CRYSTALLINE VITAMIN B_{12} REQUIREMENT OF THE YOUNG DAIRY CALF

I. Development of a Vitamin B_{12} Deficient Synthetic Milk

II. The Crystalline Vitamin B_{12} Requirement of the Young Dairy Calf

by

CHARLES A. LASSITER

A Thesis

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy

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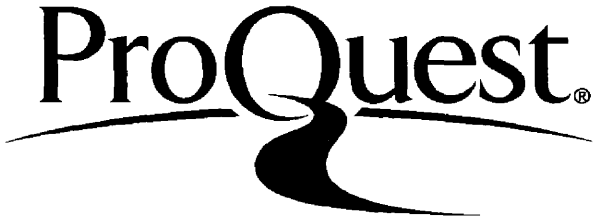

VITA

Charles Albert Lassiter was born in Murray, Kentucky February 20, 1927. He received his secondary education in the public schools of Calloway County, Kentucky. He was on active duty in the United States Naval Reserve from April, 1945 until August, 1946. He attended Murray State College and was awarded the degree of Bachelor or Science in Agriculture by the University of Kentucky in 1949. He was awarded a Graduate Teaching Assistantship in the Animal Husbandry Department of the University of Kentucky and received the degree of Master of Science in Agriculture from that institution in 1950. At that time he was awarded a Graduate Research Assistantship in the Dairy Department of Michigan State College. He held this appointment for two years while partially completing the requirements for the degree of Doctor of Philosophy. In June 1952, he accepted a position in the Dairy Section of the University of Kentucky as Assistant Professor of Dairying and Assistant Dairy Husbandman.

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AN ABSTRACT

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Crystalline Vitamin B_{12} Requirement of the Young Dairy Calf

Fourteen newborn dairy calves were used as experimental subjects to develop a synthetic milk deficient in vitamin B_{12}. The synthetic milk was composed of "alpha" protein, d,l-methionine, glucose, lactose, lard, lecithin, and salts and was supplemented with all known required vitamins with the exception of vitamin B_{12}. The components were homogenized into a concentrate containing 26.3 percent dry matter. The vitamin B_{12} deficient ration supported life but did not promote growth. However, if supplemented with either an APF concentrate or crystalline vitamin B_{12} normal or slightly sub-normal growth was promoted.

It was found that calves fed such a synthetic milk required d,l-methionine in addition to that contained in the "alpha" protein. The d,l-methionine requirement of the dairy calf was found to be more than 0.15 percent but less than 1.0 percent of the ration.

Twenty-three dairy calves were fed the vitamin B_{12} deficient ration supplemented with 0, 10, 20, 40, and 80 micrograms of crystalline vitamin B_{12} per kilogram of dry matter consumed for the 6-week period from 3 to 45 days of age in an attempt to establish the crystalline vitamin B_{12} requirement of the young dairy calf. The average daily gain

of Groups I, II, III, and IV was -0.10, 0.20, 0.20, and 0.65 pound per day, respectively. Incomplete data were obtained on Group V. It was found that the young calf required vitamin B_{12}. The principal symptoms of a vitamin B_{12} deficiency observed were growth cessation, lack of appetite, general poor condition, muscular weakness, and a white-spotted kidney condition. Calves fed a ration deficient in vitamin B_{12} tended to have greater concentrations of hemoglobin, red blood cell volume, and red blood cell counts in the blood but the lack of vitamin B_{12} did not appear to have any effect on plasma calcium, inorganic phosphorus, magnesium, or ascorbic acid.

Preliminary results indicated that the crystalline vitamin B_{12} requirement of the dairy calf was more than 20 micrograms but not more than 40 micrograms of vitamin B_{12} per kilogram of dry matter consumed.

Evidence was obtained which indicated that some batches of "alpha" protein were not satisfactory sources of protein for the young dairy calf. Results were obtained which indicated that some batches of "alpha" protein were deficient in one or more required nutrients or contained a toxic factor. Calves fed rations containing this protein consistently died within the first two weeks of life. Such protein was not found to be deficient in the amino acids methionine or lysine. The lecithin content of the synthetic

milk was not observed to be the toxic or deficiency factor encountered when feeding such rations. A rat growth study showed that the toxic or deficiency factor was definitely associated with the "alpha" protein in the synthetic ration.

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INTRODUCTION

The development of a satisfactory milk replacement for the raising of future herd replacements is a matter of utmost economic importance to dairymen. Many attempts to develop a calf ration which would equal or surpass milk in its ability to support growth of young calves have been made but few have resulted in even moderately satisfactory calf growth. Many of the more successful milk replacements have included milk by-products in relatively large proportions, thus replacing less milk than commonly believed. More complete knowledge of the nutritive requirements of the young calf might pave the way for the use of less expensive ingredients in milk replacements.

Synthetic milks have been formulated but, due to the high cost of purified compounds, they are far from being the solution for the economic problem. The synthetic milks have been used very successfully in ascertaining certain of the fundamental nutritional requirements of the young calf. These rations have usually contained casein or other milk protein. Workers in various fields have found that casein is a good source of vitamin B_{12}, thus rendering synthetic milks containing casein useless for the study of vitamin B_{12} deficiency.

This investigation was initiated in an attempt to formulate a synthetic milk, without the use of protein of animal origin, which would be nutritionally adequate for the young calf and to use the ration formulated in the determination of the vitamin B_{12} requirement of the young calf.

PART I

DEVELOPMENT OF A VITAMIN B_{12} DEFICIENT SYNTHETIC MILK

REVIEW OF LITERATURE

Adaptability of Soybeans as a Source of Protein for Animal Consumption

Osborne and Mendel (1917) were the first workers to show that raw soybeans when fed as the sole source of protein in the ration of the rat were unsatisfactory. When raw soybeans were used as the only protein source the rats grew very little and presented rather poor condition. However, these same workers were able to show that if the soybeans were heated in an electric oven at 110° Centigrade for four hours a few of the rats showed increases in growth, while the others showed no increase. These workers attributed this difference to greater consumption of the heated meal by the rats which grew on this ration. In an attempt to further increase the nutritive value of soybeans these workers cooked the soybeans for three hours and found that soybeans processed in this manner increased the growth rate of all the rats. It was further shown that soybeans were relatively poor source of both calcium and chlorine.

Robison (1930) showed that the cooking of raw soybeans improved their feeding value for pigs and also stated that the cooking process slightly increased the digestibility of the protein. Vestal and Shrewsbury (1932) confirmed the

earlier observations of Robison concerning the effect of cooking on raw soybeans for both swine and rats.

Mitchell and Smuts (1932) reported that raw soybeans were deficient in cystine for the white rat. When the soybeans were supplemented with cystine a marked increase in growth occurred. Other workers (Jackson and Block, 1932) observed that methionine, like cystine, was capable of stimulating growth in rats subsisting on a basal ration poor in cystine. These workers further suggested that cystine and methionine make up a freely interconvertible system of which only one member is necessary and that each could substitute for the other to some degree.

Shrewsbury et al. (1932) demonstrated that the addition of 2.25 to 5.0 percent crude casein to a corn-soybean ration improved the growth rate of both rats and swine. Cooked soybeans were definitely superior to raw soybeans although cooked soybeans had slightly less nutritive value than an equivalent amount of crude casein. These same workers found that the addition of 3.0 percent dried yeast to a corn-soybean ration did not improve it. Shrewsbury and Bratzler (1933) in an attempt to explain the beneficial effects of cooking soybeans showed that soybeans were deficient in cystine and, since both cooking and the addition of cystine to this ration improved its nutritive value, suggested that cooking might serve to make the cystine more available.

Hayward et al. (1936a, 1937) found that a soybean oil meal of the expeller type, one which was processed at low temperature for a long period of time, contained protein of low nutritive value and was similar in nutritive value to raw soybeans. Soybean oil meals prepared at medium or high temperature for short periods of time contained proteins which had about twice the nutritive value of raw soybeans. These workers expressed the same belief as Shrewsbury and Bratzler (1933) that heating causes some essential protein factor to become available which before was unavailable. Later these same workers (Hayward et al., 1936b) showed that either the addition of 0.3 percent cystine or the application of heat practically doubled the nutritive value of soybean protein, but the addition of cystine to heated soybeans did not further improve the feeding value of the protein for rats. These observations confirmed the earlier beliefs of Mitchell and Smuts (1932).

Johnson et al. (1939) in an attempt to confirm the earlier work of Hayward et al. (1936b) conducted sulfur and nitrogen balances on rats fed heated soybeans. It was shown that the raw soybeans contained a sulfur-nitrogen complex which was absorbed but could not be used for tissue building. It was also found that treating the soybeans with the proper heat treatment made this complex available to the animal.

Rose et al. (1936) showed in rat studies that of the sulfur-containing amino acids that methionine was the indispensable one rather than cystine. Methionine alone stimulated growth of rats fed a raw soybean ration low in cystine but when cystine was substituted for methionine the rats failed to grow or did so at a reduced rate. These workers further suggested that cystine might be able to substitute for methionine to a limited extent. Hayward and Hafner (1941) presented similar results with chicks. It was found that 0.3 percent cystine supplemented soybeans to a limited extent, but that 0.3 percent methionine was a better supplement for soybeans. A combination of both cystine and methionine produced no better results than methionine alone. These workers concluded that raw soybeans were deficient in available cystine and contained a suboptimal amount of methionine. It was further thought that the heat treatment of soybeans improved the protein as a whole as well as making cystine available.

Klose and Almquist (1941) showed that methionine was essential for the growth of the chick. Neither cystine nor homocystine alone could replace the growth-promoting effects of methionine. However, homocystine and choline together could replace methionine in the ration of the chick. These observations were made on chicks fed a purified ration with the protein arachin from peanuts as the source of protein.

Marvel *et al.* (1944) confirmed the choline-methionine relationship proposed by Klose using a basal diet of corn and soybean oil meal. Almquist *et al.* (1942) reported that even heat-treated soybean protein fed at a 20 percent level in the diet was still slightly deficient in methionine for growing chicks. As the result of many years of work on the amino acid requirements of chicks, Almquist (1947) was able to establish the quantitative requirement for sulfur-containing amino acids for the chick. It was found that the chick's requirement for sulfur-containing amino acids was 0.9 percent of the ration. The methionine requirement of the chick was found to be 0.9 percent of the ration in the absence of cystine and 0.5 percent in the presence of 0.4 percent cystine.

After 25 years of investigation workers in various fields had been able to improve the nutritive value of raw soybeans by two principal methods, treating them with heat (Osborne and Mendel, 1917) and supplementing them with methionine (Rose *et al.*, 1936). However, a growth-inhibiting substance was shown to be present in soybeans (Ham and Sandstedt, 1944). These workers isolated a substance in unheated soybeans which greatly retarded the activity of trypsin *in vitro*. The trypsin inhibitor was destroyed by autoclaving the soybean oil meal. A factor very similar to this one, if not identical with it, caused a reduction in chick growth when raw soybeans were fed. The existence of such a substance in raw soybeans

was confirmed by Bowman (1945). Almquist and Merritt (1952) showed that as little as five percent raw soybeans caused maximum anti-trypsin growth reduction in chicks. The inhibition was overcome by adding 0.1 percent trypsin to the ration or by cooking the soybeans.

Since cooking or treating raw soybeans with heat improved their nutritive value the problem arose as to the effect of overheating soybeans (Clandinin et al., 1946). These workers showed that the heating of soybean flakes in an autoclave at 15 pounds pressure for more than 3 3/4 minutes had adverse effects on the nutritive value of the meal. These effects, however, could be corrected by the addition of known vitamins and amino acids. These results showed an apparent destruction of vitamins and amino acids during such heat treatment. These same workers later (1947) showed that overheated soybean oil meal was deficient in available lysine and methionine. Slight destruction of both arginine and tryptophane was also demonstrated.

Riesen et al. (1947) showed that trypsin inhibitor was not the only factor involved when comparing the nutritive value of raw and properly cooked soybeans. It was concluded that a decrease in nutritive value was associated with overheated soybeans because there was decreased liberation of the essential amino acids. Clandinin et al. (1948) concluded after several years work on the proper heat treatment of raw

soybeans for chicks that maximum nutritive value could be obtained either by heating at 15 pounds pressure for four minutes or by heating for 45 minutes at four pounds pressure.

Byerly et al. (1937) observed seasonal variation in the hatchability of eggs from hens fed rations containing no animal protein. It was observed that eggs produced from hens fed such a ration consistently had a lower hatchability percentage during the winter months. However, eggs produced from hens exposed to sunlight had a higher hatchability percentage during these months. Bird et al. (1946) observed that the eggs from hens fed a ration which contained 30 percent soybean oil meal as the sole source of protein hatched poorly, thus confirming the earlier findings of Byerly (1937). These workers found that over a 43-week period only 66 percent of the fertile eggs produced by soybean oil meal fed hens hatched, while 84 percent of the eggs from hens fed a sardine meal ration hatched. There was a high rate of mortality even in the hatched chicks from the soybean oil meal fed hens. These results were in agreement with the earlier findings of Heuser (1946). These workers further observed that if the soybean oil meal rations were supplemented with five percent cow (or steer) manure, 10 percent sardine meal, or 10 percent dried milk that the low hatchability condition was corrected.

Bird and Marvel (1943) observed that if hens on a low riboflavin ration were fed the feces of hens fed a riboflavin supplemented ration a marked increase in the hatchability of the eggs from the low riboflavin groups occurred. Hammond (1942) observed that the growth of chicks being fed a high plant-protein ration could be increased if a small amount of cow manure were added to their rations. The cow manure was collected and dried at 45° Centigrade for 24 hours. Heuser et al. (1946) reported that the inclusion of three percent fish meal into a chick starting ration containing soybean oil meal as the sole source of protein increased growth, decreased mortality, and increased feed efficiency. This beneficial effect appeared to be additive rather than supplementary, thus indicating that it was not due chiefly to the addition of essential amino acids.

Rubin and Bird (1946a) started investigations in an attempt to purify the hatchability factor. It was observed that incubated cow manure was definitely a carrier of the factor. These workers found that the cow manure factor was not identical with the Lactobacillus casei factor (from liver, yeast, or fermentation residues), factors U, R, or S, vitamins B_{10} or B_{11}, synthetic folic acid, or pyracin lactone.

Cary et al. (1946) presented evidence for an unidentified factor (X) which was required for rat growth when rats were

fed soybean oil meal as the sole source of protein. It was found that commercial casein, Sherman Vitamin A-Free casein, SMA Labco casein, and casein prepared by centrifugation from milk contained varying amounts of the unidentified factor (X). However, if casein were subjected to 10 six-hour extractions with hot alcohol the factor was removed. It was further found that liver extract was also a good source.

Zucker et al. (1948) reported a nutritional factor necessary for rat growth which was associated with animal protein sources. These workers found that the deficiency revealed itself most strikingly after the normal lactation period and it was characterized by a marked growth reduction, high mortality, high blood urea, and a low white blood cell count. These workers were of the opinion that the factor was very similar, if not the same, as the "nutritional factor X" of Cary and the cow manure factor of Rubin and Bird. Zucker et al. (1948) proposed the name of "zoopherin" for this unidentified nutritional factor for rats.

Krider et al. (1948) showed that the addition of six B-complex vitamins (thiamine, riboflavin, pyrodoxine, niacin, pantothenic acid, and choline) to a corn-soybean oil meal ration for pigs improved survival, growth rate, red blood cell counts, and hemoglobin values. However, pigs fed this supplemented ration still gained weight at a suboptimal rate as compared to pigs fed a similar ration containing animal

protein. The addition of 1.5 percent of an AB liver extract (*Lactobacillus casei* factor) produced an increase in growth in addition to that produced by the six water-soluble vitamins. The addition of crystalline pteroylglutamic acid did not produce a growth response. Therefore the AB liver extract contained some factor essential for maximum growth of pigs other than the *Lactobacillus casei* factor.

Rickes *et al.* (1948a) and Smith (1948a) announced almost simultaneously the isolation of the active principal concerned in the treatment of Addison's pernicious anemia. Rickes *et al.* (1948a) proposed the name of vitamin B_{12} for this new substance. Ott *et al.* (1948) soon afterward showed that vitamin B_{12} possessed APF (Animal Protein Factor) activity when added to a 40 to 70 percent soybean oil meal ration for chicks raised from hens fed an all-plant ration. These workers postulated that vitamin B_{12} might be the same as the APF factor.

Lillie *et al.* (1948) showed that a soybean oil meal type ration supplemented either with vitamin B_{12} or cow manure gave essentially the same growth response in chicks. These workers assumed then that the active principal in the cow manure factor was vitamin B_{12}. Linstrom *et al.* (1949) reported that the addition of 1.0 percent of a vitamin B_{12} concentrate gave as good growth in chicks as the addition of 3.0 percent fish solubles. These workers also reported

that vitamin B_{12} produced a beneficial effect on the hatchability of eggs produced by hens being fed rations containing soybean oil meal as the sole source of protein.

Hale and Lyman (1949) reported that the addition of a vitamin B_{12} concentrate to a basal ration composed mainly of corn and soybean oil meal increased growth gains in pigs 31 percent over the unsupplemented group. The vitamin B_{12} supplement also increased efficiency of feed utilization. Neumann et al. (1949) showed that the addition of vitamin B_{12} to a soybean protein "synthetic milk" improved growth, hematopoesis, and the general well-being of the pigs. The addition of a manure factor produced similar results.

Purified Rations -- Soybean Type

Johnson et al. (1940) reported the use of a purified ration in studying the growth requirements of calves. This ration contained casein and lactalbumin as the sources of protein. When this ration was fed to young calves subnormal growth resulted. The cause of the slow growth was attributed to the calves' lack of appetite for the ration and periods of digestive upsets. Wiese et al. (1947a) and Johnson et al. (1947) developed a synthetic milk ration which apparently supported normal growth of calves until the calves were 12 weeks of age. These rations contained casein as the source of protein as did the earlier rations of Johnson and associates (1940).

Wiese et al. (1947b) produced a riboflavin deficiency and Johnson et al. (1948) produced a thiamin deficiency in the calf using a synthetic milk developed by Wiese et al. (1947a). Draper and Johnson (1952a) found the quantitative requirement of the calf for riboflavin to be 1.05 micrograms per gram of dry matter using a synthetic milk which contained casein as the source of protein. In addition to these investigations the Illinois workers (Wiese et al., 1946) have found that the calf requires biotin, (Johnson et al., 1950b) demonstrated that pyridoxine is required by the calf and (Johnson et al., 1947) showed that pantothenic acid is required by the calf. The synthetic milks used in all of these studies consisted essentially of cerelose, casein, lard, minerals, vitamins, and water.

Flipse et al. (1948) confirmed the earlier work of Wiese (1946) that the new-born calf requires biotin. These workers further showed that an interrelationship exists between biotin and potassium in the treatment of paralysis in calves due to the lack of biotin in the calf's ration. Flipse (1948) compared the relative nutritive value of corn starch, dextrin, and corn sugar as sources of carbohydrates in synthetic milks for calves. Flipse et al. (1950a, 1950b) further investigated carbohydrate sources for the calf by studying the separate nutritive values of glucose, starch, corn syrup, and lactose and the influence of lactose on the nutritive value of starch

and corn syrup in synthetic milks. These workers showed that the inclusion of a small amount of lactose in such rations was very beneficial. It was also shown that starch as the principal source of carbohydrate constituted a poor nutrient for the calf. Glucose and corn syrup yielded fair results but were not as beneficial as lactose in the ration of the new-born calf. All of these investigations by Flipse (1948) and Flipse et al. (1948, 1950a, and 1950b) employed synthetic milks of which the protein source was crude casein.

Neumann et al. (1948) developed a synthetic milk for baby pigs containing an isolated protein of the soybean, "alpha" protein, which was shown to be deficient in vitamin B_{12} for the baby pig. The addition to the ration of an antipernicious anemia liver extract produced a growth response. Johnson and Neumann (1949) later showed that vitamin B_{12} and an antipernicious anemia liver extract produced the same growth response in pigs maintained on the "alpha" protein, vitamin B_{12} deficient ration. Neumann et al. (1950) described the symptoms of vitamin B_{12} deficiency in the baby pig fed an "alpha" protein synthetic milk. These workers also established the quantitative requirement of the baby pig for vitamin B_{12} when fed as a concentrate. The solids in the synthetic milk used in these studies were composed of 29.4 percent "alpha" protein, 0.6 percent d,l-methionine, 30.9 percent glucose, 30.8 percent lard, and 8.3 percent mineral salts. These

materials were homogenized with water into a synthetic milk containing 13.0 percent solids and 4.0 percent fat. In addition the ration was supplemented with all the known fat and water-soluble vitamins except vitamin B_{12}. Nesheim and Johnson (1950) and Nesheim et al. (1950) established the quantitative crystalline vitamin B_{12} requirement of the baby pig.

Johnson et al. (1951) reported the use of an "alpha" protein synthetic milk in producing vitamin B_{12} deficiency in the calf. Apparently the composition of the ration was similar to the synthetic-vitamin B_{12} deficient ration used with pigs as reported by Neumann et al. (1948). Draper et al. (1952b) described vitamin B_{12} deficiency in the calf utilizing a ration identical to that used by Neumann et al. (1948) in producing vitamin B_{12} deficiency in the baby pig.

For a more comprehensive review of the literature on the general subject of vitamin B_{12}, the reader is referred to Part II of this manuscript.

EXPERIMENTAL PROCEDURE

Selection of Animals

The animals fed the experimental rations are recorded in Table 1. All of the 14 experimental calves were males with the exception of two females, one each fed Rations 7 and 10. A system of random allotment was used in assigning calves to the rations as the calves were born into the College experimental herd. Calves were also obtained from the main College herd and from a local farmer. These calves followed the same system of allotment to rations as the calves from the College experimental herd. The only prerequisite to assignment was normal health and appearance.

Feeding and Management

Because of necessity, calves were started on the experiment in all seasons of the year. Any possible differences due to prenatal nutrition were minimized by the allotment of calves from the various herds to all of the experimental rations whenever possible. Calves were permitted to remain with their dams for 48 hours following parturition. Each calf was then placed in an individual pen, fasted for 24 hours, weighed, and started on the synthetic milk rations. The calves were fed twice daily by nipple pail at a rate

TABLE 1

ASSIGNMENT OF ANIMALS

Ration	No. of calves	Breed distribution and herd no.
1	2	C-783 Ayrshire C-784 Holstein
2	2	C-788 Brown Swiss C-791 Holstein
3	1	C-798 Holstein
4	1	C-803 Holstein
5	1	C-809 Holstein
6	1	C-810 Holstein
7	1	C-814 Holstein (Female)
8	1	C-816 Ayrshire
9	1	C-817 Holstein
10	3	A-95 Holstein (Female) C-820 Holstein C-821 Holstein

calculated to meet the recommended nutrient allowance of the National Research Council (1945).

The constituents of each of the experimental rations fed are listed in Table 2. In addition to the components listed, each calf received (a) at the time it was placed on experiment and at weekly intervals thereafter, a capsule containing 70,000 I. U. of vitamin A (Shark liver oil) and 10,000 I. U. of vitamin D (viosterol), and (b) a daily dosage of 20 milligrams of thiamin hydrochloride, 20 milligrams of calcium pantothenate, 20 milligrams of riboflavin, 20 milligrams of pyridoxine hydrochloride, 10 milligrams of vitamin K (2 methyl napthoquinone), 0.04 milligram of biotin, 200 milligrams of inositol, and 3 grams of choline chloride. These vitamins were prepared in a stock solution, stored in amber glass under refrigeration, and administered orally to the calves once daily.

The calf pens were bedded with wood shavings. No hay or dry feed was fed.

Preparation of Feed

The synthetic milk was prepared by a modification of the procedure of Wiese et al. (1947a) with the exception of the rations which contained the 50 percent-protein soybean oil meal. Due to the limited refrigeration facilities available, the synthetic milk was prepared once or twice weekly and stored

TABLE 2

COMPONENTS OF RATIONS FED

Ingredients	Ration									
	1	2	3	4	5	6	7	8	9	10
	(Percent)									
50% Protein soybean oil meal	34.5	34.5	--	--	--	--	--	--	--	--
"Alpha" protein	--	--	24.5	24.5	24.0	19.5	24.5	24.75	24.85	24.90
Crude casein	--	--	--	--	--	5.0	--	--	--	--
d,l-Methionine	0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.25	0.15	0.10
Glucose (cerelose)	19.5	19.5	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Corn syrup	20.0	20.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Lactose	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lard	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Soya lecithin	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salts[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Furfural	0.5	0.5	--	--	--	--	--	--	--	--
APF supplement[2]	--	1.5	1.5	--	--	--	--	--	--	--
Streptomycin	--	0.1	0.1	--	--	--	--	--	--	--
Crystalline vitamin B_{12}[3]	--	--	--	B_{12}	B_{12}	B_{12}	B_{12}[4]	B_{12}	B_{12}	B_{12}

[1] Salts mixture was composed of 40 parts calcium phosphate (secondary), 8 magnesium oxide, 5 potassium phosphate (mono - H), 7 potassium phosphate (di - H), 5 sodium chloride, 5 potassium chloride, 10 potassium sulfate, 1.98 ferric citrate, 0.04 manganese sulfate, 0.04 copper sulfate, 0.04 cobalt sulfate, and 0.05 potassium iodide.

[2] APF supplement contained 3.5 milligrams of vitamin B_{12} per pound.

[3] 80 micrograms of vitamin B_{12} per kilogram of dry matter consumed.

[4] 80 micrograms of vitamin B_{12} per kilogram of dry matter consumed injected intramuscularly.

as a liquid concentrate. The frequency of preparation depended upon the number of calves on trial at any particular time. Rations 1 and 2 were prepared as follows: 81 grams of sodium bicarbonate and 27 grams of calcium hydroxide were dissolved in 39.6 pounds of water at about 60° Centigrade. A heavy duty electric stirrer was used and 6.2 pounds of soybean oil meal and 40 grams of d,l-methionine were added with constant agitation. Stirring was continued for 15 to 20 minutes to insure complete mixing. Near the end of the agitation period 1.4 pounds of lard and 164 grams of lecithin were added to the solution. The other ingredients of the ration, 3.5 pounds of glucose, 1.8 pounds of lactose, 3.6 pounds of corn syrup, and 0.8 pounds of salts, were added to the soybean oil meal-lard suspension and agitation was continued until complete mixing occurred. Since the particle size of this solution would not permit homogenization an attempt was made to reduce the particle size of the mixture by agitating it in a Waring Blendor for a period of 2 to 3 minutes.

Rations 3 to 10 were prepared as follows: 4.5 pounds of "alpha" protein were washed with tap water twice to remove an anti-vitamin of thiamin. Hot water (60°C.) was then added to bring the water content of the milk up to the required amount. The other ingredients were then added as previously

described. The entire liquid concentrate was then homogenized at 3,000 pounds pressure.

The synthetic milks thus prepared, contained 26.3 percent dry matter and were stored under refrigeration in this form. At feeding time one part of the concentrate was added to about 1 1/2 parts of hot water and the milk was fed at 85° to 95° Fahrenheit.

Criteria for Evaluation of the Nutritive Value of the Rations

Evaluation of the response of the calves to the experimental rations was based upon daily observations on the health and general appearance, growth rate, blood analyses, and post-mortem examinations.

Health and general appearance. Observations were made at least once per day with regard to the general condition, appetite, consistency of the feces, and the general reaction of the animals.

Growth rate. A record of feed consumption and refusal was maintained. Each calf was weighed between the hours of 11:00 A.M. and 1:00 P.M. on the day it was placed on experiment and on the third, seventh, tenth, fourteenth, seventeenth, twenty-first, twenty-fourth, and twenty-eighth days of the trial. Feed adjustments were made once per week according to the changes in body weight.

Blood analyses. Two test tubes of blood were collected from the jugular vein of each calf once weekly. One tube contained lithium citrate and the other contained potassium oxalate as anticoagulant. Determinations for red blood cell volume (hematocrit) by the method of Wintrobe (1942, p. 201), and hemoglobin by the method of Sanford (1933) were made on the whole blood. The blood plasma was analyzed for calcium by the method of Shohl (1922), inorganic phosphorus by the method of Fiske and SubbaRow (1925), magnesium by the method of Briggs (1922) as modified by Duncan et al. (1935), and ascorbic acid by the method of Mindlin and Butler (1938).

Post-mortem examinations. All animals which died during the trial or were killed at the end of the experimental period were subjected to gross post-mortem examinations. Histological sections were made of selected organs from representative animals and of any organ or tissue which appeared abnormal on gross inspection.

RESULTS

The growth response of the calves to the 10 experimental rations is shown in Table 3. As is shown in Table 3 the growth rate of the calves fed Ration 1 was entirely unsatisfactory. The growth rates of calves fed Rations 2, 3, 4, 6, and 9 might be considered normal for calves fed synthetic milk rations.

TABLE 3

GROWTH RESPONSE OF CALVES FED THE VARIOUS EXPERIMENTAL RATIONS

Ration	Average starting weight	Average gain over 28 day period	Average daily gain	Increase over starting weight
	lb.	lb.	lb.	%
1	93.0	-1.7	-0.06	-1.80
2	98.5	18.0	0.64	18.27
3	115.0	22.0	0.79	19.13
4	102.0	21.0	0.75	20.58
5	87.0	2.5	0.09	2.87
6	100.5	19.0	0.68	18.91
7	84.0	10.5	0.38	12.50
8	90.5	Died at 13 days of age		
9	86.0	20.0	0.71	23.26
10	92.5	7.2	0.26	7.78

The calves fed the vitamin B_{12} deficient ration, Ration 1, did not increase in weight but appeared to be normal in other respects. As is shown in Table 3 the addition of an APF supple-

ment and streptomycin to Ration 1 caused a marked increase in the growth rate of the calves fed Ration 2. The growth rate and general appearance of the calves fed Ration 3 indicated that the 50 percent protein soybean oil meal could be satisfactorily replaced by "alpha" protein as a source of protein in the ration of the dairy calf. The growth rate of the calf fed Ration 4 showed that the observed increase in the growth rate of the calf fed Ration 3 was due partly, if not entirely, to the vitamin B_{12} content of the APF supplement.

As was shown by the growth rate and general appearance of the calf fed Ration 5 the basal ration did not appear to be deficient in d,l-methionine. The basal ration did not appear to be deficient in any unidentified factor that might be supplied by crude casein as is shown by the growth data of the calf fed Ration 6. The intramuscular injection of crystalline vitamin B_{12} appeared to depress the growth rate of the calf fed Ration 7. The growth rate and general appearance of the calves fed Rations 8, 9, and 10 indicated that d,l-methionine was not depressing the growth rate of the calves during the early days of life.

The average blood analyses of the calves which were fed the different experimental rations are shown in Table 4. The blood data for all of the experimental calves appeared to be within normal limits.

TABLE 4

AVERAGE BLOOD ANALYSIS OF THE CALVES BY RATIONS

Ration	Hemo-globin	RBCV	Ca	Inorg. P	Mg	Ascorbic acid
	gm. %	%	mg. %	mg. %	mg. %	mg. %
1	11.76	27.9	10.3	6.83	2.33	0.373
2	13.64	34.5	10.2	7.41	2.47	0.404
3	12.28	33.5	9.6	7.95	2.82	0.303
4	9.97	26.8	10.0	6.89	2.12	0.205
5	11.41	29.9	10.7	6.47	2.27	0.264
6	13.58	37.5	10.4	6.94	2.13	0.162
7	11.68	31.9	10.1	6.96	2.28	0.184
8	10.99	30.5	11.9	8.83	3.27	0.347
9	12.46	34.7	10.4	6.68	2.39	0.376
10	10.92	29.8	9.9	7.29	2.31	0.215

DISCUSSION

An inspection of the growth data of the calves which were fed Ration 1 as noted in Table 3 indicates that this ration was not suitable for new-born calves. Both calves appeared to be normal and ate well throughout the experimental period. The feces of both calves tended to be slightly loose when compared to feces of calves receiving whole milk. Although the calves fed Ration 1 did not increase in body weight, they exhibited exceptional vigor, appetite, and general condition. Ration 1 was nutritionally complete as far as was known with the exception of vitamin B_{12}.

Ration 2 was the same as Ration 1 with the exception that it was supplemented with 1.5 percent of an APF supplement and 0.1 percent of crystalline streptomycin. The APF supplement contained 3.5 milligrams of vitamin B_{12} per pound of supplement. It should be noted that either the APF supplement or streptomycin produced a sharp increase in the growth rate of the calves. Both of these calves exhibited good growth and condition throughout the 28-day experimental period. These calves gained an average of 0.68 pound per day which was still below the Ragsdale standard (Ragsdale, 1934) but it probably could be considered good growth for calves being fed synthetic-milk rations.

"Alpha" protein was substituted for the 50 percent protein soybean oil meal in Rations 3 through 10. It was believed that the use of this protein would further identify the nutrients in the synthetic milk rations. Since only 50 percent of the soybean oil meal was protein, it was possible that the other portion of the soybean oil meal could be furnishing a factor or factors required by the young calf. The use of the "alpha" protein enabled the homogenization of the complete ration which improved the physical nature of the milk.

It was found that "alpha" protein contained a factor which, when not removed by washing with water, acted as an anti-vitamin of thiamin in the calf. Calf C-798 was the first calf fed the "alpha" protein synthetic milk. The protein was not washed and within three days the calf exhibited typical vitamin B_1 deficiency symptoms of general muscular weakness and head retraction. This calf was given 100 milligrams of thiamin hydrochloride subcutaneously and two grams per day orally for three days. The calf was able to stand within two hours after the thiamine was injected and appeared to be normal within two days. The "alpha" protein was washed twice with tap water throughout the remainder of the trial and there was no further evidence of the anti-vitamin activity of the protein. The sodium content of the unwashed "alpha" protein was determined with the Perkin-Elmer Flame Photometer and was

found to contain 520 milligrams of sodium per 100 grams of "alpha" protein. The excessive quantities of sodium or of a compound containing sodium might have been acting as the anti-vitamin.

The feces of calf C-798 and all of the other calves receiving rations with "alpha" protein as the source of protein were very fluid and black in color. This diarrhea did not seem to be infectious or to be injurious to the calves in any manner.

Beginning with the calf fed Ration 4 all of the vitamin B_{12} was supplied by crystalline vitamin B_{12} rather than by the APF supplement. The purpose of Ration 4 was to determine whether the increase in growth rate of calves fed Rations 2 and 3 was due to the vitamin B_{12} content of the APF supplement or the antibiotic streptomycin supplementation of these rations. The growth rate of the calf fed Ration 4 was about the same or slightly faster than the growth rate of the calf fed Ration 3 which indicated that the vitamin B_{12} content of the APF supplement rather than streptomycin was causing the increased growth rate of the calves. Calf C-803 at the end of the experimental period appeared to be normal in appetite, health, and general condition. The feces of this calf were loose and black in color as were the feces of the calf fed Ration 3.

The average daily gain of Rations 2, 3, and 4 could all be considered normal or slightly below the Ragsdale standard

(Ragsdale, 1934). However, an inspection of the weekly gains of these groups (Appendix, Table 15) will indicate that the calves gained in weight slightly for the first week on trial, remained constant for about 10 days, and then gained normally or slightly faster than normal for the last 10 to 14 days of the trial. It was believed that possibly the ration lacked some factor(s) which was stored at birth and which was also synthesized by the rumen bacteria after the rumen started functioning at about three to four weeks of age. In an attempt to correct this condition calves fed Rations 5, 6, 7, 8, 9, and 10 were subjected to various treatments.

Calves fed Ration 5 received the basal ration plus one percent d,l-methionine. As is shown in Table 3 the growth rate of the calves fed Ration 5 was very poor which might have indicated that the extra methionine was exhibiting an inhibitory effect on the growth rate of the calf. At the end of the experimental period Calf C-809 exhibited very poor condition and had very severe diarrhea. It was established with this ration that the basal ration was not deficient in d,l-methionine.

Ration 6 (Calf C-810) was the same as the basal ration with the exception that five percent of the "alpha" protein was replaced by five percent of crude casein. It was thought that this amount of crude casein would be enough to supply any unidentified factors without changing the amino acid

balance of the ration significantly. However, the growth curve of the calf fed this ration was very similar to those of calves fed rations previously discussed and it was concluded that the casein did not supply the calf with any unidentified factors not contained in "alpha" protein.

Ration 7 was devised to test the affect of adding additional vitamin B_{12} to the ration. For this reason calf C-814 received daily intramuscular injections of 80 micrograms of vitamin B_{12} per kilogram of solids consumed. Nesheim and Johnson (1950) showed that swine utilize vitamin B_{12} about 50 percent more efficiently when it was injected intramuscularly than when administered orally. As is shown in Table 3 the average daily gain of this calf was not as great as the daily gains of Rations 2, 3, 4, or 6. After about two weeks of the injections the calf became very irritable and nervous. Toward the end of the experimental period this condition improved but the animal exhibited a rough hair coat and poor general condition. Unfortunately only one calf was fed this ration and it cannot be stated with certainty that the growth rate of this calf would be typical of the reaction to this ration. However, since the extra vitamin B_{12} did not produce a growth stimulus it was assumed that the lag in growth between the seventh and eighteenth days was not due to the lack of vitamin B_{12}.

Since the supplementation of Ration 5 with 0.5 percent of extra d,l-methionine apparently produced a depression of growth it was believed that d,l-methionine might be inhibiting growth during the early stages of life. Because of this, Rations 8, 9, and 10 contained 0.25, 0.15, and 0.10 percent d,l-methionine, respectively, replacing the original 0.5 percent.

Calf C-816, Ration 8, was fed a ration containing 0.25 percent d,l-methionine. This calf grew normally for the first seven days of the experimental period, but on the eighth day became very weak, thin, and drowsy. The calf received vitamin B_1 therapy, but failed to respond and finally died on the ninth day of the experimental period. Post-mortem examination revealed a patent foramen ovale and although the ductus arteriosus was somewhat constricted it was still patent. The liver had a slight orange tinge and the contents of the gall bladder were very thick and cohesive. The death of the animal was attributed to circulatory disturbances probably not produced by the ration.

Ration 9 contained 0.15 percent d,l-methionine. Inspection of the growth data in Table 3 indicates that the calf grew at about the same rate as the calves which received 0.5 percent d,l-methionine in their rations. However, this calf was maintained on the ration six weeks instead of four weeks to follow the growth rate beyond the regular experimental period. The growth of the calf from the fourth to the sixth

week was rather poor which might have indicated that the body stores of methionine were depleted at about four weeks of age and that the ration did not contain enough methionine to meet the calf's requirement for that amino acid.

The calves fed Ration 10 were supplied with an even smaller amount of d,l-methionine, 0.10 percent of the ration, for the first two weeks of the experimental period. At the end of the two week period it became evident that the ration did not contain sufficient methionine because the calves lost an average of approximately 10 pounds in body weight during this period. After two weeks on this ration the d,l-methionine content of the ration was increased from 0.10 percent to 0.5 percent of the ration. The calves began to increase in body weight and to improve in health and general condition. During the next four weeks the calves gained about 0.75 pounds per day.

Based upon the results of Rations 9 and 10 it was evident that the calf's requirement for d,l-methionine under the conditions of this experiment was more than 0.15 percent of the ration and an allowance of 0.5 percent of the ration was used in additional research reported in Part II of this manuscript.

The lag in the growth curve during the first ten days of life of calves fed the "alpha" protein-synthetic ration was still unexplained. However, beginning with Ration 5 the calves were weighed on the first, second, fourth, seventh,

tenth, and fourteenth days of the experimental period. An examination of the growth data for calves fed these rations (Appendix--Table 15) indicates that the calves consistently weighed four to five pounds more on the second day than on the first day of experiment. These calves made very little gain for the first 14 days of the experimental period, but thereafter gained normally when the ration was nutritionally adequate. Most of the increase in weight was probably due to increased fill rather than growth since the calves were fasted 24 hours before being weighed and placed on the experimental rations. Assuming this to be true it appeared that the calves increased in weight very little, if any, during the first 14 days on trial but gained normally thereafter.

An inspection of the blood data in Table 4 indicates no significant differences among the various experimental rations.

Since Ration 1 did not promote growth, but normal growth was promoted when this ration was supplemented with either an APF supplement or crystalline vitamin B_{12} in the presence of adequate d,l-methionine, it was considered to be vitamin B_{12} deficient. Therefore, it was postulated that this ration would be suitable to establish the crystalline vitamin B_{12} requirements of the young dairy calf.

SUMMARY

Fourteen new-born dairy calves were allotted to ten experimental rations and fed various synthetic milks utilizing a 50 percent protein soybean oil meal or "alpha" protein as the source of protein in these rations. The calves were placed on these experimental rations at 3 days of age and retained for a 28-day feeding trial. These rations supported life when supplemented with d,l-methionine, but did not promote growth. The supplementation of these rations with either an APF supplement containing vitamin B_{12} or crystalline vitamin B_{12} promoted sub-normal to normal growth of the calves.

The predominant symptom of vitamin B_{12} deficiency in the dairy calf appeared to be the lack of growth. The lack of vitamin B_{12} had no effect on hemoglobin content or red blood cell volume of whole blood or the calcium, inorganic phosphorus, magnesium or ascorbic acid contents of the blood plasma.

The d,l-methionine requirement of the dairy calf under the conditions of this experiment appeared to be more than 0.15 percent, but less than 1.0 percent of the ration. An allowance of 0.5 percent of the ration appeared to be sufficient to promote normal growth and general health of the dairy calf when the ration was supplemented with 80 micrograms of vitamin B_{12} per kilogram of solids consumed.

Since crystalline vitamin B_{12} supplementation promoted good growth of calves fed the vitamin B_{12} deficient rations it was postulated that these rations could be used in establishing the crystalline vitamin B_{12} requirements of the young dairy calf.

PART II

THE CRYSTALLINE VITAMIN B_{12} REQUIREMENT OF THE YOUNG DAIRY CALF

REVIEW OF LITERATURE

Isolation and Characterization of Vitamin B_{12}

Whipple and Robscheit-Robbins (1925) reported the development of an experimental anemia in dogs which was curable by the feeding of beef liver. This work inspired Minot and Murphy (1926) to test such a diet on the treatment of patients suffering from Addison's pernicious anemia, a disease described by Addison (1849) more than 70 years previously. These workers treated 45 patients with a special diet high in protein, iron, and liver. All of the patients showed a decrease in severity of the pernicious anemia symptoms. These same workers later (1927) showed that liver itself was the active principal in the diets. Mammalian liver (200 grams per day) was given in 105 cases of pernicious anemia for varying lengths of time with beneficial effects. An increase in red blood cell count resulted in every case. Chon et al. (1928) showed that the active pernicious anemia principal in liver could be extracted with water, preferably at a faintly acid pH and that purification from proteins and other substances could be brought about by adding ethanol up to a concentration of 70 percent. This procedure resulted in "Chon's fraction G" which was active in pernicious anemia patients when injected. These early findings led to the inevitable conclusion that an active anti-pernicious anemia factor existed in liver.

Since 1926 numerous chemists, including Dakin *et al.* (1936) and SubbaRow and Jacobson (1936), have attempted to isolate and identify the active principal in liver responsible for the cure of pernicious anemia. Castle and Townsend (1929) set forth their well-known theory of the "intrinsic" and "extrinsic" factors concerned in pernicious anemia. These workers proposed that these factors, one present in normal gastric secretions and the other in certain foods, of which liver was an outstanding example, interacted to form a substance which was essential for the development of mature erythrocytes. Castle *et al.* (1944) showed that crude casein was a carrier of the "extrinsic" factor. Extraction of casein with hot alcohol removed the factor. The alcohol extracted casein plus all the members of the vitamin B-complex did not reconstitute the "extrinsic" factor found in the crude casein. These workers at this time proposed that the "extrinsic" factor was a thermostable component of the vitamin B-complex as yet unidentified.

Shorb (1947) reported that *Lactobacillus lactis* Dorner (ATCC 8000) failed to grow in an amino acid medium containing all the synthetic B-complex vitamins, or when supplied with either clarified tomato juice or certain liver extracts. But growth did occur when tomato juice plus the liver extracts were added to the media. The liver factor (LLD) was found in high concentrations in refined liver extracts but

low in such products as Wilson Liver fraction L, brewer's yeast, tomato juice, and yeast. It was also reported that crude casein was inactive in this regard. Shorb suggested that the LLD factor might be related to the animal growth factor.

In the meantime investigators in the animal nutrition field were accumulating evidence for the existence of a factor or factors closely associated with animal proteins which was needed for reproduction and growth of rats, pigs, and chickens. Byerly et al. (1937) reported that there was a seasonal variation in the hatchability of eggs produced by hens fed an all-plant ration. The hatchability dropped to a rather low value in the winter months and increased as the warmer seasons approached. Bird and Marvel (1943) observed that if hens on a low riboflavin ration were fed the feces of a riboflavin supplemented group the hatchability of the eggs produced by the low riboflavin group increased. Hammond (1942) reported that dried cow manure added to an all-plant riboflavin-deficient ration increased the growth of chicks. Heuser et al. (1946) showed that the inclusion of three percent fish meal into an all-plant ration increased the growth response to soybean oil meal. Bird and associates (1946) observed that the hatchability of eggs produced by soybean oil meal fed hens was lower than eggs produced by hens fed sardine meal. This condition was corrected by the

inclusion of five percent cow (or steer) manure, 10 percent sardine meal or 10 percent dried milk in the ration. These workers found kidney damage, uratic deposits in the ureters, and distended gall bladders on post-mortem examination.

Rubin and Bird (1946a) stated in their first report of a chick growth factor in cow manure that the factor was not identical with any of the previously reported growth factors such as the folic acid complex. It was shown that folic acid prevented anemia in the chicks but did not promote good growth, whereas the cow manure factor did promote good growth. These workers also showed that solubilized liver was a good source of the factor but butyl fermentation solubles were not. Rubin and Bird (1946b) showed that the cow manure factor was stable to heat in the dry state at 100° Centigrade for one hour, would not dialyze through cellophane, was moderately soluble in water and ethyl alcohol, but was insoluble in ether and chloroform. It was shown further by these same workers (1947) that the factor was transmitted from the hen to the chick through the egg. The factor was present in the egg yolk and was acetone insoluble. Rubin et al. (1947) showed that the cow manure factor improved hatchability as well as growth of chicks produced from hens fed an all-plant ration. These workers expressed the belief that the same dietary factor in cow manure affected both hatchability and growth. It was thought that fish meal contained the same

factor as did cow manure. Bird et al. (1948) further chemically characterized the chick growth factor in cow manure. It was found that it was soluble in water at pH 3.0 if the protein was previously removed, soluble in 80 percent acetone and was extractable by ammoniacal ethanol. The factor was stable when autoclaved for two hours in neutral solutions but was destroyed by autoclaving for one hour with 2N acid. There was some evidence of destruction by standing in slightly alkaline solutions.

McGinnis et al. (1947) showed that an unidentified chick growth factor was produced in the feces of hens after the feces were incubated for 72 hours at 30° Centigrade. However, if the feces were frozen immediately after excretion there was little or none of the factor present. These results indicated that the production of this factor in the feces of hens occurred after the feces were voided by the hen and not to any extent in the digestive tract. McGinnis and Carver (1947) showed that chicks hatched from hens fed a ration low in the growth factor and the chicks themselves fed the ration grew poorly and showed excessively high mortality. When this ration was supplemented with fish meal, growth was promoted and the mortality was prevented. Evidence was also presented which showed that the factor was transmitted from the hen to the egg, thus confirming the earlier work of Rubin and Bird (1947). The feeding of degydrated

alfalfa did not permit the storage of the unidentified factor or factors in the egg.

Cary et al. (1946) reported the existence of an unidentified factor (X) necessary for rat growth when the rats were fed a ration containing soybean oil meal as the sole source of protein. Crude and Labco casein and liver contained varying amounts of the factor. However, the factor could be removed from casein by extraction with hot alcohol. Hartman and Cary (1946) reported that milk, cheese, beef and pork muscle, and egg yolks contained Cary's unidentified factor (X). It was also found that the potency of egg yolks seemed to vary with the ration of the hen. Hartman et al. (1949b) reported that the unidentified factor (X) for rats might be synthesized by the microorganisms of the digestive tract of the rat. These workers found that the addition of a single dose of rat feces to rats being fed a ration deficient in the unidentified nutritional factor (X) promoted growth, thus indicating intestinal synthesis.

Zucker et al. (1948) reported that rats required a nutritional factor which was associated with animal proteins. These workers called the factor zoopherin and stated that it appeared to be very similar to the nutritional factor (X) of Cary and the cow manure factor of Rubin and Bird. Later Zucker and Zucker (1948a) found that alfalfa leaf meal, dried grass, and young fresh grass did not contain animal protein

factor activity. These same workers (1948b) also reported that zoopherin was widely distributed among various lower animal forms.

Krider et al. (1948) found that the addition of six crystalline B vitamins to a basal ration of corn and soybean oil meal for pigs increased survival, growth rate, red blood cell counts, and hemoglobin values. However, the addition of 1.5 percent of an AB liver extract (Lactobacillus casei factor) increased growth even more. These workers attributed the increase in growth to some factor other than the Lactobacillus casei factor in the liver extract because crystalline folic acid did not produce the growth response.

Schaefer et al. (1948b) reported that fox require an unidentified factor essential for growth and hemoglobin production. These workers (1948a) also reported that mink required an unidentified factor for normal nutrition. Fresh raw liver and whole milk corrected the deficiency in both fox and mink.

On April 16, 1948 Rickes et al. (1948a) announced the isolation in minute amounts of a biologically active, pure, crystalline material clinically active in the treatment of pernicious anemia from a clinically active liver concentrate. This material which crystallized in the form of small red needles was tentatively named vitamin B_{12}. On April 24, 1948, just eight days after the announcement by Rickes et al. (1948a),

Smith (1948a) reported the purification of two amorphous forms of the anti-pernicious anemia factor from liver. While this material was not as pure as the crystalline material of Rickes et al. (1948a) it exhibited many of the same physical and chemical properties. Smith used four tons of ox liver to isolate about one gram of the active pernicious anemia principle. Smith et al. (1948) later stated that Smith's (1948a) original product was the same as the one described by Rickes et al. (1948a) and also suggested the name, vitamin B_{12}.

Shorb (1948) working in conjunction with Rickes et al. showed that vitamin B_{12} was either wholly or partly responsible for the LLD growth activity observed for liver extracts.

Rickes et al. (1948b) reported that vitamin B_{12} contained cobalt and that the cobalt-complex nature of vitamin B_{12} was an outstanding property of the new vitamin. This finding was confirmed by Smith (1948b) and it was shown that about four percent of the vitamin molecule was cobalt. Smith also stated that if each molecule of vitamin B_{12} contained one atom of cobalt that the minimum molecular weight of vitamin B_{12} would be about 1600. Smith found the molecular weight to be 1550-1750 by X-ray crystallography and about 3,000 by diffusion methods. It was also found that the molecule contained three atoms of phosphorus. Rickes et al. (1948c) reported that vitamin B_{12} was obtainable from a new source,

Streptomyces griseus fermentation. Like vitamin B_{12} from liver it contained cobalt, phosphorus, had comparable activity for growth of Lactobacillus lactis, and had APF activity for chicks. Brink et al. (1949) found that each molecule of vitamin B_{12} contained one atom of cobalt, one atom of phosphorus, and was levorotatory. The molecule was not a peptide since hydrolysis of the vitamin did not liberate alpha amino acids. Alkali fusion of vitamin B_{12} indicated the presence of certain cyclic five membered nitrogen-containing compounds including pyrrols.

Other forms of vitamin B_{12} have been isolated. Kaczka et al. (1949) demonstrated the existence of vitamin B_{12a} and Licktman et al. (1949), the existence of vitamin B_{12b}. However, Kaczka et al. (1951) demonstrated that vitamin B_{12a} and vitamin B_{12b} were identical and contained a hydroxyl group in place of a cyanide radical in the vitamin B_{12} molecule. These workers suggested the name of cyanocobalamin for vitamin B_{12} and hydroxocobalamin for vitamins B_{12a} and B_{12b}. Buchanan et al. (1950) isolated vitamin B_{12c} from culture broths of Streptomyces grieseus. These workers showed that vitamin B_{12a}, B_{12b}, and B_{12c} were equally as active as vitamin B_{12} when measured by animal growth response. Smith (1951) found that vitamin B_{12c} differed from vitamin B_{12} only in having a nitrite group in place of the cyano group. Smith also showed that if the nitrite group was removed from vitamin B_{12c} that vitamin B_{12d} was formed. Lewis

et al. (1952) showed the presence of another active form of vitamin B_{12} in rat feces. The compound had growth-promoting properties for Lactobacillus leichmannii and the chick but not for the rat. When inorganic cobalt was fed to rats an increased production of the factor occurred in the intestinal tract of the rat. The new active form of vitamin B_{12} and vitamin B_{12} appeared separate on a paper chromatogram and had a different absorption spectrum.

Tove et al. (1950) reported that mink required a methanol soluble factor for growth. Fish solubles were a good source of the factor but dried distillers solubles or dried whey were not. These workers found that vitamin B_{12} and the methanol soluble factor had many of the same properties, but the methanol soluble factor seemed to contain another factor or factors in addition to vitamin B_{12}.

Groschke et al. (1950b) found that incubated pig manure contained large quantities of vitamin B_{12} whereas fresh pig manure did not. Miller and Groschke (1950) showed that incubated horse manure was another potent source of vitamin B_{12}.

Bickoff et al. (1950) showed the existence of vitamin B_{12} like growth factors for Lactobacillus leichmannii in alfalfa. However, when the vitamin B_{12} was destroyed by alkali digestion the results showed that 85 percent or more of the original activity was due to factors other than

vitamin B_{12}. These factors did not replace vitamin B_{12} for chick growth.

Wright et al. (1948) showed that thymidine was able to replace vitamin B_{12} for Lactobacillus lactis (ATCC 8000). Thymine under the same conditions was inactive. These workers proposed that vitamin B_{12} acts in a co-enzyme system in carrying out reactions concerned with the conversion of thymine to thymidine because in the presence of thymidine vitamin B_{12} was no longer required by Lactobacillus lactis. These workers expressed the belief also that one of the troubles in pernicious anemia might be the inability to synthesize certain nucleosides. Folic acid was found to increase thymine, thus explaining the value of folic acid in some cases of pernicious anemia. The effects of large amounts of thymine in pernicious anemia might be explained on the same basis.

Kitany et al. (1949) showed that the desoxyribosides of a number of purines and pyrimidines were found to be active in replacing the vitamin B_{12} requirement for Lactobacillus leichmannii. Folkers (1950) demonstrated that 5,6-dimethylbenzimidazole was a degradation product of vitamin B_{12}. Riboflavin has a structure similar to this compound.

Trenner et al. (1950) reported that vitamin B_{12} and ascorbic acid were incompatable in the same solution. It was found that when these two vitamins were in solution together that there was some destruction of vitamin B_{12} activity, par-

ticularly vitamin B_{12a}. A competitive antagonist of vitamin B_{12} was reported by Beiler et al. (1951). The antagonist of vitamin B_{12} was produced by treating the vitamin with strong acid with hydrogen peroxide. Indications were that the cyanide-cobalt complex was attacked.

Vitamin B_{12} -- Clinical Aspects

Addisonnian pernicious anemia was first described by Addison (1849). It was found that it was a type of anemia characterized by a reduced number of red blood cells of abnormally high hemoglobin content. The red blood cells themselves were large, immature forms. Degeneration of the spinal cord nerve trunks was frequently associated with the disease. Little advancement was made in the treatment of pernicious anemia until Minot and Murphy (1926) reported that large amounts of liver relieved the symptoms. From this time until the isolation of vitamin B_{12} in 1948 it was mostly a problem of concentration of the active principal in liver as has already been described.

Since folic acid was known to be important in red blood cell formation and function some workers thought that the anti-pernicious anemia factor and folic acid might be the same. However, Wilkinson (1946) stated that they were not the same and Spies and Stone (1947) showed that synthetic folic acid neither prevented or relieved subacute combined

degeneration of the cord in pernicious anemia, whereas certain types of liver extracts did both.

West (1948) showed that the crystalline vitamin B_{12} of Rickes (1948a) produced positive hematological activity in three cases of pernicious anemia. West obtained increases in reticulocyte counts, red blood cell counts, and hemoglobin. Ungley (1948) reported similar results utilizing large doses of liver or one of Smith's (1948a) highly purified extracts. Berk et al. (1948a, 1948b) reported that vitamin B_{12} when injected was effective in relieving both the nervous symptoms and the hematological symptoms of pernicious anemia. There were no allergic reactions as had been found when liver extracts were used, thus showing that vitamin B_{12} was not the cause of the allergic reactions. One patient had acquired the nervous symptoms while being treated with folic acid. Vitamin B_{12} therapy quickly relieved this condition thus showing again the ineffectiveness of folic acid in curing the nervous phase of the disease. Berk et al. (1948b) showed that vitamin B_{12} was actually the "extrinsic" factor concerned with pernicious anemia as had been proposed by Castle (1929) earlier. These workers showed that 5 micrograms of vitamin B_{12} given orally had little if any effect on reticulocytosis. However, when the same amount of vitamin B_{12} was given with 125 to 150 milliliters of normal gastric juice the reticulocyte rise was then present. These results indicated that gastric juice (intrinsic factor) was necessary

for the optional utilization of the relatively small amount of vitamin B_{12}. These workers further suggested that the function of the intrinsic factor of normal gastric juice was to facilitate the absorption by the intestine of vitamin B_{12}, rather than to react with the extrinsic factor as hitherto had been assumed.

Spies et al. (1948a) reported that vitamin B_{12} was effective in treating tropical sprue. Spies et al. (1948b) and Bethell (1950) showed that vitamin B_{12} was effective in treating nutritional macrocytic anemia and non-tropical sprue as well as pernicious anemia. Spies et al. (1948c), Hall and Campbell (1948) and Jones et al. (1949) confirmed the findings of Berk (1948b) that vitamin B_{12} was effective in relieving the symptoms of spinal cord degeneration in pernicious anemia. Patel (1948) showed that two cases of tropical macrocytic anemia were cured by treatment with Smith's (1948a) anti-pernicious anemia factor. Erf and Wimmer (1949) reported that the factor from liver and the one from Streptomyces griseus fermentation were closely related if not identical for the treatment of pernicious anemia.

Goldsmith (1949) and Schieve and Rundles (1949) reported that folic acid and vitamin B_{12} both produced a positive reticulocytosis in pernicious anemia patients but that vitamin B_{12} produced a more complete recovery. Spies et al. (1949) reported that the animal protein factor was effective in

treating the macrocytic anemia of pernicious anemia, nutritional macrocytic anemia, tropical sprue, and nutritional glossitis.

Wolf et al. (1950) showed that there was no evidence for any chemical reaction when vitamin B_{12} was incubated with normal gastric juice for 22 hours although such a mixture was effective when fed to patients with pernicious anemia. These results supported the hypothesis presented by Berk (1948b) that the intrinsic factor exerted its effect in promoting the absorption of small amounts of vitamin B_{12}. However, Bird and Hoevet (1951) presented evidence to indicate that the intrinsic factor actually bound vitamin B_{12} compounds. Meyer et al. (1950) reported that the oral administration of an extract of swine duodenal mucosa and a suboptimal amount of vitamin B_{12} produced some reticulocyte response but that it was suboptimal.

Due to the great inconvenience and social laws prohibiting the employment of restricted diets for human beings very little information is available on the growth-promoting effects of vitamin B_{12} when fed to human beings. However, Wetzel et al. (1949) reported the effects of vitamin B_{12} therapy on 11 children, three of whom exhibited growth failure although they were already receiving a well-balanced diet. The children were given 10 micrograms of vitamin B_{12} per day in addition to the well-balanced diet. Five of the 11 children

responded dramatically to a single dose of vitamin B_{12}. The most dramatic general effects of vitamin B_{12} were shown by a boy with severe allergic bronchitis, whose sleep had been interrupted regularly for 12 months by asthmatic attacks and whose desire and ability to eat had been greatly diminished by wheezing. The growth response in this case was accompanied by a remarkable alleviation of the asthmatic symptoms, which completely vanished during the first week of vitamin B_{12} administration. After the vitamin B_{12} administration physical vigor and alertness increased, better general behavior and a greatly improved appetite existed.

Dedichen and Laland (1949) showed that the pure anti-anemia factor, vitamin B_{12}, had no effect upon the leucocyte count in a normal person thus showing that vitamin B_{12} was concerned principally with the red blood cells. Vijayaraghavan and Dunn (1951, 1952) showed that vitamin B_{12}, B_{12a}, and B_{12b} were equally effective in increasing red blood cell counts in mice made anemic by phenylhydrazine. However, thymidine, a degradation product of vitamin B_{12}, exhibited little or no anti-anemia activity.

The question of the metabolism of orally administered vitamin B_{12} has been of particular interest. Chow (1951) found that giving vitamin B_{12} orally over a period of several months or in a single massive dose did not result in the appearance of vitamin B_{12} activity in the urine, thus indicat-

ing very poor absorption. However, intravenous administration resulted in vitamin B_{12} activity in the urine. Jutton and Parsons (1951) confirmed these findings. Lang et al. (1952) showed that when vitamin B_{12} was injected that nearly all of the vitamin was excreted within eight hours.

The effectiveness of very minute amounts of vitamin B_{12} in the treatment of pernicious anemia has been of considerable interest. Dameshek (1949) commenting on the extraordinary potency of vitamin B_{12} expressed himself as follows: "In these crowded days when one therapeutic miracle succeeds another in rapid succession, the appearance of a new substance with almost incredible therapeutic effects inspires but little excitement..... The isolation of vitamin B_{12} in the research laboratories of Merck and Company in this country and almost simultaneously in the Glaxo Laboratories in England is the most recent case in point. Here is a substance that, when given to a patient suffering from pernicious anemia, results in a maximal reticulocyte response following a single injection of 5 to 10 thousandths of a milligram (0.000005 Gm.)! Has there ever been in the history of medicine a more potent material, microgram for microgram?"

The Role of Vitamin B_{12} in Animal Nutrition

Hartman et al. (1949a, 1951) listed crude casein, dried skim milk, and some kinds of cheese as particularly good sources of vitamin B_{12} for the rat, whereas, cereal grains,

oil meals, and yeast were very poor sources of the vitamin. Rats maintained on a vitamin B_{12} deficient ration exhibited poor growth and lactation. Under some conditions it appeared that intestinal synthesis of the vitamin occurred, particularly on a riboflavin deficient ration. Stern et al. (1949) found that rats fed a ration poor in vitamin B_{12} grew poorly and showed little or no liver basophilia, whereas, those which received vitamin B_{12} or liver grew well and showed considerable cytoplasmic basophilia in their liver cells. Emerson et al. (1949) demonstrated that rats were able to store vitamin B_{12} during the suckling period because such rats grew normally for about two months after weaning on a vitamin B_{12} deficient ration but thereafter grew rather poorly. Dryden et al. (1951) reported that if rats were fed a vitamin B_{12} supplemented ration the litter size was not affected but the average body weight of the young was increased. Meyer et al. (1951) reported that the addition of as little as 0.1 microgram of vitamin B_{12} per day per rat to the rations of rats being fed a pork ration helped to overcome a conception lag previously noted when feeding such rations. Dryden et al. (1952) reported that there was a high rate of mortality of rats within the first few days after birth if the mothers were fed a ration consisting of alcohol-extracted casein as the source of protein. Vitamin B_{12} supplementation tended to prevent the occurrence of the

phenomenon. There was no difference in feed consumption noted between the deficient mothers and supplemented mothers during gestation.

Ruegamer et al. (1948) found that neither niacin nor folic acid therapy had any effect on a macrocytic anemia existing in dogs fed a niacin deficient ration. However, liver extracts were effective in restoring normal blood picture and general health of the animals. Best results were obtained when the extracts were administered in combination with folic acid.

Ott et al. (1948) and Nichol et al. (1949a, 1949b) showed that crystalline vitamin B_{12} had animal protein activity when fed to chicks receiving a basal ration of 40 to 70 percent soybean oil meal. Lillie et al. (1948) showed that the factor in cow manure and vitamin B_{12} produced essentially the same growth response in chicks. Vitamin B_{12} was effective when injected indicating that its effect upon the chick was direct and was not mediated through the intestinal flora. Lindstrom et al. (1949) reported that crystalline vitamin B_{12} had a very beneficial effect on the hatchability of eggs produced by hens fed a corn-soybean oil meal ration. Richardson et al. (1949, 1950) showed that the addition of vitamin B_{12} to a ration utilizing cottonseed meal as the sole source of protein had a beneficial effect on the growth of chicks. Lillie et al. (1949b) showed that if the eggs produced by

hens fed a vitamin B_{12} deficient ration were injected with the vitamin that the chicks grew faster, exhibited lower mortality, and feathering was better. Briggs et al. (1949), Carver and McGinnis (1950), Sherwood and Couch (1950), Couch et al. (1950c), Groschke and Evans (1950a), Olcese et al. (1950a), Petersen et al. (1950a, 1950b), Reed and Couch (1950a), Machlin et al. (1950), and Peeler et al. (1951) all showed that the addition of vitamin B_{12} to an all-plant ration increased the growth rate of chicks and increased the hatchability of eggs produced by hens receiving such rations. Lillie et al. (1949a) and Singsen and Matterson (1950) showed that turkeys required vitamin B_{12} for growth. Stokstad et al. (1950) demonstrated that vitamin B_{12b} had the same biological value as vitamin B_{12} for chicks. Menge et al. (1951) were able to show that the removal of the yolk sac of day old chicks prevented the transmission of vitamin B_{12} or a vitamin B_{12} active substance from the dams to the chicks. Halbrook et al. (1950a, 1950b) showed that built-up poultry house litter contained vitamin B_{12} and APF activity as measured by chick growth. Later these same workers (1950c) found that one year old built-up poultry house litter contained 261 millimicrograms of vitamin B_{12} per gram, whereas new litter contained only 1 millimicrogram of vitamin B_{12} per gram of litter. Nichol et al. (1949b) reported that no change in the hemoglobin level accompanied the growth response

to injected vitamin B_{12} when chicks were fed a corn-soybean oil meal diet. Mushett and Ott (1949) found that chicks fed a 70 percent soybean oil meal ration showed fewer and less severe cases of gizzard erosion when supplemented with vitamin B_{12}. The vitamin B_{12} deficient chicks exhibited larger hearts and livers but smaller spleens on post-mortem examination. Olcese et al. (1950b) reported that the reduction in the hatchability of eggs produced by hens fed a vitamin B_{12} deficient ration was due to embryonic mortality which was usually greatest about the 17th day of incubation. Gassner et al. (1950) found that cockerels fed a 70 percent sesame meal ration exhibited marked reduction in the development of the combs and testes. It was found that the addition of vitamin B_{12} to the basal ration prevented this condition.

Johnson and Neumann (1948) reported that an antipernicious anemia liver extract stimulated growth in baby pigs being fed an "alpha" protein-type synthetic ration. This growth response was not as pronounced when the extract was added to a casein-type synthetic ration. These workers suggested that the factor present in the liver extract was either identical or very closely related the animal protein factor. The same workers (1949) showed that the antipernicious anemia liver extract growth factor (reticulogen) and vitamin B_{12} were the same. Johnson and Nesheim (1949) produced both folic acid and vitamin B_{12} deficiencies using a

soybean synthetic milk in pigs. Vitamin B_{12} and folic acid either alone or together produced increases in growth and reticulocytosis. They concluded that the baby pig required both of the vitamins but that the two deficiencies might be overlapping. Hale and Lyman (1949) supplemented a basal ration of corn and soybean oil meal with a vitamin B_{12} concentrate (Merck and Company) for pigs and obtained a 31 percent increase in growth over that with the basal ration. A greater efficiency of feed utilization was also reported. Anderson and Hogan (1949) reported similar results when a similar ration was fed to weanling pigs. Hogan and Anderson (1949) removed six baby pigs from their mother at two days of age and placed them on a vitamin-free casein synthetic ration. Three of the pigs received vitamin B_{12} injections for 30 days. In the following four-week period the three supplemented pigs gained 26.8 pounds and the three unsupplemented pigs gained 15.9 pounds. During the next six-week period one pig in the control group died, another did not gain consistently, and the other began to lose weight. The injection of the third pig with 15 micrograms of vitamin B_{12} caused it to gain at a moderate rate. Luecke et al. (1949a, 1949b) reported that the addition of a vitamin B_{12} concentrate to a basal ration of corn and soybean oil meal increased the growth rate of weanling pigs 53 percent in one trial and 38 percent in a second trial. However, it was

pointed out by the authors that the test material used was a concentrate and it would be difficult to attribute all of the increase in growth rate to its vitamin B_{12} content. On the contrary Heidebrecht et al. (1949) were unable to obtain a significant increase in growth of pigs fed an all-plant ration when supplemented with vitamin B_{12}. Colby and Ensminger (1950a) and Powick et al. (1951) reported similar results when baby pigs were fed a casein-type synthetic ration.

Cartwright and Wintrobe (1949) produced a nutritional macrocytic anemia in swine which was partially curable by vitamin B_{12} therapy but fully curable by folic acid administration.

Neumann et al. (1949) described the symptoms of vitamin B_{12} deficiency in the pig. These workers found that baby pigs fed a vitamin B_{12} deficient ration were irritable, sensitive to touch, sluggish, and exhibited weakness in the rear quarters. Johnson et al. (1950a), Anderson and Hogan (1950a, 1950b), Cunha et al. (1950a, 1950b), Cunha (1950), Dyer et al. (1950), Luecke et al. (1950), Shefchik et al. (1950), Miller et al. (1951), Squibb and Solazar (1951), and Sure (1951) all reported that the addition of either crystalline vitamin B_{12}, a vitamin B_{12} concentrate, or an APF supplement to basal rations of corn and soybean oil meal increased growth and had beneficial effects in swine. Dyer et al.

(1951) and Barrick et al. (1950) reported that the nutritive value of cottonseed meal for swine could be improved by supplementing it with vitamin B_{12}.

Much of the experimental work in ruminants with vitamin B_{12} has been designed to relate the vitamin with cobalt metabolism. The essentiality of cobalt in ruminants was established by Neal and Ahmann (1937a, 1937b). With the discovery of vitamin B_{12} Hale et al. (1949, 1950) proposed the theory that cobalt deficiency in sheep might be due to the lack of vitamin B_{12} synthesis in the rumen. The rumen contents of cobalt deficient and cobalt supplemented sheep were assayed by the chick growth method for vitamin B_{12}. The rumen contents of the cobalt supplemented groups consistently contained more vitamin B_{12}. The addition of vitamin B_{12} to the rumen contents of the unsupplemented groups improved chick growth. Injected vitamin B_{12} had no effect on cobalt deficiency but oral administration of vitamin B_{12} partially corrected the deficiency. Becker et al. (1949) were not able to confirm the results that vitamin B_{12} when fed to sheep suffering from a cobalt deficiency cured the deficiency. These workers found no evidence that vitamin B_{12} was an important intermediate in cobalt metabolism in lambs. Becker and Smith (1951) and Smith et al. (1951) later showed that the injection of an antipernicious anemia liver extract cured cobalt deficiency

in sheep and that vitamin B_{12} was an important intermediate in cobalt metabolism. Abelson and Darby (1949) showed that radioactive cobalt might be incorporated into the vitamin B_{12} molecule. It was further found that rumen microorganisms synthesized vitamin B_{12} and that the feces of ruminants were a good source of the vitamin. Colby et al. (1950b) found that the addition of an APF concentrate to the ration of young lambs produced no effect on growth rate during the suckling period. Koch and Smith (1951) showed that vitamin B_{12b} was as effective as vitamin B_{12} in curing cobalt deficiency in sheep. Harper et al. (1951) found that the addition of cobalt to the ration of ewes produced a significant increase in the vitamin B_{12} content of the ewe's milk. These workers postulated that this was the direct result of increased vitamin B_{12} synthesis by the rumen microorganisms.

Williams and Knodt (1949) and Wallace et al. (1949) advocated the inclusion of some source of animal protein, particularly dried skim milk, in milk substitutes for young dairy calves. Reece (1950) reported the first experimental results concerning the importance of vitamin B_{12} in the ration of the calf. Although Reece (1950) was principally interested in the influence of thyroprotein feeding on weight gains of dairy calves some evidence was presented which indicated that the vitamin might be of importance in the nutrition of the young calf. Rusoff and Haq (1950, 1951)

found that neither the addition of an APF supplement (Merck's No. 3 APF) to the ration of calves or the injection of calves with vitamin B_{12} had any beneficial effects. Williams and Knodt (1950, 1951) and Bloom and Knodt (1951) published similar results when an APF supplement was added to a milk replacement for calves. Loosli and Wallace (1950) found that the addition of an APF supplement (Lederle's) to the ration of dairy calves increased the growth rate about 20 percent. However, it was suggested that the increase in growth might have been due to the antibiotic, aureomycin, content of the APF supplement rather than its vitamin B_{12} content since crystalline aureomycin produced about the same results. Hibbs and Pounden (1950) fed an APF supplement to dairy calves which had been fed milk for seven to nine weeks and found no significant beneficial effect attributable to the supplement. On the contrary, Johnson *et al.* (1951) employing an "alpha" protein synthetic milk found that calves did require vitamin B_{12}. Draper *et al.* (1952b) described a study of vitamin B_{12} deficiency in the calf. These workers found that the lack of vitamin B_{12} caused cessation of growth, poor appetite, and in a few cases the calves exhibited incoordination. An examination of the peripheral nerves of these calves revealed varying degrees of demyelination and the distribution of bone marrow cells indicated a low proportion of the myeloid series. In some of the vitamin B_{12} deficient

calves growth was resumed following liver extract or crystalline vitamin B_{12} therapy. Other animals failed to respond to such therapy. These workers expressed the belief that the ration was possibly deficient in factors other than vitamin B_{12} which were required for normal growth and development of the calf.

Carlson et al. (1949) expressed the belief that fish meal, some APF supplements, and some batches of dried brewer's yeast contained a factor(s) in addition to vitamin B_{12} which increased the growth rate of chickens. Stokstad and Jukes (1950) found that a fermentation product of Streptomyces aureofaciens promoted the growth of chicks being fed a ration adequate in vitamin B_{12}. Crystalline aureomycin produced similar results. Hill and Branion (1950), Reed and Couch (1950b), Norris et al. (1950), and Stokstad and Jukes (1951) all confirmed the observations of Carlson et al. (1949) that some APF supplements contained a factor(s) in addition to vitamin B_{12} which stimulated the growth rate of chicks. Cunha et al. (1949a) and Burnside et al. (1950) published evidence that some APF supplements contained a factor(s) in addition to vitamin B_{12} which stimulated the growth rate of pigs.

Metabolism and Mode of Action of Vitamin B_{12}

du Vigneaud *et al.* (1939), while studying the utilization of "labile methyl groups" by rats, observed that choline enabled the rat to utilize homocystine. However, it was noted that an occasional rat would grow on the homocystine ration without choline. These workers suggested that the explanation of this phenomenon was refection. Several years later after the discovery of vitamin B_{12} Schaefer *et al.* (1949b) observed that vitamin B_{12} supplementation markedly reduced the choline requirement of the chick. Gillis and Norris (1949a) showed that vitamin B_{12} reduced the chick's requirement for all methylating compounds and suggested that one of the prime functions of vitamin B_{12} was concerned with the methylating process of the body. These workers (1949b) reported similar results for an APF supplement but did not establish to which fraction of the APF supplement it was due. Schaefer *et al.* (1949a, 1950) and McCormick and Drill (1950) reported that vitamin B_{12} greatly reduced the incidence and severity of renal hemorrhage in weanling rats fed rations deficient in choline. Cunha *et al.* (1949b) showed that vitamin B_{12} replaced the methionine needs of the pig. Patrick (1950) observed similar results in chicks. Dinning *et al.* (1950) showed that methionine in the absence of vitamin B_{12} or betaine in the presence of vitamin B_{12} could function in the production of leucocytes in the rat thus

suggesting that the vitamin may be essential for the utilization of the methyl groups of betaine. Jukes et al. (1950) showed that vitamin B_{12} might enhance the conversion of homocystine to methionine in the chick. Stekol and Weiss (1950) observed this same phenomenon in rats. Bennett et al. (1951) showed that rats fed a ration free of "labile methyl groups" but containing homocystine, folic acid, and vitamin B_{12} grew well. If the folic acid were removed, growth still occurred but not at a maximal rate, thus suggesting that vitamin B_{12} was the principal factor. These workers further proposed that folic acid and vitamin B_{12} were responsible for the conversion of homocystine to methionine, possibly functioning in enzyme systems. Schaefer and Knowles (1951) proposed a similar theory for the function of vitamin B_{12} in the methylating processes of the body. du Vigneaud and associates (1950) while working with rats in a "germ free" environment showed that the synthesis of "labile methyl groups" probably occurred in the animal tissue rather than by action of the intestinal bacteria.

Burns and McKibbin (1951) found that the dog's requirement for methylating compounds was dependent upon the vitamin B_{12} content of the ration as had been reported in other species of animals. Dinning et al. (1951), Dubnoff (1951), Gillis and Norris (1951), Hale and Schaefer (1951), Jukes and Stokstad (1951a, 1951b), Schaefer et al. (1951), Strength

et al. (1951), Liener and Schultze (1952), and Travers and Cerecedo (1952) presented evidence of the function of vitamin B_{12} in the methylating processes of the body. However, Lucas et al. (1951) presented incomplete results indicating that vitamin B_{12} had no effect on the liver lipids in experiments with rats. Stekol et al. (1952) using radioactive compounds concluded that vitamin B_{12} or its physiological derivative was involved in the synthesis of serine from glycine.

Hartman et al. (1949c) reported that vitamin B_{12} deficiency might even be fatal to rats fed a ration exceptionally high in protein. Based on this finding these workers postulated that vitamin B_{12} plays a role in the ability of the normal mammal to utilize large amounts of protein. Charkey et al. (1950) found that the non-protein nitrogen level in the blood of vitamin B_{12} deficient chicks was higher than that of vitamin B_{12} supplemented chicks. Thomas and Cheng (1950) observed that vitamin B_{12} supplemented rats grew as fast, if not faster, than rats receiving rations much higher in protein. Abbot (1951) found that vitamin B_{12} increased the utilization of nitrogen as well as phosphorus in rabbits. Rose and Schweigert (1952) observed that the amounts of desoxyribonucleic acids and ribonucleic acids in the livers of rats fed vitamin B_{12} deficient rations were lower than those for the supplemented rats. Alexander and Backlar (1951) reported that in a vitamin B_{12} deficiency that the rate of nucleic acid synthesis in the liver was reduced.

Ling and Chow (1951) found that vitamin B_{12} deficient rats had a much smaller amount of body fat, higher water content, and a lower blood sulfhydryl content. These results suggested that vitamin B_{12} plays a role in carbohydrate, fat, and protein metabolism. These same workers later (1952) found that vitamin B_{12} deficient rats had a lower carbohydrate reserve and utilized injected glucose very poorly. Cuthbertson and Thornton (1951) found that large amounts of lactose produced a growth retardation in rats. However, vitamin B_{12} administration could counteract this reduction in growth.

Emerson (1949) reported that vitamin B_{12} counteracted the growth retarding effect of thyroid powder when fed in conjunction with a ration void of animal protein. Betheil and Lardy (1949) obtained similar results. Lewis *et al.* (1950) found that vitamin B_{12} would not counteract the effects of hyperthyroidism when rats were fed basically a sucrose-casein ration but the vitamin was effective when the rats were fed a corn-soybean oil meal ration. These workers suggested that other required factors might be supplied by intestinal synthesis. Meites (1950a, 1950b, 1950c) and Libby and Meites (1952) showed that vitamin B_{12} supplementation reduced the growth retarding effects of thiouracil in rats and chicks. Watts *et al.* (1951) obtained similar results with rats. However, Greer (1951) was unable to confirm the findings of Meites (1950a) and Watts (1951). Ershoff (1949)

reported the existence of another antithyrotoxic factor for the rat in addition to vitamin B_{12}. Menge and Combs (1950) and Machlin et al. (1951) found that vitamin B_{12} decreased the severity of glycine toxicity in chicks. Hardin and Hove (1951) demonstrated that the severity of d,l-methionine toxicity was more pronounced in the absence of vitamin B_{12}.

Scott et al. (1949) obtained results which indicated that vitamin B_{12} was required in addition to p-pyracin for the enzymatic release of folic acid from pteroylhepaglutamate. Dietrich et al. (1951) observed that vitamin B_{12} given orally to chicks increased the liver storage of both folic acid and the citrovorum factor. Yacowitz et al. (1950, 1951) presented evidence that vitamin B_{12} had a sparing effect on the pantothenic acid requirement of the chick. Evans et al. (1951) found that chicks on a vitamin B_{12} deficient ration had higher pantothenic acid contents of the livers than chicks maintained on a vitamin B_{12} supplemented ration. These workers concluded that vitamin B_{12} aided in the transfer of pantothenic acid from the liver for its use elsewhere in the chick's body.

Register et al. (1949) found that beef muscle contained about twice as much vitamin B_{12} as did pork muscle. This difference was attributed to the synthesis of the vitamin by microorganisms in the rumen of cattle. Lewis et al. (1949) found that the kidney was the chief storage organ of vitamin

B_{12} in the rat. Couch et al. (1950b) confirmed these findings in the chick and Richardson et al. (1951) obtained similar results in rats. Couch and Olcese (1950a), Scheid et al. (1950) and Schweigert et al. (1951) showed that the vitamin B_{12} content of the liver was increased as the level of vitamin B_{12} was increased in the ration. Chow et al. (1951) found that when vitamin B_{12} was given orally there was very little, if any, excreted in the urine unless extremely large amounts were given. On the other hand, when the vitamin was injected subcutaneously nearly quantitative excretion occurred. These results suggested very poor absorption of the vitamin when given orally. Barbee and Johnson (1951), Register and Sarett (1951), Yamamoto et al. (1951), Chow et al. (1950), and Lang et al. (1951) obtained very similar results. Davis and Chow (1951) reported that the vitamin B_{12} activity of rat feces could be increased by feeding aureomycin. This finding was explained on the basis of the effect of the antibiotic on the intestinal flora of the rat. Since the chemical structure of vitamin B_{12} is related to the chemical structure of riboflavin and other compounds, Cooperman et al. (1952) were interested in the growth promoting effects of these compounds when fed to rats and chicks being fed a vitamin B_{12} deficient ration. These workers showed that 1-alpha-d-ribofuranosido-5,6-dimethylbenzimidazole and riboflavin possessed some growth promoting ability but they

did not increase the liver and kidney content of vitamin B_{12}.

Collins et al. (1951) found that cow's milk contained 6.6 micrograms of vitamin B_{12} per liter and colostrum was observed to contain more than normal milk. Anthony et al. (1951a, 1951b) found the vitamin B_{12} content of mature cow's milk to be about 7.0 micrograms per liter. These same workers found that calves at birth had a normal blood level of vitamin B_{12}. However, Jersey calves had lower blood levels than either Holstein or Guernsey calves. Jersey, Holstein, and Guernsey blood levels of vitamin B_{12} were found to be 0.72, 1.05, and 1.03 micrograms per liter, respectively.

Vitamin B_{12} Requirement of Various Species

The vitamin B_{12} requirement of the various species as reported in the literature is shown in Table 5.

Stokstad et al. (1949) found that the oral administration of vitamin B_{12} was about 50 percent as effective as the injected vitamin. Oleson et al. (1950c) found that if aureomycin were fed in addition to vitamin B_{12} that the requirement for the vitamin was between 2.1 and 4.2 micrograms of vitamin B_{12} per kilogram of ration for growing chicks. Catron et al. (1950) reported that the feeding of aureomycin lowered the requirement of growing-fattening swine for vitamin B_{12} but they did not establish a new requirement. The studies conducted

TABLE 5

VITAMIN B_{12} REQUIREMENT OF VARIOUS SPECIES

Species	Method of admin.	Requirement	Purpose	Reference
Chick	Oral	30 ug./kg. diet	Growth	Ott et al. (1948)
"	"	15 ug./kg. diet	"	Stokstad et al. (1949)
"	Injected	0.3 ug./week	"	Stokstad et al. (1950)
"	Oral	11 ug./kg. diet	"	Briggs et al. (1950)
"	"	8 ug./kg. diet	"	Milligan & Combs (1950)
"	"	4 ug./kg. diet	Hatchability	Milligan & Combs (1950)
Pig	"	10 ug./kg. diet	Growth	Catron & Culbertson (1949)
"	"	10 ug./kg. diet	"	Lepley et al. (1949)
"	"	50 ug./kg. diet	"	Neumann et al. (1950)
"	"	20 ug./kg. diet	"	Nesheim & Johnson (1950)
"	Injected	0.6 ug./kg. B.W.	"	Nesheim & Johnson (1950) & Nesheim et al. (1950)
"	Oral	15 ug./kg. diet	"	Anderson & Hogan (1950c)
"	"	11 ug./kg. diet	"	Richardson & Blaylock (1950) & Richardson et al. (1951)
"	"	11 ug./kg. diet	"	Vohs et al. (1951)

by Neumann *et al.* (1950), Nesheim and Johnson (1950), and Nesheim *et al.* (1950) were made utilizing an "alpha" protein synthetic milk. The author was able to find no reference to the vitamin B_{12} requirement of the calf.

EXPERIMENTAL PROCEDURE

Selection of Animals

The composition of the experimental groups is shown in Table 6. All of the 23 experimental calves were Holstein males with the exception of one Holstein female in Group II and one Brown Swiss male in Group I. The selection of the experimental animals and their allotment to the five experimental groups was accomplished by the same procedure as was followed in Part I. Calves were placed on experiment three days after birth and retained for a 42 day feeding trial. All calves that died during the experimental period and other selected animals were subjected to post-mortem examination.

TABLE 6

COMPOSITION OF EXPERIMENTAL GROUPS

Group	No. of animals	Breed distribution	Average starting weight
			lbs.
I	3	2 Holstein 1 Brown Swiss	98.0
II	3	3 Holstein	98.0
III	3	3 Holstein	99.0
IV	4	4 Holstein	94.0
V	10	10 Holstein	100.5

Feeding and Management

The experimental animals were fed and managed essentially the same as described in Part I. The calves were permitted to remain with their dams for 48 hours following parturition, but were not fasted prior to being placed on the experimental rations. The experimental trial period was not started until the calves were three days of age. The calves received two feedings of the synthetic milk to insure the obtaining of the true weight of a calf before starting the experimental period.

All the calves were given three intramuscular injections of 500,000 units of procaine penicillin in oil during the first week of the experimental period. These injections were given on the second, fourth, and sixth days after birth. The incidence of pneumonia in the dairy experimental barn had been quite severe just prior to the start of this trial and because of this all calves were treated with penicillin in an attempt to protect the calves during the first few days of life.

The formula for the basal ration is noted in Table 7. The five experimental rations are identical with the exception of the amount of crystalline vitamin B_{12} which was varied. Groups I, II, III, IV, and V received 0, 10, 20, 40, and 80 micrograms of crystalline vitamin B_{12}, respectively,

TABLE 7

BASAL RATION

	%
"Alpha" protein[1]	29.5
d,l-Methionine	0.5
Glucose (Cerelose)	25.0
Corn syrup	20.0
Lactose	10.0
Soya lecithin	2.0
Lard	8.0
Salts[2]	5.0

[1] "Alpha" protein was washed twice with tap water to remove the anti-vitamin of thiamine.
[2] Salt mixture was the same as fed in Part I.

per kilogram of dry matter consumed. The vitamin B_{12} was added to the water-soluble vitamins and administered orally from a test tube once each day following the morning feeding. In addition to the fat soluble vitamins fed in Part I, 150 milligrams of mixed tocopherols were given to each calf each week. The calves received the same vitamins of the B-complex and in the same amounts as outlined in Part I with the addition of 20 milligrams of thiamine hydrochloride and 416 micrograms of crystalline folic acid per day.

Preparation of Feed

The synthetic milk was prepared as outlined in Part I for the "alpha" protein rations. The synthetic milk contained 26.3 percent dry matter and was fed as previously stated.

Criteria for Establishing the Crystalline Vitamin B_{12} Requirement of the Dairy Calf

Evaluation of the vitamin B_{12} requirement of the dairy calf was based upon daily observations on the health and general appearance, growth rate and efficiency of feed utilization, blood analyses, and post-mortem examinations.

Health and general appearance. Observations were made at least once per day with regard to the appetite, general condition, consistency of the feces, and the general reaction of the animals.

Growth rate and efficiency of feed utilization. An accurate record of the feed consumption and refusal was maintained. Each calf was weighed between the hours of 11:00 A.M. and 1:00 P.M. on the day it was placed on experiment and on the first, third, seventh, tenth, seventeenth, twenty-first, twenty-fourth, twenty-eighth, thirty-first, thirty-fifth, thirty-eighth, and forty-second days of the experimental period. Feed adjustments were made once per week according to the changes in body weight.

Blood analyses. The blood was analyzed for the same constituents as outlined in Part I with the exception that red blood cell counts by the method of Wintrobe (1942, p. 211) were made once weekly.

Post-mortem examinations. All animals which died during the experimental period or were sacrificed at the end of the

trial were subjected to gross post-mortem examination. Histological sections were made of selected organs from representative animals and of any organ or tissue which appeared abnormal upon gross inspection.

Comparative Nutritive Value of Two Batches of "Alpha" Protein as Determined by Rat Growth

The nutritive value of two batches of "alpha" protein was determined by a rat growth study. The purpose of this study is outlined in other parts of this manuscript.

The composition of the two experimental groups is shown in Table 8 and the formulae for the rations used are presented in Table 9. The rats were maintained on the experimental rations for six weeks. These animals were housed two per cage and weighed three times weekly.

TABLE 8

COMPOSITION OF EXPERIMENTAL RAT GROUPS

Group	No. of rats	Average starting weight
		grams
A	4	69.0
B	4	69.0

TABLE 9

FORMULAE OF RAT RATIONS

Ingredients	Ration A	Ration B
	%	%
"Alpha" protein[1]	29.7	---
"Alpha" protein[2]	---	29.7
d,l-Methionine	0.3	0.3
Glucose (Cerelose)	45.0	45.0
Lactose	10.0	10.0
Lard	5.0	5.0
Salts[3]	5.0	5.0
Cod liver oil	5.0	5.0
Vitamin mixture[4]		

1 "Alpha" protein from batch used in Ration A was from the same batch as was fed in the first experiment (Part I) and as fed to Groups I, II, III, and IV of this experiment.

2 "Alpha" protein from batch that was fed to Group V (Part II).

3 Salts as listed in Table 2 of Part I.

4 Vitamin mixture composed of 2.0 grams of choline chloride, 56 mg. of calcium pantothenate, 20 mg. of nicotinic acid, 10 mg. of riboflavin, 8 mg. thiamine hydrochloride, 4 mg. pyridoxine hydrochloride, 0.8 mg. vitamin K (2-methyl-napthoquinone), and 141 ug. of crystalline vitamin B_{12} per kilogram of ration.

RESULTS

The chemical analyses of the ingredients used in the experimental rations are presented in Table 10 and that of the basal ration (liquid concentrate) in Table 11.

TABLE 10

CHEMICAL ANALYSES OF INGREDIENTS OF RATION

Ingredients	Water	Ash	Crude fiber	Ether extract	Protein	N-free extract
	%	%	%	%	%	%
"Alpha" protein	9.05	1.57	0.00	0.17	88.50	0.71
Lactose	1.53	0.12	0.00	0.58	0.66	97.11
Cerelose	9.10	0.05	0.00	1.35	0.31	89.19
Corn syrup	26.44	0.81	0.00	2.15	0.18	70.42

TABLE 11

CHEMICAL ANALYSIS OF BASAL RATION

Constituent	Percent
Moisture	73.69
Protein	8.13
Crude fiber	0.00
Ether extract	2.75
Ash	1.67
Nitrogen-free extract	13.76
Ca	0.247
P	0.220
Mg	0.736
K	0.238
Mn (Mg/kg.)	3.44
Co (Mg/kg.)	2.45
Fe (Mg/kg.)	86.30
Cu (Mg/kg.)	6.15

As is shown in Table 6 the starting weights of Groups I, II, III, and V were nearly the same. The average starting weight of the calves in Group IV was slightly less than that of the other four groups.

The average growth rate and efficiency of feed utilization of the five experimental groups are shown in Table 12. The average daily gains of Groups I, II, III, and IV were -0.10, 0.20, 0.20, and 0.65 pound per day, respectively. Incomplete data were obtained on Group V due either to a deficiency or toxic factor existing in the "alpha" protein which was fed to this group. This was the only group fed this batch of protein because the calves in this group were started on experiment after those in the other four groups had been completed.

TABLE 12

GROWTH AND FEED UTILIZATION

Group	Average starting weight	Average daily gain	Increase over starting weight	Gain/D.M. consumed
	lb.	lb.	%	lb./lb.
I	98.0	-0.10	-9.80	---
II	98.0	0.20	8.48	0.124
III	99.0	0.20	8.40	0.132
IV	94.0	0.65	28.84	0.399
V	100.0	Incomplete data		

The growth curves of calves in Groups I, II, III, and IV are presented in Figures I, II, III, and IV, respectively. The average growth curves of all groups are shown in Figure V by 10-day periods.

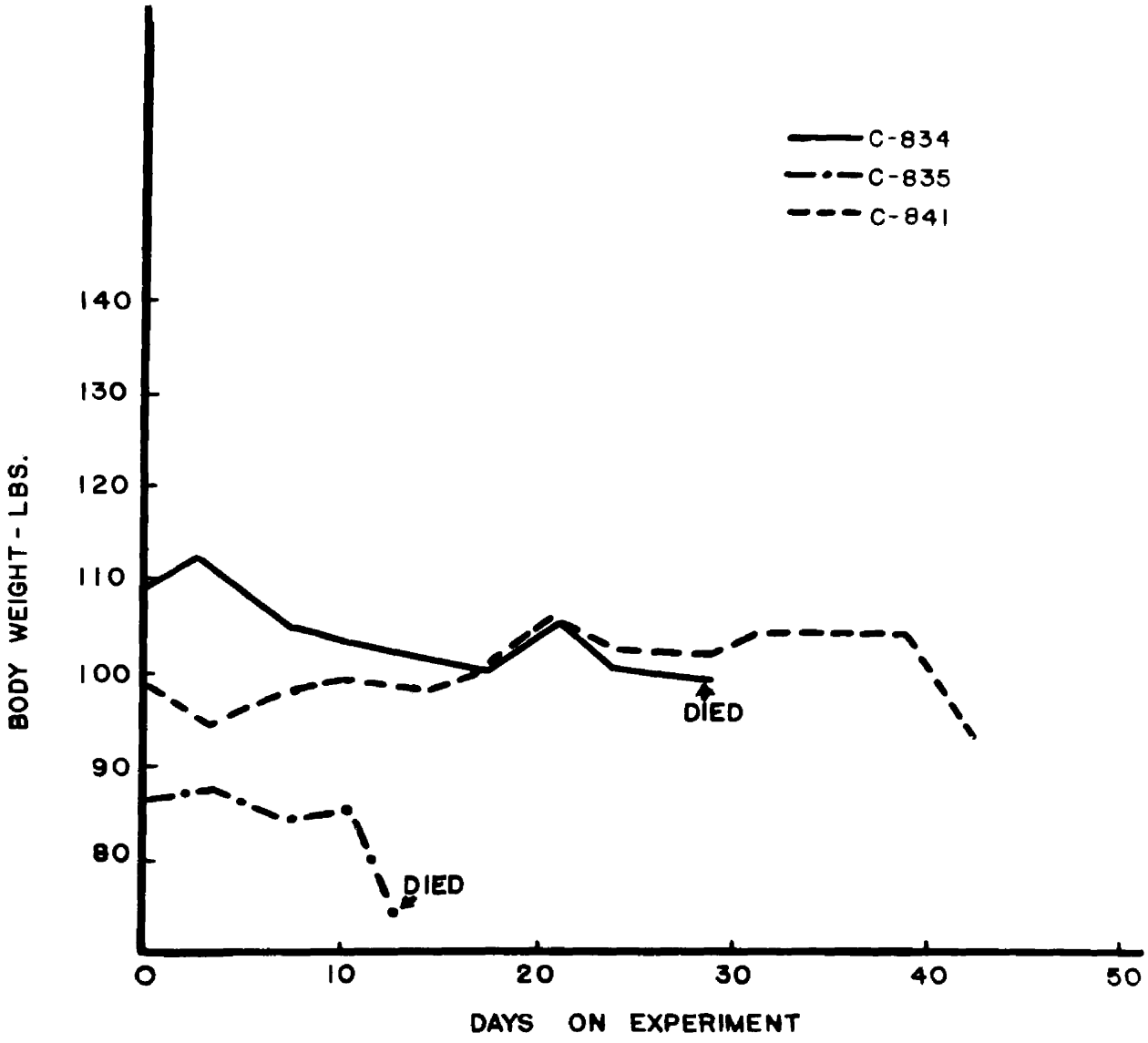

FIG. I. GROWTH CURVES -- GROUP I

NO VITAMIN B_{12} / KG. D. M.

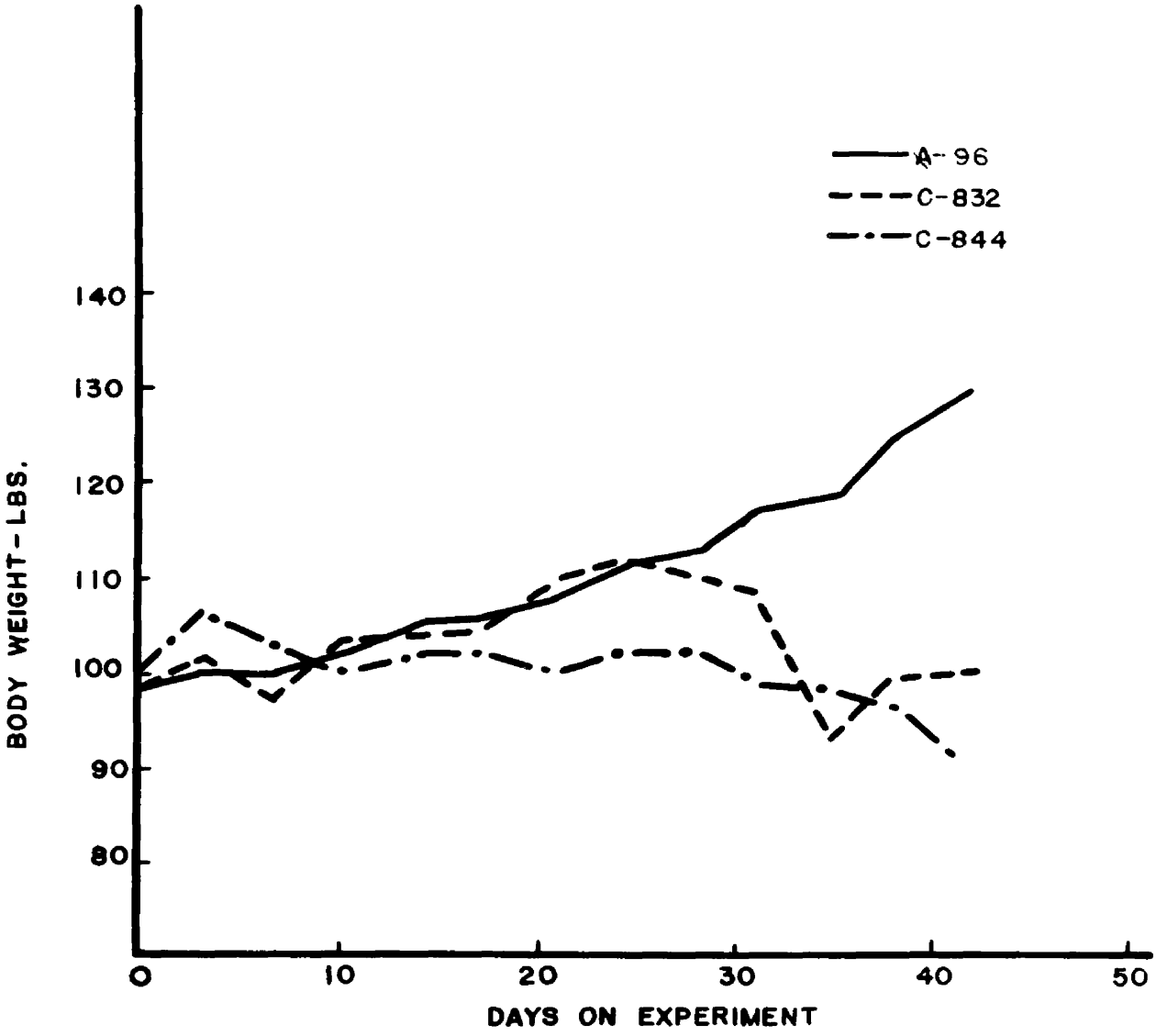

FIG. II. GROWTH CURVES -- GROUP 2

10 μG. VITAMIN B_{12} / KG. D. M.

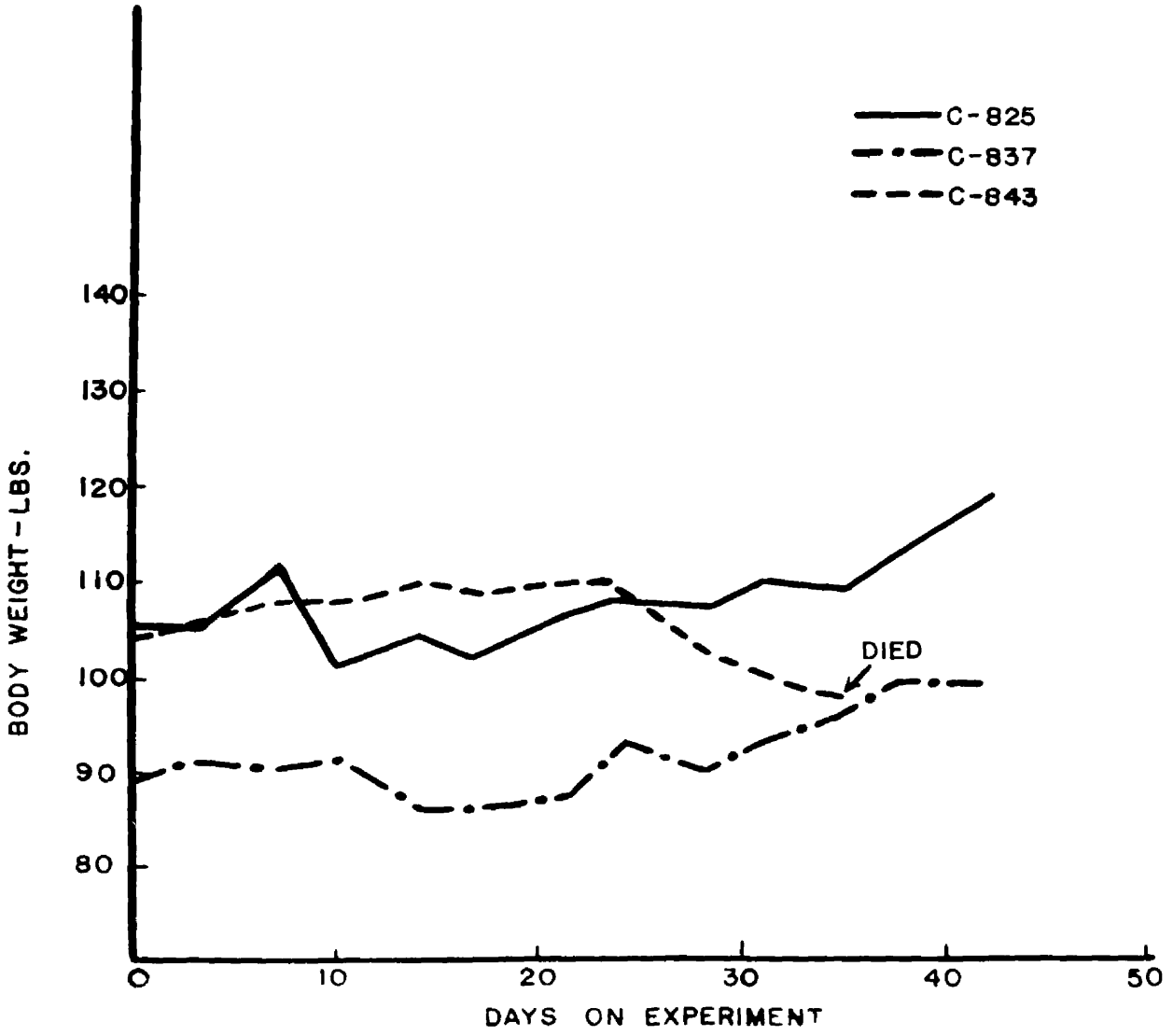

FIG. III. GROWTH CURVES -- GROUP 3

20 μG. VITAMIN B_{12}/KG. D.M.

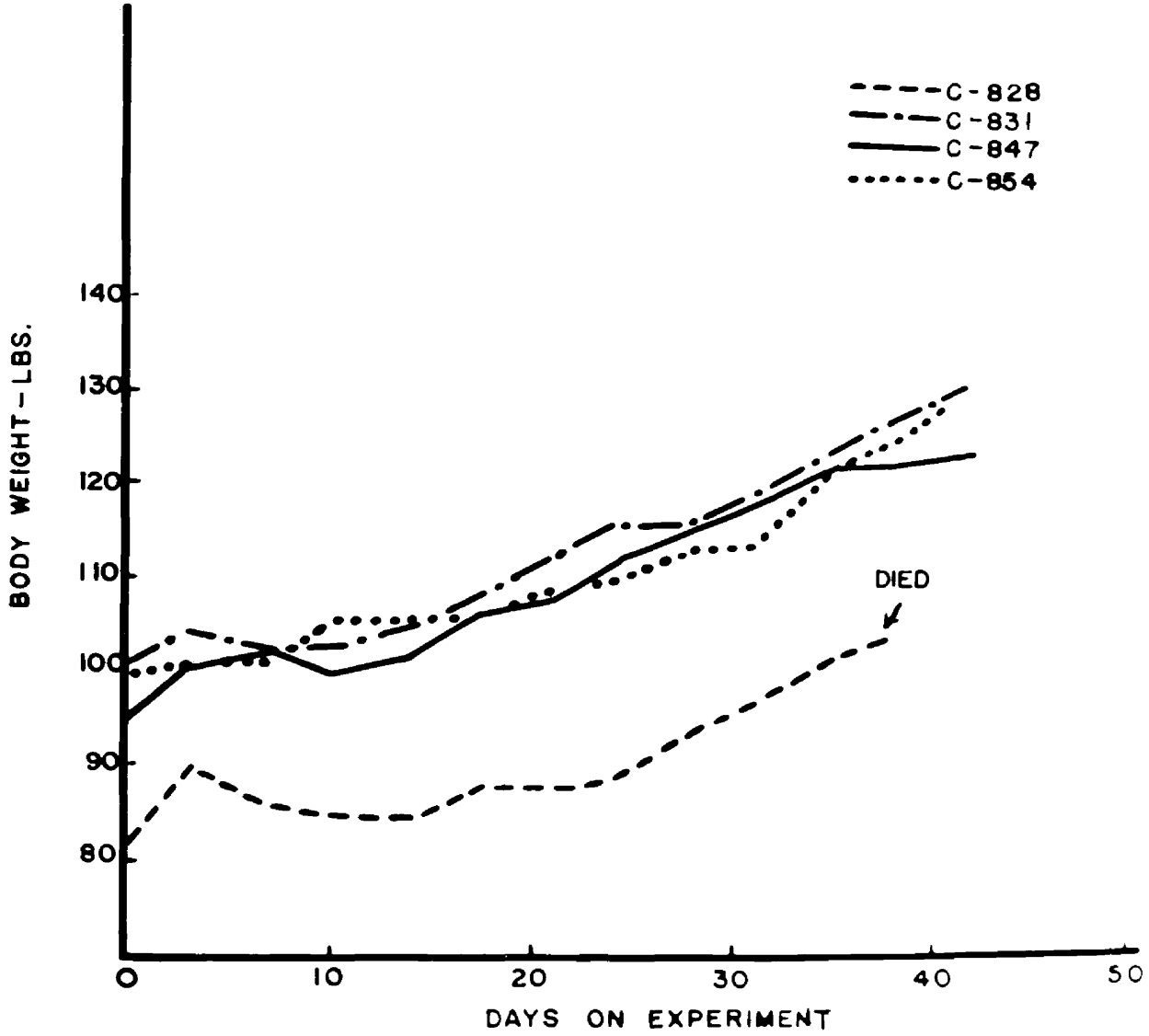

FIG. IV. GROWTH CURVES -- GROUP 4

40 μG. VITAMIN B_{12}/KG. D M.

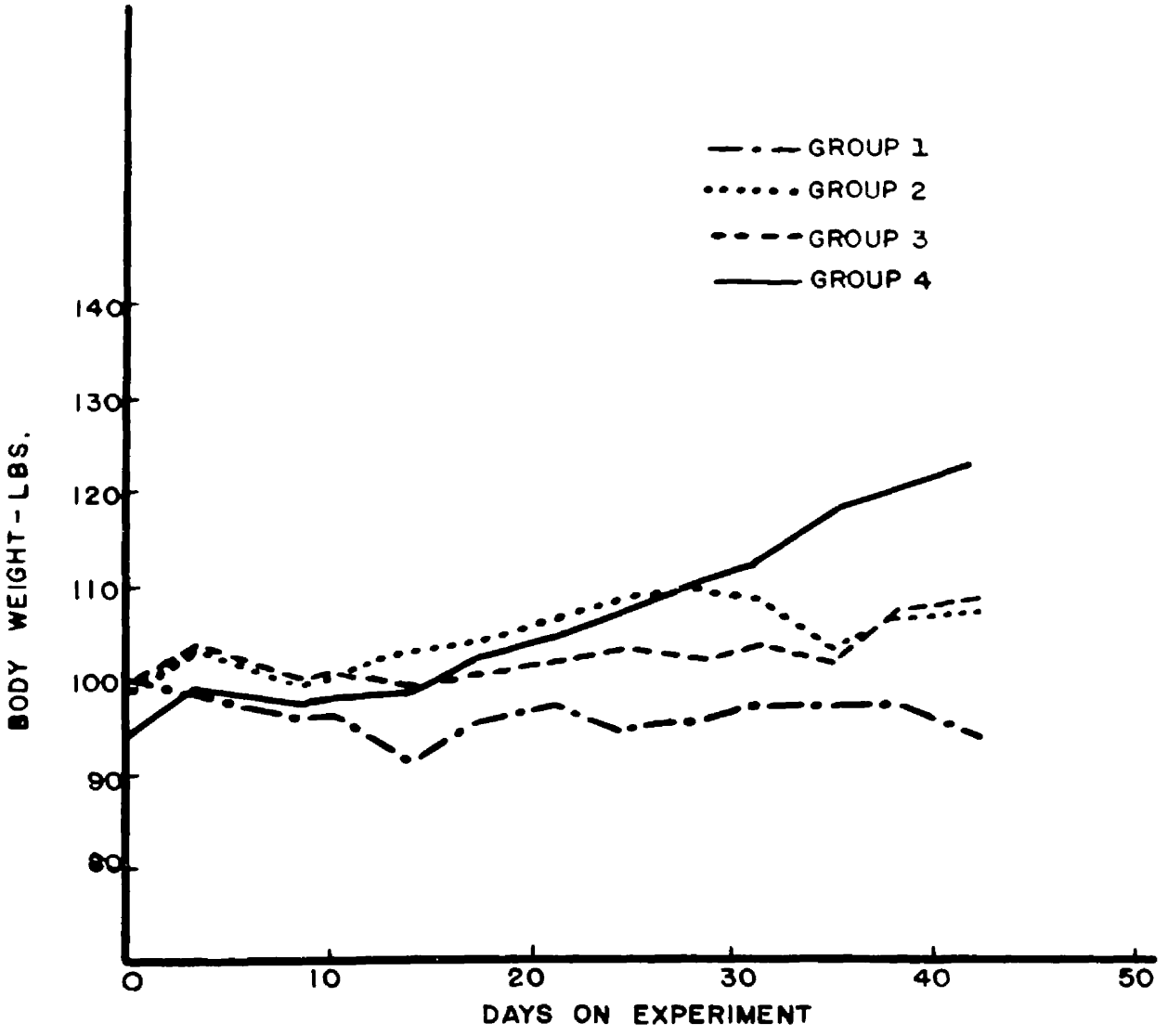

FIG. V. GROWTH CURVES -- ALL GROUPS

The growth data of Group I shows that this ration was deficient in vitamin B_{12} as were similar rations developed in the first experiment (Part I). Group I received the basal ration without any vitamin B_{12} supplementation. Two of the three calves receiving the vitamin B_{12} deficient ration died before the six-week experimental period was completed. Calf C-835 died 16 days after being started on the deficient ration. This calf appeared to be in normal health for the first 10 days of the trial but thereafter lost weight, had a poor appetite, and exhibited very poor condition. The calf seemed to possess a very low resistence to disease as evidenced by symptoms of bronchitis and pneumonia during the last week of life. The calf was treated with sulfa drugs on the seventh day of the experiment and the respiratory trouble seemed to be cleared up. The symptoms reappeared in six days and persisted throughout the remainder of the trial. Upon post-mortem examination the mediastinal lymph nodes were somewhat enlarged and congested and the trachea and bronchi contained a frothy fluid mixed with purulent material. The cause of death was established as bronchopneumonia.

Calf C-834 presented very much the same growth curve and general condition as C-835. This calf appeared to be normal for the first seven days of the trial but after that time it began to lose weight until death occurred on the

thirtieth day of the trial. The calf weighed ten pounds less on the thirtieth day of the experimental period than on the first day of the trial. As was found with calf C-835 the resistence to disease was very low. The calf showed symptoms of pneumonia on the 11th day of the experimental period. The calf was given sulfa therapy for 15 days before the symptoms were relieved. Post-mortem examination revealed congestion of the lymph nodes, areas of marked congestion and hemorrhagic spots scattered throughout the length of the intestinal tract.

The third calf, C-841, in Group I survived the experimental period, but was in extremely poor condition and was nearly void of appetite at the end of the trial. During the last week of the period it ate only seven of fourteen meals. For the first 28 days of the trial the calf did not gain in body weight but otherwise appeared to be normal. However, during the remainder of the trial the calf gradually lost weight and became progressively weaker. At the end of the trial the calf was very emaciated, showed general muscular weakness, possessed very little energy, and had a very poor appetite. The calf was killed at the end of the trial and the carcass was in very poor flesh and the kidneys were somewhat enlarged and spotted with white foci ranging up to 3 millimeters in diameter. The renal lymph nodes were enlarged and congested as had been found with the other two calves in the group.

When the vitamin B_{12} deficient ration was supplemented with crystalline vitamin B_{12} an increase in the growth rate of the calves occurred. All of the calves in Group II lived the entire length of the experimental period. Two of the calves, C-832 and C-844, presented very similar growth curves. The calves were able to maintain their body weight over the six-week trial and the general condition of these calves at the end of the six-week trial was greatly improved over that of the calves in Group I. Calf C-844 was killed at the end of the period and the white-spotted kidney condition was present but not to the extent that it was found in the calves in Group I. Calf A-96 made exceptionally good growth throughout the experimental period. The reason for the sharp increase in growth rate of this calf could not be fully explained. It was possible that the calf was born with an extra large storage of vitamin B_{12}. Another explanation was that the calf received an additional supply of vitamin B_{12}. An examination of the feeding records of the experimental herd revealed that a casein-type synthetic ration was being fed at the same time that this calf was on experiment. It was possible that contamination from this ration occurred. The growth data for this calf are included in the average for Group II as shown in Table 12 and Figure V.

The average daily gain of the calves in Group III was much greater than that for Groups I or II if the growth data

of calf A-96 were eliminated from the average shown in Table 12 for Group II. Two of the three calves in this group survived the six-week experimental period. Calf C-843 grew normally for the first two weeks of the trial but stopped growing and finally died the thirty-sixth day of the experimental period. The calf was in fair condition, exhibited no muscular weakness, but during the latter part of the trial had poor appetite. Post-mortem examination revealed the white-spotted kidney condition previously described and light yellowish areas in the liver. The other two calves in Group III exhibited none of the vitamin B_{12} deficiency symptoms that have been described except poor growth. These calves exhibited limited appetite but not to the extent noted in Groups I and II.

When the basal vitamin B_{12} deficient ration was supplemented with 40 micrograms of vitamin B_{12} per kilogram of dry matter consumed a sharp increase in growth rate occurred when compared to the growth rates of Groups I, II, and III. The calves in Group IV increased 28.84 percent over their starting weight and made an average daily gain of 0.65 pounds per day. The growth rate of this group was still somewhat below that of the Ragsdale standard (Ragsdale, 1934) but was probably good growth for calves being fed synthetic milk rations. The general condition and appetite of all of the calves in this group were excellent throughout the experimental period. Calf C-828 was the only calf that died during

the experimental period and it died on the 42nd day of the period. The calf grew at a rate just less than the average of the group but in other respects appeared to be normal. Post-mortem examination revealed no indication of the cause of death. Calf C-847 was killed at the end of the experimental period and none of the previously described symptoms of the vitamin B_{12} deficiency were found. At the end of the experimental period the calves in Group IV were in excellent general condition, showed extremely good vigor and thriftiness, possessed excellent appetites and their body weight was increasing normally.

One of the outstanding symptoms observed of a vitamin B_{12} deficiency was the lack of appetite. It was found that the calves in Groups I, II, III, and IV averaged refusing 13, 7, 5, and 5 feeds per calf for the 42-day period, respectively. As the amount of vitamin B_{12} supplementation increased the appetite of the calves also improved.

Figures VI, VII, and VIII show calves C-841, C-844, and C-854, representative animals of Groups I, II, and IV, respectively, at six weeks of age. The improved condition and general appearance of calf C-854 over that of calves C-841 and C-844 may be observed.

The weekly blood analyses of the plasma constituents are presented in Table 13. Figure IX shows the red blood cell volumes, hemoglobin concentrations, and red blood cell counts of Groups I, II, III, and IV by 10-day periods.

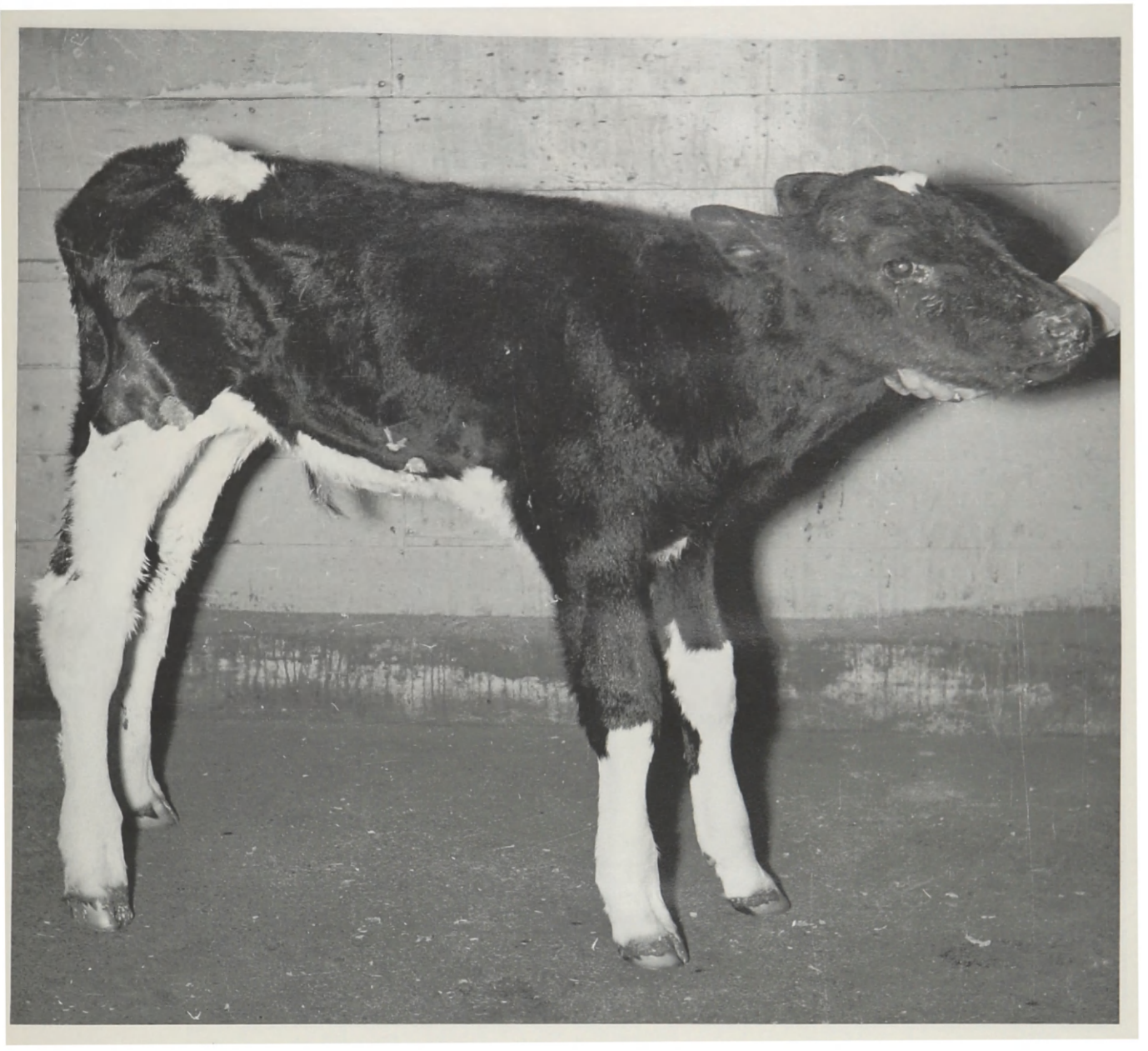

Fig. VI. Calf C-841 -- Group I -- 0 ug. vitamin B_{12}/kg. D.M.

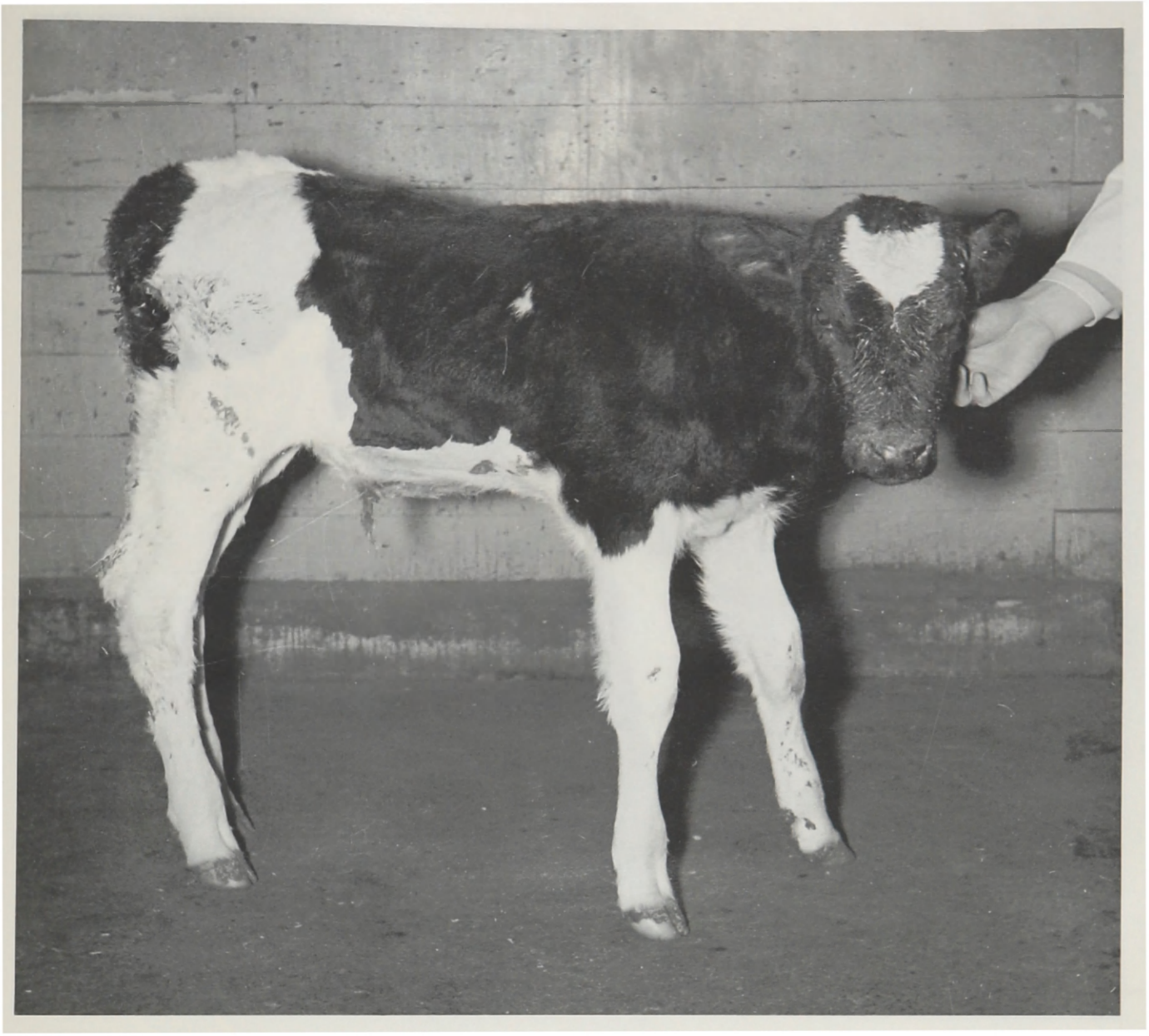

Fig. VII. Calf C-844 -- Group II -- 10 ug. vitamin B_{12}/kg. D.M.

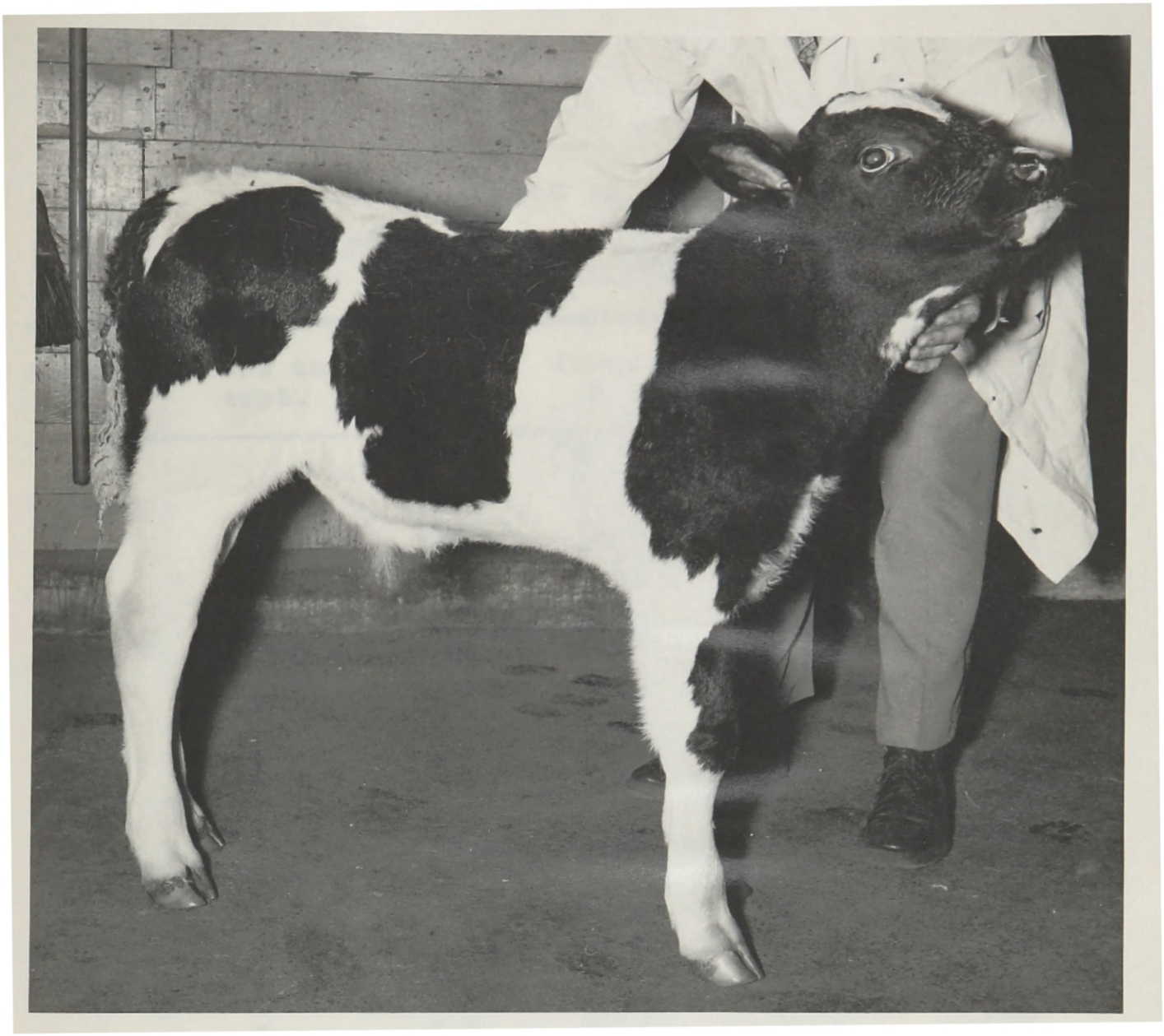

Fig. VIII. Calf C-854 -- Group IV -- 40 ug. vitamin B_{12}/kg. D.M.

TABLE 13

WEEKLY ANALYSES OF BLOOD PLASMA CONSTITUENTS

Group	Weeks on expt.	Ca	Inorg. P	Mg.	Ascorbic acid
		mg. %	mg. %	mg. %	mg. %
I	1	10.1	7.38	2.90	0.367
	2	9.7	7.14	2.34	0.276
	3	9.1	7.42	2.03	0.273
	4	9.2	7.08	2.23	0.200
	5	9.3	6.99	2.29	0.151
	6	9.2	6.86	2.14	0.246
	Average	9.4	7.15	2.32	0.252
II	1	9.7	7.16	2.31	0.214
	2	9.9	7.45	1.93	0.193
	3	10.1	8.62	2.47	0.317
	4	9.7	7.57	2.18	0.228
	5	9.5	7.34	2.10	0.193
	6	9.7	6.88	2.01	0.188
	Average	9.8	7.50	2.17	0.222
III	1	10.5	8.39	2.65	0.299
	2	9.5	6.86	2.03	0.244
	3	9.7	6.79	2.13	0.267
	4	9.0	6.37	2.19	0.237
	5	9.5	8.02	2.31	0.283
	6	9.4	6.01	2.14	0.162
	Average	9.6	7.07	2.24	0.249
IV	1	9.7	7.31	2.20	0.215
	2	9.6	6.79	2.99	0.232
	3	9.8	6.90	2.00	0.228
	4	9.4	7.05	2.13	0.219
	5	9.4	7.07	1.97	0.184
	6	9.2	6.52	2.46	0.201
	Average	9.5	6.94	2.29	0.213
V	Incomplete data				

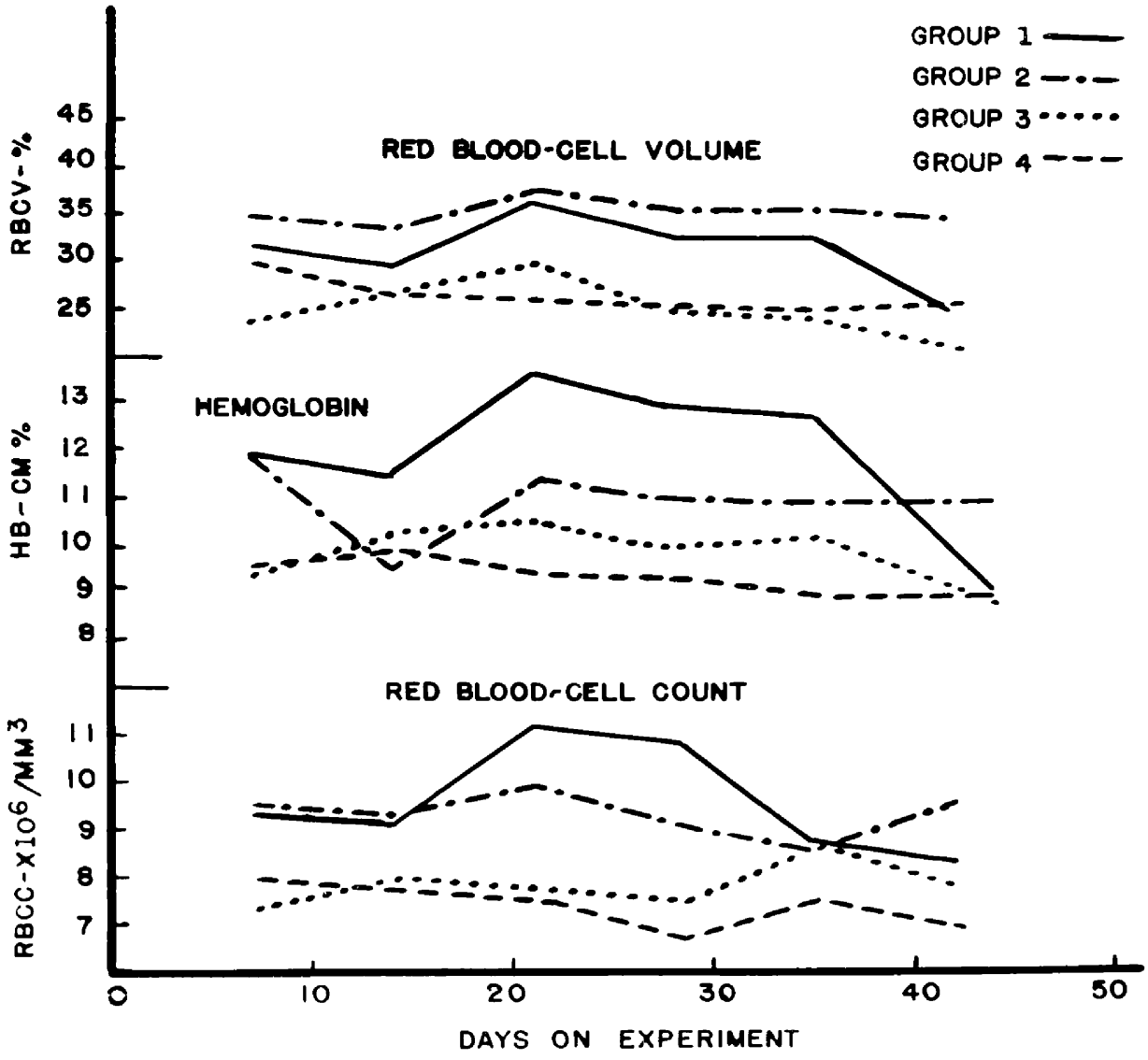

FIG. IX. CHANGES IN SOME BLOOD CONSTITUENTS OF THE EXPERIMENTAL CALVES

As is shown in Table 12 the plasma content of calcium, inorganic phosphorus, magnesium, and ascorbic acid for all groups appeared to be normal and within the normal range of variation. The red blood cell volumes, hemoglobin contents, and red blood cell counts of the whole blood of the vitamin B_{12} deficient calves were consistently higher than adequately vitamin B_{12}-supplemented calves.

As noted previously, the data on the calves in Group V was incomplete due to the calves' inability to survive on the ration. Since the only variation made in the ration was a change of batches of "alpha" protein, two groups of rats were used to ascertain the relative nutritive value of those two batches. The average growth rate and feed efficiency of the two experimental rat groups are shown in Table 14 and growth curves of both groups of rats are presented in Figure X.

TABLE 14

GAIN AND FEED UTILIZATION--RAT EXPERIMENT

Group	Starting weight	Average daily gain	Gain/D.M. consumed
	gms.	gms.	gms./100 gm.
A	69.0	2.45	43.1
B	69.0	1.55	31.9

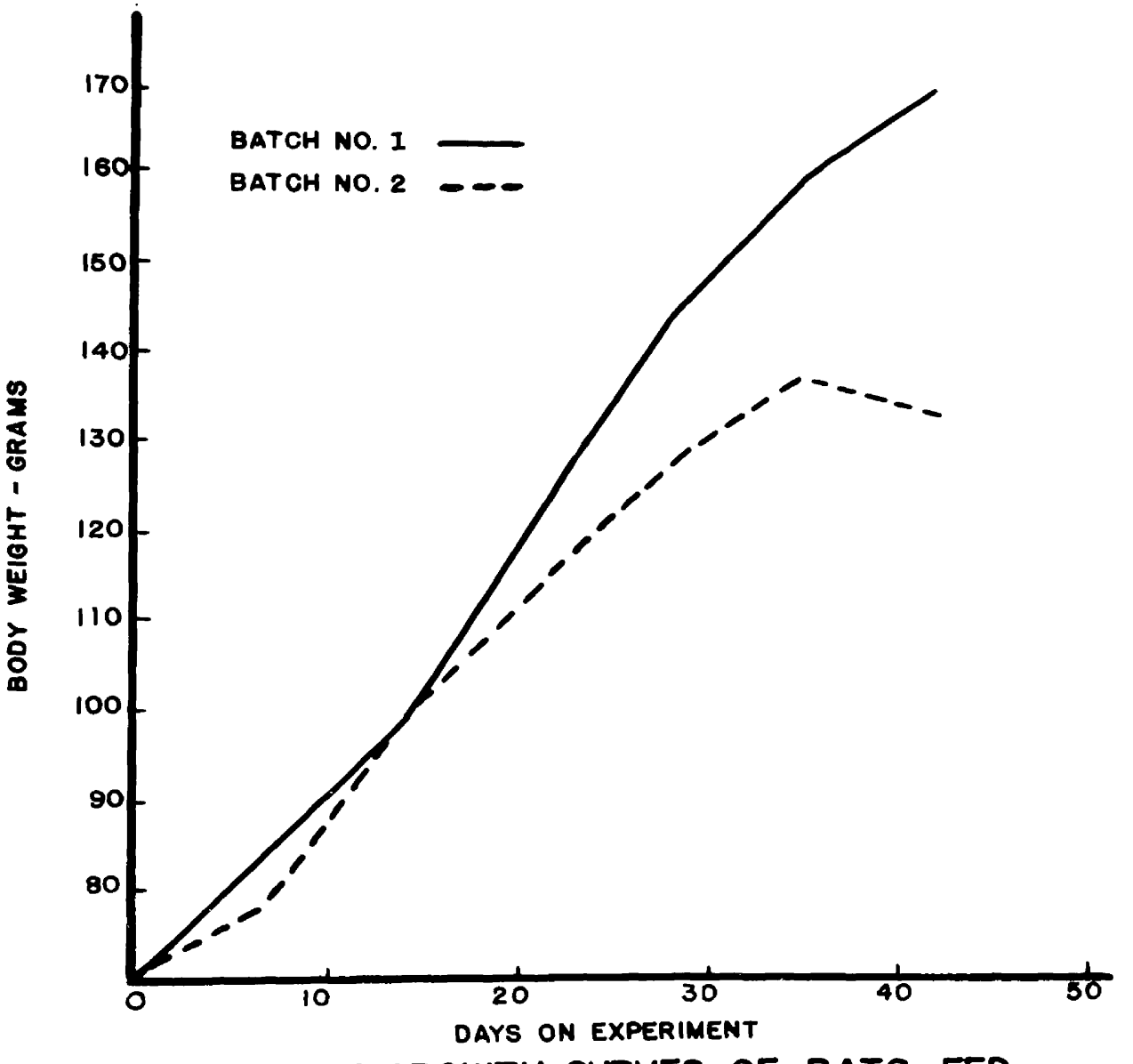

FIG. X. GROWTH CURVES OF RATS FED TWO BATCHES OF "ALPHA" PROTEIN

As is shown in Table 14 and Figure X there was a decided difference in the growth rates of the two groups of rats. Group A gained at the rate of 2.45 grams per day whereas Group B only gained 1.55 grams per day. As is shown in Figure X the two groups gained at about the same rate for the first 15 days of the experimental period but thereafter Group A gained at a much faster rate.

DISCUSSION

The basal ration used in this study contained approximately one-third as much ether extract as did a similar "alpha" protein synthetic milk used by Draper *et al.* (1952b) in studying the vitamin B_{12} deficiency of the calf. However, Flipse *et al.* (1950a, 1950b) showed that young dairy calves increased normally in body weight and appeared to be in good general health when fed a synthetic milk containing the same amount of ether extract as did the basal ration used in this study. The vitamin B_{12} deficient ration developed by Draper *et al.* (1952b) and the ration reported in this study were comparable in other respects with one exception. These workers did not mention the presence of an anti-thiamin substance in the "alpha" protein. It is difficult to understand why this condition was not encountered because the thiamin content of the ration fed and the basal ration used were comparable with those in this study. In fact, the calves' daily intake of thiamin in the study reported herein probably was slightly greater. However, as reported in Part I, when the anti-thiamin substance was not removed by washing with water the calves exhibited typical thiamin deficiency symptoms within 3 to 4 days after being fed such a ration.

The symptoms of a vitamin B_{12} deficiency in the calf were described by Draper et al. (1952b). These workers found that the lack of vitamin B_{12} caused a cessation of growth, poor appetite, and in some cases the calves exhibited incoordination. The symptoms observed in this study closely paralleled those described by these workers. However, the white-spotted kidney condition was not found by these workers. White-spotted kidneys were reported by Moore and Hallman (1936) as a symptom of vitamin A deficiency. However, it is believed that the ration used in this study was adequately fortified with vitamin A since other typical symptoms of a vitamin A deficiency did not exist and because this condition was not found when the vitamin B_{12} deficient diet was supplemented with adequate vitamin B_{12}. Draper et al. (1952b) also found that calves could be raised to twelve weeks of age without cessation of growth.

The appearance of the light yellowish areas in the liver of calf C-843 possibly indicated some fatty infiltration of the liver. Ling and Chow (1951) found that vitamin B_{12} plays an important role in lipid metabolism in rats.

Johnson and Nesheim (1949) showed that vitamin B_{12} supplementation to pigs existing on a vitamin B_{12} deficient ration increased red blood cell counts. There were no indications in this study that the lack of vitamin B_{12} caused a low red blood cell count or that vitamin B12 supplementation

increased the red blood cell counts of calves being fed a vitamin B_{12} deficient ration. The only explanation of the high red blood cell volume, hemoglobin content, and red blood cell counts of the whole blood of the vitamin B_{12} deficient calves is that these calves were slightly dehydrated with concurrent hemoconcentration.

It should be noted that all of the experimental groups have been discussed except Group V. The purpose of this group was to determine whether the crystalline vitamin B_{12} requirement of the young dairy calf was higher than 40 micrograms of vitamin B_{12} per kilogram of dry matter consumed. When the experiment was started it was believed that four groups would be sufficient to determine the requirement. It was later noted that Group V was needed and, as a result, the calves in Group V were fed the basal ration after most of the other groups had been completed. The calves were allotted to Group V but none of these calves finished the six-week experimental period. In fact, all of the calves died within the first 13 days of the trial. All the ten calves presented similar growth patterns and general condition. The calves appeared to be normal for the first four or five days of the trial, but beginning at about the sixth day of the experimental period the calves began to decrease in body weight. Many of the calves lost as much as 10 to 15 pounds in the short period of two to three days. The calves

appeared to be in a very dehydrated condition. Surprisingly, the calves never did lose their appetite. Most of the calves upon post-mortem examination showed slight congestion and hemorrhage of the intestinal tract, yellowish livers, and enlarged kidneys and gall bladders. However, none of the above findings could definitely be named as the cause of death. Most of the calves passed feces four to five days before death which were grayish in color and semi-liquid in consistency. The feces of the calves of Groups I, II, III, and IV were usually black in color by the time they were one week of age.

Although the calves in Group V did not live it is proposed that the crystalline vitamin B_{12} requirement of the young dairy calf is not more than 40 micrograms of vitamin B_{12} per kilogram of dry matter consumed since the calves in Group IV grew at a rate equal to the growth rate of calves in experiment one (Part I) which received 80 micrograms of vitamin B_{12} per kilogram of dry matter consumed.

A comparison of the rations fed in Part I and this experiment shows that the principal difference between the rations was the amount of protein which they contained. A new supply of "alpha" protein was fed to Group V. The other four groups had been fed the same batch of "alpha" protein as had been fed to the calves in Part I. These facts made a study of the relative nutritive value of the new batch of "alpha" protein advisable.

Since Clandinin et al. (1946) showed that overheated soybean oil meal was deficient in available lysine, calf C-865 (Appendix--Table 17) was fed Ration V supplemented with eight grams of lysine hydrochloride per day. The growth rate and general condition of this calf was approximately the same as that of the calves in Group V and it died six days after being placed on the ration. The ration did not appear to be deficient in the amino acid lysine.

The calves, C-866 and C-868, (Appendix--Table 17) received Ration V with the exception that a new source of methionine was used. One of the calves, C-866, survived on the ration for three weeks and at the end of this time appeared to be normal in every respect and made normal gains during this period. However, calf C-868 presented about the same toxicity or deficiency symptoms as did the calves in Group V. The calf was losing weight and was removed from the ration after being fed the ration for six days. The results with this calf showed that the ration was not deficient in methionine. It was not clear why calf C-866 was able to survive on the ration.

Calf C-867 (Appendix--Table 17) received Ration V with the exception that the soya lecithin was removed from the ration diet and replaced by lard. The purpose of this ration was to determine whether the soya lecithin contained toxic principles due to deterioration. However, this calf died

after being fed the ration for six days which indicated that the lecithin portion of the ration was not the toxic or deficiency factor present in this ration.

Since it was not possible to determine the toxic or deficiency factor in Ration V with calves two groups of rats were fed experimental rations shown in Table 9 to compare the nutritive value of the two batches of "alpha" protein. It may be observed that the two rations were identical with the exception of the "alpha" protein included in them. Group A received the "alpha" protein from the batch which was used for the calves in experiment one (Part I) and the calves in Groups I, II, III, and IV in this experiment. Group B was fed "alpha" protein from the batch that was used for the ten calves in Group V.

There was a very marked difference in the growth rate of the two groups of experimental rats. Group A gained an average of 2.45 grams per day as compared to 1.55 grams per day for Group B. For the first 15 days of the experimental period there was very little difference in the growth rates of the two groups. However, from the 15th day of the trial until the end of the experimental period, Group A gained body weight at a much faster rate than Group B. During the last seven days of the trial Group B lost some body weight. At the end of the trial the rats in Group B were losing weight, were in poor condition, and had rather poor appetite. Un-

fortunately, the trial had to be stopped because the supply of "alpha" protein for Ration A was exhausted. If the trial were to have been conducted longer, it is doubtful whether the rats in Group B would have lived. At the end of the trial the rats in Group A were in excellent condition and showed no evidence of a deficiency or toxic factor in their ration.

Draper et al. (1952b) produced a vitamin B_{12} deficiency in the dairy calf utilizing a ration very similar to the ration employed in this study. These workers showed that the calf required vitamin B_{12} but these workers also expressed the belief that the vitamin B_{12}-supplemented rations were still deficient in a factor or factors required by the young dairy calf since some of the vitamin B_{12} supplemented calves did not respond to the vitamin B_{12} therapy. The general condition of the calves and the post-mortem findings of these workers very closely paralleled the findings in this study. Draper et al. (1952b) particularly observed distended gall bladders on post-mortem examination of these calves. It is believed that the same deficiency or toxic factor that was found in the study conducted by these workers was the factor encountered in this study. Under the conditions of this experiment "alpha" protein did not always appear to be a suitable source of protein for the young dairy calf.

SUMMARY

Twenty-three new-born dairy calves were allotted to five experimental groups and fed an "alpha" protein synthetic milk deficient in vitamin B_{12}. The basal ration was supplemented with 0, 10, 20, 40, and 80 micrograms of crystalline vitamin B_{12} per kilogram of dry matter consumed in an attempt to establish the crystalline vitamin B_{12} requirement of the young dairy calf. The average daily gains of calves on the 0, 10, 20, and 40 microgram levels were -0.10, 0.20, 0.20, and 0.65 pound per day, respectively. Incomplete data were obtained on Group V.

It was found that the dairy calf requires vitamin B_{12}. Growth cessation, lack of appetite, general poor condition, muscular weakness, and a white-spotted kidney condition were symptoms found associated with vitamin B_{12} deficiency in the young dairy calf.

Preliminary results indicated that the crystalline vitamin B_{12} requirement of the dairy calf is more than 20 micrograms but not more than 40 micrograms of vitamin B_{12} per kilogram of dry matter consumed.

Evidence was also obtained which indicates that "alpha" protein is not always satisfactory as a source of protein for the young dairy calf.

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APPENDIX

TABLE 15

GROWTH DATA -- PART I

Days on Expt.	Calf No.	
Ration 1	C-783	C-784
	lbs.	lbs.
0	103.0	93.0
7	106.0	95.0
14	104.0	87.0
21	107.0	88.5
28	108.5	82.0
Ration 2	C-788	C-791
0	113.5	83.5
7	121.5	93.0
14	119.0	95.5
21	122.0	96.0
28	130.0	103.0
Ration 3	C-798	
0	115.0	
3	130.0	
7	127.5	
10	131.5	
14	130.0	
17	123.5	
21	127.0	
24	133.0	
28	137.0	
Ration 4	C-803	
0	102.0	
3	107.0	
7	108.0	
10	111.0	
14	112.0	
17	111.5	
21	122.0	
24	123.0	
28	118.5	

TABLE 15 (Cont.)

Days on Expt.	Calf No.
Ration 5	C-809
	lbs.
0	87.0
1	90.5
3	91.0
7	91.0
10	89.0
14	82.0
17	80.0
21	79.5
24	86.5
28	89.5
Ration 6	C-810
0	100.5
1	104.0
3	109.5
7	108.0
10	105.0
14	109.0
17	112.0
21	114.0
24	121.0
28	119.5
Ration 7	C-814
0	84.0
1	85.0
3	88.0
7	85.5
10	84.0
14	87.5
17	87.0
21	87.0
24	89.0
28	94.5

TABLE 15 (Cont.)

Days on Expt.	Calf No.		
Ration 8	C-816		
	lbs.		
0	90.5		
1	91.0		
3	97.0		
7	83.0		
10	Died		
Ration 9	C-817		
0	86.0		
1	91.0		
3	95.5		
7	95.0		
10	94.5		
14	96.0		
17	97.0		
21	99.0		
24	102.0		
28	106.0		
31	102.0		
35	109.5		
38	113.0		
42	110.0		
Ration 10	A-95	C-820	C-821
0	91.0	110.5	87.5
1	95.5	110.0	85.0
3	96.0	111.0	81.0
7	89.5	104.5	79.0
10	93.0	100.0	79.0
14	95.5	104.5	78.5
17	98.0	102.0	80.0
21	100.0	106.0	82.0
24	105.5	106.5	84.0
28	106.0	108.0	85.5
31	106.5	108.5	86.5
35	107.5	116.0	86.0
38	108.5	120.0	88.5
42	110.0	126.5	91.0

TABLE 16

GROWTH DATA -- PART II

Group I	Calf No.		
Days on Expt.	C-834	C-835	C-841
	lbs.	lbs.	lbs.
0	109.0	86.0	98.5
1	109.0	85.5	97.0
3	112.0	87.0	95.5
7	107.0	84.0	98.0
10	104.0	85.0	99.0
14	101.0	72.5	98.0
17	100.0	Died	100.0
21	105.0		104.0
24	100.0		102.0
28	99.0		102.0
31	Died		104.0
35			104.5
38			104.0
42			93.0
Group II	**A-96**	**C-832**	**C-844**
0	98.0	97.5	100.0
1	100.5	97.5	103.0
3	100.0	101.0	107.0
7	100.0	97.0	104.0
10	102.0	102.0	100.0
14	105.0	103.0	102.0
17	106.0	102.5	102.0
21	108.0	110.0	100.0
24	111.5	112.5	102.0
28	113.5	110.0	102.0
31	117.0	108.0	99.0
35	119.0	93.0	98.0
38	125.0	99.0	96.0
42	130.0	100.0	90.0
Group III	**C-825**	**C-837**	**C-843**
0	105.0	89.0	104.0
1	105.0	91.0	106.0
3	111.0	92.0	106.0
7	101.0	90.0	108.0
10	104.0	91.0	108.0
14	102.0	86.0	110.0
17	106.0	86.0	109.0

TABLE 16 (Cont.)

Group III (cont.)	Calf No.		
Days on Expt.	C-825	C-837	C-843
	lbs.	lbs.	lbs.
21	108.0	87.0	110.0
24	107.0	93.0	110.0
28	110.0	90.0	103.0
31	113.0	92.0	100.0
35	109.0	96.0	98.0
38	113.0	98.0	Died
42	119.0	98.0	

Group IV	C-828	C-831	C-847	C-854
0	82.0	101.5	95.5	100.0
1	82.5	101.0	102.0	101.0
3	90.5	105.0	101.0	101.0
7	86.0	103.0	103.0	101.0
10	85.0	103.0	100.0	106.0
14	82.0	104.5	102.0	106.0
17	88.0	108.0	106.0	105.0
21	88.0	112.5	108.0	109.0
24	89.0	116.0	112.0	110.0
28	94.0	116.0	115.0	113.0
31	97.0	119.0	118.0	114.0
35	102.0	124.0	122.0	122.0
38	104.0	127.0	122.0	125.0
42	Died	130.0	123.0	130.0

Group V	C-840	C-845	C-846	C-848	C-849
0	101.0	114.0	105.0	93.0	114.0
1	99.0	115.0	105.0	95.0	123.0
3	97.0	Died	110.0	97.0	123.0
7	95.5		99.0	96.0	117.0
10	Died		88.0	88.0	98.0
14			Died	Died	Died

Group V (cont.)	C-850	C-851	C-855	C-856	C-858
0	88.0	117.0	88.0	102.0	90.0
1	89.0	119.0	87.0	102.0	92.0
3	90.0	120.0	85.0	106.0	95.0
7	90.0	116.0	Died	Died	75.0
10	82.0	106.0			64.0
14	Died	Died			Died

TABLE 17

GROWTH DATA OF CALVES C-865, C-866, C-867, AND C-868

Days on expt.	Calf No. C-865	C-866	C-867	C-868
	lb.	lb.	lb.	lb.
0	86.0	87.0	100.0	96.0
1	88.0	87.0	101.0	98.0
3	86.0	91.0	99.0	99.0
4	82.0	91.0	98.0	99.0
5	73.0	88.0	91.0	91.0
6	Died	86.0	85.0	89.0
7		87.0	Died	Removed
10		90.0		
14		90.0		
17		94.0		
21		98.0		

TABLE 18

RAT GROWTH DATA

Group A Days on expt.	Rat No. 1	2	3	4
	gms.	gms.	gms.	gms.
0	77.0	74.0	63.0	60.0
3	74.0	74.0	62.0	66.0
5	95.0	80.0	65.0	70.0
7	102.0	88.0	71.0	75.0
10	103.0	92.0	77.0	81.0
12	120.0	107.0	80.0	86.0
14	127.0	112.0	75.0	82.0
17	142.0	128.0	92.0	106.0
19	128.0	118.0	98.0	106.0
21	136.0	128.0	108.0	120.0
24	152.0	143.0	115.0	128.0
26	147.0	137.0	123.0	140.0
28	158.0	152.0	127.0	139.0

TABLE 18 (Cont.)

Group A (cont.) Days on expt.	Rat No. 1	2	3	4
	gms.	gms.	gms.	gms.
31	172.0	169.0	137.0	152.0
33	170.0	165.0	142.0	156.0
35	175.0	156.0	150.0	158.0
38	173.0	145.0	141.0	144.0
40	188.0	164.0	163.0	164.0
42	197.0	166.0	162.0	160.0
Group B	**5**	**6**	**7**	**8**
0	78.0	75.0	64.0	58.0
3	82.0	75.0	63.0	61.0
5	89.0	77.0	62.0	68.0
7	91.0	80.0	69.0	73.0
10	99.0	88.0	72.0	67.0
12	108.0	101.0	78.0	80.0
14	116.0	108.0	82.0	89.0
17	114.0	111.0	91.0	99.0
19	111.0	121.0	101.0	104.0
21	121.0	129.0	101.0	104.0
24	127.0	143.0	110.0	112.0
26	127.0	144.0	114.0	116.0
28	135.0	150.0	114.0	114.0
31	132.0	144.0	123.0	126.0
33	126.0	142.0	130.0	133.0
35	138.0	152.0	128.0	133.0
38	139.0	148.0	126.0	139.0
40	150.0	170.0	136.0	144.0
42	134.0	138.0	128.0	136.0

TABLE 19

WEEKLY BLOOD ANALYSES -- EXPERIMENTAL CALVES

Calf no.	Week	Whole Blood			Plasma			
		RBCC	Hb	RBCV	Ascorbic acid	Ca	Inorg. P	Mg
		$10^6/MM^3$	gm.%	%	mg. %	mg. %	mg. %	mg. %
C-783	1	---	7.73	21.0	0.497	11.3	6.82	2.77
	2	---	8.67	23.0	0.440	11.0	7.86	2.72
	3	---	8.90	23.0	0.488	10.3	6.82	2.03
	4	---	8.97	23.0	0.667	9.5	6.82	2.07
	5	---	9.03	23.0	0.693	11.2	6.82	2.40
C-784	1	---	15.50	42.5	0.257	10.5	7.06	2.84
	2	---	15.50	43.0	0.192	9.4	7.43	2.07
	3	---	14.45	37.5	0.101	9.9	5.85	2.14
	4	---	13.95	39.5	0.200	9.5	6.17	2.00
C-788	1	---	13.70	34.5	0.515	10.2	8.17	3.03
	2	---	13.00	32.0	0.277	9.9	6.82	2.77
	3	---	13.70	33.0	0.326	---	6.59	2.62
	4	---	12.90	32.0	0.320	9.5	6.90	2.82
C-791	1	---	13.70	36.0	0.434	10.0	7.64	2.31
	2	---	14.37	38.5	0.420	10.4	6.70	2.00
	3	---	15.00	36.0	0.371	11.5	9.02	1.86
	4	---	12.83	34.0	0.567	10.3	7.43	2.29
C-798	1	---	13.43	38.5	0.294	9.9	9.02	3.12
	2	---	11.23	31.5	0.442	9.2	8.54	1.52
	3	---	11.67	32.0	0.197	9.6	6.74	1.95
	4	---	12.77	32.0	0.279	9.8	7.52	4.70
C-803	1	---	10.93	29.0	0.289	10.4	7.95	2.52
	2	---	10.43	29.5	0.142	10.5	6.59	1.93
	3	---	10.13	26.0	0.184	9.5	6.63	2.08
	4	---	9.40	25.0	0.220	10.0	7.22	2.04
	5	---	8.97	24.5	0.188	9.5	6.06	2.05
C-809	1	---	11.60	30.5	0.479	11.9	9.26	2.44
	2	---	11.15	30.0	0.212	10.5	5.53	2.13
	3	---	11.60	32.0	0.182	9.9	5.03	2.05
	4	---	11.30	27.0	0.182	10.4	6.06	2.44
C-810	1	---	14.55	38.5	0.126	11.1	6.82	2.51
	2	---	13.25	38.5	0.083	10.5	6.44	2.05
	3	---	13.25	39.0	0.182	10.0	7.18	1.80
	4	---	13.25	34.0	0.255	9.8	7.31	2.17

TABLE 19 (Cont.)

Calf no.	Week	Whole Blood			Plasma			
		RBCC	Hb	RBCV	Ascorbic acid	Ca	Inorg. P	Mg
		$10^6/MM^3$	gm.%	%	mg. %	mg. %	mg.%	mg.%
C-814	1	---	12.60	33.5	0.223	9.6	6.25	2.60
	2	---	12.20	34.0	0.186	10.4	7.02	1.91
	3	---	11.43	31.5	0.173	10.3	6.63	2.00
	4	---	10.50	28.5	0.154	9.9	7.95	2.59
C-816	1	---	10.13	27.0	0.712	12.7	9.02	2.52
	2	---	11.05	34.0	0.381	11.0	8.58	4.01
C-817	1	---	13.17	32.5	0.538	11.5	6.06	2.44
	2	---	12.70	37.5	0.381	10.0	5.88	2.02
	3	---	12.83	37.0	0.208	10.6	6.25	2.00
	4	---	12.45	36.0	0.343	10.1	7.02	2.29
	5	---	11.15	30.5	0.309	9.9	8.17	3.21
	6	---	9.83	26.0	0.328	9.6	6.82	2.75
	7	---	9.90	26.5	0.355	9.7	6.90	1.86
A-95	1	---	10.50	28.5	0.253	11.8	7.90	2.08
	2	---	10.93	33.5	0.328	11.4	8.04	2.59
	3	---	11.23	28.0	0.184	10.4	8.77	2.70
	4	---	11.43	29.5	0.305	9.1	8.26	2.23
	5	---	11.23	29.5	0.216	9.8	7.95	2.14
	6	---	10.63	29.5	0.222	10.8	8.97	3.34
C-820	1	---	10.07	25.0	0.278	9.5	6.40	2.22
	2	---	11.50	33.0	0.292	9.8	6.82	2.05
	3	---	11.30	30.5	0.225	9.2	6.51	2.22
	4	---	10.80	29.0	0.241	9.0	8.08	2.31
	5	---	9.77	27.0	0.231	9.3	6.44	2.51
	6	---	10.20	27.0	0.193	10.1	7.68	2.34
C-821	1	---	10.57	29.5	0.247	9.8	7.22	2.16
	2	---	10.93	31.5	0.206	9.6	6.21	2.00
	3	---	11.23	30.5	0.164	9.2	6.06	2.22
	4	---	10.50	29.0	0.099	9.7	7.26	2.16
	5	---	9.77	27.0	0.161	9.2	5.60	1.93
	6	---	9.77	26.5	0.115	9.8	7.47	2.14
C-834	1	13.87	16.90	47.0	0.309	10.9	8.30	3.16
	2	11.67	16.40	44.0	0.182	8.2	6.25	2.60
	3	14.27	17.00	47.5	0.337	9.2	7.86	2.14
	4	13.14	16.00	41.0	0.162	8.5	7.14	2.22
	5	9.99	15.90	43.0	0.054	8.8	7.73	1.65

TABLE 19 (Cont.)

Calf no.	Week	Whole Blood			Plasma			
		RBCC	Hb	RBCV	Ascorbic acid	Ca	Inorg. P	Mg
		$10^6/MM^3$	gm. %	%	mg. %	mg.%	mg.%	mg.%
C-835	1	6.02	8.47	23.0	0.453	9.7	6.82	2.07
	2	7.88	8.13	23.5	0.320	10.5	7.64	1.97
C-841	1	6.47	10.50	25.5	0.604	11.1	5.36	2.60
	2	8.16	10.43	27.5	0.340	9.7	7.03	2.48
	3	7.71	10.50	25.0	0.325	10.4	7.52	2.44
	4	8.05	10.43	26.0	0.208	9.0	6.98	1.91
	5	8.91	10.30	26.0	0.238	9.8	7.02	2.24
	6	7.72	9.77	24.5	0.247	9.7	6.25	2.92
	7	8.32	9.10	25.5	0.246	9.2	6.86	2.14
A-96	1	---	11.00	29.0	0.245	11.1	7.43	2.22
	2	7.47	11.60	32.0	0.243	9.6	6.25	3.01
	3	8.87	10.50	30.0	0.220	10.2	6.06	2.07
	4	9.17	12.00	33.0	0.209	10.8	8.30	2.37
	5	9.73	11.60	33.0	0.259	10.3	7.68	2.22
	6	8.33	10.57	29.0	0.258	10.2	7.86	2.52
	7	10.55	12.45	34.0	0.316	9.8	7.90	2.20
C-832	1	12.04	15.00	45.0	0.175	9.8	7.18	2.00
	2	---	---	---	0.137	9.4	7.18	1.79
	3	12.77	13.70	39.5	0.251	10.4	7.82	1.72
	4	10.55	12.90	36.5	0.179	9.5	6.25	2.07
	5	11.44	14.45	41.0	0.142	9.6	7.73	2.07
	6	11.03	12.27	33.0	0.125	9.8	5.53	2.00
C-844	1	7.40	7.33	20.5	0.445	11.0	8.49	2.85
	2	9.44	9.33	28.0	0.224	9.8	8.04	1.91
	3	10.04	8.75	26.5	0.222	10.2	8.30	1.93
	4	8.11	8.67	25.5	0.491	9.1	9.73	3.31
	5	7.55	8.53	24.5	0.246	9.4	8.77	2.26
	6	6.33	8.07	23.5	0.179	8.6	6.44	1.72
	7	7.17	8.33	23.5	0.124	9.5	7.22	1.84
C-825	1	---	10.3	26.5	0.197	9.7	7.02	2.14
	2	---	11.67	29.0	0.161	9.2	5.88	1.93
	3	---	10.63	35.5	0.167	9.6	6.58	2.29
	4	8.77	10.70	26.0	0.277	8.9	6.66	2.00
	5	8.23	9.40	24.5	0.247	9.5	8.04	1.91
	6	8.13	8.90	22.5	0.137	9.4	5.92	2.01

TABLE 19 (Cont.)

Calf no.	Week	Whole Blood			Plasma			
		RBCC	Hb	RBCV	Ascorbic acid	Ca	Inorg. P	Mg
		$10^6/MM^3$	gm. %	%	mg. %	mg. %	mg. %	mg.%
C-837	1	6.70	9.70	26.0	0.262	11.4	9.17	2.79
	2	7.77	10.07	29.0	0.233	9.3	7.64	2.01
	3	7.90	10.63	28.0	0.144	8.9	5.95	1.87
	4	6.71	10.27	25.5	0.212	8.7	6.59	1.72
	5	6.70	10.87	25.0	0.221	9.0	7.68	2.43
	6	7.78	8.67	21.0	0.186	9.5	6.06	2.27
C-843	1	7.00	8.27	20.5	0.437	10.5	9.07	3.12
	2	8.00	9.10	24.5	0.337	10.0	7.06	2.14
	3	8.87	10.57	28.0	0.490	10.5	7.43	2.22
	4	7.41	9.20	25.0	0.223	9.3	5.85	2.84
	5	10.26	10.43	27.5	0.381	9.2	8.35	2.60
C-828	1	---	10.43	29.0	0.228	10.0	7.22	2.44
	2	7.44	10.50	29.0	0.251	9.9	6.63	2.59
	3	8.68	10.50	29.5	0.157	10.2	6.63	2.00
	4	---	---	---	0.173	9.7	8.44	2.14
	5	7.42	8.97	27.0	0.251	9.6	7.43	1.86
	6	6.61	8.00	24.0	0.179	8.6	5.40	2.00
C-831	1	9.11	11.15	33.5	0.247	9.5	6.82	1.93
	2	8.50	11.60	31.0	0.341	10.3	7.35	2.05
	3	7.56	10.50	29.0	0.277	10.2	7.22	1.71
	4	6.25	10.13	26.5	0.197	9.0	5.71	1.86
	5	8.04	9.20	24.5	0.125	9.8	7.02	2.14
	6	5.45	9.77	25.5	0.160	9.4	6.82	3.12
C-847	1	6.07	7.50	22.5	0.230	9.7	8.77	2.29
	2	7.57	8.27	23.0	0.223	9.0	6.66	3.03
	3	6.75	7.67	21.0	0.254	9.4	7.02	2.22
	4	6.96	8.75	26.0	0.297	9.0	7.06	2.44
	5	6.91	9.33	25.0	0.187	9.0	7.02	1.86
	6	6.89	8.75	25.0	0.194	9.1	6.29	1.95
C-854	1	6.96	9.47	27.0	0.253	11.2	7.82	2.62
	2	8.70	9.03	26.5	0.154	9.6	6.44	2.13
	3	7.58	9.77	25.5	0.114	9.2	6.51	4.31
	4	---	10.00	26.5	0.223	9.3	6.74	2.07
	5	7.85	9.27	25.5	0.207	9.9	7.02	2.07
	6	7.71	8.33	24.0	0.174	9.2	6.82	2.00
	7	8.98	9.07	28.0	0.269	9.8	7.57	2.75

TABLE 19 (Cont.)

Calf no.	Week	Whole Blood			Plasma			
		RBCC	Hb	RBCV	Ascorbic acid	Ca	Inorg. P	Mg
		$10^6/MM^3$	gm. %	%	mg. %	mg. %	mg. %	mg. %
C-840	1	9.49	13.10	36.0	0.107	9.0	6.44	2.60
C-845	1	7.99	11.60	30.0	0.571	11.5	7.43	2.69
C-846	1	7.95	12.00	32.0	0.355	11.3	8.54	2.70
	2	6.59	10.87	31.0	0.249	9.6	5.88	2.14
C-848	1	9.71	13.7	35.0	0.310	10.7	6.21	2.22
	2	9.82	14.45	38.0	0.429	9.2	7.64	3.10
C-849	1	9.51	12.35	34.0	0.294	11.1	7.77	2.69
	2	9.57	12.35	34.0	0.311	9.6	5.88	5.08
C-850	1	6.42	9.47	29.5	0.310	9.6	7.06	2.49
	2	8.46	10.13	34.5	0.258	9.4	6.82	2.40
C-851	1	6.86	9.77	26.5	0.238	9.6	7.02	2.67
	2	7.43	10.07	31.0	0.250	9.3	6.82	2.07
C-855	1	11.37	15.10	48.0	0.258	10.2	9.02	2.52
C-856	1	9.92	16.30	48.0	0.328	9.1	7.64	2.60
C-858	1	7.66	8.33	24.5	0.210	9.5	9.47	2.13
	2	11.65	12.63	35.0	0.458	10.0	12.39	4.41
C-865	No data							
C-866	1	---	12.10	34.5	0.255	9.2	7.47	2.13
	2	---	10.43	29.5	0.184	9.6	8.26	2.14
	3	---	10.20	29.0	0.266	9.3	8.73	2.22
C-867	1	---	13.25	39.0	0.402	8.5	6.44	2.69
C-868	1	---	12.33	33.5	0.274	9.0	6.82	7.79

TABLE 20

FEED CONSUMPTION OF THE EXPERIMENTAL RAT GROUPS

Week	Group A		Group B	
	Lot 1	Lot 2	Lot 1	Lot 2
	gms.	gms.	gms.	gms.
1	117.0	100.0	103.0	95.0
2	156.0	129.0	137.0	116.0
3	182.0	162.0	143.0	138.0
4	180.0	176.0	171.0	138.0
5	177.0	189.0	178.0	136.0
6	155.0	185.0	158.0	122.0
Total	967.0	941.0	890.0	745.0