt.												
	0	4.9	9.4	18.3	26.2	34.8	41.2	57.5	72.2	84.9	99.1	112.	122. 129.
	Metabolizable Calories												
T_1	2868	2967	2952	2974	2941	2979							
	2984	2951	2923	2959	2991	2979							
T_2	2936	2918	2970	2950	2960	2979							
	2910	2930	2948	2953	2978	2979							
						2979	2948	2962	3003	2996	2993		
T_3						2979	2976	2995	2994	2995	2987		
						2979	3023	2983	3007	3004	3003		
T_4						2979			3004	3012	2992		
Av.	2924	2941	2948	2959	2967	2979	2982	2980	3002	3002	2994		
	Body Wt. Gains (gms.)												
T_1	-2.6	13.8	10.6	18.6	19.2	24.4							
	8.1	9.9	13.7	19.9	18.1	24.4							
T_2	7.0	10.3	13.8	15.0	20.0	24.4							
	4.1	4.7	9.6	15.0	13.8	24.4							
T_3						24.4	29.5	29.5	38.0	42.5	31.7		
						24.4	17.3	26.0	28.5	23.9	31.7		
T_4						24.4	22.6	29.8	28.4	39.6	34.2		
						24.4	35.5	24.4	38.6	38.1	39.6		
T_5								30.8	35.4	31.3	38.9	40.5	41.8
								28.5	38.5	35.1	36.1	35.8	42.8
Av.	4.1	9.7	11.9	17.1	17.8	24.4	26.2	28.2	32.6	35.1	35.4	38.1	42.3
	Liver Vitamin Content (mcg./gm. - wet)												
	20.5	21.2	29.9	31.5	35.3	34.5	26.3	42.9	38.4	38.4	49.1	48.4	

APPENDICES

APPENDIX I. CONTINUED

Table 5.--Niacin

	Daily Vitamin Intake mcg./100 gms. of body wt.										
	0	106.	216.	298.	389.	515.	650.	789.	894.	1086.	1289.
	Metabolizable Calories										
T_1	2760	2808	2816	2771	2831	2826					
	2815	2757	2841	2765	2828	2887					
T_2	2804	2840	2836	2861	2881	2865					
	2840	2835	2864	2842	2858	2847					
T_3						2857	2837	2856	2861	2871	2862
						2854	2836	2797	2806	2854	2866
T_4						2832	2923	2907	2912	2911	2933
						2880	2902	2912	2984	2913	2930
Av.	2805	2810	2839	2810	2850	2856	2874	2868	2891	2887	2898
	Body Wt. Gains (gms.)										
T_1	10.3	15.7	26.8	33.3	28.0	19.3					
	17.2	24.3	9.5	27.6	31.0	42.0					
T_2	16.4	24.8	27.0	30.0	31.9	35.4					
	19.4	23.5	21.1	23.4	30.5	26.1					
T_3						31.0	27.1	23.2	26.7	35.5	30.1
						30.5	24.9	23.7	31.4	31.8	30.1
T_4						31.8	41.9	29.1	30.2	27.4	25.2
						29.6	31.3	32.9	23.0	24.1	43.0
Av.	15.8	22.1	25.0	28.6	30.3	30.7	31.3	27.2	27.8	29.2	32.1
	Liver Vitamin Content (mcg./gm. - wet)										
	33.3	50.4	71.9	77.0	84.4	80.0	91.2	82.2	90.6	90.6	82.2

APPENDIX II. THE ANALYSIS OF VARIANCE OF THE EXPERIMENTAL DATA

Table 1. Metabolizable energy values.

	Source	df	SS	MS	F
Pyridoxine:	Total	45	34,273		
	Treatments	10	24,719	2471.9	8.2 **
	Residual	35	10,554	301.5	
Thiamine:	Total	41	130,158		
	Treatments	9	114,959	12773.0	27.1 **
	Residual	32	15,199	475.0	
Riboflavin:	Total	47	79.,351		
	Treatments	10	43,188	4318.8	4.4 **
	Residual	37	36,163	977.4	
Pantothenic Acid:	Total	45	40,898		
	Treatments	10	25,653	2565.3	5.9 **
	Residual	35	15,245	435.6	
Niacin:	Total	47	106,296		
	Treatments	10	46,201	4620.1	2.86 *
	Residual	37	60.095	1624.0	

* Significant (P = .05).

** Significant (P = .01).

APPENDIX II. CONTINUED

Table 2.--Vitamins and vitamin intake levels as influencing oxygen consumption

Source	d.f.	S.S.	M.S.	F.
Total	188	1430.41		
Vitamins	5	363.88	72.80	20.56**
Levels (within vitamins)	29	394.73	13.61	3.84**
Residual (error)	167	590.61	3.54	

**Significant P = .01.

Table 3.--Vitamins and time of day as influencing oxygen consumption

Source	d.f.	S.S.	M.S.	F.
Total	54	416.96		
Time	3	19.35	6.45	3.41*
Vitamins	5	223.76	44.75	23.68**
T x V	15	23.00	1.53	- -
Days (within vitamins)	8	107.309	13.41	7.09**
Residual (error)	23	43.521	1.89	

*Significant P = .05.

**Significant P = .01.