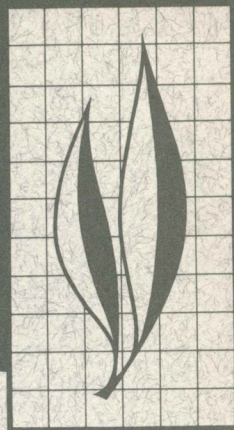


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The Effect of Certain B-Vitamin Supplements on the Survival and Performance of Calves Fed a High-Carbohydrate, Low-Fat Diet

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When milk-replacer diets are being compounded for calves, a problem may be encountered with regard to the source of energy. Foreign fats are not always tolerated well and carbohydrates sometimes present certain limitations.

This report stems from an experience in which dextrose was used as the main source of energy in a diet composed basically of non-fat milk solids. Neuromuscular abnormalities similar to symptoms of thiamine deficiency developed; and as more cases were observed, it became apparent that biotin deficiency symptoms also were probably involved. Further work indicated that neither thiamine nor biotin alone, nor the two in combination, were effective in preventing the syndrome. Finally it was found that an increased intake of several B-vitamins in combination with relatively massive increases of thiamine and biotin offered good protection against development of the symptoms, and was reasonably effective in eliminating the condition once it had developed.

This paper presents detailed descriptions of the characteristic deficiency syndrome and a summary of results of certain treatments that were instituted in attempts to prevent or cure the abnormal condition with B-vitamin therapy.

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The Effect of Certain B-Vitamin Supplements on the Survival and Performance of Calves Fed a High-Carbohydrate, Low-Fat Diet¹

INTRODUCTION

IN SOME preliminary studies to test the acceptability of certain semi-synthetic diets, calves fed a low-fat, high-carbohydrate diet developed symptoms which suggested thiamine deficiency (Ronning and Griffith, 1954). These symptoms did not develop in calves with comparable diets in which the carbohydrate was lower and the fat content higher. This has been confirmed further in more recent work in which portions of the carbohydrate of the diets have been replaced isocalorically with fat (Benevenga and Ronning, 1963).

Cunningham and Loosli (1954a, 1954b) reported that calves, kids and lambs fed purified fat-free diets containing high levels of cerelese developed similar symptoms except when their diets were supplemented with lard or hydrogenated coconut oil. Lambert *et al.* (1954) maintained calves on fat-free diets for 8 to 12 weeks without the development of such severe difficulties, although a milder syndrome, which included hair loss, skin lesions, and retarded growth, was observed. The diets used by these workers involved a somewhat lower carbohydrate content, which was in the form of lactose and higher levels of B-vitamins than those used by

Cunningham and Loosli (1954a, 1954b).

The relatively higher thiamine requirement of animals fed high-carbohydrate diets and the "thiamine-sparing action" of fat are widely accepted (Evans and Lepkovsky, 1929; Stirn *et al.*, 1939).

Since the symptoms encountered in the preliminary studies of Ronning and Griffith (1954) were so strikingly similar to those characteristic of thiamine deficiency (Johnson *et al.*, 1948), it was of interest to attempt to control the problem with thiamine administration rather than with fat supplementation. Further observations, however, soon showed that the dietary deficiency was not a simple one (Ronning and Benevenga, 1960). Through further work, evidence has accumulated which suggests that a complicated B-vitamin deficiency may be involved, with thiamine and, possibly, biotin being of key importance. Detailed descriptions of the characteristic deficiency syndrome observed and a summary of the results of certain treatments instituted in attempts to prevent or cure the condition with B-vitamin therapy, are presented in this paper.

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EXPERIMENTAL PROCEDURE

Male Holstein and Jersey calves were used in this study as they became available from the University herd during the years 1957–1962. After suckling their dams for 2–4 days, they were fed twice daily a semi-synthetic milk containing approximately 14 per cent solids at the rate of 12 per cent of liveweight. The composition of the solids fed was: cerelese, 38 per cent; dried skim, instant powder, 34 per cent; and dried whey, 28 per cent. Vitamin A, 5,000 IU, vitamin D, 500 IU, and 3.3 gm of a salt mixture were added per pound of dry solids. The salt mixture was composed of trace-mineralized salt, 82.5 per cent; MgO, 9.4 per cent; $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 5\text{H}_2\text{O}$, 7.6 per cent; and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.5 per cent. The dry ingredients were mixed with water at 100° F just prior to the feeding. The proximate analysis of the diet as fed is presented in table 1. Early in the study, the calves were fed whole milk for a few days while being taught

to drink, but later they were placed directly on the experimental diet.

Initially, calves were fed the diet without supplements; as soon as specific symptoms appeared, various therapeutic measures were taken in attempts to cure the calves. With later calves, specific oral supplementation was planned, with the objective of preventing the development of symptoms.

The calves were examined carefully at least twice daily, and descriptions of their general condition and symptoms of

TABLE 1
COMPOSITION OF THE HIGH-CARBOHYDRATE, LOW-FAT DIET

Ingredient	Dry matter	Diet as fed (Water added)
	per cent	per cent
Crude protein.....	17.4	2.38
Ether extract.....	0.3	0.04
Nitrogen free extract..	75.4	10.32
Ash.....	6.9	0.94
Total solids.....	13.68



Fig. 1. Calf with ataxia of forelimbs.

any abnormalities were recorded in case histories, abstracts of which are presented in the Appendix. Calves exhibiting the syndrome were kept under observation as long as they survived or until they could be declared normal. Those which did not develop an acute syndrome either spontaneously or in apparent response to prophylactic measures were kept under observation until it seemed reasonably certain that their progress was normal. Occasionally it was necessary to terminate observations because of a shortage in dietary supply or on account of other factors.

Deficiency symptoms. The varied nature of certain abnormal conditions and the apparent susceptibility of the calves to gastroenteric infections complicated diagnosis of the nutritional status of the animals. However, a rather specific characteristic syndrome can be described.

Calves usually exhibited deficiency symptoms after about nine days on the unsupplemented diet, although some became distressed sooner. The most characteristic symptoms reflected two differ-

ent conditions which interfered with locomotion.

One condition was characterized by ataxia, which was especially obvious in the forelimbs (fig. 1). When the calves attempted to walk, their forelegs would buckle, and they would fall to their knees or sometimes pitch forward and fall. In the more severe cases, calves were unable to rise or to stand without aid. In the recumbent position, especially following exhaustion from attempts to walk, the forelegs frequently were extended, with the head held rigidly at an angle higher than normal or retracted along the shoulder. These symptoms were strikingly similar to those associated with thiamine deficiency in calves described by Johnson *et al.* (1948).

The other condition appeared to be paralytic abasia peculiar to the rear legs (fig. 2). In the early stages of development of this symptom, calves walked with a staggering motion of the hind quarters. As the distress became more severe, there appeared to be complete

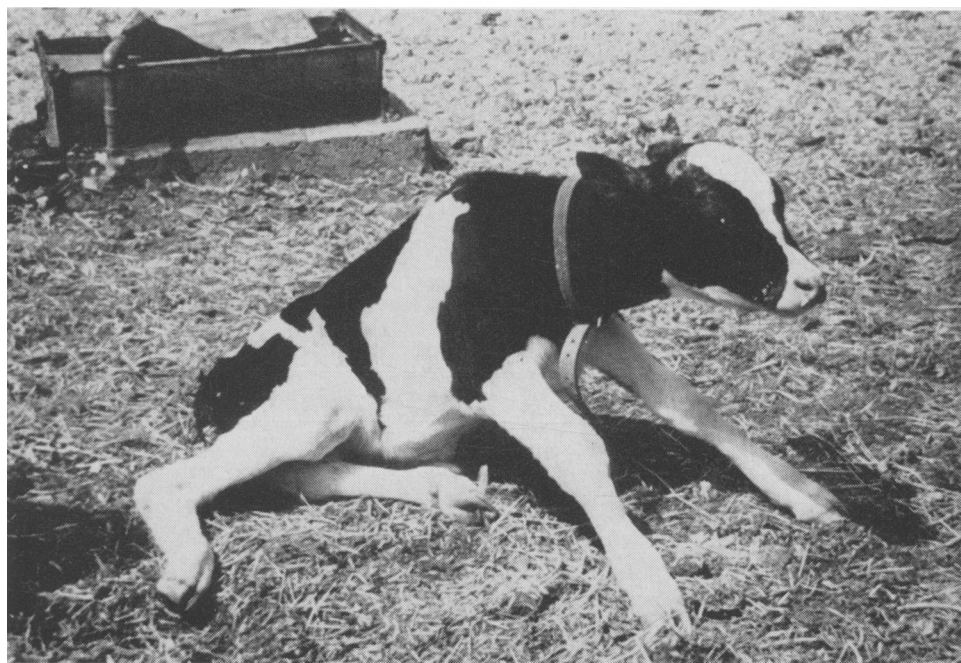


Fig. 2. Calf with ataxia of rear limbs.

paralysis of the hind limbs, so that the calves often ended in a "sitting" position and were unable to rise. This situation appeared to be identical with the description of biotin deficiency by Wiese *et al.* (1946).

Either of these two conditions might be predominant in the early development of the deficiency symptoms; in most cases, however, the two conditions became equally manifest as the calves' distress increased. Eventually the calves became very lethargic; they frequently exhibited a rapid, continual chewing and grinding action of the jaws. At this stage, there appeared to be excessive retention of fluid in the digestive tract. Without effective treatment, survival at this stage was very unlikely.

Anorexia was prevalent and appeared to be associated with dysphagia which, if not too severe, could be alleviated somewhat by allowing the calf to cup its tongue around the feeder's finger while drinking. Two calves in this condition died of pneumonia as a result of the aspiration of fluid into the lungs. As calves became more apathetic, they lost all interest in food.

These calves were very susceptible to diarrhea, which frequently developed into severe or fatal enteritis. Administration of antibiotics in the feed at low levels or in therapeutic amounts did not always satisfactorily control this difficulty. Several post-mortem diagnoses indicated acute gastroenteritis, or terminal pneumonia, associated with the

enteric conditions. No inferences could be drawn as to the cause of the diarrhea. It could have been related to several factors, such as the curdling properties of the semi-synthetic milk, growth of gastrointestinal pathogens due to ideal media and conditions, or it could have been caused simply by decreased resistance as a consequence of dietary stress. Diarrhea frequently seems to be a companion of nutritional deficiencies. At any rate, the prevalence of diarrhea complicated the study. Johnson *et al.* (1948) encountered similar problems when studying thiamine deficiency in calves.

Therapy and supplementation. Treatments resorted to in attempts to cure animals after the development of specific symptoms involved intravenous administration of B-vitamins, singly or in combination. Later, oral administration was resorted to, with the objective of protecting calves from the deficiency. Initially, these treatments were based mainly on trial and error. As more experience was gained, therapeutic measures were preplanned; even then, devious treatments often were resorted to on an "instant-plan" basis in a desperate search for combinations which might relieve acute conditions. The various vitamin mixtures used are identified by number and their contents described in table 2.

The calves fall into four general groups in accordance with intended treatment. In Group I, nine calves were

TABLE 2
VITAMIN SUPPLEMENTS GIVEN TO CALVES

Vitamin	Mix #1*	Mix #2†	Mix #3‡	Mix #5*	Mix #9‡
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml
Thiamine HCl.....	2	26	2.6	20	12.5
Riboflavin.....	1	2.6	1	1.3
Pyridoxine HCl.....	2	2.6	2	1.3
Ca pantothenate.....	2	5.2	2	2.6
Biotin §.....	0.2	0.4	0.04	0.4	0.2
Choline Cl.....	20	1040	20	520.0
Niacin.....	1	1

* Intravenous mixture, in physiological saline.

† Oral mixture in distilled water, administered at a rate of 0.25 ml per 2 pounds of diet.

‡ Oral mixture in distilled water, administered at a rate of 0.25 ml per pound of diet.

§ Dissolved in minimum 95 per cent ethanol prior to combination in final mixture.

fed the experimental diet without supplementation until symptoms developed and judgment could be rendered about possible specific deficiencies. At that time, 10–20 mg thiamine HCl and/or 1–2 mg biotin were administered intravenously via the jugular vein. These amounts were in accordance with those previously reported as adequate to correct specific deficiency symptoms (Johnson *et al.*, 1948; Wiese *et al.*, 1946). The frequency of administration varied, and occasionally additional injections of a B-vitamin mixture were used. This mainly involved mixture #1, which was a combination of thiamine HCl, riboflavin, pyridoxine HCl, Ca pantothenate, biotin, choline Cl and niacin in amounts based on levels used successfully for remission of specific deficiencies of each vitamin (Hopper and Johnson, 1955; Johnson *et al.*, 1948, 1950, 1951a, 1951b; Warner and Sutton, 1948; Wiese *et al.*, 1946, 1947a).

In Group II, three of five calves were from the beginning given oral supplements of vitamin mix #2, which furnished 3.25 mg thiamine and 0.05 mg biotin per pound of diet, amounts approximately ten times those that have been used successfully in purified diets (Wiese *et al.*, 1947b). The diet for the two other calves was supplemented orally with vitamin mix #3, which contained thiamine HCl, riboflavin, pyridoxine HCl, Ca pantothenate, biotin and choline Cl at levels used successfully in synthetic diets by other workers (Wiese *et al.*, 1947b). Intravenous ad-

ministrations of various combinations of vitamins were resorted to frequently in attempts to combat the generally negative response of the animals to the preplanned supplementary regimen.

In Group III, nine calves were fed the diet without supplement until specific symptoms could be diagnosed. At this time the animals were treated by intravenous administration of vitamin mix #5, and daily oral supplementation with vitamin mix #9 was initiated. Mix #5 was the same as mix #1, except for increased concentrations of thiamine and biotin. Mix #9, administered at a rate of 0.25 ml per pound of diet, furnished ten times as much thiamine and biotin—but the same amounts of the other vitamins—as mix #3, which had been administered at a rate of 0.25 ml per 2 pounds of diet to Group II, but without successful results. From time to time, on the basis of the condition of various animals, it appeared advisable to administer additional amounts of mix #5 and, occasionally, single injections of thiamine or biotin.

In Group IV, 15 calves were fed the diet from the beginning, with daily oral supplementation with vitamin mix #9. Deviations from planned treatment often involved adjustments in concentration of specific vitamins in hope of improving the condition of calves which were not responding entirely satisfactorily. At times, when calves did not appear to be adequately protected by the oral supplement, vitamin mix #5, or thiamine and biotin singly, were administered intravenously.

RESULTS AND DISCUSSION

Of 18 calves (Groups I and III) fed the experimental diet without any supplemental B-vitamins, 13 developed the typical symptoms described. Only three (24E, Group I; 18E and 77E2, Group III) survived for significant lengths of time without developing typical, acute symptoms; however, these calves had persistent diarrhea, and frequently ex-

hibited dullness and erratic appetite. Two others did not exhibit typical symptoms but died, probably from pathogenic infections as indicated by autopsy (33E, Group I, pneumonia; and 94E, Group III, generalized microbial invasion).

Some calves in Group I, notably 30E, 34E, and 36E, appeared to respond to

the administration of thiamine and biotin but suffered relapses within 2–14 days. Further treatment of these calves with a mixture of B-vitamins (mix #1) administered at relatively low levels apparently was without benefit. One calf (72E) in this group recovered, and judged on the basis of its performance, its condition may have improved as a result of vitamin therapy. This animal was given a high-level supplement of thiamine and biotin on the fifteenth day of observation, when, for the first time, it was unable to rise. On the following day, the calf appeared more alert but was still weak, and because of poor appetite was given a small amount (1 kg) of whole milk and an injection of B-vitamin mixture #1 intravenously. On the following day, oral supplementation with high levels of thiamine and biotin (mix #2) was initiated. During the next ten days, an additional 2 mg of biotin was administered intravenously, after which the animal improved dramatically. It would appear that this calf benefited materially from the relatively high levels of vitamin administration. The single feeding of milk cannot be discounted as contributing to the calf's improvement, but it seems unlikely that such a small amount would by itself elicit the rather dramatic response observed. Since the calf appeared entirely normal, observations were discontinued in order to conserve the limited supply of diet for completion of critical experiments with other calves.

The oral supplementation used in Group II appeared to be without benefit. All the calves developed deficiency symptoms within 5–11 days. The performance of this group was complicated by infections, three of the calves becoming afflicted with salmonellosis, and one with mixed septicemia.

Of the nine calves in Group III, six developed typical symptoms within 2–12 days, one (94E) died without exhibiting typical symptoms, and two (18E, 77E2) survived and grew without exhibiting acute symptoms. Of the six that

did display typical symptoms, three (17E, 79E2, and 80E2) responded to the intravenous administration of vitamin mix #5 within 2–4 days, but several injections seemed to be necessary before apparent cure was effected. Thereafter, the calves were maintained for significant lengths of time without further complications, supported only by oral supplementation of the diet with vitamin mix #9. One calf (76E2) showed some improvement after treatment was initiated but did not recover its strength and died nine days after initial treatment. Autopsy indicated complications. Another calf (86E) died suddenly without response on the day after vitamin therapy was initiated. The sixth animal (89E) of the treated group seemed to respond but had several relapses, and in general its performance was not encouraging. Modifications of the oral supplement and in the intravenously-administered vitamins did not appear to be beneficial to the animal. It did survive, however, until the forty-first day of observation, when it was sacrificed. Autopsy indicated gastroenteric and pulmonary complications.

Eleven of the 15 calves in Group IV survived and demonstrated reasonable growth. The other four (13E, 24E2, 79E and 81E) died within 7–17 days, and all were shown, on autopsy, to be infected with intestinal pathogens.

At the present stage in this study it is quite clear that when calves are fed this basic low-fat, high-carbohydrate diet, they have little or no chance of developing normally nor, indeed, of surviving. It is equally clear that when the diet is supplemented with a certain mixture of B-vitamins, the performance of the animals is improved materially.

There is evidence suggesting that thiamine and biotin may be of key importance in the supplementary regimen. The symptoms displayed by calves in the deficient state were strikingly similar to those described for calves suffering from these specific vitamin deficiencies. Some deficient animals appeared to

respond to therapy with these two vitamins, although the effect was not sustained.

The mixtures of B-vitamins at normal therapeutic levels used initially and administered intravenously (mix #1) or orally (mix #3), failed to cure or to protect the animals. After increases in the levels of thiamine and biotin in these mixtures, there was, however, curative response when the vitamins were given by intravenous injection (mix #5), and marked protective response when they were orally administered (mix #9). Still, a complicated deficiency is apparent from the limited response to

oral and intravenous administration of thiamine and biotin only, even at high levels. Continuing work (Benevenga, Baldwin and Ronning, 1964) should clarify this interesting nutritional problem. The order of importance and the quantitative definitions of specific members of the B-vitamin mixture now used (mix #9) and the potential modifying effect of fat need to be studied further. Better facilities have decreased complications due to extraneous environmental stresses, and means for biochemical measurements have enabled more specific, objective diagnoses of the deficiency syndrome.

SUMMARY

Calves fed a low-fat, high-carbohydrate diet developed a characteristic syndrome which included anorexia, lethargy, incoordination of the forelimbs, paralysis of the rear legs and, ultimately, death. These calves were very susceptible to diarrhea, which frequently developed into severe and often fatal enteritis.

The symptoms suggested thiamine or biotin deficiencies, but intravenous injections of these vitamins alone at levels

up to 100 mg and 2 mg, respectively, brought about at best only transitory relief. Oral supplementation with very high levels of these two vitamins alone was of no apparent benefit. However, the syndrome appeared to be alleviated by intravenous injection or prevented by oral supplementation with mixtures of relatively high levels of thiamine and biotin in combination with certain other B-vitamins and choline.

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APPENDIX

CASE HISTORY ABSTRACTS

Calf no.	Observations and Treatments
	<i>Group I:</i> Placed on diet at about one week of age; diet not supplemented; B-vitamin therapy as indicated, according to condition of calf observed.
24E	<i>Day 1</i> , 59 lb.; <i>days 1-4</i> : diarrhea, treated with terramycin; <i>day 6</i> : apparent recovery; <i>day 56</i> : intestinal distention; <i>days 58 and 59</i> : diarrhea; <i>days 59-99</i> : poor appetite, intermittent anorexia; <i>day 96</i> : offered hay and grain; <i>day 100</i> : death. The calf gained about 0.5 pound per day. An autopsy revealed hepatic fibrosis, peritonitis, and a small ulcer just distal to the pylorus.
28E	<i>Day 1</i> , 56 lb.; <i>day 12</i> : diarrhea; <i>day 18</i> : uncoordinated, 10 mg thiamine HCl administered iv (intravenously); <i>day 19</i> : bloated, indolent, diarrhea, 1 mg biotin given iv; <i>day 20</i> : extensive fluid retention in the rumen, bloated; unable to stand, stupor; <i>day 21</i> : died.
30E	<i>Day 1</i> , 71 lb.; <i>day 10</i> : weak, uncoordinated, 10 mg thiamine HCl given iv; <i>day 11</i> : 10 mg thiamine HCl, iv; <i>day 12</i> : diarrhea, very morbid, unable to rise, 1 mg biotin given iv; <i>day 13</i> : very much improved, walked normally, only slight ataxia of rear limbs; <i>day 14</i> : weak, diarrhea, treated with terramycin; <i>day 27</i> : regressed, weak, uncoordinated; <i>day 35</i> : lethargic, muscular tremor; <i>day 36</i> : 2 ml vitamin mix #1, iv; <i>day 38</i> : labored breathing, 2 ml vitamin mix #1, iv; <i>day 39</i> : anorexia; <i>day 40</i> : died. Gained about 0.5 pound per day until final week of life, when it lost 10 pounds.
33E	<i>Day 1</i> , 52 lb.; <i>day 8</i> : poor appetite, weak; <i>day 10</i> : diarrhea, poor appetite, treated with terramycin; <i>day 11</i> : condition about the same, treated with terramycin; <i>day 12</i> : anorexia, labored breathing, temperature 98.8, heart rate 100, respiration 58, lungs were very congested, died.
34E	<i>Day 1</i> , 78 lb.; <i>days 10-13</i> : diarrhea, bloat, anorexia; <i>days 31-32</i> : poor appetite, diarrhea treated with terramycin, uncoordinated and lethargic; <i>days 33-38</i> : poor appetite; <i>day 39</i> : A.M., weak, uncoordinated, unable to rise, 1 mg biotin given iv; P.M., diarrhea, bloated, 10 mg thiamine HCl, iv; <i>day 40</i> : A.M., refused diet, very weak, morbid, 20 mg thiamine HCl, iv; P.M., 2 mg biotin, iv; <i>day 41</i> : much improved, up, moving around, stronger, 2 ml vitamin mix #1, iv; <i>day 43</i> : anorexia, 2 ml vitamin mix #1, iv; <i>day 44</i> : anorexia; <i>day 45</i> : dysphagia, stupor, 2 ml vitamin mix #1, iv, calf died. Virtually no gain. Autopsy indicated inhalation pneumonia.
35E	<i>Day 1</i> , 56 lb.; <i>days 10-36</i> : very poor appetite, completely refusing diet on intermittent days; <i>day 39</i> : refused diet, could not rise, uncoordinated, 10 mg thiamine HCl and 1 mg biotin given iv; <i>day 40</i> : anorexia, lethargy, died. Virtually no gain.
36E	<i>Day 1</i> , 87 lb.; <i>days 2-6</i> : poor appetite, slight diarrhea, slight ataxia of rear limbs; <i>day 31</i> : anorexia; <i>day 32</i> : A.M., anorexia, lethargy, uncoordinated, labored respiration, 1 mg biotin given iv; P.M., refused diet, bloated, 10 mg thiamine HCl, iv; <i>day 33</i> : A.M., improved, stronger, could get up and move around; P.M., poor appetite; <i>days 34-38</i> : condition unchanged, ap-

Calf no.	Observations and Treatments
	petite improved, 2 ml vitamin mix #1 and 10 mg thiamine HCl, iv, diarrhea treated with terramycin; <i>day 41</i> : died. Very little gain in weight in first 21 days, then 1 pound per day.
40E	<i>Day 1</i> , 56 lb.; <i>days 6-7</i> : anorexia; <i>day 9</i> : anorexia, ataxia, unable to rise, 10 mg thiamine HCl and 1 mg biotin given iv; <i>day 10</i> : no change; <i>day 11</i> : died. Autopsy indicated possible inhalation pneumonia, ulcerations in fundic area of abomasum and ileum, hemorrhage in pericardium at apex of heart.
72E	<i>Day 1</i> , 55 lb.; <i>day 13</i> : slight anorexia; <i>day 14</i> : indolent; <i>day 15</i> : unable to rise, rear limbs very weak, 2 mg biotin and 100 mg thiamine HCl given iv; <i>day 16</i> : physically weak, more alert, refused diet, gave 1.0 kg milk, 2 ml vitamin mix #1 administered iv; <i>day 17</i> : weak, ataxic, initiated daily oral supplementation with vitamin mix #2, considerable improvement by evening; <i>day 18</i> : much improved; <i>day 22</i> : much stronger, uncoordinated; <i>day 23</i> : 2 mg biotin, iv; <i>day 26</i> : much stronger and frisky, appetite normal; <i>day 27</i> : observations discontinued.
Group II: Placed on diet at about one week of age; diet supplemented daily with oral administration of B-vitamin mix #2 or #3; intravenous treatments with B-vitamins as indicated by progress of each calf.	
73E	<i>Day 1</i> , 58 lb.; given vitamin mix #2; <i>day 11</i> : lethargic, unable to rise, 2 mg biotin, iv; <i>day 12</i> : no change in condition; <i>day 13</i> : much improved; <i>day 14</i> : regressed, lethargic, unable to rise, 2 ml vitamin mix #1, iv; <i>day 15</i> : stupor, 3 mg epinephrin, im (intramuscularly), 5 ml vitamin mix #1, iv; <i>day 16</i> : A.M., continual chewing action of jaws, 5 ml vitamin mix #1, iv; P.M., condition improved; <i>day 17</i> : much improved, stronger; <i>day 18</i> : still weak and unable to rise; <i>day 19</i> : died. Autopsy indicated acute bronchopneumonia, possibly due to inhalation and salmonellosis.
74E	<i>Day 1</i> , 79 lb.; given vitamin mix #2; <i>day 9</i> : anorexia, lethargy; <i>day 10</i> : A.M., anorexia, ataxia, 2 mg biotin and 40 mg thiamine HCl, iv; P.M., unable to rise, 2 ml vitamin mix #1, iv; <i>day 11</i> : much improved, up and able to move around; <i>day 12</i> : anorexia, lethargy; <i>day 13</i> : condition improved; <i>day 14</i> : weaker, 5 ml vitamin mix #1, iv; <i>day 15</i> : condition same; <i>day 16</i> : A.M., lethargic and uncoordinated; P.M., anorexia, 100 mg thiamine HCl administered; <i>day 17</i> : poor appetite, appeared stronger; <i>day 18</i> : same; <i>days 19, 20 and 23</i> : anorexia, appeared weaker; <i>day 24</i> : poor appetite; <i>day 26</i> : anorexia, 100 mg thiamine HCl administered iv; daily oral supplementation changed to vitamin mix #3; <i>day 27</i> : stronger, anorexia, 2 mg biotin, iv; <i>day 28</i> : weak; <i>day 29</i> : poor appetite; <i>day 30</i> : anorexia, stupor, unable to rise, 5 ml vitamin mix #5 given iv; oral supplementation changed to vitamin mix #9; <i>day 31</i> : A.M., able to rise unaided, given 3.5 pounds of milk, and 100 mg thiamine HCl, iv; P.M., poor appetite; <i>day 32</i> : died. Autopsy indicated mixed septicemia and lung congestion.
75E	<i>Day 1</i> , 107 lb.; given vitamin mix #2; <i>day 6</i> : poor appetite, lethargic, 2 ml vitamin mix #1, iv; <i>day 7</i> : indolent; <i>day 8</i> : poor appetite; <i>day 9</i> : A.M., lethargic, 2 mg biotin, iv; P.M., unable to rise; <i>day 10</i> : appears physically stronger but indolent; <i>day 11</i> : A.M., unable to stand, torpid, 2 ml vitamin

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mix #1, iv; P.M., appeared stronger, 2 mg biotin, iv; *day 12*: anorexia, torpid, 5 ml vitamin mix #1 given iv; *day 13*: died. Autopsy indicated salmonellosis.

76E *Day 1*, 75 lb.; given vitamin mix #3; *day 6*: poor appetite; *day 7*: A.M., very weak and indolent, uncoordinated, 100 mg thiamine HCl, iv; P.M., unable to rise, poor appetite, 2 mg biotin, iv; *day 8*: died. Autopsy indicated salmonellosis.

77E *Day 1*, 57 lb.; given vitamin mix #3; *day 5*: poor appetite, lethargic; *day 6*: A.M., weakness and ataxia of rear limbs, 100 mg thiamine HCl, iv; P.M., poor appetite, indolent; *day 7*: died. No autopsy.

Group III: Placed on diet at 2 to 4 days of age, not supplemented; as symptoms developed, each calf was treated with vitamin mix #5, iv; this was followed by oral supplementation daily with vitamin mix #9.

17E *Day 1*, 67 lb.; diarrhea, poor appetite; *day 2*: A.M., extremely weak, unable to rise, 5 ml vitamin mix #5 given iv; P.M., weakness and ataxia of rear limbs, seemed alert; 2 ml vitamin mix #5, iv; initiated daily oral supplementation with vitamin mix #9; *day 3*: very much improved, tried to get up by itself, rear limbs still ataxic; 5 ml vitamin mix #5 given iv; *day 4*: stronger, able to rise; *day 5*: much improved; *day 13*: biotin removed from oral supplement; *day 28*: trial ended, calf looked normal, was alert, playful. Gained nearly 0.75 pound per day.

18E *Day 1*, 89 lb.; *days 1-8*: poor appetite, lethargic; *days 8-28*: nearly normal, frequent diarrhea, trial ended. Overall gain nearly 1 pound per day, most weight gained in the final two weeks of trial.

76E2 *Day 1*, 100 lb.; *days 1-6*: indications of diarrhea; *day 6*: A.M., diarrhea, P.M., indolent; *day 7*: A.M., unable to rise, 5 ml vitamin mix #5 given iv; P.M., seemed improved, initiated daily oral supplementation with vitamin mix #9; *day 8*: seemed stronger, 5 ml vitamin mix #5 given iv, both A.M. and P.M.; *day 9*: still weak but able to rise, 5 ml vitamin mix #5 given iv; *day 10*: poor appetite; *days 11-13*: condition about the same; *day 13*: rumen distended, indolent; *day 14*: same, noted continuous grinding of teeth; *day 15*: A.M., weaker, had difficulty in getting up, 5 ml vitamin mix #5 given ip (intraperitoneally); P.M., condition same, 5 ml vitamin mix #5, iv; *day 16*: stupor, labored breathing, died. Autopsy indicated fibrinous pneumonia, ulcerations in the posterior ventral compartment of rumen, and the fundic portion of abomasum.

77E2 *Day 1*, 69 lb.; *day 5*: slightly weak; *day 9*: slight ataxia; *days 10-12*: alert, diarrhea; *days 13-17*: voluminous liquid diarrhea; *days 18-19*: indolent, increased ataxia of rear limbs; *day 20*: indolent, diarrhea; *day 21*: improved, more alert; *days 22-27*: marked improvement, more active; *days 28-35*: gradual regression; *day 35*: lethargic condition due to diarrhea; *days 36-39*: continual improvement; *days 40-80*: appeared normal in all respects; *day 80*: trial ended. Gained about 0.5 pound per day, but gain was variable from week to week.

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- 79E2** *Day 1*, 88 lb.; *day 9*: lethargic, gaseous distention in lower intestinal tract; *day 10*: unable to rise, 5 ml vitamin mix #5 given iv, at 5 P.M. and 10 P.M.; *day 11*: A.M., anorexia, indolent, 5 ml vitamin mix #5 given iv; P.M., poor appetite, condition much improved, able to rise, initiated daily oral supplementation with vitamin mix #9; *day 12*: poor appetite, 5 ml vitamin mix #5 given iv, still weak; *day 13*: improved, much stronger, 5 ml vitamin mix #5, iv; *day 14*: same; *day 15*: A.M., same; P.M., anorexia; *day 16*: started giving 4 pounds of water at noon daily; *days 17-19*: much improved, stronger; *days 20-21*: partial anorexia; *days 22-52*: continual improvement, appeared nearly normal, exhibited light intermittent diarrhea; *day 52*: trial ended. Overall gain about 0.25 pound per day, but lost weight through day 26; from days 27 through 50 gained about 0.5 pound per day.
- 80E2** *Day 1*, 91 lb.; *day 3*: diarrhea; *day 6*: started giving about 4 pounds of water at noon daily; *day 7*: weak; *day 9*: A.M., indolent, unable to rise, poor appetite, 5 ml vitamin mix #5 given iv; P.M., bloated, 5 ml vitamin mix #5 given iv; initiated daily oral supplementation with vitamin mix #9; *day 10*: A.M., poor appetite, 5 ml vitamin mix #5, iv; P.M., stupor; *day 11*: A.M., lying down with head retracted, forelimbs rigidly extended; A.M., and noon, 10 ml vitamin mix #5, ip; P.M., 5 ml vitamin mix #5, iv, poor appetite, appeared stronger; *day 12*: A.M. and P.M., 5 ml vitamin mix #5, iv; *days 13-45*: condition improved, gained strength, appeared normal; *day 45*: trial ended. No gain in weight; lost weight through day 25 and recovered weight loss by day 45.
- 86E** *Day 1*, 82 lb.; *days 8-11*: diarrhea, poor appetite, becoming weak, exhibited difficulty rising; *day 12*: A.M., diarrhea, indolent, unable to rise, 5 ml vitamin mix #5 given iv; *noon*, condition worse; P.M., anorexia, 5 ml vitamin mix #5, iv; *day 13*: died.
- 89E** *Day 1*, 85 lb.; *day 2*: inadvertently given vitamin supplement; *days 6-7*: indolent, poor appetite; *day 8*: initiated daily oral supplementation with vitamin mix #9, modified by doubling riboflavin and pyridoxine concentration; *day 9*: indolent, ataxic; A.M., and P.M., 5 ml vitamin mix #5 given iv; P.M., slightly improved; *day 10*: A.M., anorexia, 100 mg thiamine HCl, iv; *noon*, 2 mg biotin, iv, 1 mg epinephrine, im; P.M., stupor, 5 ml vitamin mix #5, iv; *day 11*: A.M., slight improvement, 5 ml vitamin mix #5, iv, A.M. and P.M.; P.M., condition worse, administered 1 mg epinephrine, im; *day 12*: A.M., improved, 5 ml vitamin mix #5 given iv; P.M., poor appetite, 4 mg biotin, iv; *day 13*: condition same, 5 ml vitamin mix #5, iv; *day 14*: A.M., condition improving, 5 ml vitamin mix #5, iv; P.M., much stronger, able to rise and walk around; *days 15-20*: continued improvement; *days 21-23*: slight regression; *day 24*: anorexia, extremely weak, doubled biotin and oral supplementation; *day 25*: poor appetite, barely able to rise; *day 26*: voluminous diarrhea; *day 27*: much weaker, unable to rise; *days 28-30*: indolent, continued poor appetite; *days 31-38*: general improvement; *day 39*: continual grinding of teeth; *day 41*: sacrificed. Autopsy indicated subacute gastroenteritis, mild inflammation of mucosa of small intestine, small abscess of the right cardiac lobe of the lung; lost about 0.3 pound per day.
- 94E** *Day 1*, 95 lb.; *day 11*: A.M., received vitamin supplement inadvertently, difficulty in rising; P.M., anorexia; *days 14-16*: lethargic; *day 22*: diarrhea,

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frisky; *day 27*: died. Autopsy indicated generalized microbial invasion especially in the kidney, liver, spleen; hyperplasia of lymphoid tissue, small intestine, and bile duct epithelial cells. Average rate of gain nearly 1 pound per day.

Group IV: Placed on diet at 2 to 4 days of age; diet supplemented daily with vitamin mix #9, given orally; modifications as indicated by progress of each calf.

- 12E** *Day 1*, 88 lb.; *days 6–10*: poor appetite, dull, occasionally grinding teeth; *days 10–39*: improved, did very well, strong, alert; *day 40*: sacrificed. Autopsy indicated good general condition, hemorrhage of pulmonary arterial wall, brown fat in retroperitoneal area but not in omentum. Gain overall about 0.8 lb. per day, over 1.0 lb. per day last two weeks of trial.
- 13E** *Day 1*, 86 lb.; *day 2*: diarrhea, persistent until death, treated periodically with terramycin; *day 8*: indolent, unable to rise; *days 8–10*: treated with 1 gm choline chloride, iv; *day 11*: A.M., anorexia; P.M., 10 mg thiamine HCl, iv; *day 12*: improved, 5 mg pyridoxine HCl, iv, A.M. and P.M.; *days 13–14*: improved, 5 mg pyridoxine HCl, iv, A.M. and P.M.; *day 15*: diarrhea, treated with terramycin A.M. and P.M.; *day 16*: voluminous diarrhea, treated with terramycin, lethargic; *day 17*: died. Autopsy indicated salmonellosis.
- 14E** *Day 1*, 100 lb.; *day 7*: diarrhea, frisky; *days 7–41*: apparently normal in all respects; *day 41*: trial ended. Overall gain about 1.25 pounds per day.
- 15E** *Day 1*, 55 lb.; *days 1–35*: apparently normal in all respects; *day 35*: sacrificed. Autopsy indicated good general condition, hemorrhage of pulmonary artery wall, brown fat in retroperitoneal area but not in omentum, renal pelvic fat normal; gain overall about 0.5 pound per day.
- 19E** *Day 1*, 69 lb.; *day 2*: A.M., poor appetite, unable to rise, 10 mg pyridoxine HCl given iv, increased pyridoxine concentration of oral supplement to 12.5 mg per ml; P.M., anorexia, diarrhea; *day 3*: slight improvement; *day 4*: A.M., regressed, 1 mg epinephrine, im, 5 ml vitamin mix #5, iv; P.M., poor appetite; *day 5*: poor appetite, indolent, diarrhea, given terramycin A.M. and P.M.; *day 6*: A.M., too weak to move; P.M., poor appetite, 5 ml vitamin mix #5, iv; *day 7*: A.M., much improved, 5 ml vitamin mix #5, iv; P.M., more improvement, tried to get up, 5 ml vitamin mix #5, iv; *day 8*: A.M., continual improvement, 5 ml vitamin mix #5, iv; P.M., able to get up and walk around, weak, 2.5 ml vitamin mix #5, iv; *days 9–13*: rapid, marked improvement, regained original strength, alert; *day 14*: removed oral supplement from diet; *days 15–26*: appeared entirely normal, frisky; *day 26*: trial ended. Overall daily gain about 0.1 pound; however, had lost much weight during first week; during final two weeks of the trial gained about 1.0 pound per day.
- 20E** *Day 1*, 63 lb.; A.M., pyridoxine concentration of oral supplement increased to 12.5 mg per ml; P.M., diarrhea, poor appetite; *days 2–3*: lethargic, unable to rise, intermittent shivering; *day 5*: improved; *day 6*: much improved, able to rise; *day 8*: pyridoxine supplement reduced to original level; *day 10*: removed biotin from oral supplement; *days 11–27*: apparently

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- normal in all respects; *day 27*: trial ended. Overall gain about 0.4 pounds per day, considerable loss during the first week, about 1.0 pound per day gain during the last two weeks.
- 23E** *Day 1*, 63 lb.; A.M., diarrhea, treated with terramycin; P.M., indolent; *day 3*: A.M., very weak; P.M., doubled dose of oral supplement; *days 4-5*: improved, near normal; *day 7*: oral supplement reduced to original level; *days 8-23*: apparently normal in all respects; *day 23*: trial ended. Overall gain about 0.4 pound per day, no gain in first week.
- 24E2** *Day 1*, 61 lb.; *day 2*: severe diarrhea; *day 3*: unable to rise, doubled dose of oral supplement; *days 4-13*: poor appetite, condition generally poor, sporadic improvements, several administrations of 2.5 to 5 ml vitamin mix #5, iv; *day 14*: died. Autopsy indicated salmonellosis.
- 79E** *Day 1*, 88 lb.; *day 3*: A.M., indolent, ataxic; P.M., poor appetite, lethargic; *day 4*: improved, still weak; *day 5*: A.M., lethargic; P.M., poor appetite; *day 6*: slight improvement; *days 9-12*: much improvement, apparently normal; *day 13*: died. Autopsy indicated granulomatous nephritis.
- 80E** *Day 1*, 58 lb.; *day 5*: becoming weak, ataxic; *days 6-8*: anorexia; *day 12*: indolent; *days 15-21* and *day 27*: diarrhea; *day 31*: trial ended. Overall gain about 0.5 pound per day.
- 81E** *Day 1*, 52 lb.; *day 3*: anorexia, lethargic; *day 5*: A.M., indolent, ataxic; P.M., unable to rise, anorexia; *day 6*: anorexia; *day 7*: died. Autopsy indicated septicemia.
- 87E** *Day 1*, 92 lb.; *day 10*: slight diarrhea; *days 19-20*: weakness in rear limbs; *days 22-40*: appeared normal; *day 41*: removed from trial. Overall gain about 0.5 pound per day, lost weight during first two weeks of the trial.
- 88E** *Day 1*, 96 lb.; *days 1-2*: indolent; *day 9*: lethargic, weakness in rear limbs; *day 10*: A.M., unable to rise, 100 mg thiamine HCl given iv; noon, 2 mg biotin, iv; P.M., poor appetite, 5 mg vitamin mix #5, iv; *day 11*: much improved, able to rise; *day 12*: condition about same, weakness in rear limbs, 5 ml vitamin mix #5 and 2 mg biotin, iv; *days 13-22*: steady improvement of condition and increasing incidence of diarrhea; *day 24*: doubled level of riboflavin, pyridoxine and biotin in oral supplement; *days 25-38*: condition appeared normal but variable; *day 39*: lethargic; *day 40*: unable to rise, ataxic; *days 41-44*: steady improvement in condition; *days 45-53*: apparently normal; *day 53*: trial ended. Overall gain 0.5 pound per day, virtually no gain to day 17, then gained about 0.75 pound per day.
- 90E** *Day 1*, 92 lb.; *days 9-19*: lethargic; *days 20-28*: seemingly normal; *day 29*: becoming weak; *day 30*: poor appetite, lethargic; *days 31-38*: intermittent diarrhea, indolent; *day 38*: trial ended. Overall gain about 0.75 pound per day.
- 92E** *Day 1*, 88 lb.; *days 8-12*: indolent, variable appetite; *days 13-15*: improvement in condition; *day 16*: A.M., very weak; P.M., doubled concentration of riboflavin, pyridoxine, and biotin in the oral supplement; *day 17*: weak, very slow eating; *days 18-26*: general improvement in condition, gained strength, ate slowly; *day 27*: P.M., anorexia; *days 28-41*: appeared stronger, slight diarrhea, ate slowly; *day 42*: diarrhea worse, condition weakening; *days 43-44*: about same, treated with enterfur in two doses for diarrhea; *day 45*: trial ended. Overall gain about 0.5 pound per day, virtually no gain from days 10 to 17.

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