THE EFFECT OF PYRIDOXINE DEFICIENCY ON GASTRIC SECRETION

BY

R. Armour Forse B. Sc., M.D., C.M.

National Research Council Fellow in Experimental Surgery and Demonstrator in Physiology.

McGill University, Montreal, Canada.

A McGill University thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

AUGUST 1950.

CONTENTS

PREFACE

This manuscript is the product of a year spent in research in the Experimental Surgical Laboratories at McGill University. It covers primarily the effect of pyridoxine deficiency on gastric secretion and blood in the dog, and the effect of Vitamin B12, dessicated liver and meat on a later deficiency syndrome. To investigate this problem it was necessary to compose a new synthetic diet and design a new gastric pouch. The experimental work and several of the methods used are original. The first part of this material has been accepted for presentation in abstract form at the Forum on Fundamental Surgical Problems during the Thirty-Sixth Annual Clinical Congress of the American College of Surgeons in Boston, Massachusetts, October 23rd to 27th, 1950.

I would like to thank Dr. Gavin Miller for giving me the opportunity to study and do research for a year in the experimental surgical laboratories at McGill University.

It is with great pleasure and sincere appreciation that I thank Dr. D. R. Webster for his

supervision, encouragement, constant interest, and advice during the course of this investigation.

I am indebted to Miss Hope Thompson for her technical assistance both in biochemistry and hematology, and to Dr. T. R. Waugh for allowing me to use his equipment.

I wish to thank Miss Carol Phillips and Mr. Paul Rouston for the innumerable services which they so cheerfully performed throughout the year, also Miss Helen Ward and Mrs. Anne Nolan for the typing of this manuscript.

It was a pleasure to work in the same laboratory with Dr. Donald Currie, Dr. Andrew Chou, Dr. Stanley Skoryna, Dr. Douglas Miller and Mr. Huntley Millar and I will remember my association with them as a happy one.

R. A. F.

ILLUSTRATIONS

CHAPTER 1

INTRODUCTION

The search for a greater knowledge of the physiology and biochemistry involved in gastric secretion has advanced very slowly for many years. Many of the possible roads of investigation have been blocked waiting for progress in special fields. However, much investigation can be done using the present knowledge to help elucidate the extremely difficult but important problems.

Any advancement in the knowledge of gastric secretion would be a valuable step forward in the understanding of this complex function and might be used to great value in many important problems including that of peptic ulcer and combined systems disease. The etiology of peptic ulcer is still unknown in spite of the very considerable investigation and wide observation conducted for many years on this common pathological condition. Theories have been advanced which variously involve hypermotility, hyperacidity, various peptic enzymes, end-artery thrombosis or spasm, body type, allergy, focal infections and

psychic trauma. However, it is probable that any one of these possible factors can be disproved in a series of ulcer patients and it is obvious that to understand a pathological state we must first know the normal state.

It was shown by Webster and Armour in 1933 (199) that the vitamin B complex was essential for the normal production of gastric secretion. Street, Cowgill, & Zimmerman, in 1941 (183) obtained suggestive evidence that the component, pyridoxine was essential for the normal production of gastric secretion. On the basis of this work it was decided to investigate the effect of pyridoxine deficiency on gastric secretion.

The investigation of the effect of pyridoxine deficiency on gastric secretion is a difficult problem. The accurate study of such a problem necessitates first obtaining healthy pouch dogs, secondly, getting these dogs deficient only in pyridoxine, and thirdly, the development of the deficiency state in the desired time. In order to study both phases of gastric secretion, at least some of the dogs must have innervated gastric pouches.

There are several methods of making innervated gastric pouch dogs (138, 4, 94, 31, 186). The method described by Cope seemed the most applicable from many points of view. However, we found this technique led to many complications and it soon had to be abandoned. The other methods possessed one or more of the many inadequacies observed in the method described by Cope, so it was necessary to design a new gastric pouch.

The second major problem was to develop a pyridoxine deficiency state in these pouch dogs. There have been many synthetic diets used to develop controlled pyridoxine deficiency states, but none recently using gastric pouch dogs. Thus it was necessary to compose a new synthetic diet, which was based on the diet used by Seeler and Silber (159). Many changes were made in this diet. The bone ash was replaced by the mineral mixture, which was increased to compose 5% of the diet. The mineral mixture was that recommended by Jones and Foster (97). The vitamin supplement was increased and all the known components of the vitamin B complex were added whether or not deficiency states had been described in dogs.

The third major problem was to accelerate the development of the pyridoxine deficiency state, so that the investigation could be carried out within a year. Three important steps were taken to achieve this end. The youngest dogs possible were used that would survive the major operation and could be trained on the stands. These dogs were prone to develop distemper and this necessitated developing a technique of immunization under the existing circumstances to prevent this complication. A high percent of protein was fed in the basal diet to accelerate the deficiency state (117, 120). The analogue desoxypyridoxine has been shown to accelerate the pyridoxine deficiency state (133, 49, & 123). Thus, it was decided to use the analogue desoxypyridoxine together with a pyridoxine deficient diet to accelerate the deficiency state in one series of the animals.

The investigation of the effect of pyridoxine deficiency on gastric secretions was carried out on five series of dogs.

Series 1. Seven dogs with Heidenhein or Cope pouches.

Series 2. Four dogs with the newly designed gastric pouches. This series was divided into four groups.

Group 1. A control dog fed the basal diet.

Group 2. Three dogs fed the pyridoxine free diet and desoxypyridoxine.

Group 3. Two dogs fed the pyridoxine diet alone.

Group 4. One dog fed the basal diet and desoxypyridoxine.

CHAPTER 11.

HISTORY

The following historical review concerning the vitamin B complex and its known role in gastric secretion, the components of the vitamin B complex and their known role in gastric secretion, pyridoxine, its isolation synthesis and the pyridoxine deficiency syndrome, desoxypyridoxine, gastric pouches, and vitamin B12 is not considered to be complete. The reports included are intended rather to bring out the important discoveries in each subject to date. The conclusions from the historical review are drawn up at the end of each section.

A. The Vitamin B Complex and Its Known Role in Gastric Secretion.

In 1915 McCallum and Davis (125, 126) demonstrated the necessity in a synthetic diet of an unknown water soluble accessory required for growth or prolonged maintenance of rats in addition to the fat soluble vitamin, previously described by them.

Voegtlin and Lake in 1918 (193), Karr in 1920 (99), and Cowgill in 1921 (33), observed

anorexia in cats and dogs with polyneuritis. Voegtlin and Meyers (193) later observed polyneuritic pigeons would void a large amount of intensely bile stained material when given water soluble B vitamin preparation and developed the hypothesis that the vitamin was concerned with stimulation of the digestive glands. They suggested the identity of vitamin B and secretin, but this could not be confirmed by Cowgill and Mendel (34).

Miyadere in 1921 (118) found a diminution of the secretion from a Pavlov pouch dog on a vitamin A and B free diet. As his experiment advanced, alcohol became a stronger gastric stimulant and rice and albumen a weaker gastric stimulant.

Danysz, Michel and Koskowski in 1922 (41) studied groups of pigeons on special diets. They found that the group fed polished rice developed a marked decrease in the volume and acidity of gastric secretion combined with almost a complete loss of peptic activity.

Cowgill, Deuel and Smith in 1925 (35) stated that the restorative action of vitamin B on

the appetite is not due to stimulation of gastric secretion, but they made no investigation of the stomach secretion.

Cowgill, Deuel and Plummer in 1926 (36) fed dogs a vitamin B deficient diet and they developed anorexia. In the mild cases of deficiency there was no remarkable change in gastric motility, but severe cases developed gastric atony. They were able to demonstrate rapid improvement by adding vitamin B to the diet.

In 1926, Farnum (53) found that feeding a beri-beri producing diet to a dog with a Pavlov pouch gradually decreased the total volume, as well as the free and the total acidity of gastric secretion. He also found that the gastric secretory mechanism of the beri-beri dog was not only less responsive to the partaking of food, but it was also appreciably more refractive to histamine hydrochloride injection. The gastric response after gastrin was modified only slightly, if at all. The composition of the diet offered these animals is not reported and Babkin in 1933 (7) states that the data is not quite conclusive, because of the great

variation in the secretory response of the pouch to histamine.

Rose, Stucky and Cowgill in 1929 (146) confirmed the disturbances of gastric motility in dogs suffering from vitamin B deficiency. They presented evidence to suggest that the water intake of the organism may be one of the factors which affects the muscular contractions of the stomach. In 1930 (147) these investigators showed the tendency to anhydraemia, whether occurring in a vitamin B deficient animal or its control, ran more or less parallel with a decrease in gastric hunger contractions. Dogs deprived of water for six days developed complete gastric atony. The atony ran more or less parallel with the anhydraemia that ensued. Administration of fluids produced a rapid and remarkable return of vigorous hunger contractions.

Gildea, Kattwinkel and Castle in 1930 (65) placed dogs on a vitamin B deficiency diet and found that there was no change in the secretory activity of the stomach when tested at intervals of a few days with subcutaneous injection of 0.5 mgm. histamine.

This work is of little, if any, value because pouch dogs were not used and a single sample of gastric juice was aspirated for examination.

Webster and Armour in 1933 (197) realized that all the results obtained by previous experiments were complicated by loss of appetite and inanition in the experimental animals, which factors might greatly affect the response of the gastric glands. They prepared three dogs with oesophagotomy gastric fistula and obstructed pylorus, and maintained them for several months on duodenal feedings. This was an excellent means of removing the complicating factors of loss of appetite and inanition present in all the previous experimental work. They removed all vitamins from the dogs' diet and noted first a diminution and then complete cessation of gastric secretion. The secretory function of the gastric mucosa returned within a few days after 10 grams of powdered yeast was added to the diet. Hence, the vitamins in yeast are necessary for the normal secretory activity of the gastric mucosa.

CONCLUSIONS

1. The vitamin B complex is an essential component of diets for animals.
2. There is no relation between vitamin B and secretin.
3. Danysz, Michel and Koskowski were the first to observe a reduction in gastric secretion in vitamin B deficiency.
4. The vitamin B complex is essential for the following:
   (a) Normal appetite
   (b) Normal gastric motility
   (c) Normal gastric secretion - both volume and acidity.
5. Anhydraemia in normal or deficient animals will result in gastric atony.
6. Webster and Armour removed the complicating factors in previous experimental work and proved the vitamins in yeast were essential for normal gastric secretion.

B. The Components of the Vitamin B Complex and their Known Role in Gastric Secretion.

Chick and Roscoe in 1927 (29) described experiments with young growing rats which confirm the views held by many investigators, especially Goldberger and his colleagues, that the water soluble vitamin contained two constituents. They suggest calling these the antineuritic vitamin and (Goldberger's P.P. factor) vitamin B. They soon became known as vitamin B1, and B2 or G.

In 1932 Sure (185) found no relation between the failure in growth and the incidence of pellagra-like symptoms in the rat. He thus suggested that vitamin B2 (G) was composed of two dietary essentials.

Cowgill and Gilman in 1934 (37) demonstrated in three out of four dogs, a diminished response of the gastric glands to stimulation with histamine during the state of deficiency in vitamin B. One animal showed no other sign of vitamin B deficiency than anorexia, and yet there was a marked decrease in the gastric secretion. They then studied a group of dogs on the Goldberger 195 (68) diet,

presumably deficient in vitamin G (B2) and demonstrated no loss of gastric function. However, in view of the uncertainties of the Goldberger diet, this component could not definitely be excluded. They concluded that the diminution of gastric secretion was due to the absence of the antineuritic or B1 component.

Gyorgy in 1934 (75) and Harris in 1935 (80) separated vitamin B2, the antidermatitis factor, into two components which they called the real vitamin B2 (flavin) and vitamin B6.

Then Birch, Gyorgy and Harris in 1935 (17) differentiated the anti-black tongue and the pellagra preventive factor from lacto-flavin and vitamin B6 (the so-called rat pellagra factor). Lepkovsky, Jukes and Krause in 1936 (106) published an excellent paper showing the dual nature of the third factor.

In 1937 Street (180) produced canine black-tongue with a synthetic diet and cured it by adding autoclaved yeast to the diet. It was characterized by anorexia, progressive decrease in weight, reddening of the oral mucosa and persistent diarrhoea.

Later, Street and Cowgill (181) cured canine black-tongue with nicotinic acid and Fouts, Helmer and Lepkovsky (55) cured human pellagra with nicotinic acid.

Fouts, Helmer, Lepkovsky, and Jukes in 1938 (56) produced vitamin B6 deficiency in dogs and demonstrated the typical hypochromic microcytic anemia which they cured with the extract left in the process of polishing rice. It was not until 1940 that Borson (20) demonstrated the relief of hypochromic microcytic anemia in vitamin B6 deficiency with synthetic vitamin B6.

Sebrell in 1938 (158), Street in 1939 (182) and Axelrod, Lipton and Elvehjem in 1940 (5) produced riboflavin deficiency in dogs. This was characterized by a sudden onset of weakness with ataxia and a varying degree of spasticity. There is bradycardia and a slowing of respiration with the development of coma in one hour and death in less than twelve hours.

McKibbin, Madden, Black and Elvehjem in 1939 (128) emphasized the importance of vitamin B6 and another factor W found in liver filtrate in the nutrition of the dog. On a diet deficient in this factor, the pups developed rapidly anorexia, decrease in weight, dermatitis and death.

In 1940 Fouts, Helmer and Lepkovsky (59) showed that adult dogs fed a synthetic diet supplemented with thiamine, riboflavin, nicotinic acid, and factor 1 (B6) developed a deficiency syndrome characterized by anorexia, decrease in weight, intermittent diarrhoea, moderate anemia, and death. This could be prevented by factor 11 in purified liver extract.

McKibbin, Black and Elvehjem in 1940 (129) presented evidence for the necessity of pantothenic acid and factor W in the nutrition of the dog. They demonstrated a growth plateau with a growth response on adding pantothenic acid to the diet. Daft, Sebrell, Babcock and Jukes in 1940 (39) reported the development of adrenal haemorrhage and necrosis in rats on a diet deficient in pantothenic acid and they prevented this condition by adding pantothenic acid to the diet.

Martin, Thompson and De Carrejel Forero in 1941 (114) concluded that inositol had a definite action on the stomach and small intestines of mice.

Schaefer, McKibbin and Elvehjem in 1941 (153) showed dogs developed growth plateaux, anorexia, and loss of weight on a choline deficient diet and they prevented this condition by adding choline to the diet.

In 1941 Morgan (119) demonstrated the effect of imbalance in the filtrate fraction of the vitamin B complex in dogs. The dogs that survived grew moderately well, but exhibited gradual depigmentation of the hair, lack of activity and elderly behaviour.

Schaefer, McKibbin and Elvehjem in 1942 (154) in studying the vitamin B complex and the nutrition of the dog demonstrated a deficiency syndrome characterized by anorexia and decrease in weight that was curable by liver extract, but not by para-aminobenzoic acid and glutamine. However, para-aminobenzoic acid is believed to be an anti grey hair factor. (Harrow 86)

In 1943 Lambooy and Nasset (102) demonstrated the inadequacy of the eight synthetic B vitamins for the nutrition of puppies. They fed young dogs a synthetic diet with only the known components of the vitamin B complex and the dogs developed varying degrees of dermatitis, loss of hair and a general unhealthy skin and coat in 75 to 125 days, and died in 100 to 150 days. This could be prevented by feeding dry whole yeast or a concentrate of yeast or liver.

Fouts, in 1943 (61) reported the development of cirrhosis of the liver in dogs fed a synthetic diet and the disappearance of the signs of the deficiency but not the fibrosis of the liver with the combined administration of large amounts of choline and powdered liver.

According to Elvehjem in 1948 (48) biotin and folic acid are not necessary for the nutrition of the dog. However, biotin has been found to be an anti-egg white injury factor and folic acid a growth factor for chicks.

During the period when most of the known components of the vitamin B complex were being isolated, there was very little research done on their effect on gastric secretion.

In 1939 Voit and Arnold (194) studied the effect of long administration of vitamin B1 in humans and found no change in either hyper or anacidity. This was not borne out by Papp (135) who found a pronounced increase in volume and concentration of gastric juice by one injection of vitamin B1.

Dyer and Roe in 1939 (46) using a new technique to study the relation of nutrition to gastric function in rats found that acute or chronic B1

deficiencies have no pathological effect on the gastric secreting cells. A diminished response to gastric stimulation did occur but only in moribund animals and this was promptly overcome by B1 therapy.

Wood, Splatt and Maxwell in 1942 (203) studied the effect of administering vitamin B1 to patients with hypo and anacidity and found no consistent change in acidity, tone or peristalsis as a result of injection of vitamin B1.

In 1941 Street, Cowgill and Zimmerman (183) maintained dogs on a vitamin B6 deficient diet, and when they developed the typical hypochromic microcytic anemia they analyzed the gastric juice. These analyzes gave a suggestion that in vitamin B6 deficiency there may be a decrease in the secretion of acid and possibly in the volume of juice secreted.

Lecoq, Chauchard and Mayoue, in 1945 (104) found vitamin B complex effective in relieving the symptomatology of acidosis and alkalosis but none of the known components of the vitamin B complex had the same effect.

Allison in 1945 (1) found in skin diseases associated with vitamin B complex deficiency that there was also a deficiency of hydrochloric acid and

that treatment with hydrochloric acid and vitamin B complex together was the superior treatment.

In 1948 Bradford, Davies, Ellis and Hughes (22) found the concentration of co-zymase and total nicotinic acid greater in cat oxyntic cells than the gastric cells. This fact was used by Paterson and Stetten in 1949 (136) in their explanation of the origin of the H-ions by the enzyme systems.

CONCLUSIONS

1. Eleven components of the vitamin B complex have been isolated.
2. Eight of the known components of the vitamin B complex are essential for the nutrition of the dog.
3. There is an unknown factor or factors in the vitamin B complex still unidentified.
4. There is evidence that this unknown factor or factors is essential in the nutrition of the dog.
5. At least one of the components of the vitamin B complex is essential for the normal production of gastric secretion.
6. The evidence indicates that vitamin B1 is not essential for the normal production of gastric secretion.
7. There is suggestive evidence to indicate that vitamin B6 is essential for the normal production of gastric secretion.

C. Vitamin B6 - Pyridoxine

The richest sources of pyridoxine are certain vegetable fats, yeast, wheat germ, legumes, meat, and meat products (21). Pyridoxine has a low toxicity and doses up to one gram per kilogram are tolerated without untoward effects (190). It may be assayed biologically by a rat test (30) microbiologically using lactobacillus casei or the yeast growth method (103, 25) and chemically by the Scudi colorimetric method (156, 157, 18).

1. ISOLATION AND SYNTHESIS

In 1926 Goldberger and Lillie (67) described the production of specific skin symptoms or dermatitis in rats which were fed a diet low in the anti-pellagric or P.P. factor. Later, investigations by Gyorgy in 1934 (75) and others showed definitely that these skin lesions were not due to the absence of the P.P. factor, but to the absence of another factor which was at that time named vitamin B6.

In 1938 Keresytesy (100) and Lepkovsky (107) starting with rice bran were able to isolate a crystalline hydrochloride with a nitrogen base having the properties of vitamin B6 as defined by Gyorgy.

Wiardi (201) in 1938 reported that Ohdake prepared vitamin B6 in 1932 and stated its formula as C8 H10 03 NHcL. However, its physiological importance was not recognized. In 1939 Stiller, Keresytery and Stephens (174), Harris, Stiller and Folker (82) reported the structure of vitamin B6, and Harris and Folker (83) and later Mowat, Pilgrim and Carlson (121) the synthesis. Reedman, Sampson and Unna (142) found synthetic and natural vitamin B6 equally effective in curing the symptoms of vitamin B6 deficiency.

2. THE PYRIDOXINE DEFICIENCY SYNDROME

In the rat:-

Hallidays and Evans in 1937 (78) worked out a diet which would induce the vitamin B6 deficiency syndrome in rats. It was characterized by arrest of growth, an estensive dermatitis, malnutrition and later death. Lepkovsky, Krause and Dimick in 1942 (108), Daniel, Kline and Folle in 1942 (40) and Patton, Karn and Longenecker in 1944 (137) have all reported on a conclusive syndrome in young suckling rats with the mothers on a pyridoxine deficient diet. In 1942, Lepkovsky and Nielsen (109) reported a green pigment producing compound in the urine of pyridoxine

deficient rats. Lepkovsky, Roboy and Haagen Smit in 1943 (110) found xanthurenic acid, a metabolite of tryptophane, in the urine of pyridoxine deficient rats. In 1941 Foy and Cerecedo (62) and in 1945 Miller and Baumann (117) observed acceleration of the pyridoxine deficiency syndrome with a high casein diet and a delay of the pyridoxine deficiency syndrome with a low casein diet.

Porter, Clark and Silber in 1948 (141) found that pyridoxine deficiency in the rat increases the excretion of xanthurenic acid seven-fold, and of kynurenin five-fold, while the excretion of kynurenic acid is not altered. This indicates interference with the conversion of kynurenin to kynurenic acid.

In 1942 Routh and Houchin (150) and in 1949 Schwartzman and Strauss (155) reported on pyridoxine deficiency in the Syrian Hampster. The latter authors described the development of a syndrome characterized by arrest of growth, decrease in food and water intake, progressive malnutrition, muscular weakness, changes of the fur and increase quantities of xanthurenic acid in the urine on a vitamin B6 deficient diet.

In the chick:-

In 1939 Jukes (98) described the symptoms of vitamin B6 deficiency in chicks. They consisted of slow growth, depressed appetite and insufficient utility of food, followed in some cases by spasmodic convulsions and death. These symptoms could be prevented by adding vitamin B6 to the diet. In 1941 Hogan and his colleagues (93) observed in addition, anemia, dermatitis, and perosis.

In the swine:-

Hawthorne in 1941 (90) observed dermatitis and later epileptiform fits in swine on a pyridoxine deficient diet. In 1943, Wintrobe (203) confirmed these observations, and also noted a microcytic hypochromic anemia and fatty liver in swine on a pyridoxine deficient diet. Cartwright in 1944 (26) found three excretory products of typtophane, metabolism, kynurenin, kyneurenic acid and xanthurenic acid in the urine of pyridoxine deficient swine. Later Cartwright (27) reported that the anemia failed to respond to iron or copper. In 1945 Cartwright and his colleagues (28) compared the microcytic hypochromic anemia of pyridoxine deficiency, with a normocytic normochromic anemia developed in

swine fed an acid hydrolysate of casein. The latter anemia was accompanied by a normal serum iron hypo or normoplastic bone marrow, and marked hypoproteinemia and oedema. Thus an imbalance in tryptophane metabolism is not likely responsible for both.

In the dog:-

In 1938 Fouts, Helmer and Lepkovsky (57) reported the development of anorexia, decrease in weight and microcytic hypochromic anemia in dogs on a vitamin B6 deficient diet. This anemia was often associated with neurological symptoms and it did not respond to increasing iron in the diet. When a rice polish extract was added to the diet there was reticulocytosis and a rapid increase in weight. All the puppies except those on liver filtrate factor developed signs and symptoms of blacktongue. In 1939 the same authors (56) cured this anemia with crystalline factor 1. (vitamin B6)

Borson and Mettier in 1940 (20) reported curing the hypochromic microcytic anemia of pyridoxine deficiency in dogs with synthetic vitamin B6, but stated the necessity of adding a factor or factors in liver filtrate for complete relief of the anemia.

In 1941 Street, Cowgill and Zimmerman (183)

studied pyridoxine deficiency in dogs. They reported the development of anorexia, loss of weight, intermittent diarrhoea, occasional vomiting, ataxia with an awkward compass gait, microcytic hypochromic anemia and a suggestion that there may be a decrease in the secretion of acid and possibly in the volume of gastric juice secreted. They state that their experiments were almost finished when the potency of nicotinic acid against canine blacktongue was announced. However, they believed their animals were receiving filtrate factor by the way they prepared their filtrate factor from liver powder. The dogs developed symptoms of incipient blacktongue which disappeared when the dosage of the concentrate was doubled.

McKibbin, Schaefer, Frost and Elvehjem in 1942 (130) studied the anemia in dogs due to pyridoxine deficiency. They found the blood plasma iron to be high and the blood copper values to be a low normal level. In 1943 Smith, Curry and Hawfield (166) studied this anemia and found that there is at least one factor possibly more in Brewer's yeast in addition to vitamin B6 which serves to stimulate haemoglobin production in the dog.

Fouts and Lepkovsky in 1942 (60) found that pyridoxine deficient dogs excreted in the urine a small amount of a compound, which can be converted into a green pigment by ferric ammonium sulphate.

In 1945 Axelrod, Morgan and Lepkovsky (6) studied the fate of tryptophane in pyridoxine deficient dogs. On either a high or a moderate protein diet kyneurin and xanthurenic acid were excreted in the urine. If dogs were on a high protein diet for one hundred days they developed nausea, anorexia and sometimes collapse after ingesting tryptophane.

Sarma, Snell and Elvehjem in 1946 (152) compared the growth and antianemic potencies of pyridoxal, pyridoxamine and pyridoxine. They showed the three compounds equally active in promoting growth and blood regeneration in dogs.

In 1946 Morgan, Goody and Axelrod (120) studied pyridoxine deficiency as it was affected by the level of dietary protein in dogs. The dogs developed the pyridoxine deficiency syndrome, anorexia, anemia, dry fur, scaliness of the skin, growth plateaux with decrease in weight and intermittent diarrhoea. The dogs receiving 45.8% casein developed the deficiency symptoms in 79 to 123 days, and those

receiving 18% casein, only after 169 to 190 days. The level of total serum proteins and albumen tended to be higher in the high-protein group than in comparable dogs fed the low protein diet. The normal dogs in the former group had 6.3 to 7.1 grams percent total serum protein and the latter 5.9 to 6.4 grams. The deficiency affected the serum protein only slightly, usually by lowering the level. The decrease which occurred in either or both albumen and globulin fractions disappeared during recovery from the deficiency.

In the monkey:-

In 1946 Greenberg and Rhinehart (70) studied pyridoxine deficiency in the Rhesus monkey. They used the modified M-3 diet Waisman (195) and noted the development of anorexia, loss of weight, decrease in activity and strength and a mild microcytic anemia. McCall, Waisman, Elvehjem and Jones (124) found the same symptoms together with ataxia, a more marked anemia and mild leucopenia.

Greenberg and Rhinehart (70) in 1946 studied xanthurenic acid excretion in pyridoxine deficient Rhesus monkeys. As early as 18 days after witholding pyridoxine there occurred marked increases in the

xanthurenic acid excretion following the feeding of tryptophane.

In the human:-

In 1939 Spies (170) reported treating patients for pellagra and beri-beri that continued to have extreme nervousness, insomnia, irritability, abdominal pain, weakness and ataxia, and these symptoms were relieved in four hours and disappeared in twenty-four hours after injection of 50 mgm. synthetic vitamin B6 intravenously. In 1940 Spies (171) reports a follow-up of twenty more cases with residual symptoms of nutritional deficiencies not relieved of synthetic nicotinic acid, thiamine hydrochloride or riboflavin, but by B6. He also demonstrated a marked reduction in the excretion of B6 in the urine of these patients.

Pyridoxine has been used with favourable results in the following clinical conditions. It is realized that there is no agreement as to the usefulness of pyridoxine in these conditions, but references are given:

Deficiency states	(148)
Acne	(96)
Cheilosis	(113)

Blood diseases (23)

Granulopenia (54)

Nervous diseases (9)

Nausea and vomiting of pregnancy (163, 92)

Pseudo hypertrophic muscular dystrophy (3)

Scborrheic dermatitis (205)

Radiation sickness (115)

In 1946 Denko and his colleagues (42) studied the excretion of the B complex vitamins in the urine and feces of seven normal adults. This showed the amazing fact that a normal individual excretes in the urine and feces far more para-aminobenzoic acid, biotin, folic acid, pantothenic acid and possibly more pyridoxine and niacin, plus their metabolites than are ingested in the diet. Daily fecal excretion exceeded the urinary excretion of all vitamins except pantothenic acid and pyridoxine.

In 1948 Hawkins and Barsky (88) studied a man who was maintained for fifty-five days on a vitamin B6 deficient diet. He developed a slight anemia, leucopenia, decrease in weight and decrease in blood pressure. There was no improvement in any of these findings with the administration of vitamin B6.

3. PYRIDOXINE AS RELATED TO COENZYME

In 1942 Snell, Guirard and Williams (167) described a base medium which supported luxuriant growth of "Streptococcus lactis" in the presence of synthetic pyridoxine, but upon which growth completely failed in the absence of added pyridoxine or of tissue extracts. By using this medium they demonstrated the presence of pseudopyridoxine in natural extracts. Excretion tests showed the pseudopyridoxine content of the urine parallelled the pyridoxine content of the ration after a time lag. It was thus concluded that pyridoxine is converted by the animal organism into a metabolite of unknown nature which possesses much greater activity for Streptococcus lactis than does pyridoxine itself. In 1943 Carpenter, Elvehjem and Strong (24) found that pseudopyridoxine did not stimulate yeast or rat growth.

Bellamy and Gunsalus (11) in 1943 replaced tryptophane with hydrolyzed gelatin and decreased the yeast extract in Streptococcus faecalis media and caused a marked decrease in the decarboxylation activity. The addition of pyridoxine and nicotinic acid restored the activity without materially affecting growth.

In 1944 Bellamy and Gunsalus (12) found the growth requirements for decarboxylase production more specific than those for maximum cell crop. Nicotinic acid and pyridoxine were among the factors required in greater concentration for the production of the tyrosine decarbosylation system than for growth.

These investigators (72) also found that the addition of pyridoxine to cell suspensions grown with suboptimal amounts of the vitamin stimulated the rate of 50 : 1 tyrosine decarbosylation and sustained the activity for a longer interval. Autoclaving the pyridoxine to convert it to pseudopyridoxine rendered the compound more active. They interpreted these results as indicating a function of pyridoxine in the decarboxylation of the amino acid.

Gunsalus and Bellamy in 1944 (71) found that a derivative of pyridoxine present in acid-autoclaved yeast extract and in pyridoxine solutions treated with cystine or dilute hydrogen peroxide functions in the decarboxylase system in proportion to its pseudopyridoxine content.

Snell in 1944 (168) found heightened

In 1945 Umbreit, Bellamy and Gunsalus (187) concluded that the naturally occurring coenzyme of the decarboxylase system is a pyridoxine derivative because it can be replaced in a variety of enzyme systems by coenzymes derived from pyridoxal and because on hydrolysis it yields a material which behaves with the tyrosine decarboxylase enzyme as does pyridoxal. Snell (169) produced evidence for the occurrence of pyridoxamine and pyridoxal in natural products.

Bellamy, Umbreit and Gunsalus in 1945 (12) stated that pyridoxine can be converted biologically and chemically into a compound of unknown structure which serves as the coenzyme of amino acid decarboxylation.

Gale and Epps (131) isolated a substance from natural products which also serves as the coenzyme of amino acid decarboxylation and they termed this material codecarboxylase. Studies have shown the similarity of the natural preparation to those prepared biologically and chemically from pyridoxal.

In 1946 Umbreit, O'Kane and Gunsalus (188) presents biological evidence in support of the

proposed mechanism of the function of pyridoxal phosphate as coenzyme of transamination. Thus pyridoxamine phosphate and pyridoxamine have been shown to be the equivalents of pyridoxal phosphate and pyridoxal respectively for transamination, though not for amino acid decarboxylation.

4. RELATION OF PYRIDOXINE TO ANTIBODY FORMATION

In 1944 Dougherty, Chase and White (45) presented almost conclusive evidence that antibodies are concentrated in lymphocytes. They showed that per unit of extractable nitrogen lymphoid tissue had significantly higher agglutinin and hemolysine titers than did the sera of the same animals.

Elrich in 1945 (47) states that the new observations as well as the old facts seem to fit into another theory of antibody formation in which the lymphocyte and possibly the granulocyte as well as the macrophage play an essential role.

In 1946 Stoerk and Eisen (176) demonstrated the supression of circulating antibodies in pyridoxine deficiency. They showed that male albino rats immunized in a state of pyridoxine deficiency developed antibody levels in the serum far below those of inanition controls (pair weighed) and full

controls. Stoerk (178) also demonstrated that in pyridoxine deficient rats the thymus weight deficit is unduly high with respect to body weight deficit.

Mushett, Stebbins and Barton (123) reported a marked leucopenia in pyridoxine deficiency with a lymphocytosis and neutropenia.

CONCLUSIONS

1. Vitamin B6 deficiency was first observed by Goldberger in 1926 and the vitamin was first named by Gyorgy in 1934.
2. Keresytesy in 1938 first isolated vitamin B6.
3. Harris, Stiller and Folker in 1939 first reported its structure and the former two, its synthesis.
4. Pyridoxine deficiency in experimental animals with insignificant changes in species is characterized by:
   - (a) Anorexia.
   - (b) Malnutrition.
   - (c) Decrease in weight.
   - (d) Arrest of growth.
   - (e) Increase xanthurenic acid and kynurenin in the urine.
   - (f) Microcytic hypochromic anemia.
   - (g) Leucopenia.
   - (h) Hypoproteinemia.
   - (i) Dermatitis.
   - (j) Dryness of hair.
   - (k) Ataxia.
   - (l) Convulsions.

(m) Fatty liver.

(n) Death.

5. High protein diets have been shown to accelerate the pyridoxine deficiency state.

6. Spies relieved several cases of a deficiency syndrome by injections of vitamin B6. This syndrome was characterized by:

(a) Nervousness.

(b) Insomnia.

(c) Irritability.

(d) Abdominal pain.

(e) Weakness.

(d) Ataxia.

7. Hawkins placed a human on a pyridoxine deficient diet and he developed slight anemia, leucopenia, decreased blood pressure and weight.

8. Pyridoxine exists in nature in the form of pyridoxine, pyridoxal and pyridoxamine.

9. Pyridoxal and pyridoxamine are known as pseudopyridoxine.

10. Pyridoxine is converted biologically into a compound of unknown structure, which serves as a coenzyme of amino acid decarboxylation.

11. The evidence indicates that pyridoxine is converted to pyridoxal which is then phosphorlated. The pyridoxal phosphate acts as the coenzyme.

12. There is biological evidence to support the proposed mechanism of the function of pyridoxal phosphate as the coenzyme of transamination.

D. Desoxypyridoxine - A Pyridoxine Antagonist.

In 1946 Ott (133) showed with chick bioassays that desoxypyridoxine (2, 4-dimethyl - 3-hydroxy - 5-hydroxymethylpyridine) is a very potent inhibitor of pyridoxine. Under the conditions of this experiment two molecules of the inhibitor were sufficient to offset the vitamin activity of one molecule of pyridoxine. This ratio was found to hold for suboptimal and optimal amounts of pyridoxine given to pyridoxine deficient chicks.

In 1947 Emerson (49) reported on the anti-vitamin B6 activity of desoxypyridoxine in the rat. When desoxypyridoxine was fed to weanling rats as an adjunct to a purified diet deficient in vitamin B6 and to the same ration supplemented with the quantity of pyridoxine in the stock diet, the time required for the development of acrodynia in the deficient group was materially decreased and the signs of depletion were aggravated. The dermatitis was noted when the ratio of desoxypyridoxine to pyridoxine was 50:1 with the animals receiving the purified diet, with the stock ration the ratio was 175:1. In adult rats there were no signs of pyridoxine deficiency in 4 months on a pyridoxine deficient

ration alone, but if 0.5 mg.% desoxypyridoxine was added to the diet the rat developed acrodynia in 55 days and the growth of the rat was depressed.

Porter, Clark and Silber in 1947 (141) studied the effect of pyridoxine analogues on tryptophane metabolism in the rat. They found that xanthurenic acid excretion was significantly increased when desoxypyridoxine was given. In both normal and deficient rats desoxypyridoxine plus tryptophane increased the excretion of kynurenin. Pyridoxine prevented the effects of desoxypyridoxine. They concluded that desoxypyridoxine interferes with some phase of tryptophane metabolism.

In 1947 Mushett, Stebbing and Barton (123) reported on the pathologic effects produced by the two analogues of pyridoxine. Feeding chicks desoxypyridoxine resulted in values for spleen weight body weight ratio which were significantly less than the value in chicks receiving the same diet without desoxypyridoxine. The histological appearance was a hypoplasia of lymphoid elements.

Puppies on a pyridoxine deficient diet and injection with 5 mgm. per Kg. of desoxypyridoxine lost weight and died within two months. They showed

a progressive decrease in erythrocyte count, hemoglobin and hematocrit. The resulting anemia was noted clearly to be microcytic and hypochromic in nature within one month. Harris (81) also observed this anemia in pups receiving desoxypyridoxine. The pups getting desoxypyridoxine as well as those on the pyridoxine free diet developed leucopenia within a month. Hawkins (89) also observed this leucopenia in pups receiving desoxypyridoxine. This was more marked in the latter group. However, whereas the pyridoxine deficient pups showed lymphocytosis and neutropenia, the desoxypyridoxine animals showed marked lymphopenia and polynucleosis. Monkeys on a natural stock ration and injected subcutaneously with a large dose of desoxypyridoxine developed microcytic anemia, leucopenia and lymphopenia.

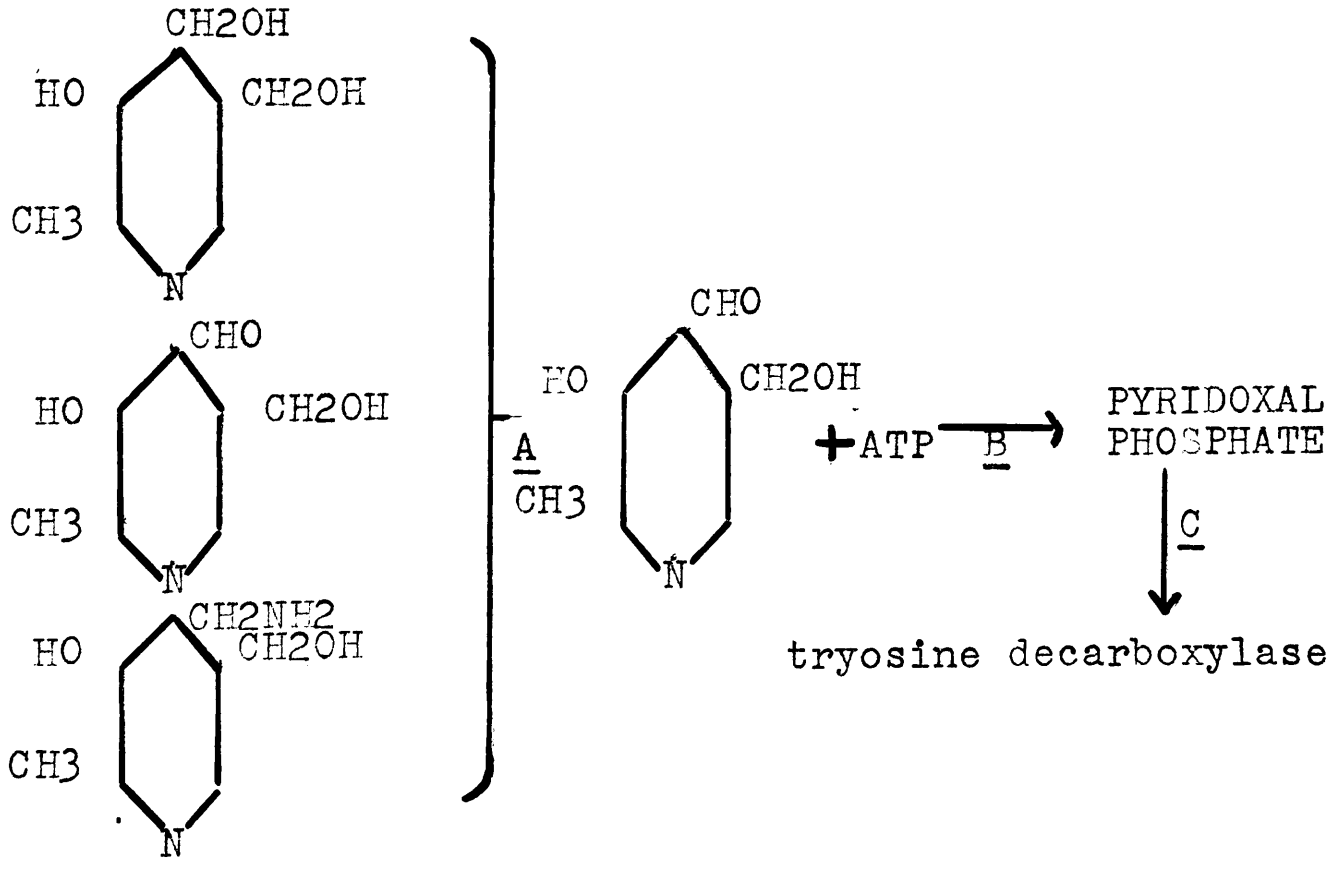
At autopsy the most striking change observed in the monkeys treated with desoxypyridoxine was atrophy of the spleen, thymus and lymph nodes. Histological sections of the spleen showed no germinal centers and only remnants of Malpighian follicles. Lymph nodes taken from many sites in the body also showed a striking diminution in lymphoid elements, particularly in the follicles. The blood forming constituents and

fat of the femoral marrow were strikingly reduced. Both erythroid and mycloid elements were affected.

In pups treated with desoxypyridoxine the lymphoid tissue showed histological evidence of atrophy. The thymus showed in addition to lymphoid hypoplasia, many areas of necrosis. Demarcation between cortex and medulla was frequently indistinct. Hassalls' corpuscles were decreased in number and showed morphological abnormalities. The marrow was relatively normal. Sudan IV stains of the adrenal glands of dogs and monkeys showed depletion in limphoid content, particularly in the zona fasciculata and zona reticularis. However, the glands were usually larger than normal. Since lymphoid tissue mass and the circulating lymphocytes are under the control of the adrenal cortical hormones (44) the question arises as to whether lymphoid atrophy results from stimulation of the adrenal gland or from a direct action of the analogues. Other signs of pyridoxine deficiency were seen in these animals. They were dryness of the hair, scaliness of the skin, tongue, lesions, hyperirritability and epileptiform convulsions. They conclude that the lymphoid atrophy is primarily due to pyridoxine deficiency, but that inanition also plays a role under the circumstances.

In 1949 Cravens and Snell (38) found that 500-1000 micrograms of desoxypyridoxine would cause 100% inhibitory effect of chick embryos. This could be prevented to a degree by supplying additional quantities of vitamin B6 or derivatives. The inhibitory effect was most marked in the first 3-4 days of embryonic life.

In 1949 Umbreit and Waddell (189) presented the following figure of the reactions of the members of the vitamin B group and suggested that desoxypyridoxine might act at A, B or C or it might be converted into desoxypyridoxine phosphate which could compete by uniting with the apo-tyrosine decarboxylase, or by displacing pyridoxal phosphate from the holoenzyme.



They then presented experimental data which indicates that desoxypyridoxine exerts its inhibiting effect by being first converted into desoxypyridoxine phosphate which then competes with pyridoxal phosphate for the apoenzyme. This conclusion offers an explanation for the observation, that in the animal desoxypyridoxine exerts its antagonistic effect primarily under conditions of restricted vitamin B6 intake.

In 1950 Mueller and Vilter (122) reported the use of desoxypyridoxine in humans. They gave doses of 60-150 mgm. intramuscularly to eight patients and noted that seborrhea-like skin lesions developed about the eyes, nose and mouth after 2 to 3 weeks. Erosions appeared in and about the mouth resembling cheilosis of riboflavin deficiency. Glossitis and stomatitis similar morphologically to the lesions of niacin deficiency were noted in about one half of the patients. One patient developed severe systemic symptoms, nausea, vomiting, weakness and dizziness. The skin mucous membrane and systemic manifestations were unchanged when a mixture containing thiamine, riboflavin and nicinamide was given, but disappeared within 48 - 72 hours after pyridoxine was administered. The inhibitory ratio of antimetabolite to metabolite in human beings

was not accurately measured, but was at least 1 : 1. The authors believed that the above lesions constitute at least part of the human syndrome of acute pyridoxine deficiency.

CONCLUSIONS

1. Desoxypyridoxine is a very potent inhibitor of pyridoxine with a ratio of 2-1 in the chick.
2. Pyridoxine in the diet prevents the effects of desoxypyridoxine.
3. Puppies on a pyridoxine deficient diet injected with 5 mgm. per kg. of desoxypyridoxine lose weight and die within two months. These pups develop microcytic hypochromic anemia that may remit slightly in 1-2 weeks only to exaserbate. They also develop a leucopenia with a marked lymphopenia and polynucleosis. The thymus shows lymphoid hypoplasia and many areas of necrosis. There is a reduction of the lipoid content of the adrenal gland.
4. Desoxypyridoxine acts by being converted into desoxypyridoxine phosphate which competes with pyridoxal phosphate for the apoenzyme.
5. Desoxypyridoxine in humans causes glossitis, erosions about the mouth, nose and eyes, stomatitis, nausea, vomiting, weakness and dizziness.

E. Gastric Pouches

In 1878 Heidenhein (91) described his well known method of making a denervated gastric pouch. A part of the stomach on the greater curvature is freed from the rest of the stomach by an incision through all layers along the longtitudinal apex of the stomach. The vagal innervation is thus completely severed, but the sympathetic fibers may remain with the blood supply on the greater curvature which is left intact to supply the pouch. The pouch is then constructed from the freed part of the stomach and the stoma at one end is brought out through the mid-line incision.

In 1910 Pavlov (138) described a modification of the Heidenhein pouch. A seromuscular bridge is left between the main stomach and pouch at the cephalad end of the pouch. The pouch is separated from the main stomach by forming the mucous membrane into two domes which are closed by stitches on the stomach side and the pouch side. Thus, some of the parasympathetic innervation is retained and this is so in a varying percent of cases.

In 1930 Armour (4) described a lesser curvature pouch made with a horseshoe incision extending across the anterior surface of the stomach with its base on the lesser curvature. It has an excellent innervation, despite the fact the incision crosses the course of the vagus nerve.

In 1938 Jemerin and Hollander (95) described the error made by Kahogue and Pavlov in the course of the posterior or right vagus nerve distribution. They dissected 20 dogs and found the vagal pattern constant and the dorsal and ventral trunks symmetrical. Above the cardia both give 1 or 2 twigs to the lower oesophagus and diaphram, and to the cardia end of the stomach. Immediately below the cardia, several fair sized branches are given off to the fundus and proximal part of the body of the stomach. The ventral then gives a large branch to the porta hepatis and the dorsal one to the coeliac plexus. Both main trunks then descend within the omental bursa parallel to the lesser curvature to about 1 inch from the pylorus. In this course, they give 3 - 5 branches passing transversely towards the greater curvature. All gastric branches run at right angles to the long axis of the stomach.

In 1938 Hollander and Jemerin (94) designed a new gastric pouch. They stated that the Pavlov pouch technique was based upon the erroneous anatomical concept that the dorsal vagal trunk runs along the greater curvature. Thus they overcame this by making an incision at right angles to the greater curvature as it approached the pyloric antrum and extended this half way across the stomach. They then cut the mucosa and submucosa on the anterior and posterior walls of the stomach from the end of that incision to a point on the greater curvature almost at the fundus. They then constructed a mucous membrance bridge along this incision of the mucous membrane and so the pouch was formed. This bridge would not only be difficult to construct, but certainly would be prone to breakdown.

Cope, McMahon, Hagstromer and Thompson in 1940 (31) described a new gastric pouch with a non leaking stoma and an intact nerve supply. This pouch was made by an incision through all layers starting along the lesser curvature at a point just to the right of the left gastric vessels and running down to one centimeter above the incisura, then across to the greater curvature and on to the

posterior wall for 1 cm. It then runs along the greater curvature to a point opposite its beginning on the lesser curvature, then back on to the anterior surface and across the stomach to the first large branch of the left vagus nerve. The mucosa and submucosa across the remaining bridge is then cut and the pouch is formed. The stoma formed at the lower end is then buried in the pyloric antrum and the abdomen is closed in layers. In three weeks the stoma is transplanted from the pyloric antrum into the flank by separating the abdominal muscles and thus a sphincteric action is obtained.

In 1942 Thomas (187) described a simplified procedure for preparing an improved Pavlov pouch. He made an incision in the anterior wall near the mid point of the greater curvature and with two vulsellum forceps he pulled out the mucosa from above and below thus inverting part of the stomach. He then cut through the mucosa and submucosa on the anterior and posterior walls and sewed them together first on the stomach side and then on the pouch side. This made a very long mucous membrane bridge which would not only be difficult to construct but prone to break down.

CONCLUSIONS

1. Heidenhein described the first denervated gastric pouch and Pavlov the first innervated gastric pouch.
2. Jemerin and Hollander in 1938 described the true course of the vagi and pointed out Pavlov's error in assuming the posterior vagus ran along the greater curvature.
3. Armour, Jemerin, Hollander and Thomas have all described innervated gastric pouches.
4. Cope and his colleagues in 1940 described a new innervated gastric pouch with a non-leaking stoma.

F. Vitamin B. 12.

In 1947 Shorb (161) found that lactobacillus lactis Dorner required the presence of two unidentified factors for growth in an amino acid medium containing all synthetic B vitamins. One factor found in tomato juice, and a heat stable factor L.L.D. found in liver and apparently concentrated in the refined extracts in almost linear relationship to their potency in remitting the symptoms of pernicious anemia. In 1948 Shorb (162) reported experimental results showing that vitamin B12 is either wholly or partially responsible for the lactobacillus lactic Dorner growth activity, and isolated vitamin B12 from liver.

Smith in 1948 (164) prepared two red pigments from ox liver and both were highly active in pernicious anemia. Smith (165) later showed the anti-pernicious anemia factor to be red needle shaped crystals containing cobalt and 3 atoms of phosphorous. Rickes and his colleagues (144) worked out the potency to be 11,000,000 lactobacillus lactic Dorner units/mg. with a 23 hour growth period. Later Rickes and his colleagues (143) used emission spectrographic analysis and showed vitamin B12 to be a cobalt

complex containing cobalt, phosphorous and nitrogen.

In 1948 Spies (172) made the first observations on the antianemic properties of vitamin B12 in pernicious anemia. He noted a peak in the reticulocyte response in 5 - 9 days with marked clinical improvement characterized by increased well being, mental alertness, strength and vigor as well as early disappearance of the soreness and burning of the mouth.

In 1948 Hall and Campbell (76) reported observations on eleven patients receiving vitamin B12 for pernicious anemia. The reticulocyte peaks ranged from 4 to 7 days and the red blood cell count rose to normal in 5 - 7 weeks. Bethell, Meyers and Neligh (16) obtained responses in pernicious anemia but not in a case of puerperal macrocytic anemia.

Shine, Ravel and Eakin (160) found that thymidine could replace vitamin B12 in the nutrition of Lactobacillus lactic Dorner and they suggested that vitamin B12 functions in the biosynthesis of thymidine.

Wright, Skeggs and Huff (205) also found

that thymidine isolated from liver could replace the requirements for vitamin B12 in certain lactic acid bacteria. Their interpretation of this fact was that vitamin B12 functions as a coenzyme in carrying out reactions concerned with conversion of thymine to thymidine, since in the presence of thymidine, the vitamin is no longer required. Thus in pernicious anemia, the primary biochemical defect may be inability to synthesize certain nucleosides particularly thymidine from parent purines or pyrimidines.

In 1948 Stone and Spies (179) found that folic acid maintains the blood levels in persons with pernicious anemia but does not offer complete therapy, as it does not prevent or relieve subacute combined degeneration or result in the healing of the mucous membrane lesions. Liver and vitamin B12 on the other hand relieves both the subacute combined degeneration and mucous membrane lesions. Berk, Brown, Finland and Castle (14) also found vitamin B12 effective in relieving subacute combined degeneration.

Berk and his colleagues (13) found that Vitamin B12 is effective orally if potentiated by

the simultaneous administration of normal human gastric juice, but not as effective as liver when given parenterally. Hence, A.P.A. (extrinsic) factor may be identical or related chemically to A.P.A. of liver which is presumably identical with vitamin B12. Gastric (intrinsic) factor is necessary for optimal utilization of vitamin B12. If there is no intrinsic factor present vitamin B12 is eliminated in the stool. Thus the intrinsic factor of normal gastric juice may function by facilitating the absorption by the intestines of vitamin B12 or chemically related compounds in the food rather than react with extrinsic factor.

Hall, Morgan and Campbell in 1949 (77) also found the intrinsic factor in Berkefield filtered pooled human gastric juice.

In 1948 Ott, Rickes and Wood (134) added vitamin B12 to purified basal diet containing 40 - 70% soybean meal as the sole source of protein and found it to exhibit animal protein factor activity in chicks. The crystalline substance elicited growth responses comparable to those obtained by supplementation with crude sources of the animal protein factor, and it is possible that

vitamin B12 is identical with or closely related to this factor.

Bethiel and Lardy (15) found vitamin B12 to be a growth factor for the hyperthyroid rat.

In 1949 Luecke and McMillan, Thorp and Bouiece (111) found significantly greater weight gains made in pigs receiving vitamin B12. However, their preparation contained impurities, so it cannot be stated definitely that the growth promoting activity was due to vitamin B12.

Emerson, Wurtz and Janett (50) state that the rat requires a factor present in liver and other animal proteins for normal growth and successful propagation. They studied the progeny of rats during gestation and lactation and found the birth weights the same, but the weaning weights of the young from mothers receiving vitamin B12 averaged 50% more than did those from the untreated mothers. The feeding of vitamin B12 to the young of control mothers resulted in body weights that were 100 grams in excess of those of the unsupplemented animals. The young of the treated mothers that were not given vitamin B12 grew for 2 months at a rate of approximately that of the young from control mothers

that were given vitamin B12. It would appear that vitamin B12 is stored during the suckling period. The red and white counts were in normal range. Emmerson (51) also found that vitamin B12 counteracts the growth retarding effects of thyroid powder when fed in conjunction with a diet devoid of animal protein. The vitamin was equally effective when administered by either the oral or subcutaneous routes.

In 1949 Wetzel, Fargo, Smith and Helikson (200) studied growth failure in school children and its response to oral vitamin B12. They concluded the amazing fact that their results speak with measurable statistical certainty of what may be termed vitamin B12 functional deficiency, that was definitely benefited by oral therapy. The growth responses were equivalent to another 100 - 240 days of regular institutional care without the help of vitamin B12.

In 1949 Hartman and Cary (87) reported that the rat requires a still unidentified growth-promoting factor. Slow growing rats fed feces showed a rapid increase in weight. This suggests that the unidentified factor called vitamin X is

synthesized by microorganisms. This increase in weight is prevented if the rats are fed sulfasuxidine. Crude preparations of vitamin B12 and cow manure are active as vitamin X.

In 1949 Stern, Taylor and Russell (173) found that the growth of weanling white rats was stimulated by inclusion of vitamin B12 or of whole dried liver in the diet, and showed considerable cytoplasmic basophilia in their liver cells. Rats which received no vitamin B12 or liver grew poorly and showed little or no liver basophilia. Popper, Koch and Syanto (139) also found that vitamin B12 prevented the disappearance of liver basophilia which occurs when soybean oil meal is the sole source of protein in the diet of rats. The basophilic hue is due to the presence of ribonucleic acid compounds in the cells and it decreases in carbon tetrachloride poisoning. This decrease in carbon tetrachloride poisoning was prevented with vitamin B12, so it can be concluded that vitamin B12 protects the liver against some poisonings.

In 1950 Sunde, Cravens, Elvehjem and Halpin (184) reported on an unidentified factor required by chicks fed practical rations. They found

this factor present in fish solubles and to a lesser degree in fish meal.

Olcese, Couch and Lyman in 1950 (132) studied the effect of vitamin B12 concentrates in the nutrition of the mature domestic fowl. The hatchability of eggs from hens fed a low vitamin B12 diet decreased to 0 in from 2 to 4 weeks and egg production was also decreased. The addition of concentrates containing vitamin B12 improved production and hatchability, but there was a tendency for the hatchability to decrease about the 9th or 10th week even when adequate vitamin B12 was fed.

Leucke, McMillan and Thorp in 1950 (112) fed 4 groups of young pigs a corn-soybean basal diet and supplemented one group with vitamin B12, one with vitamin B12 and streptomycin and one with dried animal protein factor. They found some slight gain in weight with the supplemented animals, but no significant difference in the ratios of lb. feed to lb. body weight gained among the 4 lots.

In 1950 Meyer, Sawitsky, Ritz and Krim (116) reported that when given orally vitamin B12 is frequently an adequate substitute for the

extrinsic factor of liver. If achlorhydria is present, orally administered vitamin B12 does not easily pass the intestinal barrier and relatively large doses are necessary for a therapeutic result. However, when a daily dose of not more than 25 micrograms of vitamin B12 is given along with 1.67 mgm. of folic acid, the hemopoietic response frequently parallels that of parenteral vitamin B12 therapy. They believe that folic acid promotes utilization of vitamin B12.

CONCLUSIONS

1. Shorb in 1947 found that lactobaccilus lactis Dorner required a heat stabile factor L.L.D. for growth, and later that this growth factor was vitamin B12.
2. Spies in 1948 found the antianemic properties of vitamin B12 in several cases of pernicious anemia.
3. Vitamin B12 relieves subacute combined degeneration and mucous membrane lesions as well as the anemia.
4. Vitamin B12 is an effective therapeutic agent in pernicious anemia when given orally in the

presence of gastric juice which contains the intrinsic factor.

5. Ott found vitamin B12 to exhibit animal protein factor activity in chicks.
6. The rat requires vitamin B12 for normal growth and successful propagation.
7. Wetzel and his colleagues demonstrated in school children what may be termed vitamin B12 functional deficiency.
8. Vitamin B12 prevents the disappearance of liver basophilia when soy bean oil meal is the sole source of protein in the diet and in tetrachloride poisoning.
9. Meyer and his colleagues found that vitamin B12 is effective orally in achlorhydria if given along with folic acid.

CHAPTER III

MATERIALS AND METHODS

A. MATERIALS:-

1. Animals - Dogs were used in this investigation. There were five pups and two adult mongrel dogs in the first series and three pups and 1 adult mongrel dog in the second series. Pups were used in order to accelerate the development of the deficiency state.

The fact pups were used that had been exposed to distemper in a dog pool brought in the problem of dealing with distemper. This was extremely difficult and was responsible for the death of three dogs in the first series. There were several techniques used to deal with this problem in the first series of animals. The most successful technique was used in the last series.

The dogs were taken from the dog pool the day following admission and exposure to distemper. In two days they were given 20 cc. of anti-canine distemper serum Lederle and then observed. During this period of observation they were pilled for worms and powdered for fleas. These procedures were repeated every two months throughout the investigation. The four dogs all developed distemper.

In two dogs the distemper was mild and in two severe. The dogs were given 30 cc. of anti-canine distemper serum immediately with the first sign of distemper and this was repeated in 24-48 hours. The two severe cases were also given 250 mgm. of aureomycin every 8 hours until subsidance of the signs and symptoms. This technique is described because of the excellent results obtained by its use.

2. Diet of Dogs - The dogs were fed a special synthetic diet (Figure 1). This diet was based on the diet used by Seeler and Silber (159). The salt mixture used was that of Jones and Foster (97) (Figure 1). The vitamin supplement was based on that used by Lambooy (102) and recommended by Hawkins (personal communication). In addition biotin, folic acid and vitamin K were added to exclude any possibility of a deficiency (Figure 1). Bordens vitamin free casein was used in order to have complete control of the vitamin intake. In order to obtain a pyridoxine free diet the pyridoxine was simply removed from the vitamin supplement. When vitamin B12 was added it was given as Rubramin parenterally or Rubofolin orally. The dogs were allowed to eat all the synthetic diet they would eat

Synthetic Diet

Constituent	Percentage	Cal. per 100 gm.	Approx. Daily Requirement
Dextrin	42	168.0	3.6 gm. or 14.4 cal./kg.
Casein	30	120.0	1.0 4.3
Cottonseed Oil	21	189.0	
Corn Oil	0.15	1.35	3.0 27.3
Cod Liver Oil	2.0	18.0	
Mineral Mixture	5.0		

Vitamin Supplements	mg./day	Mineral Mixture	Percentages
Thiamine	1.5	Sodium Chloride	14.6
Riboflavin	1.5	Potassium Phosphate, Monobasic	40.8
Nicotinic Acid	30.	Magnesium Sulphate	6.0
Calcium Pantothenate	10.	Calcium Carbonate	40.0
Choline Chloride	200.	Ferric Sulphate	2.83
Folic Acid	0.3	Potassium Iodide	0.083
Biotin	0.3	Manganese Sulphate	0.467
Para-Amino-Benzoic Acid	8.	Zinc Chloride	0.02726
Alpha Tocopherol	2.8	Copper Sulphate	0.04994
Inositol	0.1 gm. | 100 gms.	Cobalt Chloride	0.00238
2-Methyl Naphthoquinone	0.002 gm. | diet		
Pyridoxine	1.5 for control		
Vitamin A in C. L. O.	and recovery		
Vitamin D			

Figure 1

Table of contents of the synthetic diet modified Seeler and Silber (159). The mineral mixture is that recommended by Jones and Foster (97), and the vitamin supplement modified from Lambooy (102).

before standardization. This amount was weighed and then fixed throughout the entire experimental period. In each case it exceeded 80 calories per pound. The amount for each dog was set and offered to him daily, and the diet not eaten was force fed.

3. Desoxypyridoxine - The desoxypyridoxine supplied by Merck & Co. was given orally after the daily diet. It was prepared by dissolving the desoxypyridoxine in distilled water, so that each c.c. contained 20 mgm. The dogs that received it were given 5 mgm. per Kg. of body weight.

4. Type of Pouches -

A. Heidenhein Pounh - One dog was prepared with this type of gastric pouch. The standard technique described above was used and the animal allowed three weeks to recover from the operation before experiments were carried out.

B. Cope Pouch - Five dogs were prepared with this type of pouch and one with a modified Cope pouch. The technique of making a Cope pouch is described above. A diagram showing the outline to cut out a Cope pouch is shown in Figure 2, and a diagram showing the completed pouch is shown in Figure 3. The modified Cope pouch was made using the technique

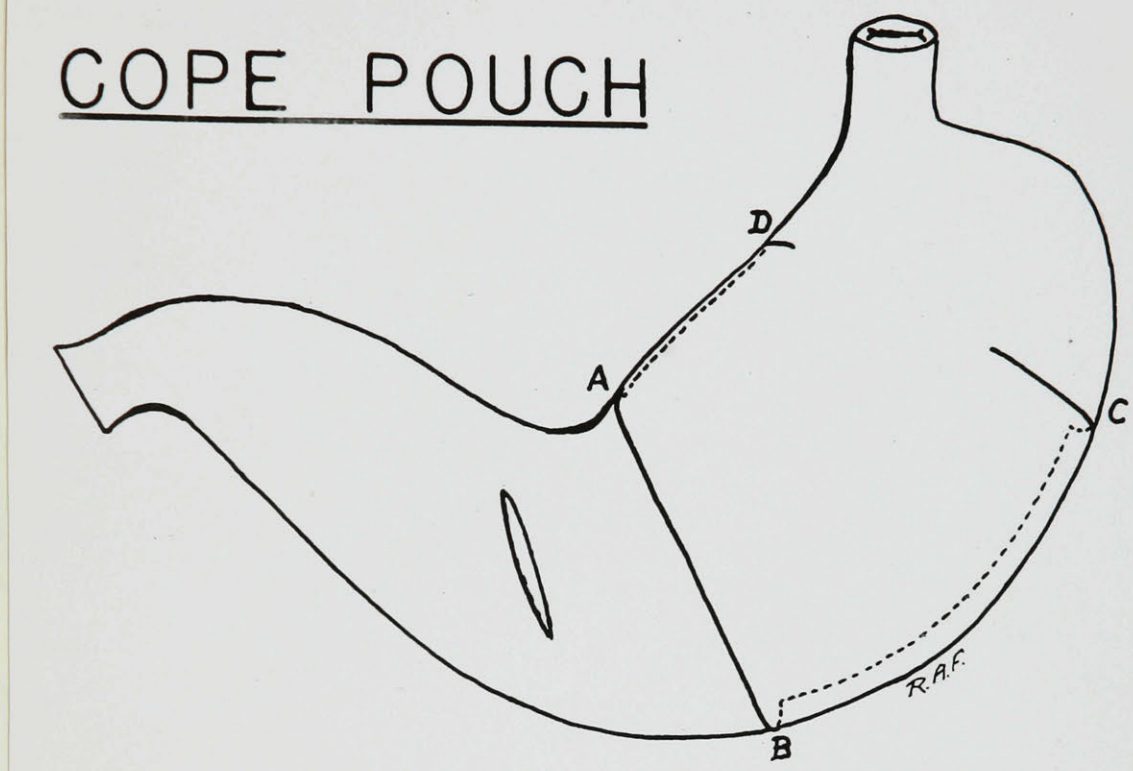


Figure 2

Diagram showing the outline of the incision to make a Cope pouch. The location of the incision in the pyloric antrum is also marked.

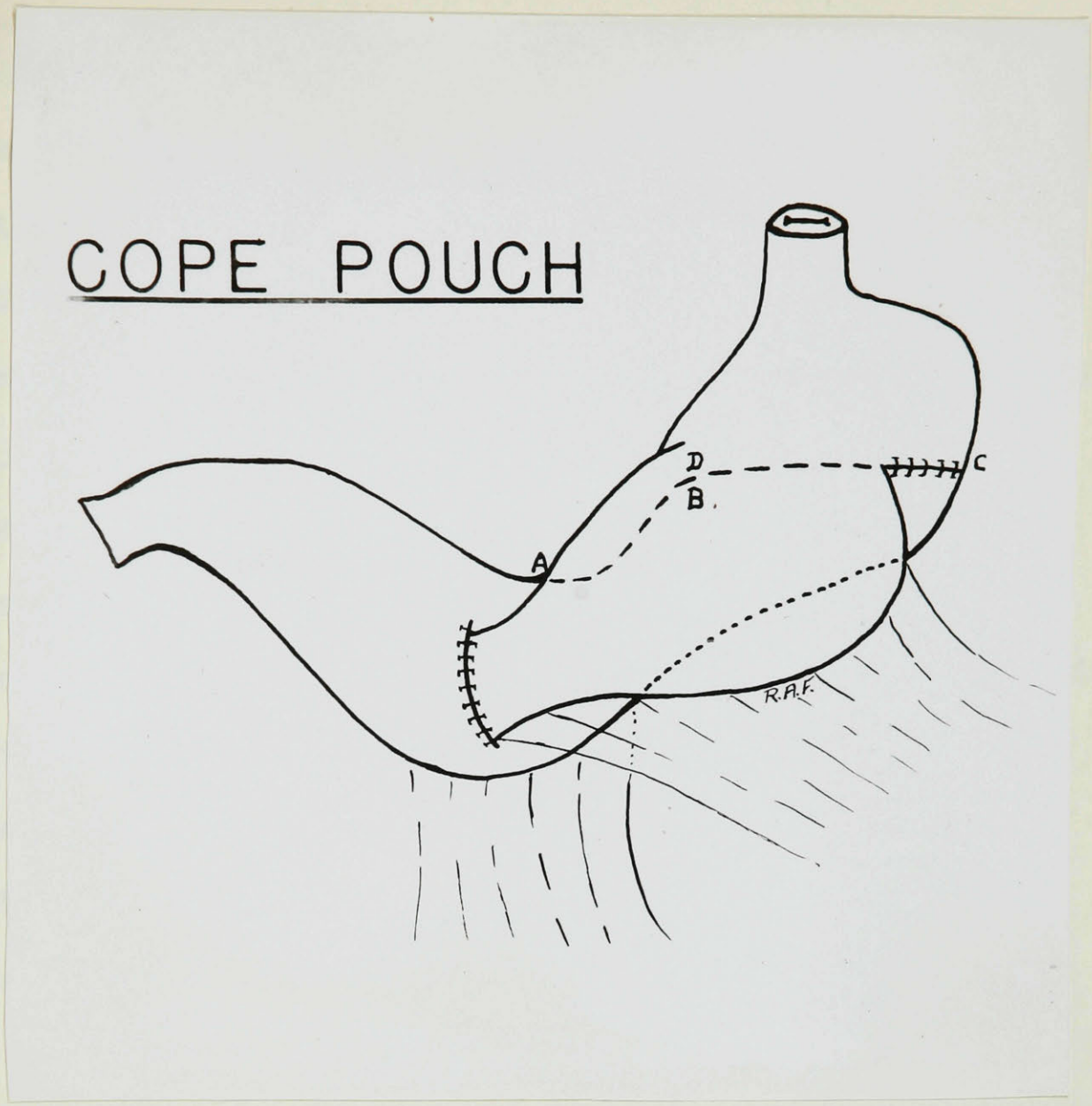


Figure 3

Diagram showing the constructed Cope pouch with the stoma placed in the pyloric antrum. The lower part of the anterior surface AB is sewn to the lesser curvature AD, and the greater curvature BC to the upper part of the anterior surface DC. Note the large size of the pouch in relation to the remainder of the stomach which is definitely stenosed.

described by Cope, with the exception that the stoma was brought out through the abdominal incision in the first stage.

C. New Gastric Pouch - Four dogs were prepared with the new gastric pouch. The dogs were allowed 3 weeks to recover from the operation before experiments were started. The technique for constructing a new gastric pouch is as follows: The abdomen is opened through an upper abdominal midline incision to the left of the falciform ligament extending from the xiphoid cartilage to one inch below the umbilicus. The stomach is then easily delivered into the wound and the left vagus nerve is located as it courses along the lesser curvature. The main branches of the left vagus that run to the body of the stomach are then located. The incision to cut out the pouch is made as in Figure 4. Care must be taken to leave the main branches of the vagus that supply this area of the stomach intact over the seromuscular bridge between A and D, Figure 4. The seromuscular bridge between A and D must be at least 1½-2 inches long to allow for adequate blood supply. The incision is made preferably with a cautery, but a scalpel may be used.

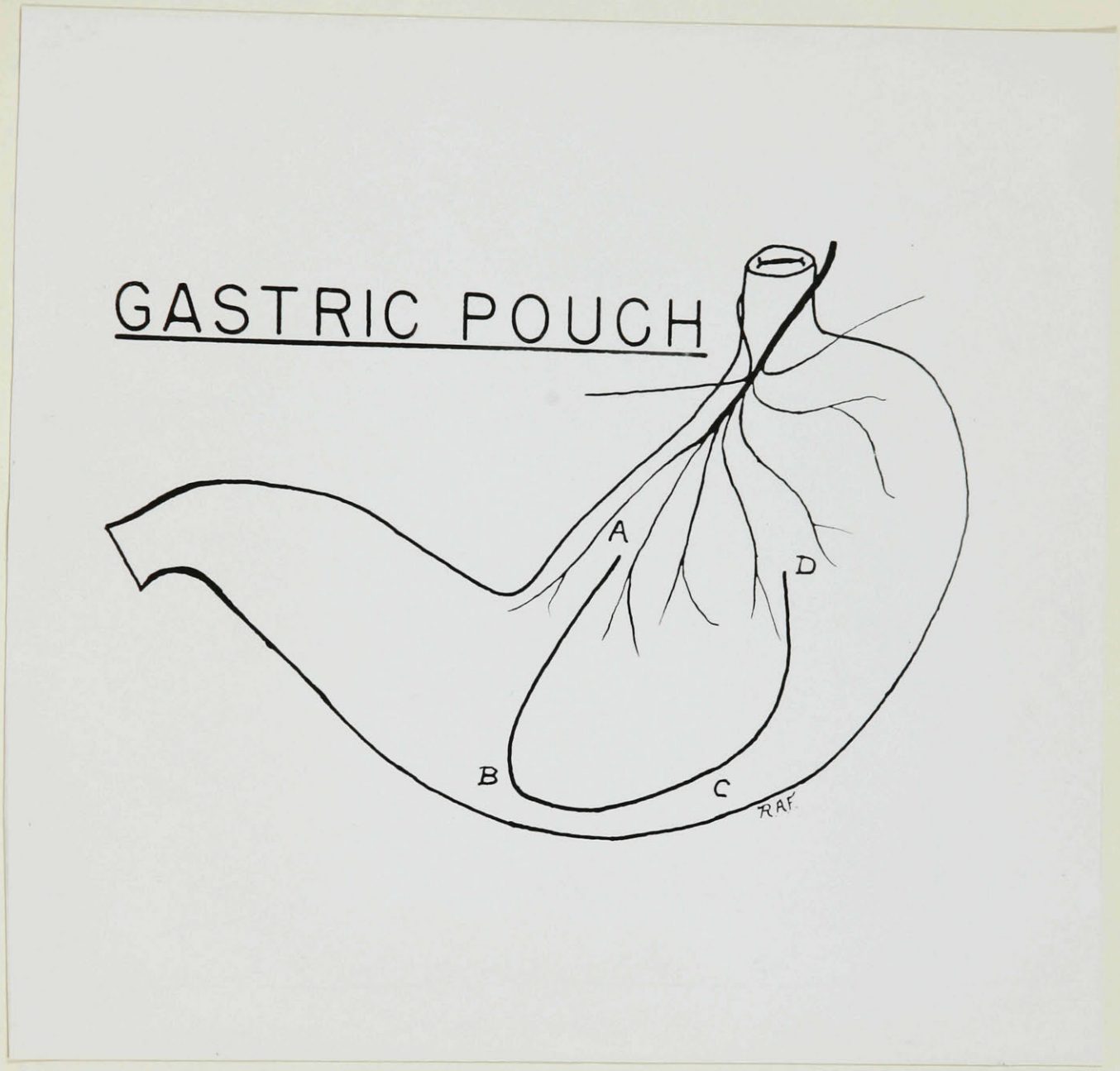


Figure 4

Diagram showing the course and distribution of the left vagus nerve and the outline of the incision to cut out the new gastric pouch, Note the relative size of the seromuscular bridge AD and the three branches of the vagus coursing over it.

It is made first through the serous and muscular layers and then the submucosa and mucosa. If a scalpel is used, the incision must be made slowly and the bleeders chiefly in the submucosa clamped and tied with number 40 cotton. The flap to make the pouch should be handled as little as possible at all times to decrease the oedema and facilitate the construction of the pouch. There should be no tension on it at any time to prevent damage to the nerves. The mucosa and submucosa across the bridge is then cut with the scalpel and if this is done with sufficient care the large veins in the submucosa can be left intact. The edge of the mucosa may be pushed back slightly, but not separated from the submucosa or it will necrose. The pouch is then constructed using 000 chromic catgut on a fused curved needle and starting on the greater curvature side at D, Figure 4. A Connell inverting stitch is used through all layers of the stomach wall where possible and the mucosa and submucosa across the bridge. Care must be taken to use very little of the flap to oppose the mucosa of the bridge in order to obtain as much length to the pouch as possible. The stomach is then reconstituted by

sewing AB to AD and BC to DC,(Figure 5). This is done to increase the breadth of the stomach in this area. The suture lines are then reinforced by a layer of interrupted stitches of No. 40 cotton. The abdomen is then closed in layers using No. 10 cotton and the stoma of the pouch is brought out through the mid point with approximately $\frac{1}{2}$ inch extending beyond the skin. If the stoma is watched carefully for 12 hours after operation and longitudinal relaxing incisions made in the serosa when necessary to relieve oedematous tension, this part of the stoma extending beyond the skin will live for some time and prevent the skin from excoriating. Using this technique four excellent pouch dogs were obtained. This represented 100% survival from the operation and all the dogs possessed excellent innervated pouches that secreted up to 150 cc. of gastric juice per day during their control period. The pouch removed from dog No. 1 Mutt at autopsy is seen in Figures 6, 7 and 8 with dark dots across the seromuscular bridge and a dark line completing the outline of the pouch. The small size of the pouch and the absence of narrowing of the stomach can be seen.

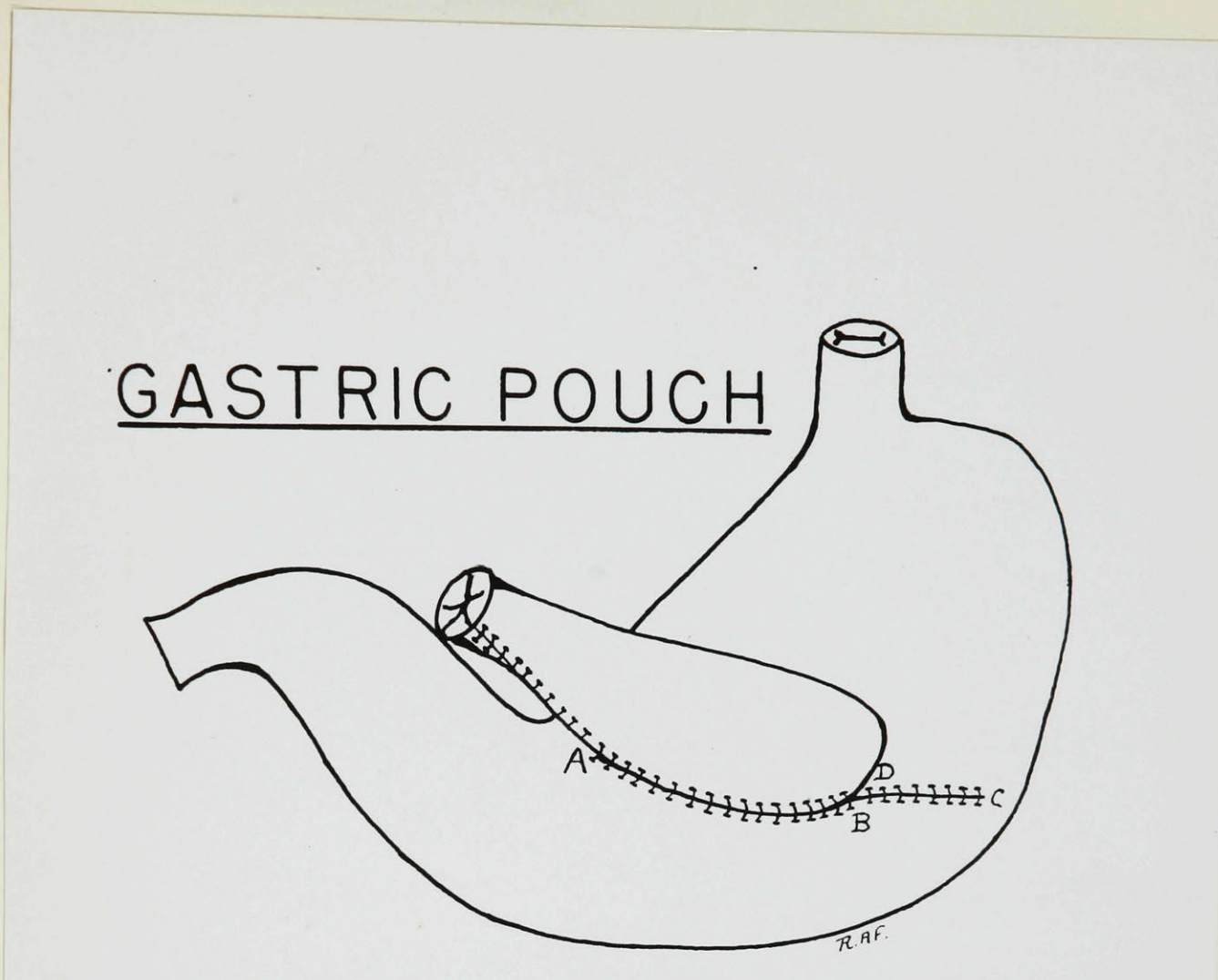


Figure 5.

Diagram showing the constructed new gastric pouch. Note the small size of the pouch in relation to the remainder of the stomach which is broadened at the site of the pouch attachment.

Figure 6 Photograph of a new gastric pouch with black dots marking the seromuscular bridge. Note the small size of the pouch in relation to the stomach.

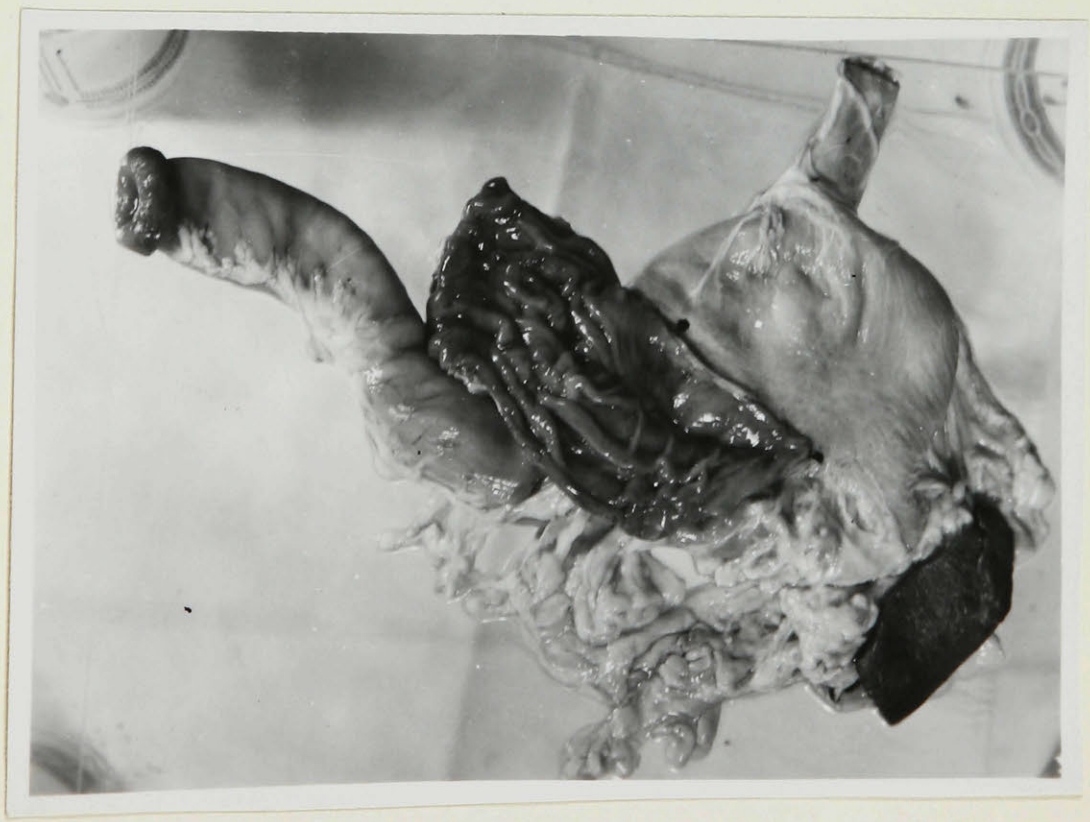


Figure 7 Photograph of new gastric pouch split open along its anterior surface.

Figure 8 Photograph of stomach behind a new gastric pouch. Note broadening of stomach instead of stenosis.

Figure 9 Photograph of stoma of dog with an indwelling catheter, two months after operation. Note absence of any excoriation.

5. Collecting Apparatus

A. Two main techniques and apparatus were used. The first consisted of several parts, Figure 10. At the top can be seen a piece of soft rubber tubing, containing numerous holes, that was pulled 1½ inches through a rubber cork. This rubber cork was cut down to fit the stoma. This part was held in position by a piece of automobile rubber tubing cut as seen in Figure 6. This was cut so that the natural curve would fit onto the dogs chest and was held in position easily with an artery clamp. The wide mouthed Erlenmeyer flask was retained in place loosely by the second piece of rubber held with an artery clamp. This apparatus was only used when the dogs were on the stand. It was a satisfactory means of collecting the secretions, but the dogs required constant care day and night to prevent excoriation around the stomas.

B. The second consisted of Malicott indwelling catheters varying in size from a French 18 to 26. The wide mouthed Erylenmeyer flask was then simply tied with cord over the dogs back. This technique was used independently by ourselves, but it was previously described by Goodman and Gilman (69).

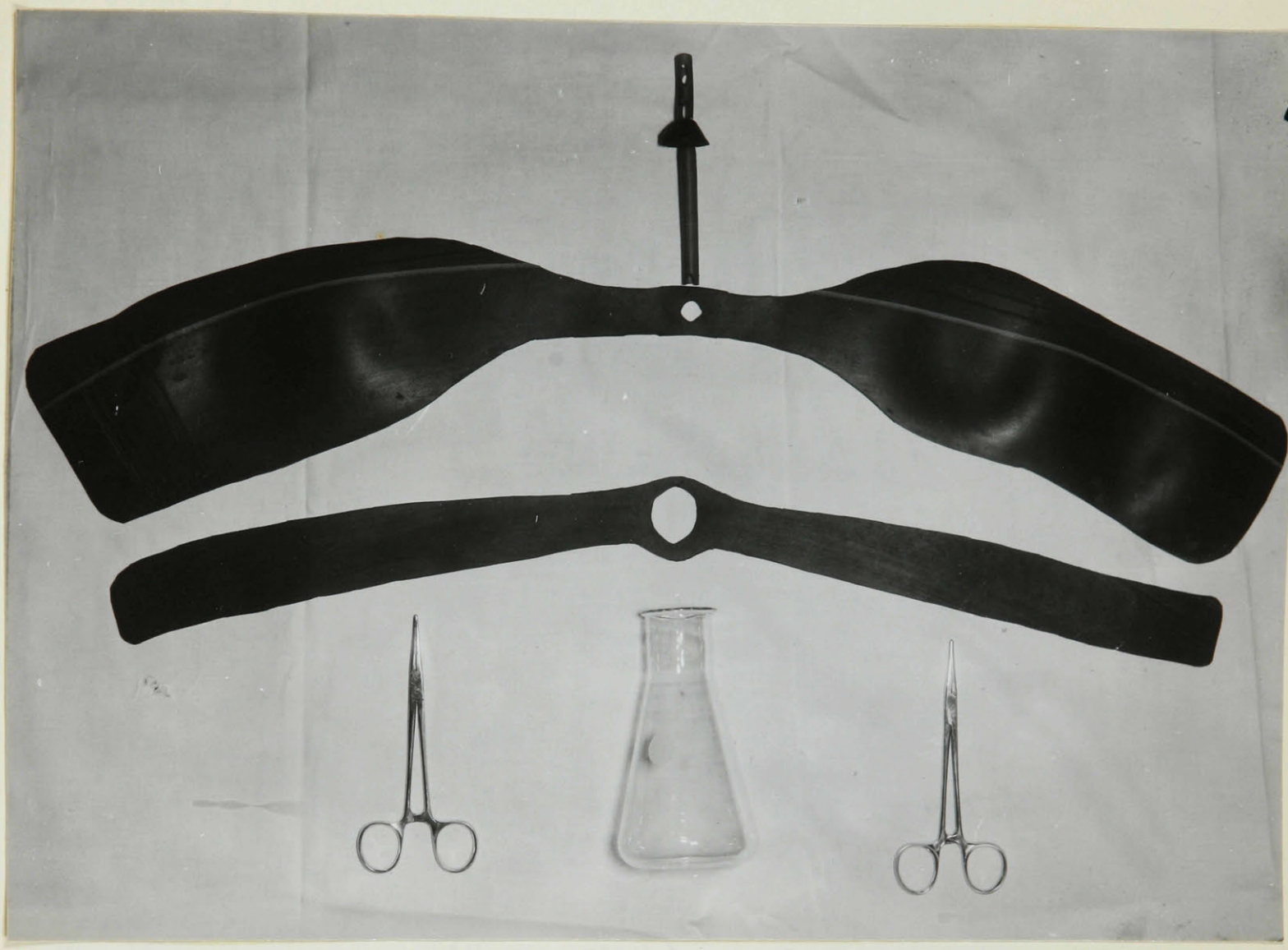


Figure 10

Photograph of collecting apparatus described in section on materials.

It was found to be a very satisfactory means of collecting the secretion and the dogs did not require the same amount of care. The indwelling catheter was found to have no significant effect on the gastric secretion. It was still possible to get a control period of 30 minutes without any secretion and histamine curves before and after its use in two dogs showed no significant variation. It was occasionally necessary to sew these catheters into the pouches for awhile to prevent the dogs from removing them at night. A typical stoma with an indwelling catheter can be seen in Figure 9. There is no excoriation around the stoma.

6. Harness - These consisted of anterior and posterior belly bands with an anterior breast strap. They were made from flour bags and if the dogs chewed the breast straps a small chain was used in its place. (Figure 12).

B. METHODS:-

1. Daily Routine of the Dogs - A very strict daily routine was adhered to in an attempt to remove all variables, so that the daily secretions could be studied and might be a good indication of the secreting function of the stomach.

On the days that the dogs were not given a histamine or insulin test they were removed from their pens and put out on the roof to run at 8.30 a.m. They were allowed to run for 30 minutes and then were brought down stairs to the office and put up on the stands. They were left on the stands for 30-40 minutes before being fed. The diet was mixed in 12 kg lots and the vitamin supplements were mixed in weekly lots for each dog and put in small tubes in a dark container in the ice box. Each day the diet for the dogs was prepared separately by mixing the weighed amount of diet with the vitamin supplement. The gastric secretion of the previous day was then added and sufficient warm water to make the diet into a mush. This diet was then given to the dogs by one of the technicians to prevent them from developing conditioned reflexes.

The dogs were force fed if they didn't eat all their daily ration. A definite technique was worked out. The dogs were held with the left hand as in Figure 11, the little finger pressed a small bit of the lip between the teeth to keep the dog from biting. The food was then forced slowly into the back of the mouth with a bulb syringe. The dog

Figure 11

Photograph of dog No 2 "Yapper", being force fed. Note method of holding lower jaw and technique of putting diet well back into mouth with large bulb syringe.

was watched carefully and allowed to stop at any sign of choking. This was a very easy and successful method of seeing that the dogs received all of their daily ration.

The desoxypyridoxine was given orally after the daily diet to those dogs receiving it. The measured amount of the solution containing 20 mgm. per c.c. was run into the dogs mouth from a pipette to be certain the dog received all the drug. The oral vitamin B12 was then given as rubofolin in a capsule to those dogs receiving it. The dogs were then given a pan of water and strapped in their harness.

It was decided to collect the secretions at 1½, 3, 5, and 6½ hours after feeding and this was done daily on every dog. Amphogel was applied around the stoma frequently each day especially when using the external collecting apparatus. The dogs were removed from the stands after 6½ hours and allowed to run on the roof for 15-30 minutes. About 3-5 cc's of amphogel was injected into the pouch and the dogs were watered and put back in their pens. When the external collecting apparatus was used it was necessary to see the dogs once or twice each

evening and apply liberal amounts of amphogel in the pouch and around the stoma. Even when the indwelling catheter technique was used it was found advisable to inject 3-5 cc's of amphogel in the pouch each night. By using this technique it was possible to prevent any excoriation around the stoma despite the large volumes of gastric juice secreted by the innervated new gastric pouches.

2. Histamine Test - It has been shown that histamine has very little positive secretory influence on the glandular cells which produce the organic components of the gastric juice, but stimulates almost exclusively the production of the fluid and acid. After the injection of histamine there is a rapid increase in volume of gastric secretion with a lowering of the pH and pepsin (66,191). It was then decided to use the histamine test along with the $1\frac{1}{2}$-5 hour secretion to study the chemical phase of gastric secretion.

The tests were carried out early in the morning to exclude any possibility of conditioned reflexes. The dogs were tied up in their pens at 6.30 a.m. and after a 30 minute control period they were given 0.5 mgm. of histamine. On the basis of

the extensive work done by Hanson, Grossman and Ivy (79) it was decided to start with 0.5 mgm. of histamine on every dog and increase the dose if necessary. It was not necessary to use more than 0.5 mgm. of histamine in any dog, for satisfactory histamine curves were obtained with that amount. The histamine was injected subcutaneously in a shaved area over the ribs and behind the shoulder. The secretions were collected every 15 minutes for 1½ hours. The volume and pH were measured and a pepsin determination done on every sample (Figure 20).

3. Insulin Test - It has been shown conclusively that injecting sufficient insulin, preferably intravenously, results in a hypoglycaemia that stimulates gastric secretion. It acts on the gastric glands through stimulation of the vagus center in the brain. Thus it was decided to use the insulin test as well as the daily secretion for the first 1½ hours to prove the pouches were innervated and study the first nervous or vagal phase of gastric secretion. The juice secreted is less acid but contains more pepsin and mucus than the juice produced by histamine stimulation. Only vagal stimulation is able to provoke the secretion of

pepsin and mucus.

The dogs were tied up in their pens at 6.30 a.m. for a 30 minute control period. A control blood sugar was taken and they were given 6 units crystalline insulin intravenously. The gastric secretion and a blood sugar was taken every 30 minutes for 2 hours. The volume and pH were measured and a blood sugar determination done. The blood sugars in every case fell below 50 mgm. percent which is considered sufficient to cause vagal stimulation (Figure 26).

4. Analysis of Gastric Secretion - The daily secretions were measured from 0-1½, 1½-3, 3-5 and 5-6½ hours and interval pH determinations were done as seen in the tables. The pH determinations were done on a standard pH meter using a buffer at pH 4. The secretions were kept to be added to the diet the following day.

The secretions collected during the histamine and insulin tests were measured and pH determinations were made immediately. Pepsin determinations were also made on the secretions collected during the histamine tests, as it was necessary to do blood sugars on the days the

insulin curves were done.

A photoelectric method was used for the determination of the peptic activity of the gastric juice. In principle, the method depends upon the use of a substrate consisting of a standardized homogenized suspension of coagulated egg white. When acted upon by pepsin, the turbidity decreases with time. It is assumed that the amount of protein digested in unit time is proportional to this decrease in turbidity, and since turbidity can be measured by means of a photoelectric colorimeter, the peptic activity of the system can thus easily be determined. Since peptic hydrolysis of protein follows a monomolecular course, and since within limits its velocity constant is a function of the concentration of pepsin, it is convenient to express peptic activity as a velocity constant. This was found to be an excellent method and gave consistantly accurate results (see graphs of histamine curves).

5. Hematology and Blood Chemistry - The standard methods were used for the blood chemistry and the red and white cell counts. The hemoglobin determinations were done on the standard Evelyn hemoglobinometer. The differential counts were done

on slides stained with Wright's stain. The size of the red blood cells was measured by a new technique described by Waugh (196). It is an ingenious method depending upon a red corona of light seen when looking through an ordinary red blood cell smear at a small light. The size of the corona varies with the size of the cells and is measured. The method was easy to use, fast and accurate.

6. Series 1 - This series consisted of seven dogs, one with a Heidenhein pouch, five with Cope pouches and one with a modified Cope pouch. These dogs developed many complications and they all died. The experimental plan was used in Series 2.

7. Series 2 - This series consisted of four dogs with new gastric pouches. The dogs were all used for more than one part of the investigation so on the basis of results these are divided into 4 groups.

8. Group 1 - This consisted of one dog kept as a control for 54 days. He was fed daily his set amount of basal synthetic diet containing vitamin B6 but no vitamin B12. He was put on the fixed daily routine and given histamine and insulin tests with the other dogs.

9. Group 2 - This consisted of three dogs that were put on the pyridoxine free diet and given desoxypyridoxine. They received this combination until they became fairly ill clinically, and it was decided not to chance loosing the dogs, so it was stopped. The first dog went for 37 days, the second 13 days and the third 28 days. The desoxypyridoxine was then stopped and the dogs were started on pyridoxine. They were given 1.5 mgm. pyridoxine daily in the diet and a booster of 5 mgm. by hypo for 2 days.

10. Group 3 - This consisted of two dogs fed their set amount of pyridoxine free diet alone. They were left on this diet until they had developed marked hypochromic microcytic anemia characteristic of pyridoxine deficiency. They were then started on 1.5 mgm. pyridoxine daily in their diet and a booster of 5 mgm. by hypo for 2 days.

11. Group 4 - This consisted of one dog fed his set amount of basal synthetic diet and given desoxypyridoxine. He was left on this combination until its effect had been studied, and the desoxypyridoxine was then stopped.

CHAPTER IV

RESULTS AND THEIR INTERPRETATION

Series 1 - This series consisted of seven dogs, one with a Heidenhein pouch, five with Cope pouches and one with a modified Cope pouch.

The dog with the Heidenhein pouch had an excellent post operative course. The dog was being standardized on the synthetic diet when the pouch prolapsed and it was impossible to replace it.

We learned from this dog the necessity of having a pouch well attached in the abdominal cavity. The next Heidenhein pouch dog made in the laboratory had the pouch sewn to the abdominal wall and it did not prolapse.

The five dogs with Cope pouches all developed complications and eventually died. (Table of results) Some of the dogs died after the first operation, some the second and some after a third operation to free the pouch from the stomach. This was performed on two dogs to prove the unavoidable complication of excoriation around a flank stoma and the stenosis with delayed emptying of the stomach with large gastric pouches.

COPE POUCHES

Dog No.	No 1.	No 2.	No 3.	No 4.	No 5.
1st Operation	x	x	x	x	x
Anaesthetic death	x				x
2nd Operation		x	x	x	
Complications			x	x	
Breakdown of mucous membrane bridge		x		x	
Excoriation around Stoma			xxx	xxx	
Revision of stoma			x	x	
Excoriation around new stoma			xxx	xxx	
Freeing of pouch from stomach		x		x	
Continuation of excoriation		xx		xx	
Inanition		xx	xxx	xx	
Distemper			x		x
Died	x	x	x	x	x

TABLE OF RESULTS

The modified Cope pouch, made by bringing the stoma out the abdominal incision at the first operation, was made in an attempt to exclude the second operation. This dog developed distemper and died. The complications developed by the Cope pouch dogs may be listed as follows:

1. Post operative anaesthetic death following a long difficult operation.
2. Anorexia.
3. Loss of weight
4. Extensive excoriation around the flank stoma, and easy breakdown of the mucous membrane bridge.
5. Anemia from bleeding around the stoma and malnutrition.
6. Inanition.
7. Demineralization due to loss of large volumes of secretion.
8. Prolonged secretion due to delayed emptying of the stenosed stomach.

We learned from the complications developed by the Cope pouch dogs that an innervated pouch must be:-

1. Simple to construct.
2. Small enough not to stenose the stomach if the stomach is properly constructed.
3. Have a good nerve supply.
4. The mucous membrane bridge must not be too large or in a position where it can be easily punctured.
5. Secrete an adequate volume of secretion.
6. The stoma must be in a ventral position so as not to lead to uncontrollable excoriation.

These facts were used in the designing and construction of the new gastric pouches.

Series 2 - This series consisted of four dogs with new gastric pouches. The dogs were all used for more than one part of the investigation, so on the basis of results, they are divided into four groups.

Group 1 - This consisted of one dog "Skippy", Figure 47, kept for 54 days as a control. He was estimated to be six months of age at the beginning of the experiment. He weighed over 10 kg. and ate 170 gm. of the synthetic diet daily together with the gastric secretions of the previous day. The dog remained healthy during this period. He was very active, ate well and gained weight. (Figure 52). There was no change in the average volume or pH of his daily gastric secretion and his blood picture remained constant. There were noted daily fluctuations in the volume of gastric secretion in all the dogs, either during control or experimental periods. Alvarey (2) noted this in humans. This variation was more marked with changes in the weather despite the fact the dogs were only out of doors $\frac{1}{2}$ - 1 hour per day. There were several periods when the volume of gastric secretion was increased on cold days. (Table next page). Roth (149) reported an increase in the volume of gastric juice secreted by humans immersed in cold water. He was able to prevent this with histaminase. There was no change in the histamine and insulin tests done at the beginning and

Table showing the changes in daily volume of gastric secretion of Dog No. 4 "chubby", and the daily mean temperature readings.

Day	Total gastric secretion	Mean temperature of day	Day	Total gastric secretion	Mean temperature of day
1	113 c.cs.	26.6 deg.C.	10	77 c.cs.	38.6 deg.C.
2	98	32.7	11	94	27.0
3	92	33.9	12	97	30.9
4	76	34.2	13	85	36
5	77	36.0	14	80	38
6	88	38.0	15	86	38
7	78	33.8	16	65	36
8	100	34.2	17	81	38
9	79	46.6	18	130	26

Note the increase in volume of gastric secretion on cold days.

end of this control period (Figures 53 and 57).

Thus it is possible to feed a six month old pup with an innervated gastric pouch secreting a large volume of gastric juice on a synthetic diet without the dog developing any signs of a deficiency providing the gastric juice is fed back to the animal.

Group 2 - This consisted of three dogs that were put on a pyridoxine free diet and given desoxypyridoxine.

The first, Dog No. 1 "Mutt" was estimated to be five months old. He weighed 15.25 kg. and was given 300 gms. of the synthetic diet daily, together with the gastric secretions of the previous day.

He ate well, was healthy and secreted large volumes of gastric juice (Figure 19). He had excellent histamine and insulin curves (Figures 20 and 26). The insulin curve and the large volume of secretion for the first 1½ hours indicate that he had a good nerve supply to his pouch.

On the 14th day he was started on a pyridoxine free diet and 80 mgm. of desoxypyridoxine. He had a precipitous fall in the volume of gastric secretion the next day (Figure 19). His appetite remained good for some time and yet the volume of

gastric secretion was remarkably reduced. The dog soon began to develop signs of pyridoxine deficiency accelerated by desoxypyridoxine. He developed small ulcers on his legs that gradually increased and later a dermatitis around his mouth, eyes and on his limbs. He also developed a stomatitis with ulcers in his mouth, followed by anorexia, loss of weight, diarrhoea, nausea, vomiting, lethargy and even ataxia (Figure 13). There was a slight increase in the pH of the daily gastric secretion (Figure 19). The effect on the histamine tests was a considerable reduction in the volume, elevation of pH and drop in pepsin value of the gastric secretion. (Figures 20, 21, 22).

The effect on the insulin curves was a slight reduction in volume and slight elevation of pH of the gastric secretion. (Figures 26 and 27).

The effect on the blood was the development of a slight microcytic hypochromic anemia, leucopenia lymphopenia, polynucleosis and eosinophilia. The anemia remitted for a few days and then started to increase.

The dog became very sick so it was decided to stop the desoxypyridoxine. In two days he had not improved and it was decided to start him on

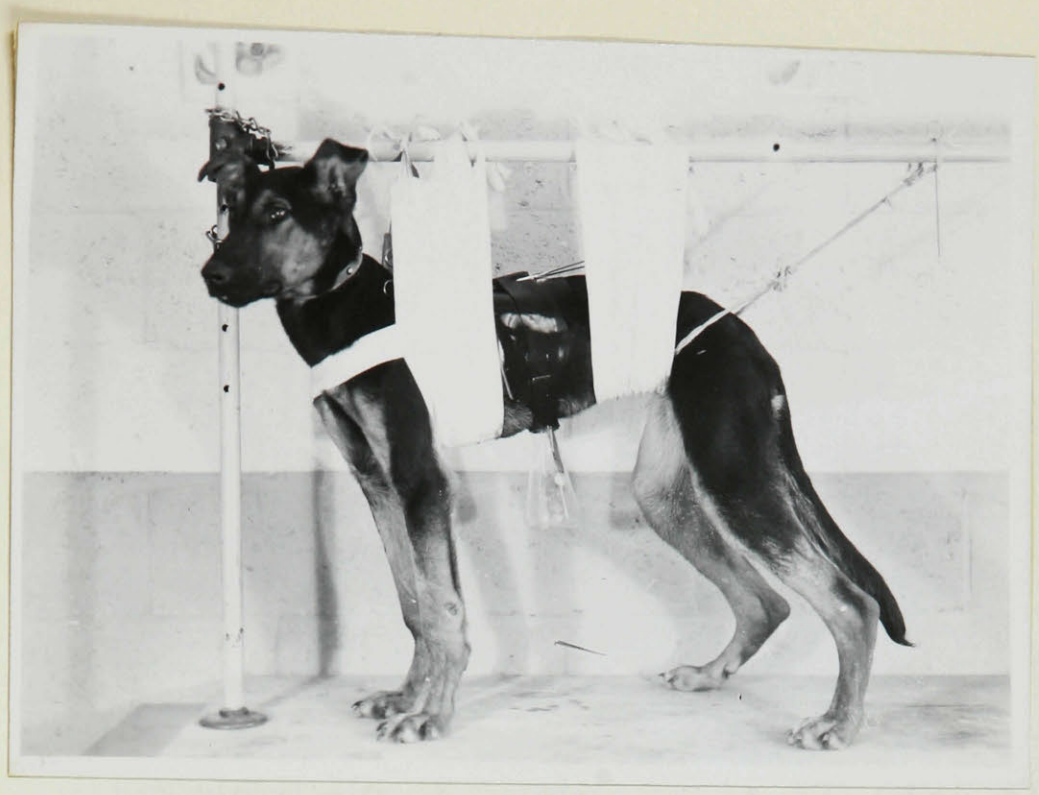


Figure 12. Photograph of dog No 1 "Mutt", 12 days after starting pyridoxine free diet and desoxypyridoxine. Note healthy appearance of dog with small ulcer on the left front leg, also note collecting apparatus and harness.

Figure 13. Photograph of dog No 1 "Mutt", 41 days after starting pyridoxine free diet and desoxypyridoxine. Note slumped listless appearance of dog, with dermatitis, especially around mouth, eyes and limbs, and large ulcers on limbs.

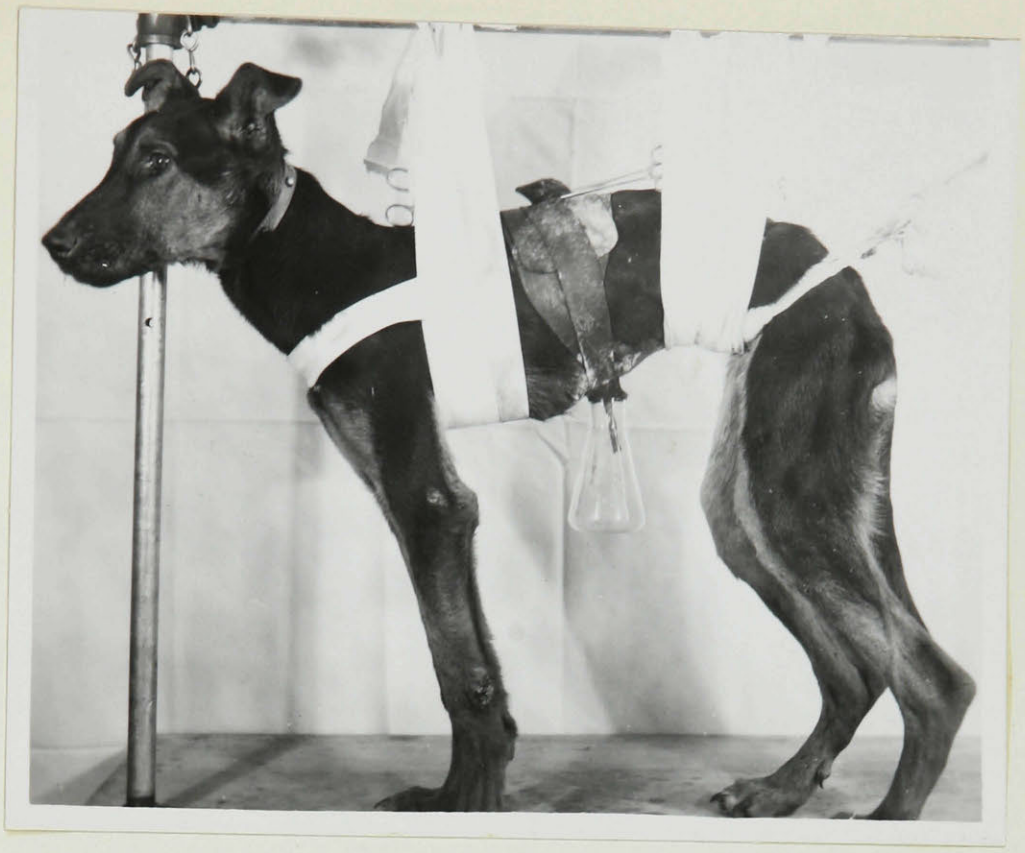


Figure 14. Photograph of dog No 1 "Mutt", 9 days after treatment with pyridoxine. Note improvement in clinical appearance, large ulcers still remain on limbs.

Figure 15. Photograph of dog No 1 "Mutt", 30 days after treatment with pyridoxine. Note extention of dermatitis and increase in ulcers on limbs.

DOG NO. - 1 - MUTT

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (Hours)
1					66	23	15	10
2					35	40	46	-
3					40	19	43	-
4					70	22	52	-
5					41	55	58	-
6					56	32	26	-
7					50	45	42	-
8					57	39	37	-
				pH	1.22	1.12	1.15	-
9					58	48	49	-
10					51	51	31	-
11					37	45	67	-
12					50	45	30	-
13					94	47	52	-
14					70	56	49	-
			Started Pyridoxine Free Diet and Desoxypyridoxine.					
15					15	10	16	20
16					32	12	31	24
17					35	15	20	14
18					35	30	11	8
19					38	17	44	-
				pH	0.88	0.94	0.92	
20		x			36	16	11	-
21		x			45	18	19	13
22		x			55	10	4	5
23		x			48	16	5	3
24		x			39	12	1	3
25		xx	x		33	15	17	4
26					28	7	10	12
27					38	25	25	30
28					31	15	16	8
29					26	16	10	10
30					34	30	31	20
31					30	23	17	10
32					29	17	13	10
				pH	0.94	0.93	1.21	1.07
33					7.5	13	9	8
34	x				38	11	16	11
35	xxxx		x		25	9	4	1
36	xxxx		x		13	5	13	5
37	xxxx		x		10	3	2	2
38	xx		xxx		19	10	17	4
39	xxxx				15	25	14	7
40	xxxx				40	26	30	20
41	xx				50	19	12	27
42	xx				26	20	14	40
43	xx				30	15	17	23
44	x				34	8	10	23
45	xx				20	8	9	6
46	xx				27	9	12	6
47	xx				45	19	19	11
48	xx				31	15	13	14
49	xx				45	19	21	28
50	xx				32	6	5	26

Figure 16.

Table of results, dog No 1 "Mutt".

DOG NO. - 1 - MUTT

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (Hours)
				pH	0.93	1.01	1.69	0.98
51	xx				8	27	7	15
				Desoxypyridoxine Discontinued.				
52	xx				14	3	3	10
53	xxxx				12	9	4	0
54	xxxx				25	8	5	7
				Started on Synthetic Diet Containing Pyridoxine.				
55	xxxx				54	15	18	38
56	xxx				53	12	20	22
57	xx				56	36	39	18
58	xx				65	59	24	31
59	xx				30	37	52	24
60					47	30	32	30
61					60	26	42	33
62					51	31	45	25
63					38	29	50	22
64	x	x			35	11	58	25
65	xx	x			39	15	35	24
66	xx	xxx			17	14	58	35
67	xx	xx			14	13	48	18
68	x	x			25	25	39	26
69	xx	x			11	12	35	40
				pH	1.13	0.93	0.82	0.83
70	xx				35	20	47	41
71	x				35	25	71	45
72					32	14	60	35
73					10	16	24	24
74					29	14	64	60
75					37	30	57	28
76					18	23	60	50
77					20	25	50	30
78					14	30	40	34
79					12	6	50	35
80					35	12	60	24
81					20	21	60	42
82					25	12	41	26
83					16	15	40	35
84					16	16	41	40
85					30	31	74	35
86					40	12	72	36
87					13	15	18	26
88					13	13	58	44
89					15	17	20	50
90					20	10	10	37
91					8	10	23	40
92					26	8	15	44
				pH	1.12	1.30	1.19	1.00
93	Started on Vitamin B-12				32	12	52	45
94					37	11	59	38
95					33	30	71	43
96	xx		xx		22	8	45	31
97	xxxx		xxx		20	10	40	30
98	xx	xxx	xxx		2	3	2	20
99	xx	xxx			27	10	40	55
100	xx	xx			27	4	4	8
				Started on Liver				

Figure 17.

Table of results, dog No 1 "Mutt".

DOG NO - 1 - MUTT

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (HOURS)
101	xx	xx			13	-	45	60
102	x	xx			20	27	40	30
103	xx	x			14	5	48	55
104	x	x			22	15	30	35
105	x	x			17	10	40	40
106	x				17	15	50	30
107	xxx				12	18	40	20
				pH	1.06	1.06	1.06	1.06
			Pouch Broken Down					
108	xxx				10	10	40	30
109	x				17	10	55	60
110					15	15	50	30
111					20	20	30	30
112					10	10	40	40
113					20	10	30	20
114					39	10	50	20
115					11	8	14	18
116					35	24	38	45
117					50	15	98	30
118					28	14	27	30
119					17	15	25	20
120					17	10	20	10
121					40	10	40	20
122					28	8	10	-
123					40	20	42	30
124					35	15	15	20
125					30	10	30	30
126					36	22	20	10
127					25	10	20	20
128					30	10	30	20
129					20	10	30	10
130					30	10	30	10
131					36	22	20	10
132					35	15	15	20
133	x				40	20	30	26
134					40	20	30	26
135					30	10	25	20
136					40	20	31	30
				pH	0.92	0.90	0.89	0.89
137					35	22	35	15
138					55	15	68	30
139					35	10	20	20
140					71	81	91	62
			Started on Meat					
141					66	38	46	68
142					48	56	67	50
143					35	50	60	45
144					49	38	61	45
145					40	20	50	30
146					25	10	50	37
147	xx				30	25	50	-
148	xx				20	40	60	15
149					20	30	50	30
150					25	16	65	40

Figure 18.

Table of results, dog No 1 "Mutt".

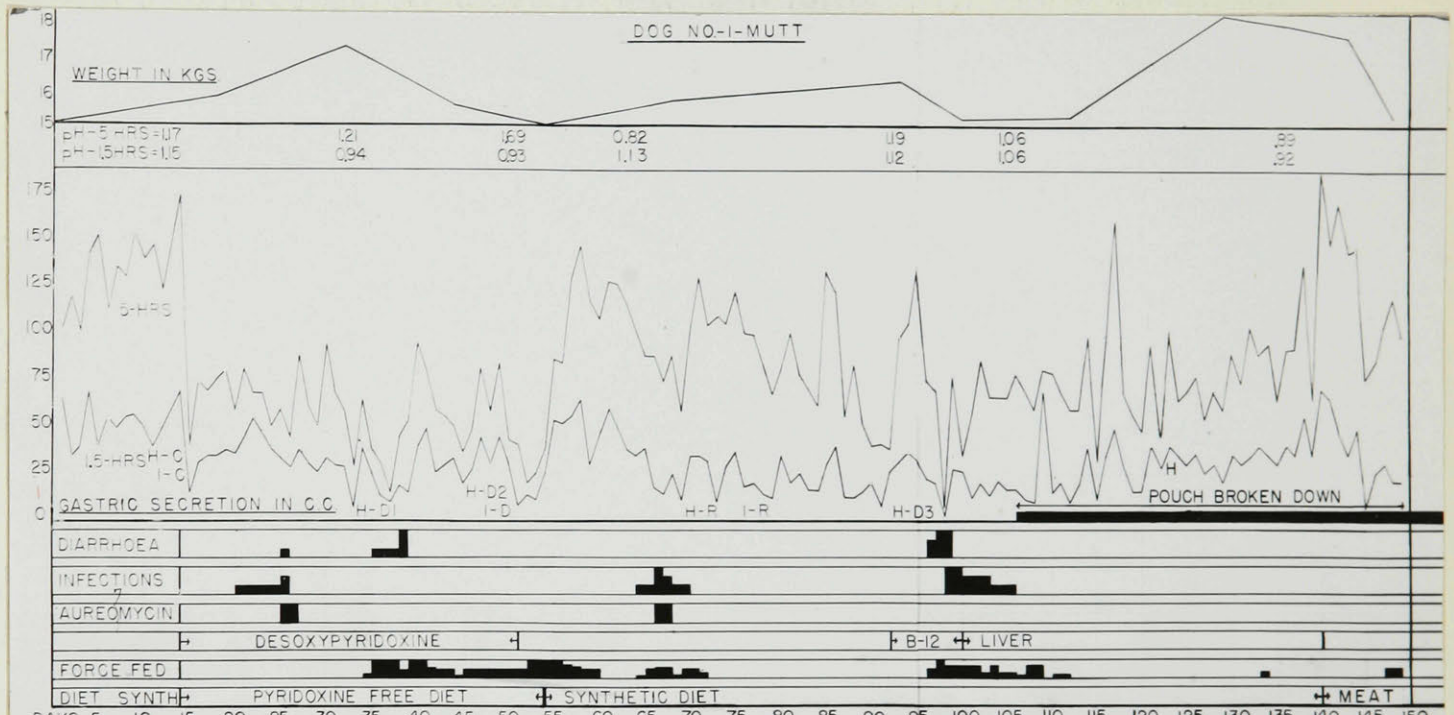


Figure 19

Composite graph of the results of dog No 1 "Mutt". Note the marked reduction in volume of gastric secretion and the rise in pH when the dog was on a pyridoxine free diet and desoxypyridoxine. The recovery to normal when pyridoxine was administered. The daily variation of gastric secretion. The effect of diarrhoea and infection on gastric secretion.

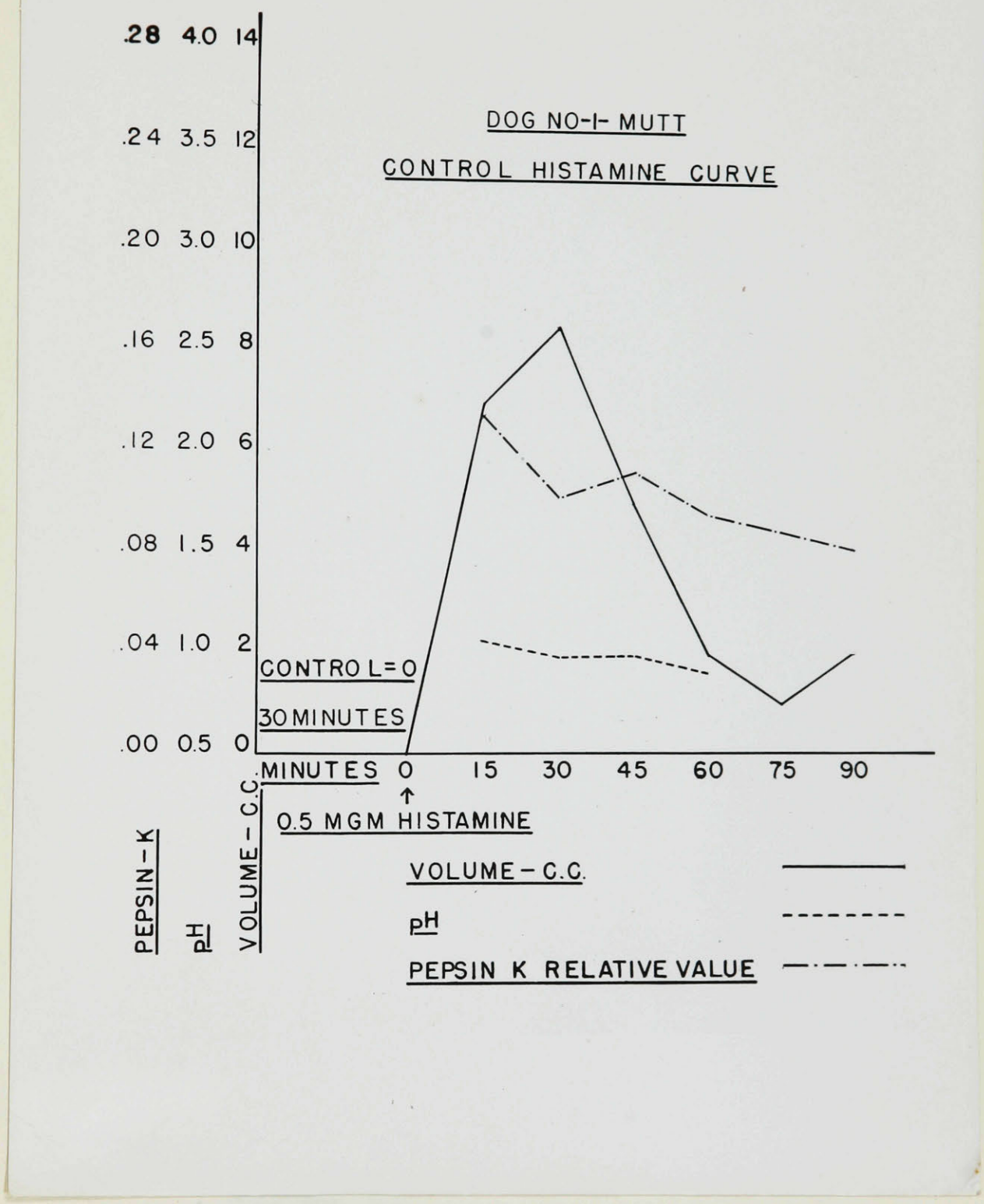


Figure 20

Note large volume (25.5 c.c.) low pH and high pepsin of the gastric secretion following injection of 0.5 mgm. of histamine.

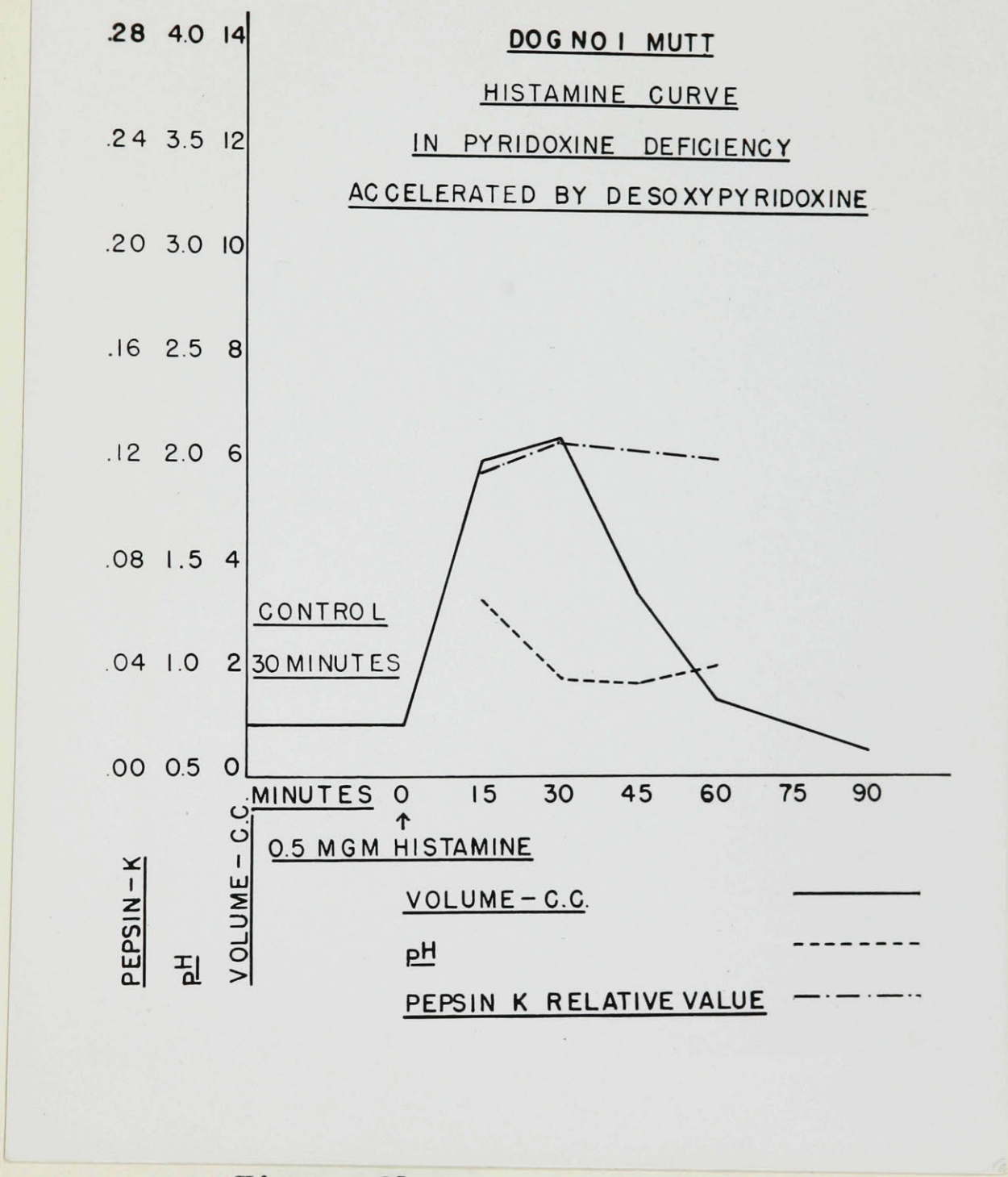


Figure 21

After 19 days on a pyridoxine free diet and desoxypyridoxine. Note the slight decrease in volume (21.0 c.c.) of gastric secretion following injection of 0.5 mgm. of histamine.

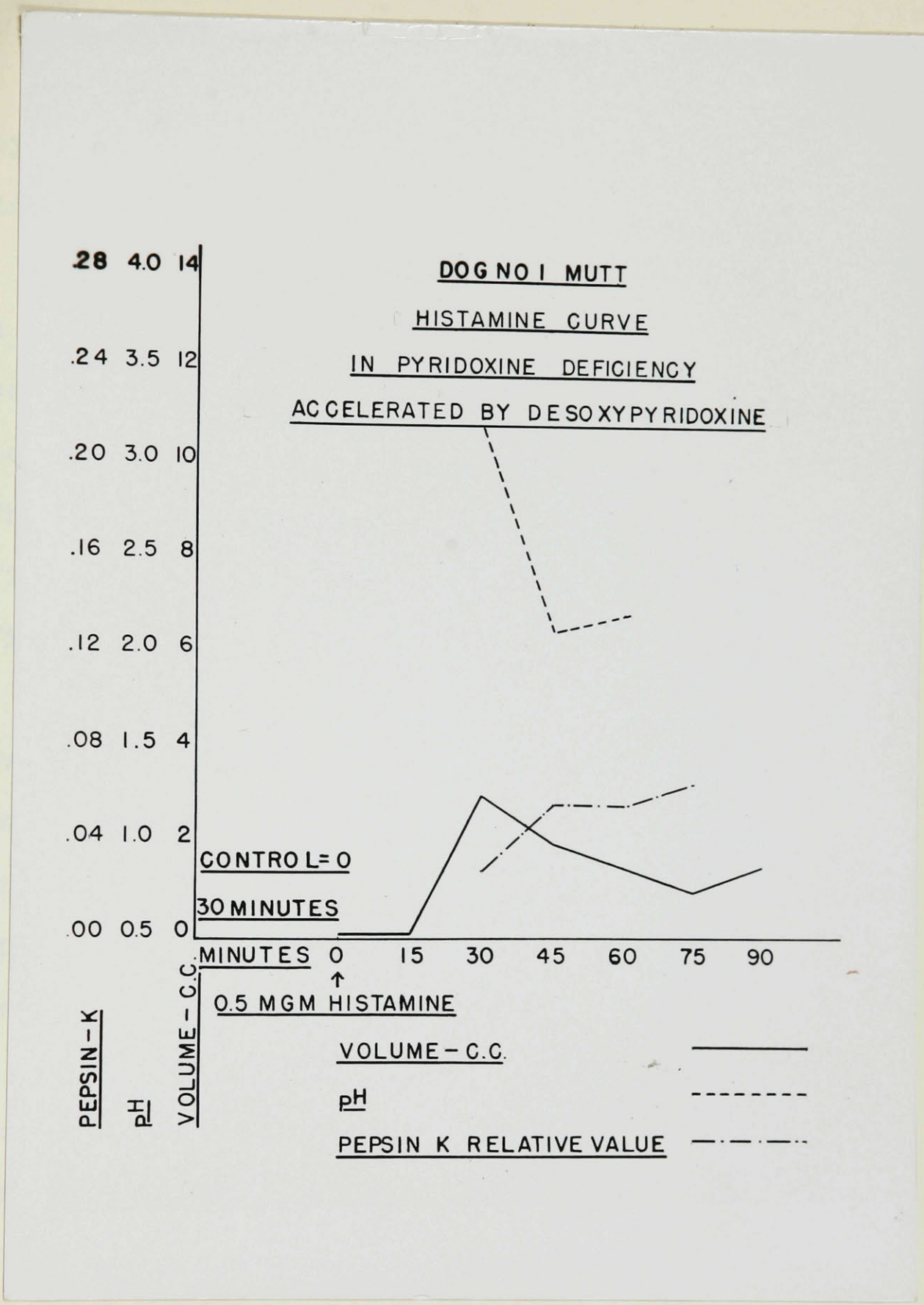


Figure 22

After 32 days on a pyridoxine free diet and desoxypyridoxine. Note the marked decrease in volume (9 c.c.) with elevations of pH and decrease in pepsin of gastric secretion.

pyridoxine. He was started on pyridoxine 1.5 mgm. in his daily diet and 5 mgm. by hypo for two days.

Administration of pyridoxine resulted in a rapid return of the volume and pH of the gastric secretion to normal. The dog also started to look better clinically and to eat his diet and gain weight. The effect on the gastric secretion can be seen in Figure 19 and on the histamine and insulin curves in Figures 23 and 28.

It is important to note that the dog had two attacks of acute upper respiratory infection and was given aureomycin. During both these attacks and one of diarrhoea there was a significant reduction in the volume and elevation of pH of the gastric secretion (Figure 19).

The dog appeared to be very susceptible to infections. This is of interest because there was a marked reduction in lymphocytes and possibly antibodies. This is further evidence to support the experimental work, indicating that antibodies are concentrated in lymphocytes.

After almost recovering from the accelerated mild pyrodixine deficiency state the dog started to show signs of developing a deficiency of some unknown

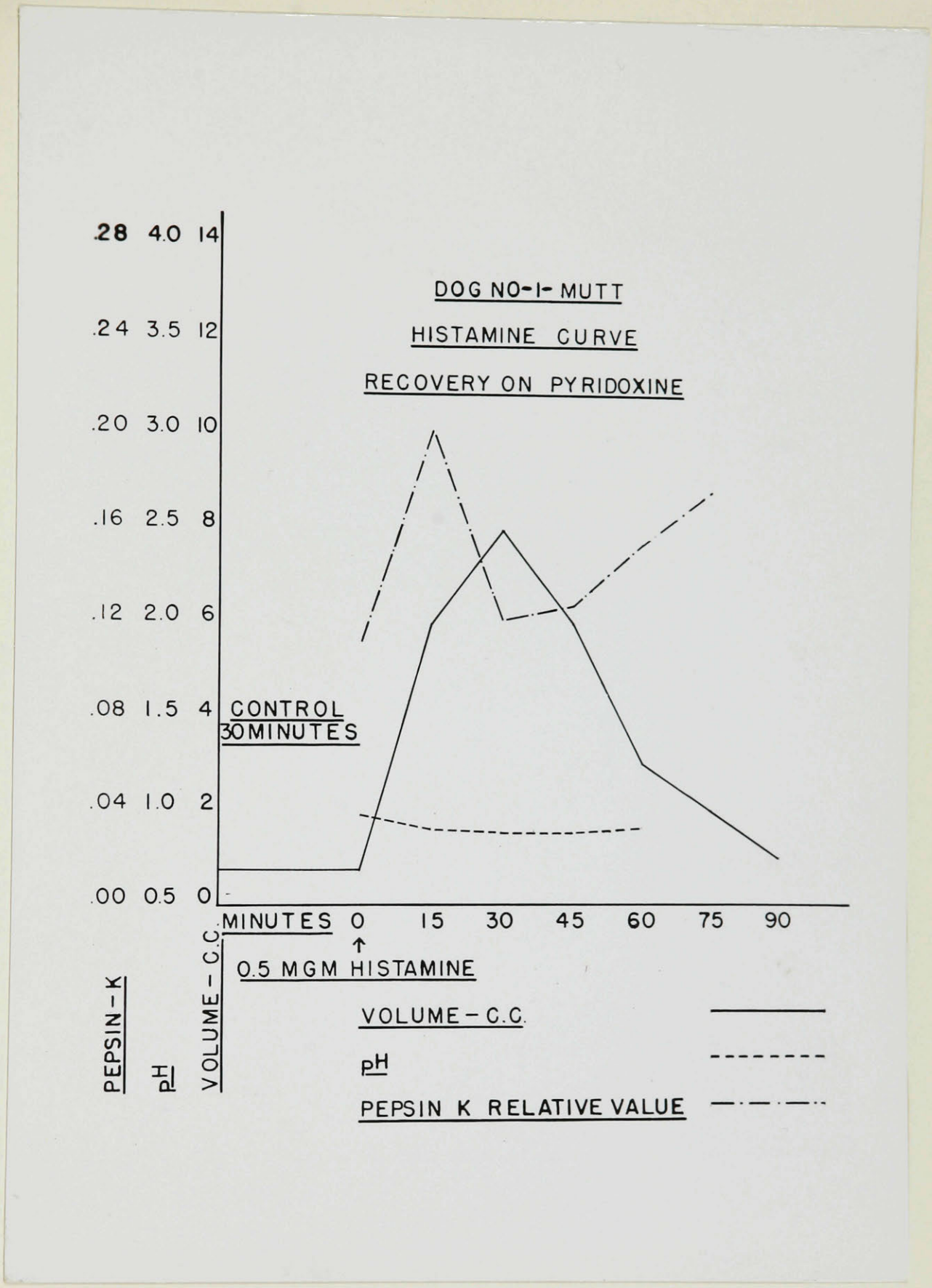


Figure 23

After 16 days on pyridoxine therapy. Note the return of the volume (26.0 c.c.) pH and pepsin of gastric secretion.

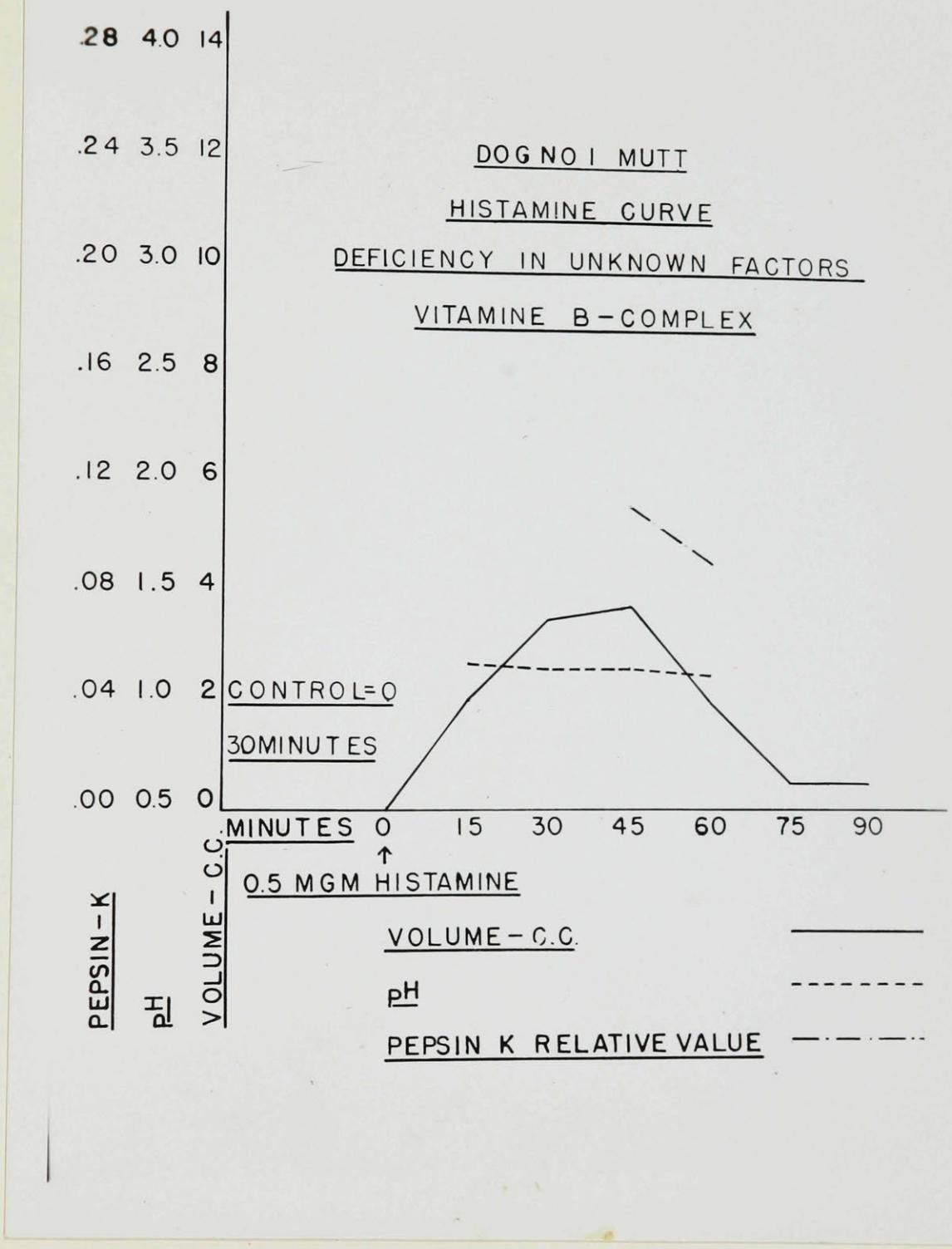


Figure 24

After 92 days on a synthetic diet there is suggestive evidence of a deficency in an unknown component of the vitamin B complex. Note the reduction of volume (12 c.c.) of gastric secretion.

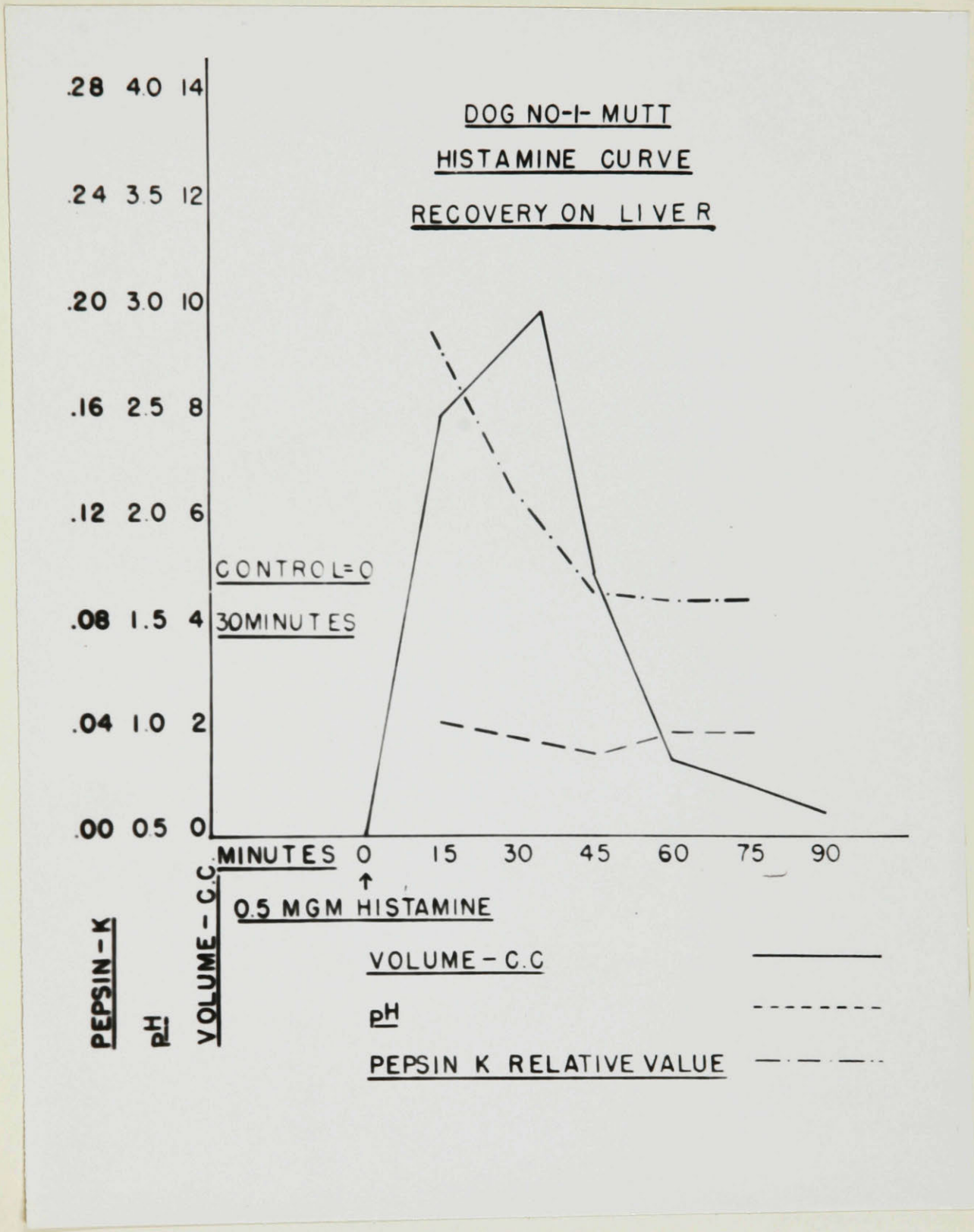


Figure 25

After 25 days on massive liver therapy. Note the return in the volume of gastric secretion (25.0 c.c.) following injection of 0.5 mgm.of histamine.

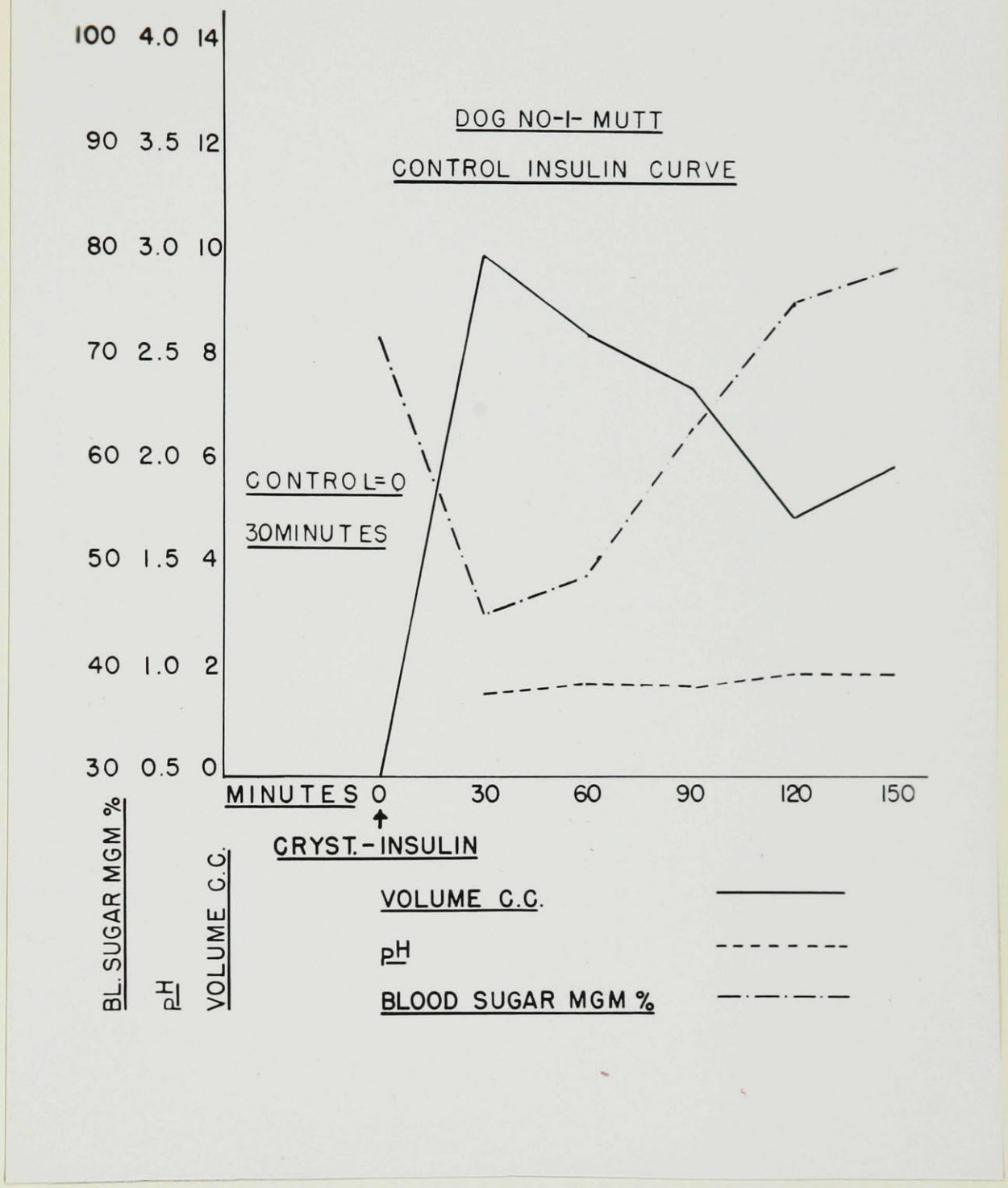


Figure 26

Note the large volume (37.c.c.) and low pH of the secretion following injection of 6 units of insulin. Also the drop in blood sugar to 46 mgm. percent.

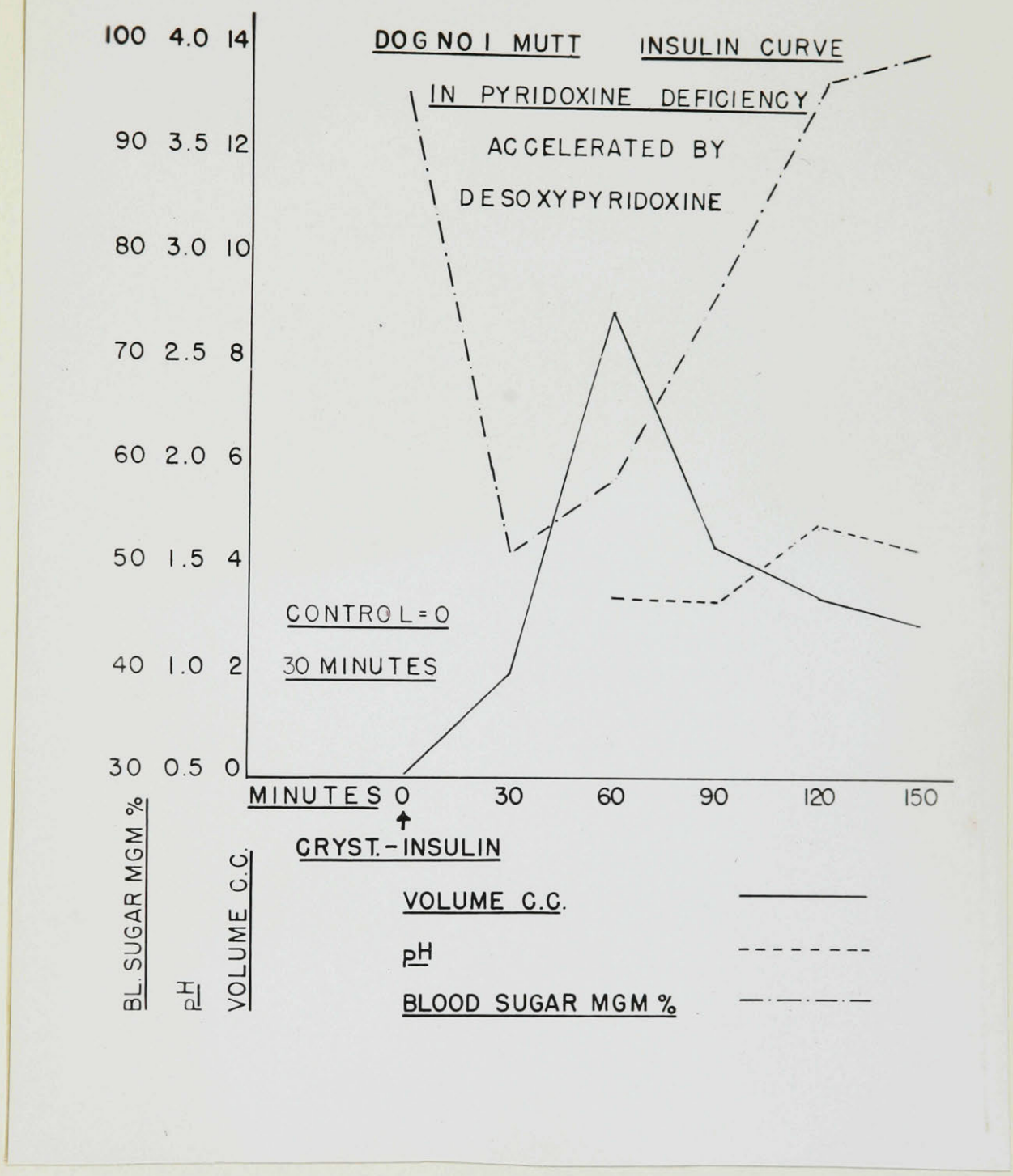


Figure 27

After 34 days on a pyridoxine free diet and desoxypyridoxine. Note the slight reduction in volume (22.8 c.c.) and elevation of pH of gastric secretion following injection of 6 units of insulin. Also the drop in blood sugar to 48 mgm. percent.

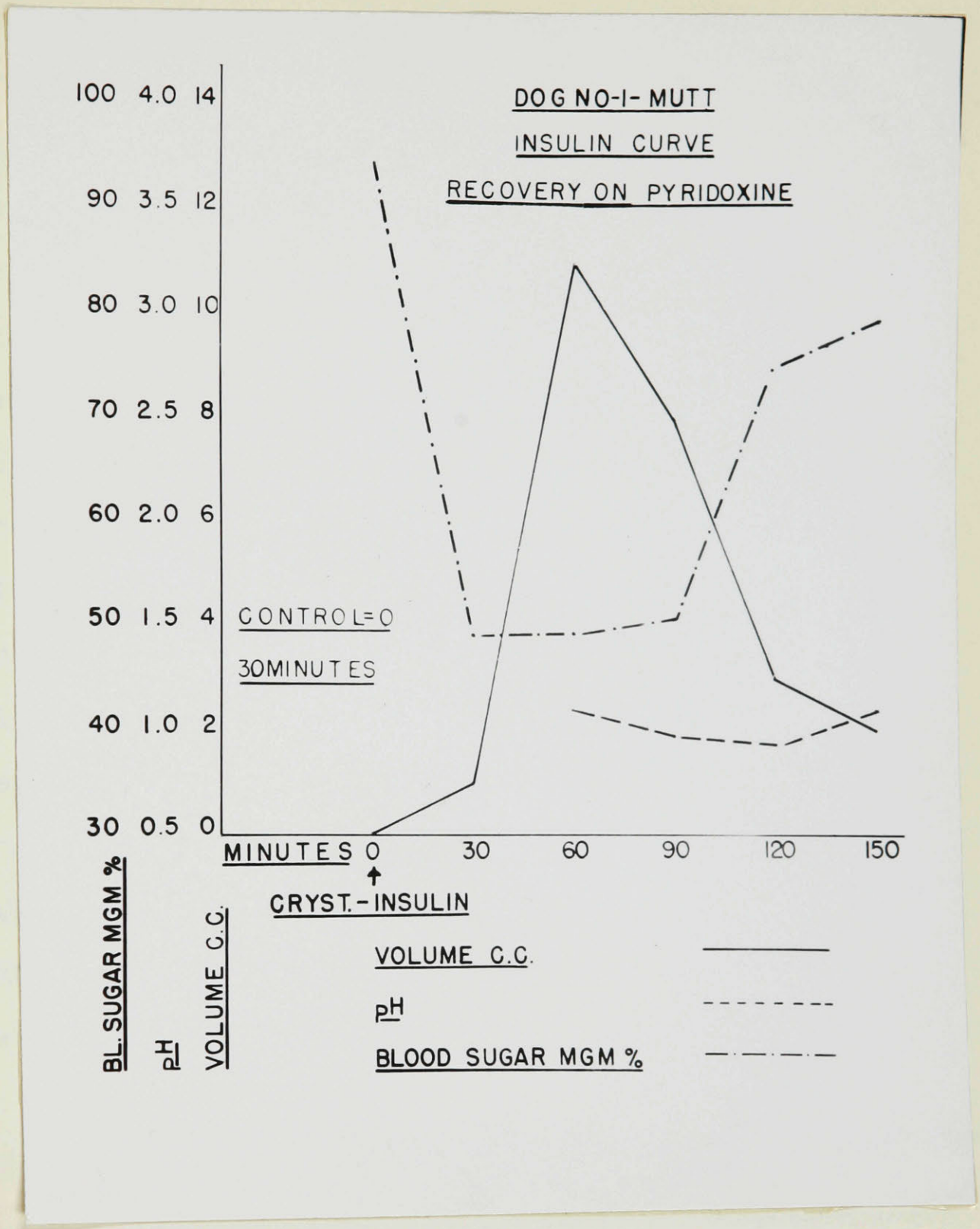


Figure 28

After 22 days on pyridoxine therapy. Note return of volume (25.5 c.c.) and pH of gastric secretion following injection of 6 units of insulin. Also drop in blood sugar to 48 mgm. percent.

component or components of the vitamin B complex. The dermatitis increased especially on his extremities and around his mouth and eyes. He developed considerable alopecia in these areas and they became very itchy. He became lethargic and although his appetite remained fairly good there was a reduction in volume and slight elevation of pH of the daily gastric secretion (Figure 19). There was also a reduction in the volume and slight elevation of pH of the gastric secretion following injection of 0.5 mgm. histamine (Figure 24). The blood chemistry showed a normal N.P.N. and a reduction in total protein to 5.73 gms. % with 2.53 gms. % albumen and 3.20 gms. % globulin. His blood sodium and chloride were normal but his potassium was slightly low (3.0 M.eq/L). There was no anemia or leucopenia associated with this deficiency. (Fig. 31).

It was decided to try the effect of vitamin B12 on the above signs of a possible deficiency state. The dog became so ill with a large abscess on his right foreleg that it was necessary to start him on dessicated liver to save his life. He was given massive doses of dessicated liver up to 80 capsules per day.

DOG NO-1- MUTT

HEMATOLOGY

DATE	HEMOGLOBIN	ERYTHROCYTE COUNT	HEMATOCRIT	ERYTHROCYTE SIZE
Dec.23	90	6,100,000	45	-
Dec.28		Operation New Gastric Pouch		
Jan.16	77	4,770,000	39	-
Jan.25	82	5,190,000	42	-
Jan.26		Started on Pyridoxine Free Diet and Desoxypyridoxine.		
Feb. 2	70	4,390,000	34	7.1
Feb. 6	75	-	-	7.1
Feb. 8	91	5,900,000	45	7.1
Feb.15	81	4,950,000	41	7.1
Feb.21	90	5,680,000	44	7.0
Feb.28	75	4,650,000	35	7.0
Mar. 5		Desoxypyridoxine Discontinued.		
Mar. 7		Started on Complete Synthetic Diet Containing Pyridoxine.		
Mar. 8	-	-	-	6.96
Mar. 16	81	5,090,000	40	6,96
Mar. 22	90	5,550,000	42	6.96
Mar. 28	77	4,380,000	38	7.1
Apr. 5	92	5,180,000	48	7.1
Apr. 11	100	6,500,000	48	7.1
Apr. 14		Started on Vitamin B - 12.		
Apr. 19	99	6,000,000	48	7.1
Apr. 22		Started on Dessicated Liver.		
Apr. 26	100	6,110,000	47	7.1
May. 3	99	6,560,000	46	7.1
May. 10	85	5,250,000	40	7.1
May. 25	84	5,490,000	38	7.1
May. 31	84	5,555,000	39	7.1
June 1		Started on meat		
June 7	107	7,260,000	49	7.1

Figure 29

Table of results.

DOG NO. - 1 - MUTT

HEMATOLOGY

DATE	LEUCOCYTE COUNT	POLYMORPHS	LYMPHOCYTES	EOSINOPHILES	MONOCYTES	BASOPHILES
Dec. 23	7,950	-	-	-	-	-
Dec. 28		Operation - New Gastric Pouch.				
Jan. 16	24,650	-	-	-	-	-
Jan. 25	14,000	86	11	3	0	0
Jan. 26		Started on Pyridoxine Free Diet and Desoxypyridoxine				
Feb. 2	12,600	84	5	10	1	0
Feb. 6	7,900	86	9	3	2	0
Feb. 8	10,700	80	8	12	0	0
Feb. 15	11,400	77	3	20	0	0
Feb. 21	9,150	79	7	14	0	0
Feb. 28	10,000	92	5	3	0	0
Mar. 5		Desoxypyridoxine Discontinued.				
Mar. 7		Started on Complete Synthetic Diet Containing Pyridoxine.				
Mar. 8	-	83	7	9	1	0
Mar. 16	28,000	88	6	6	0	0
Mar. 22	12,400	71	14	15	0	0
Mar. 28	25,000	72	10	18	0	0
Apr. 8	14,900	79	5	15	1	0
Apr. 11	16,800	76	8	15	0	1
Apr. 14		Started on Vitamin B-12.				
Apr. 19	21,250	81	7	11	1	0
Apr. 22		Started on Dessicated Liver.				
Apr. 23	19,950	79	6	15	0	0
May . 3	10,850	71	13	13	3	0
May 10	16,450	74	7	17	1	1
May 25	14,500	78	5	17	0	0
May 31	17,000	82	6	12	0	0
June 1.		Started on Meat				
June 7.	21,900	91	2	4	2	1

Figure 30

Table of results.

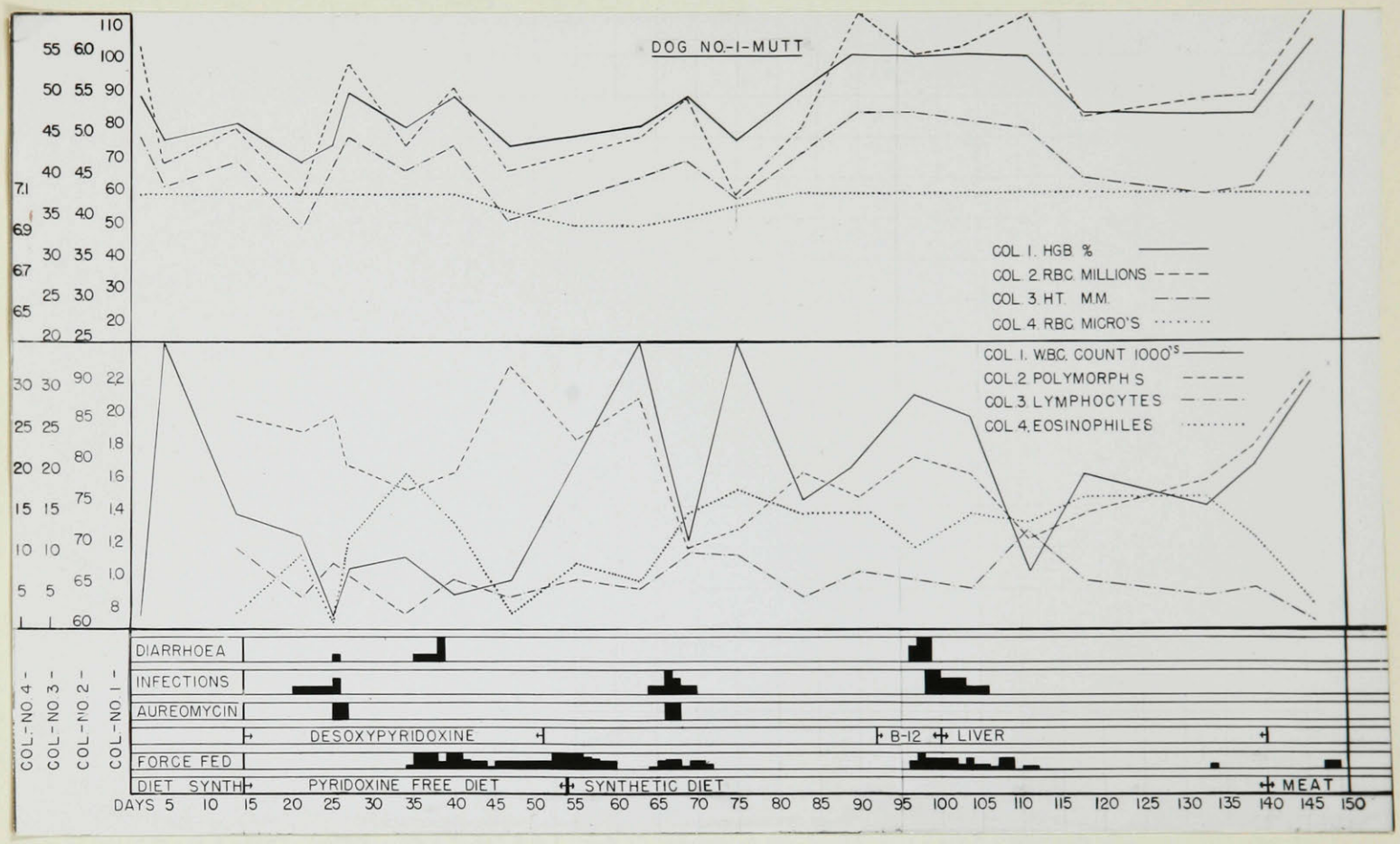


Figure 31

Composite graph of the hematological results of dog No 1 "Mutt". Note the slight hypochromic anemia, leucopenia, lymphopenia and polynucleosis on a pyridoxine free diet and desoxypyridoxine and the recovery on pyridoxine. Also note that liver had no significant effect on the blood picture.

After recovering from the infection, the dog started to recover from the deficiency state. Unfortunately the mucous membrane bridge broke down and the volume and pH of the gastric secretion has little significance. There were days when it remained clear and on those days the volume and pH were normal. A histamine curve 25 days after starting liver was normal (Figure 25).

The dog remained lethargic and inactive. The dermatitis continued with an increase in the itchiness and alopecia. The dog had gained 3 kg. in weight in 20 days, but he had considerable oedema of his legs and abdomen. His blood chemistry showed a normal N.P.N. a total protein of 5.20 gms. % with 1.77 gms. % albumin and 3.43 gms. % globulin. There was a slight but insignificant fall in the red cell count and hemoglobin. It was thus decided to try meat in an attempt to save the animal and to see its effect on this possible deficiency state.

The dog was started on meat which he ate very well for a few days; there was an improvement in his blood picture and he lost his oedema. However, despite these facts, he became very ill and it was believed the dog had cirrhosis of the liver. He

started to become dehydrated so he was sacrificed and autopsied. The autopsy revealed no other organic disease than the dermatitis. The liver, kidney, heart, adrenals, pancreas, pituitary, thyroid and all other organs were normal.

The second dog in this group was Dog No. 2 "Yapper" (Figure 32). He was a brother to "Skippy" and was estimated to be six months of age at the beginning of the experiment. He weighed 13.4 kg. and ate 240 gms. of the synthetic diet daily together with the gastric secretions of the previous day.

He ate well, was healthy and secreted large volumes of gastric juice (Figure 36). He had excellent histamine and insulin curves (Figures 37 and 41). The insulin curve and the large volume of secretion for the first 1½ hours indicate that he had a good nerve supply to his pouch.

On the 14th day he was started on a pyridoxine free diet and 68 mgm. of desoxypyridoxine. There was a gradual marked decrease in the volume and a very slight elevation of the pH of the gastric secretion. The volume continued to decrease for 13 days, when the dog developed lethargy, anorexia

Figure 32

Dog No 2 "Yapper". Note method of collecting gastric secretion with an indwelling catheter in stoma of pouch.

DOG NO - 2 - YAPPER

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (HOURS)
1					24	32	25	18
2					31	50	38	-
3					30	17	61	-
4					34	27	47	-
5					29	32	46	-
6					44	27	23	-
7					36	40	30	-
8					37	39	42	-
				pH	1.08	1.10	1.11	1.11
9					45	30	48	-
10					46	36	72	-
11					42	35	65	-
12					32	51	42	-
13					43	59	77	-
14					30	38	54	-
	Started on Pyridoxine Free Diet and Desoxypyridoxine							
15					25	60	61	-
16					18	34	64	-
17					31	55	34	25
18					23	12	45	33
19					8	20	40	30
20					13	29	34	25
21					14	12	43	30
22	x				17	24	30	23
23	x				10	4	40	23
24	x				14	8	12	14
25	xxx				13	17	27	18
26	xxx				14	10	17	11
27	xxxx				5	5	5	6
	Desoxypyridoxine Discontinued							
28	xxxx		xx		10	7	0	0
29	xxxx				12	10	9	5
30	xxxx				8	9	22	10
31	xxxx				14	11	24	12
32	xxxx				9	13	20	6
				pH	1.03	1.57	1.04	1.14
33	xxxx				8	7	13	22
34	xxxx				14	14	24	8
35	xxxx				15	8	15	13
36	xxxx				7	15	19	10
37	xxxx				11	15	19	20
38	xxxx				18	20	36	27
39	xxxx				19	34	38	28
40	xxxx				10	24	33	23
41	xxxx				25	30	32	23
42	xxxx				12	17	21	42
43	xxxx				13	17	26	25
44	xxxx				21	23	41	25
45	xxxx				14	14	39	8
46	xxxx				13	34	38	14
47	xxxx				11	11	31	26
48	xxxx				14	16	31	26
49	xxxx				15	17	53	15
50	xxxx				21	26	24	30
				pH	0.92	0.88	0.83	0.82

Figure 33

Table of results.

DOG NO - 2 - YAPPER

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½	(HOURS)
51	xxxx				13	28	53	30	
52	xxxx				15	17	53	15	
53	xxxx				8	15	10	20	
54	xxxx				11	18	32	30	
55	xxxx				17	18	39	27	
56	xxxx				21	10	11	20	
57	xxxx				15	7	20	13	
58	xxxx				12	8	14	36	
59	xxxx				14	15	24	16	
60	xxxx				27	17	31	24	
61	xxxx				30	30	43	30	
62	xxxx				22	18	40	25	
63	xxxx				28	27	35	28	
64	xxxx				26	45	33	34	
65	xxxx				12	35	45	32	
66	xxxx				16	26	42	17	
67	xxxx				17	16	18	30	
68	xxxx				10	20	17	40	
69	xxxx				12	3	3	2	
				pH	1.38	1.80	2.02	-	
70	xxxx		x		6	2	7	18	
71	xxxx				9	6	13	12	
72	xxxx				12	8	17	21	
73	xxxx				11	8	16	10	
74	xxxx				12	15	15	-	
	Started on Complete Synthetic Diet Containing Pyridoxine								
75	xxxx				24	10	36	22	
76	xxxx		x		10	13	20	26	
77	xxxx		xxx		26	21	20	24	
78	xxxx	xxx	xxx		8	5	25	11	
79	xxxx	xxx	xxx		23	5	25	11	
80	xxxx	xxx	xxx		10	10	80	43	
81	xxxx		xxx		18	11	54	37	
82	xxx		xxx		19	22	51	31	
83	xxxx				13	22	35	32	
84	xxxx				26	24	25	31	
85	xxxx				27	31	50	30	
86	xxx				29	28	53	29	
87	xxx				21	23	38	28	
88	xxx				22	26	46	24	
89	xxxx				22	25	40	40	
90	xxxx				19	22	44	31	
91	Starved				24	31	27	20	
92	"				25	20	20	5	
				pH	1.13	1.03	1.00	1.32	
93	"				20	24	15	5	
94	xxxx				20	10	18	15	
95	xxx				23	23	25	22	
96	xxxx				17	21	19	18	
97	xxxx				26	25	35	20	
98	xxxx				21	24	24	22	
99	xxxx				24	24	34	20	
100	xx				37	29	21	26	
101	xx				27	17	40	27	
					Started on Vitamin B - 12				
102	xxx				19	24	37	24	

Figure 34

Table of results.

DOG NO - 2 - YAPPER

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½	(HOURS)
103	xxx				35	32	23	18	
104	xx				33	21	21	16	
105	xx				22	17	40	26	
106	xx				23	38	38	15	
107	xxx				27	27	45	45	
				pH	1.32	0.93	0.92	-	
108	xxx				22	39	34	10	
109	xxx				24	27	49	45	
110	xxx				33	19	45	31	
111	x				24	30	47	23	
112	x				34	30	40	37	
113	xxx				34	20	48	14	
114	xxx				27	23	35	37	
115	xxx				27	16	36	43	
116	xxx				30	20	37	22	
117	x				27	29	36	21	
118	x				46	25	24	25	
119	xx				21	25	37	24	
120	xx				20	22	52	21	
121	xx				20	40	29	20	
122	x				22	25	47	20	
123	x				24	28	40	15	
124	x				33	33	55	22	
125	xx				13	31	43	20	
126	x				25	35	78	27	
127	x		x		21	22	57	25	
128	x		x		25	25	38	20	
129	xxx		xxx		36	28	35	25	
130	xx		x		14	18	40	20	
131	xx		x		33	39	43	25	
132	xx				20	18	50	27	
133	x				25	42	38	25	
134	x				27	38	40	25	
135	x				23	25	48	31	
136	x				25	12	54	25	
				pH	1.06	0.96	0.90	0.92	
137	x				37	32	59	17	
138	x				30	28	58	25	
139	xx				34	28	47	20	
140	x				27	38	47	25	
141	x				42	28	59	30	
142	x				38	41	54	33	
143	x				36	25	66	17	
144	x				34	41	48	17	
145	x				14	57	43	20	
146	x				25	38	50	30	
147	x				15	31	56	20	
148	x				16	35	49	23	
149	x				23	35	49	23	
150	x				23	35	50	25	
151	xx				22	28	45	31	
152	xx				24	32	50	27	
153	xx				26	36	66	31	
				pH	0.99	0.91	0.91	0.94	
154	xx				31	29	54	40	

Figure 35

Table of results.

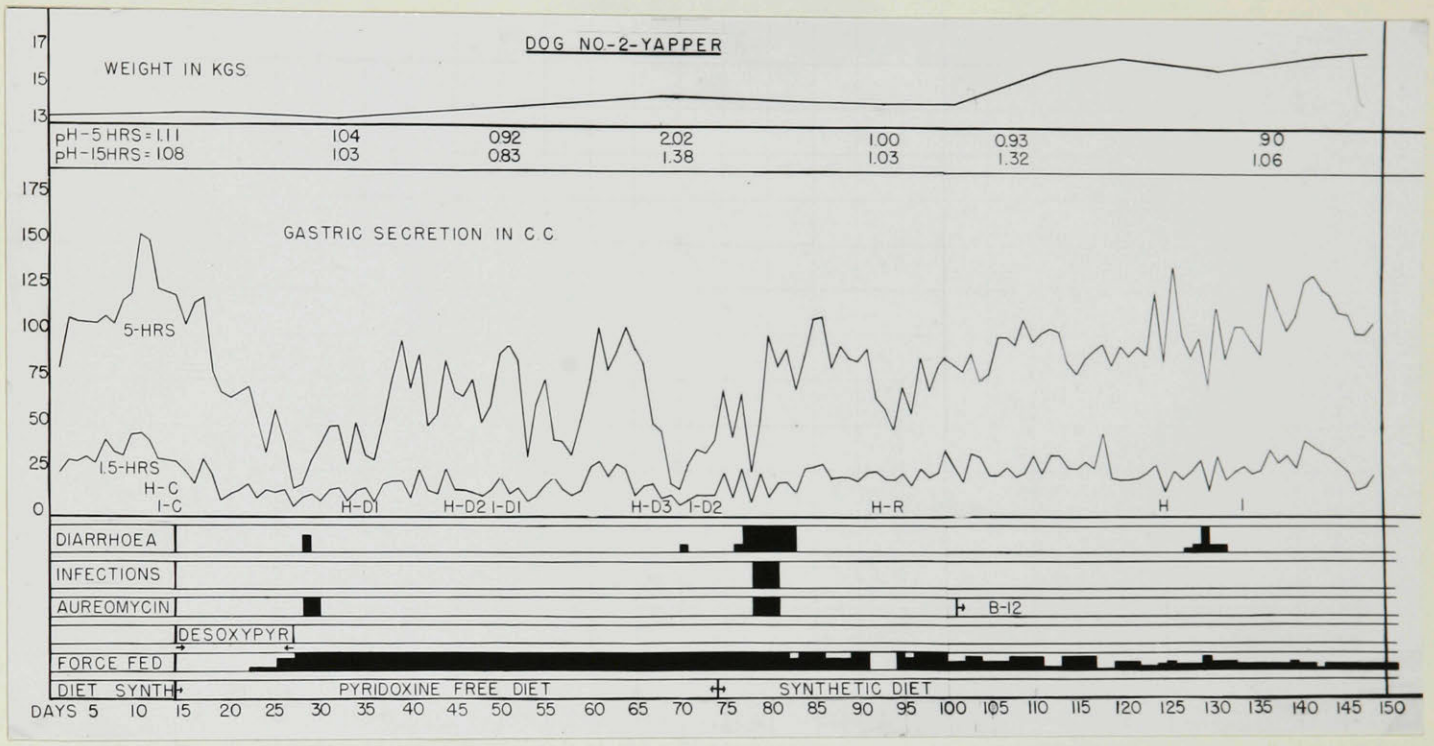


Figure 36

Composite graph of the results of dog No 2 "Yapper". Note the marked reduction in volume of gastric secretion on a pyridoxine free diet and desoxypyridoxine. The reduction in volume and elevation of pH on the pyridoxine free diet alone. The recovery on pyridoxine. The improvement in appetite and marked gain in weight on vitamin B 12.

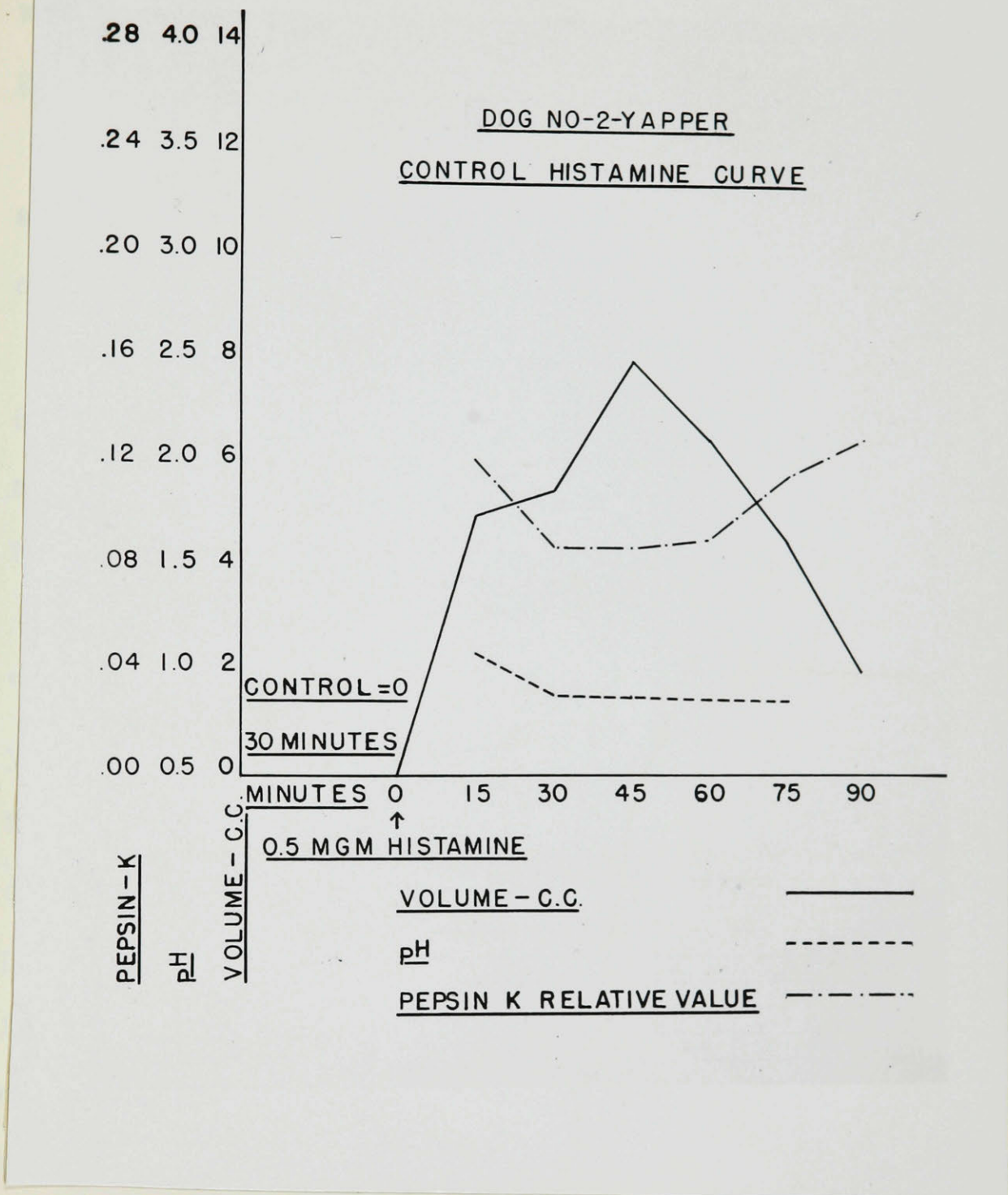


Figure 37

Note large volume (31.5 c.c.) low pH and pepsin of the gastric secretion following injection of 0.5 mgm. of histamine.

and finally nausea, vomiting and diarrhoea. There was a very slight decrease in the total volume of gastric secretion in a histamine test (Figure 38).

In that short time the dog did not develop anemia, but he did develop a leucopenia, lymphopenia and polynucleosis.

The dog became so ill it was decided to discontinue the desoxypyridoxine and leave him on a pyridoxine free diet alone. This was done and the results will be discussed in Group 3.

The third dog in this group was Dog No. 4 "Chubby" (Figure 48). She was estimated to be one year old at the beginning of the experiment. She weighed 13 kg. and ate 200 gms. of the synthetic diet daily together with the gastric secretions of the previous day. The dog remained healthy and was used first to study the effect of desoxypyridoxine alone on gastric secretion. She was then stabalized and used to study the effect of a pyridoxine free diet and desoxypyridoxine on gastric secretion. She was given a pyridoxine free diet and 65 mgm. of desoxypyridoxine daily. There was a gradual decrease in the volume of gastric secretion (Figure 64). The dog became slightly lethargic, developed anorexia and a mild

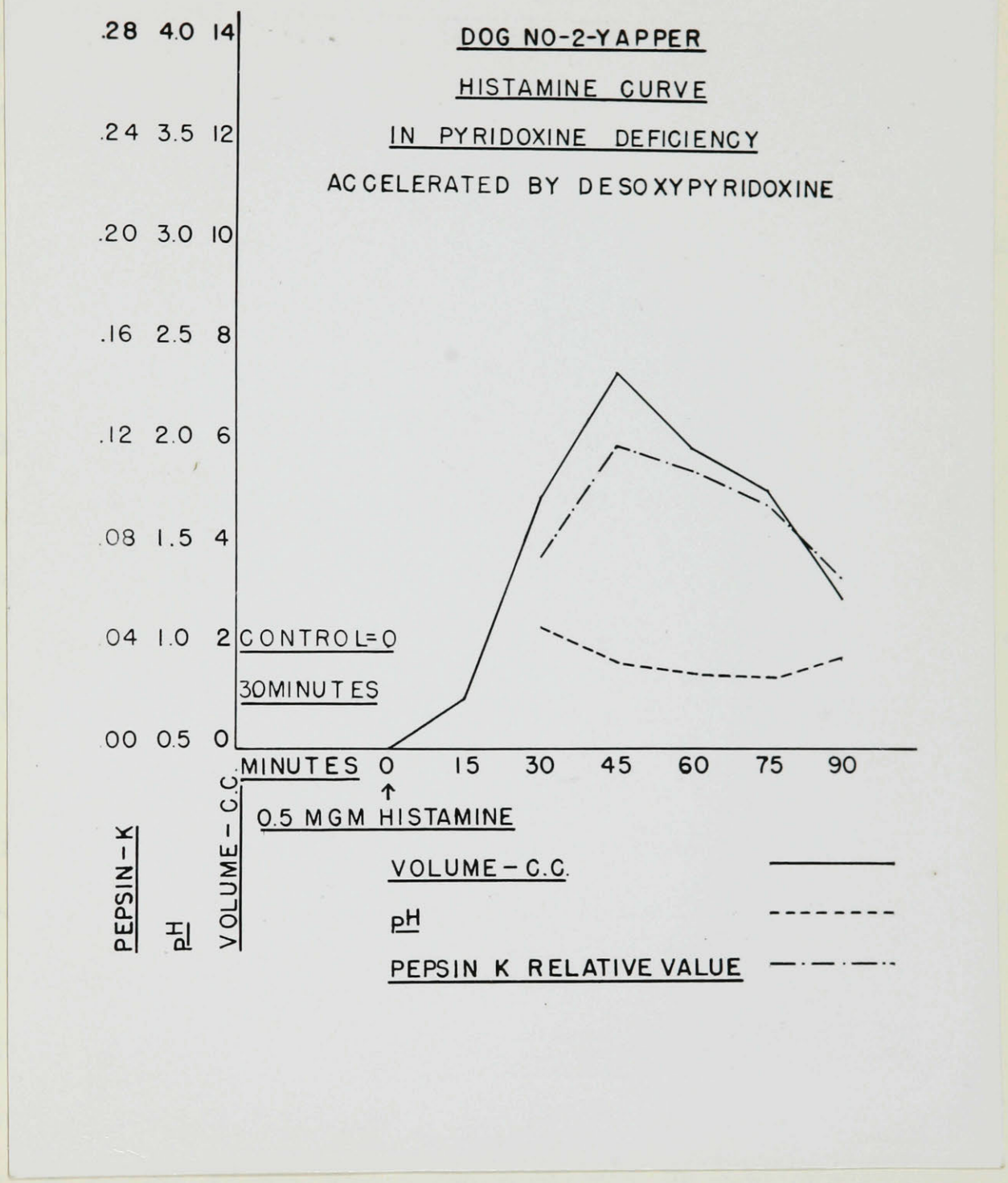


Figure 38

After 19 days on a pyridoxine free diet and desoxypyridoxine. Note the very slight decrease in volume (27.0 c.c.) of gastric secretion.

dermatitis on its legs and face.

The effect on the blood was the development of a mild microcytic hypochromic anemia, slight leucopenia, lymphopenia and polynucleosis.

The effect on the histamine curve was a slight reduction in volume (Figure 65). There was no significant change in the insulin curves.

The desoxypyridoxine was discontinued and she was started on pyridoxine 1.5 mgm. in her daily diet and 5 mgm. by hypo for 2 days. There was a rapid return of the gastric secretion, blood and clinical condition of the dog to normal (Figures 64 and 68).

It has thus been shown in 3 dogs with innervated gastric pouches, that there is a marked reduction in the daily volume with a slight elevation of pH of the gastric secretion on a pyridoxine free diet and desoxypyridoxine. These dogs developed an accelerated clinical picture of pyridoxine deficiency with added signs and symptoms due to the toxicity of desoxypyridoxine. The dermatitis is worse with more marked lethargy, anorexia and even nausea and vomiting. There is a microcytic hypochromic anemia that may remit for a short period only to progress

later. There is a leucopenia, marked lymphopenia and polynucleosis.

Group 3 - This consists of two dogs fed a pyridoxine free diet alone.

The first dog in this group was Dog No. 2 "Yapper". He was used in the second group to study the effect of a pyridoxine free diet and desoxypyridoxine on gastric secretion. After the desoxypyridoxine was discontinued he was fed 240 gms. of a pyridoxine free diet with the gastric secretion of the previous day. The daily volume of gastric secretion increased for a few days, but started to decrease again as the dog developed more signs and symptoms of pyridoxine deficiency. This decrease in volume became quite marked after 50 days on the diet and was accompanied by an elevation of pH (Figure 36). The dog continued to be lethargic, listless and have marked anorexia.

There was a slight reduction in the volume of gastric secretion in the histamine and insulin curves (Figures 37, 39, 41 and 42). There was a fairly marked microcytic hypochromic anemia, lucopenia, lymphocytosis and neutropenia.

The experiment was continued as long as it

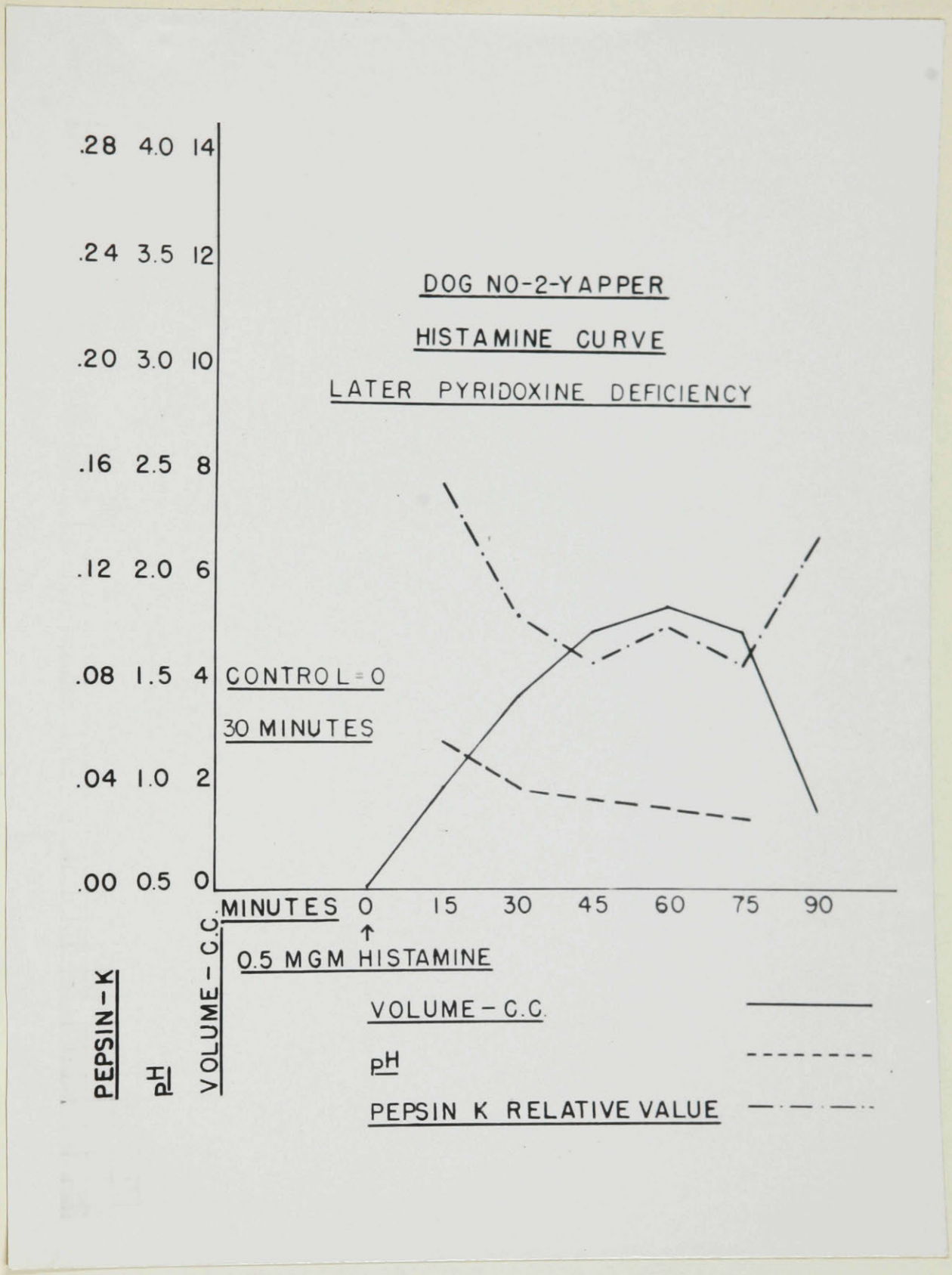


Figure 39

After 56 days on a pyridoxine free diet. Note the slight decrease in volume (23.0 c.c.) of gastric secretion.

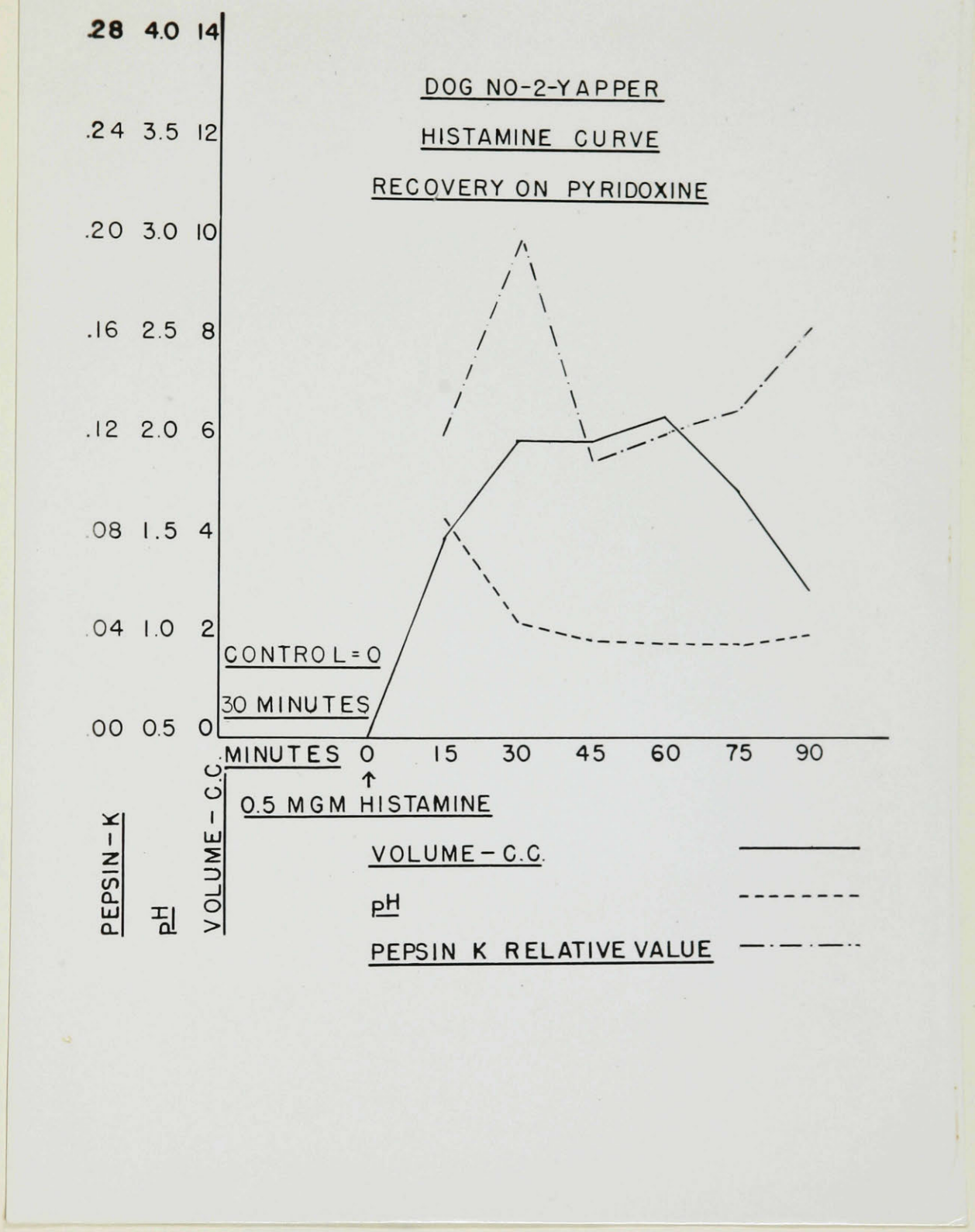


Figure 40

After 18 days on pyridoxine therapy. Note the return of the volume (30.0 c.c.) of gastric secretion.

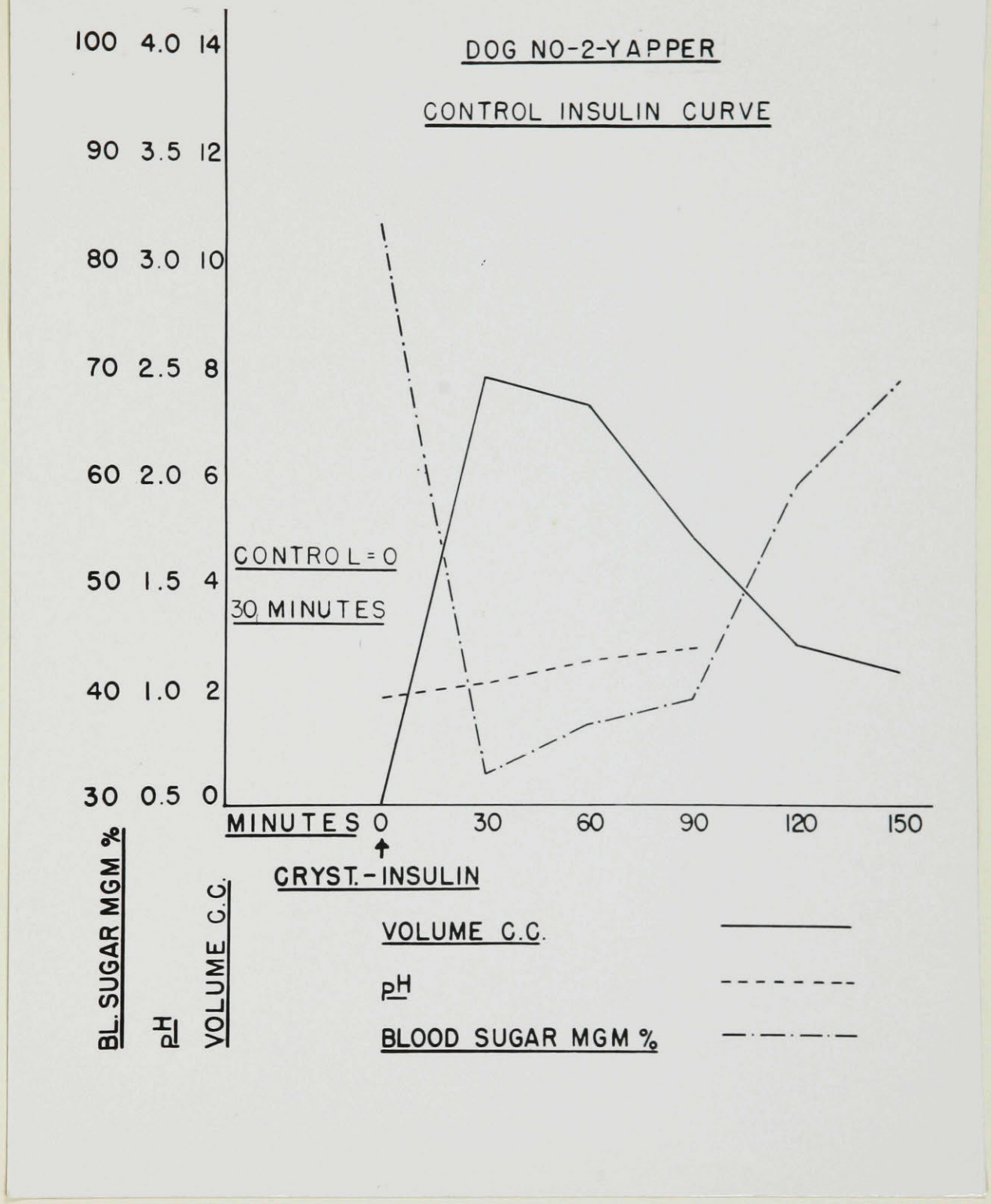


Figure 41

Note the large volume (26.0 c.c.) and low pH of the gastric secretion following injection of 6 units of insulin. Also the drop in blood sugar to 33 mgm. percent.

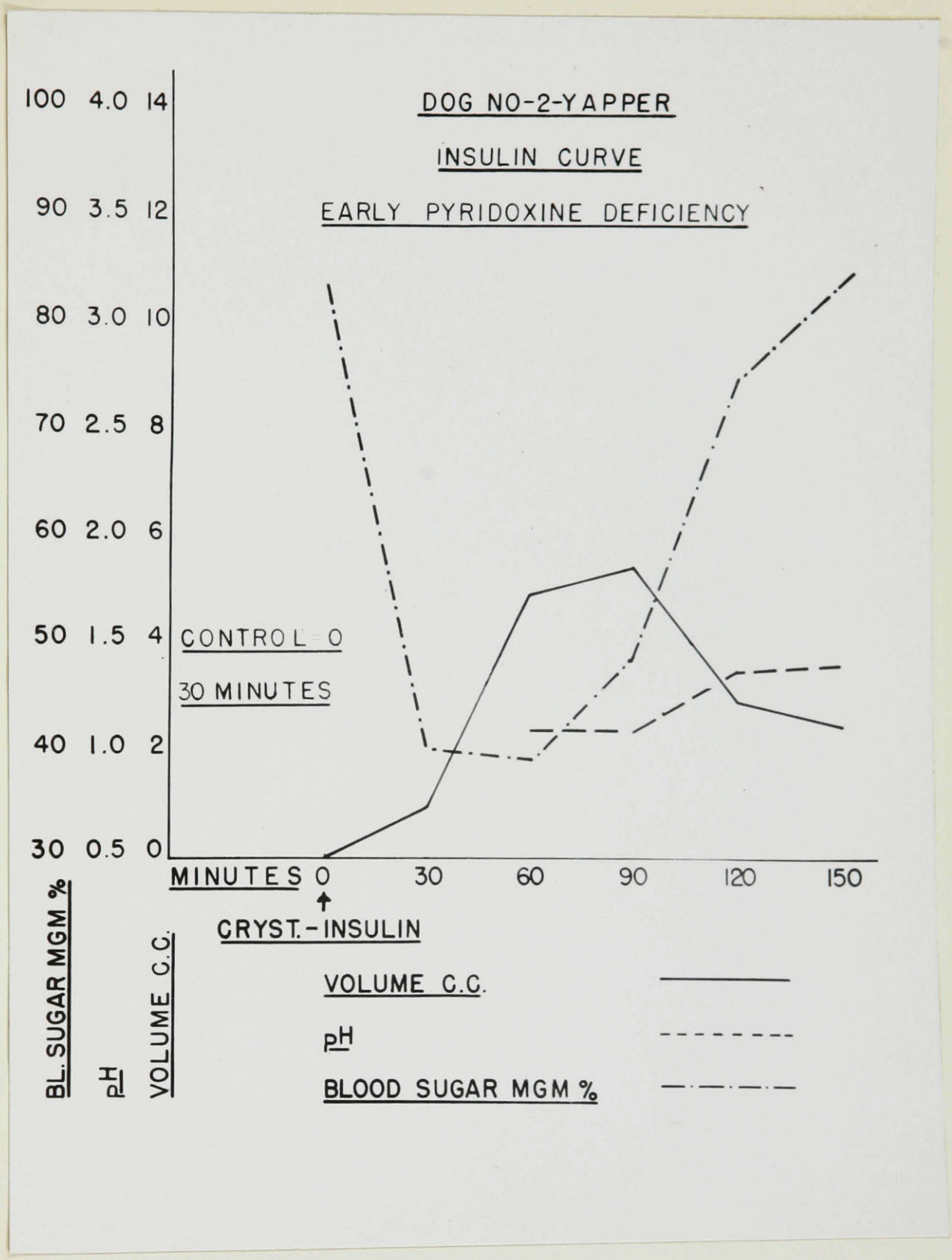


Figure 42

After 58 days on a pyridoxine free diet. Note decrease in volume (17.0 c.c.) of gastric secretion and drop in blood sugar to 41 mgm. percent.

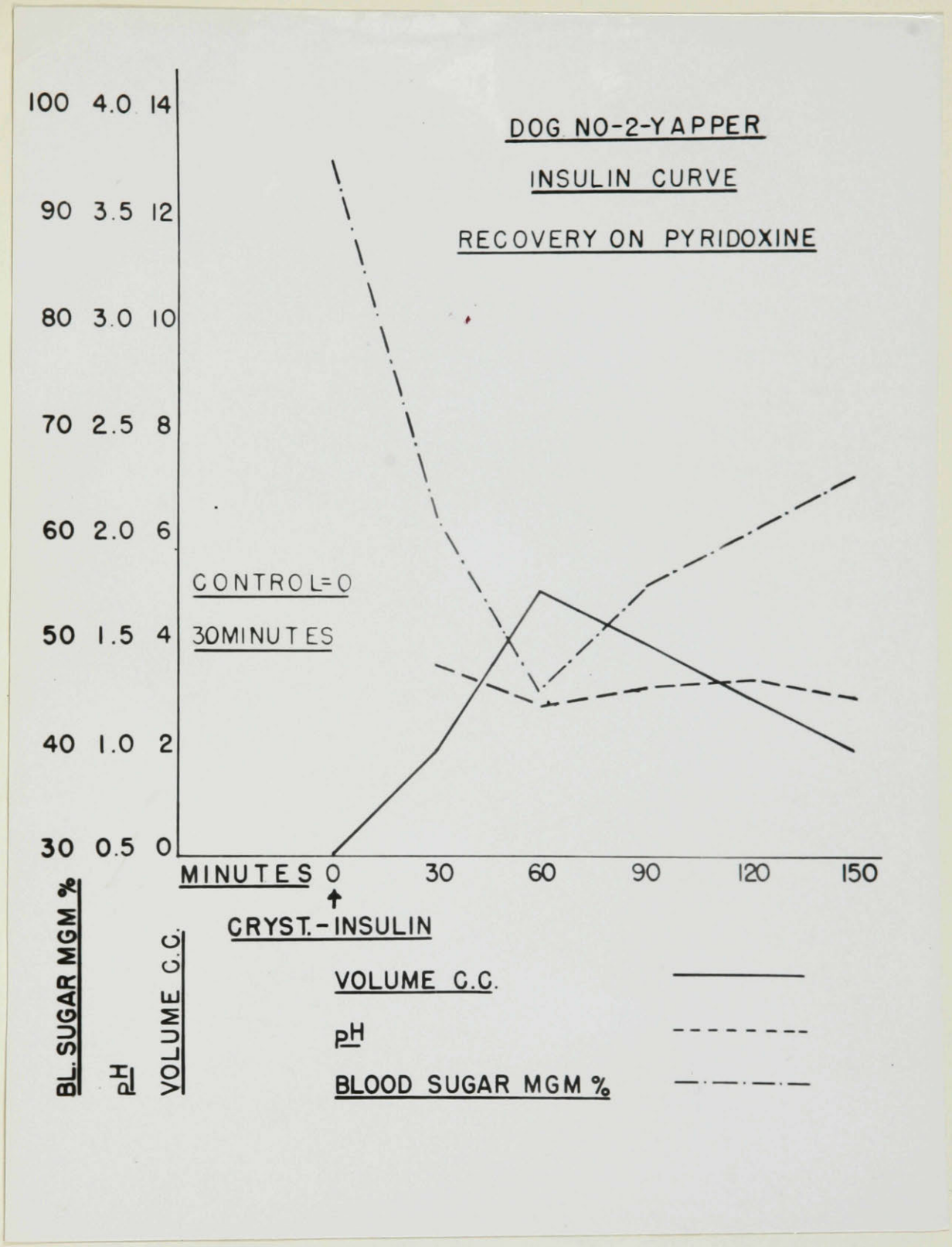


Figure 43

After 62 days on pyridoxine. Note there is no change in volume (17.0 c.c.) of gastric secretion.

DOG NO-2- YAPPER

HEMATOLOGY

DATE	HEMOGLOBIN	ERYTHROCYTE COUNT	HEMATOCRIT	ERYTHROCYTE COUNT
Dec.23	92	5,570,000	47	
Jan. 3	90	5,340,000	39	
Jan. 6		Operation New Gastric Pouch		
Jan.16	68	3,750,000	36	
Jan.25	77	4,330,000	41	
Jan.26		Started on Pyridoxine Free Diet and Desoxypyridoxine		
Feb. 2	77	5,100,000	39	7.1
Feb. 8	86	5,080,000	41	6.96
Feb. 8		Desoxypyridoxine Discontinued		
Feb.15	68	3,620,000	35	6.96
Feb.21	69	4,030,000	37	6.96
Feb.28	62	4,000,000	32	6.96
Mar. 8	53	3,990,000	27	6.89
Mar.16	49	4,000,000	26	6.89
Mar.22	37	3,350,000	21	6.89
Mar.27		Started on Complete Synthetic Diet Containing Pyridoxine		
Apr. 4	74	4,500,000	38	6.89
Apr.11	76	5,270,000	39	6.96
Apr.19	90	5,950,000	44	6.96
Apr.23		Started on Vitamin B-12		
Apr.25	82	5,500,000	41	7.0
May. 2	82	5,000,000	40	7.0
May. 9	82	5,000,000	39	7.0
May.16	80	5,110,000	38	7.1
May.23	95	5,880,000	41	6.96
May.30	82	5,060,000	39	6.98
Jun. 7	81	5,330,000	36	-

Figure 44

Table of results.

DOG NO. - 2 - YAPPER

HEMATOLOGY

DATE	LEUCOCYTE COUNT	POLYMORPHS	LYMPHOCYTES	EOSINOPHILES	MONOCYTES	BASOPHILES
Dec. 23	18,700	-	-	-	-	-
Jan. 3	11,680	-	-	-	-	-
Jan. 5	8,600	-	-	-	-	-
Jan. 6		Operation - New Gastric Pouch				
Jan. 16	16,800	-	-	-	-	-
Jan. 25	12,100	92	4	2	2	0
Jan. 26		Started on Pyridoxine Free Diet and Desoxypyridoxine.				
Feb. 2	6,500	83	4	12	1	0
Feb. 8	10,550	95	2	1	2	0
Feb. 8		Desoxypyridoxine Discontinued.				
Feb. 15	17,300	89	7	4	0	0
Feb. 21	13,500	86	7	6	1	0
Feb. 28	15,200	88	9	2	1	0
Mar. 8	10,500	75	17	7	1	0
Mar. 16	9,700	80	10	10	0	0
Mar. 22	11,800	87	9	4	0	0
Mar. 27		Started on Complete Synthetic Diet Containing Pyridoxine.				
Apr. 4	7,200	74	14	10	1	1
Apr. 11	9,550	70	11	18	1	0
Apr. 19	8,900	68	18	13	1	0
Apr. 23		Started on Vitamin B-12				
Apr. 25	9,750	65	19	15	1	0
May. 2	15,000	81	7	12	0	0
May. 9	8,500	64	23	11	1	1
May. 16	13,100	77	13	10	0	0
May. 23	10,900	71	19	10	0	0
May. 30	14,650	64	30	6	0	0
June 7	12,950	78	15	7	0	0

Figure 45

Table of results.

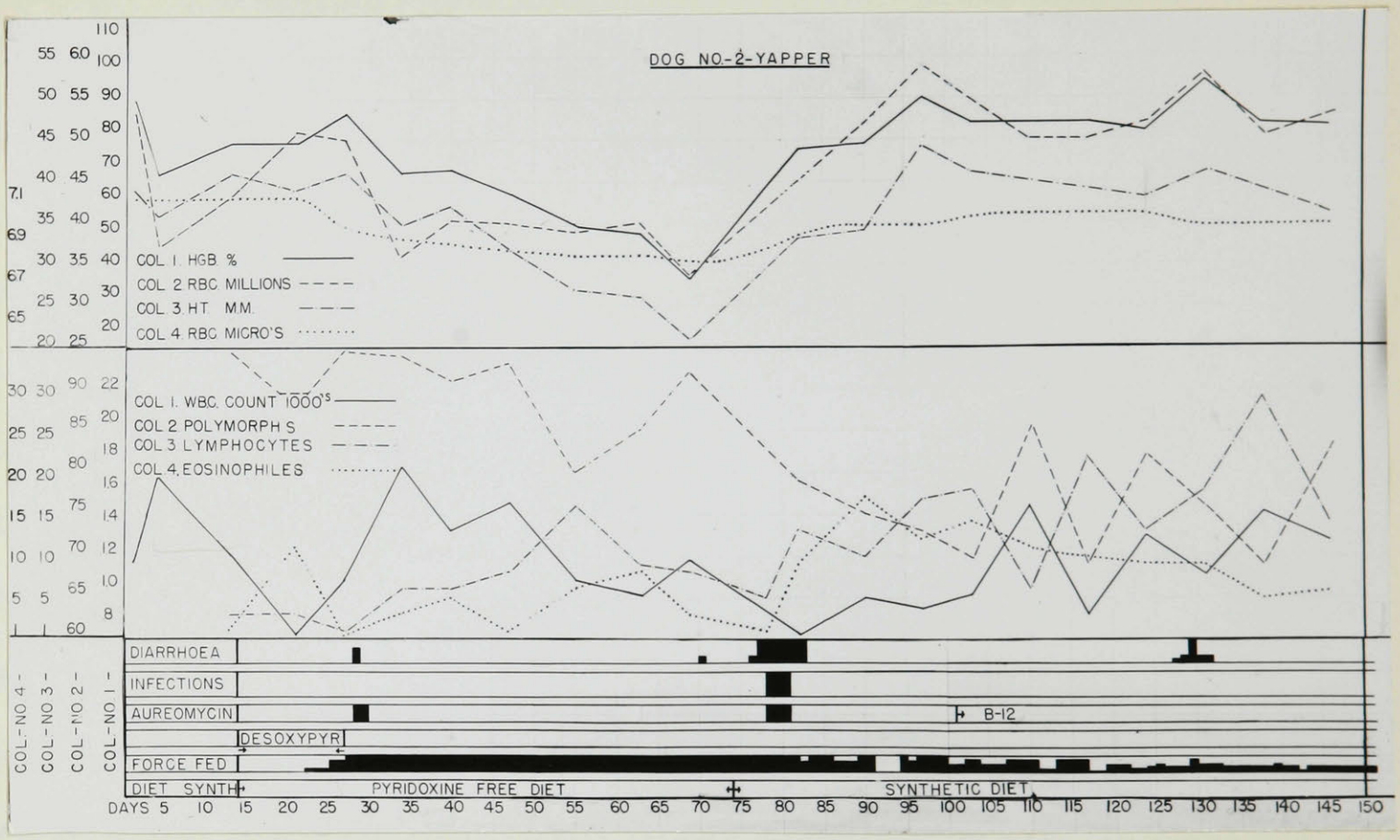


Figure 46

Composite graph of the hematological results of dog No 2 "Yapper". Note the leucopenia, lymphopenia and polynucleosis on pyridoxine free diet and desoxypyridoxine and marked microcytic anemia in pyridoxine deficiency as well as a marked leucopenia.

was considered reasonably safe for the dog and then the dog was started on pyridoxine. He was given 1.5 mgm. of pyridoxine in his daily diet and 5.0 mgm. by hypo for 2 days. He was improving slightly but in 5 days developed an acute upper respiratory infection and diarrhoea. He had been given aureomycin when he was very sick with diarrhoea during the first experiment, and he was given it again with this infection. This fact is important, in that aureomycin kills some of the bacteria in the bowel that aid in the synthesis of vitamins. The red blood cells and hemoglobin rose to a satisfactory level, but the dog continued to have lethargy, anorexia, a slight dermatitis and alopecia on his face and limbs. (Figure 46). He developed a growth plateau. The daily volume of the gastric secretion increased, but did not return to normal (Figure 36). However, the volume of secretion in the histamine and insulin tests returned to normal (Figures 40 and 43).

Three weeks after starting the pyridoxine the dog continued to have what appeared to be a deficiency in an unknown factor or factors of the vitamin B complex. He had a slight decrease in the daily volume of gastric secretion around the 95th day

but no reduction in the histamine and insulin curves (Figure 36). A similar but more marked deficiency picture in dog No. 1 "Mutt" was treated with vitamin B12 for a few days and then liver in an attempt to save the dogs life. This therapy saved the dog, but he continued to have his deficiency syndrome.

It was decided to try vitamin B12 on Dog No. 2 "Yapper". He was given 2 micrograms of rubramin (vitamin B12) by hypo daily for 3 weeks. There was a very striking improvement in this dog. He became active, started to eat some of his diet and soon looked much better clinically. He gained several pounds of weight in a few days (Figure 36). The daily volume of gastric secretion increased slightly to what appeared to be normal and remained normal (Figure 36). The histamine and insulin curves were normal. The red and white blood cell counts and hemoglobin were normal before vitamin B12 was started according to Copley, and there was no change in the blood picture (32).

The dog developed diarrhoea so it was decided to increase the vitamin B12 to 6 micrograms per day. The proteins returned to normal. The total proteins was 6.53 gms. % with 4 gms. % albumin. This

fact is not too significant as a hypoproteinaemia with a reduction chiefly in the albumen has been demonstrated in pyridoxine deficiency. "Skippy" and "Chubby" developed a hypoproteinaemia which was relieved by pyridoxine therapy alone. The dog continued to gain weight and be well clinically until the end of the year.

It is interesting to note that "Skippy" and "Chubby" did not develop this deficiency syndrome. "Chubby" should not be considered as she was an adult dog and was only fed the synthetic diet for 3 months.

It was then shown that two dogs, "Mutt" and "Yapper", fed a synthetic diet developed what appeared to be a deficiency syndrome in a factor or factors of the vitamin B complex. The second dog "Skippy", fed the same diet, did not develop this deficiency syndrome. These dogs were all used for experiments on pyridoxine deficiency. In the first two dogs the pyridoxine deficiency was accelerated with desoxypyridoxine. These two dogs were also given aureomycin on two occasions, which could have been responsible for the deficiency syndrome as described above. A satisfactory therapeutic trial was not given B12 in the first dog "Mutt", but

massive doses of liver did not relieve the syndrome. The dog was started on meat, but this was not fed long enough to consider its therapeutic effect. On the other hand, the possible deficiency syndrome was successfully treated with vitamin B12 in the second dog.

The second dog in this group was Dog No. 3 "Skippy". He was used as a control for 57 days in Group 1. He was then started on 170 gms. of a pyridoxine free diet together with his gastric secretion of the previous day.

The dog gradually developed the signs and symptoms of pyridoxine deficiency. He became lethargic and developed a slight dermatitis on his face, ears and legs. Later he developed slight anorexia, but for the first 57 days his appetite was so good that his diet was increased twice. First it was increased from 170 to 180 gms. and then to 190 gms.

The daily volume of his gastric secretion gradually decreased and the pH rose despite the increase in diet (Figure 52). There was a slight reduction in the volume of the histamine and insulin curves but no significant change in pH (Figures 53, 54, 55, 57 and 58).

Figure 47. Dog No 3 "Skippy". Note healthy condition of dog after 71 days on synthetic diet also the collecting apparatus.

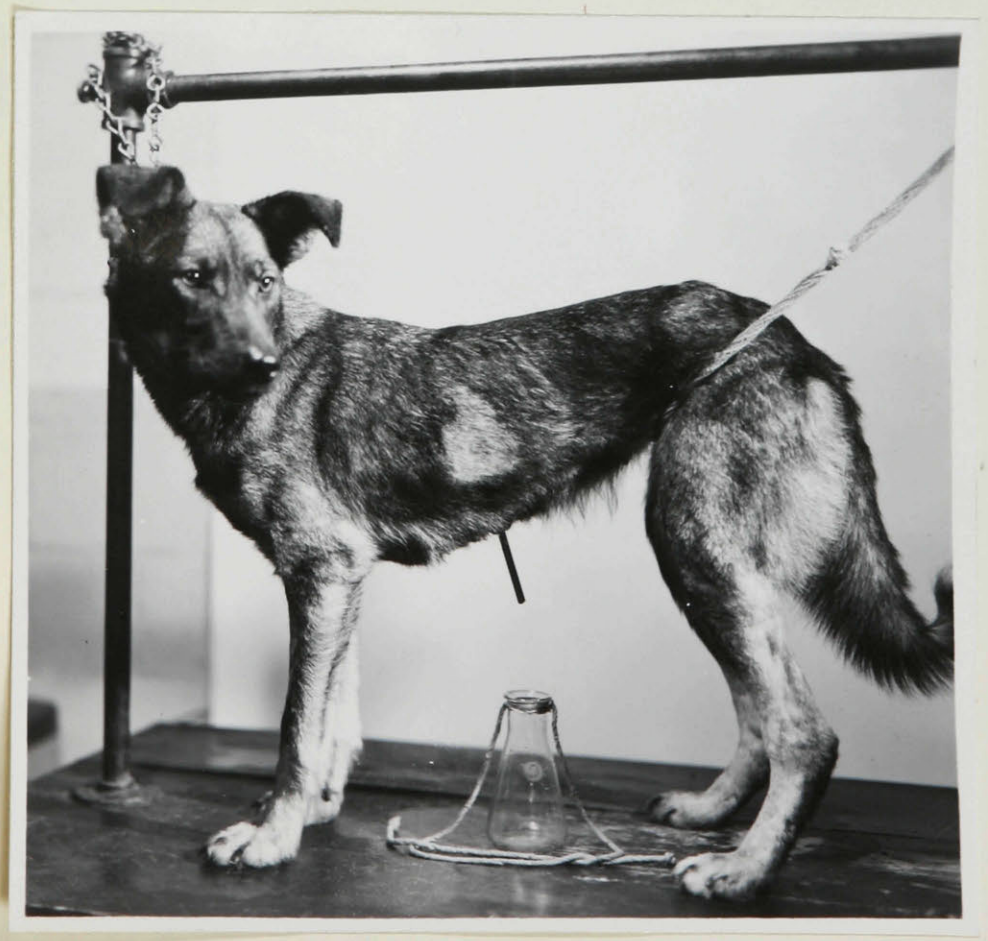


Figure 48. Dog No 4 "Chubby". Note healthy condition of dog after 6 months on synthetic diet also the indwelling catheter.

DOG NO-3-SKIPPY

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (HOURS)
1					38	34	46	-
2					17	24	20	-
3					53	32	20	-
4					20	--	--	-
5					20	15	42	-
6					52	25	18	-
7					30	36	28	-
8					27	30	30	15
9					38	21	30	20
				pH	0.88	0.88	0.87	0.87
10					38	22	20	15
11					33	36	45	26
12					42	24	25	7
13					39	28	42	10
14					27	28	44	9
15					40	38	34	7
16					22	29	26	16
17					30	30	25	23
18					25	27	31	23
19					22	36	25	19
20					35	30	40	11
21					20	20	26	17
22					19	35	28	9
23					15	48	28	20
24					19	25	34	7
25					33	30	26	15
26					25	21	33	14
27					17	24	27	15
28					19	18	18	18
29					31	38	30	12
30					32	30	23	10
31					28	48	37	20
32					23	44	33	22
33					31	44	26	25
34					30	33	33	23
				pH	0.83	0.79	0.81	0.79
35					31	26	30	13
36					31	25	27	14
37					24	29	36	16
38					19	27	45	19
39					32	30	34	10
40					31	38	31	19
				pH	0.91	0.88	0.92	0.94
41		Stoma	Revised		-	-	-	-
42		"	"		-	-	-	-
43					27	28	29	20
44	x				38	34	34	15
45	x				23	36	31	20
46	x				27	20	27	21
47	x				32	29	39	20
48					29	19	13	14
49					20	20	8	14
50					43	25	19	12
51					35	20	34	22

Figure 49

Table of results.

DOG NO - 3 - SKIPPY

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (HRS)
52					37	20	28	16
53					40	27	25	10
54					30	18	18	7
55					32	12	18	10
56					32	35	25	18
57					35	25	33	15
		Started on a Pyridoxine Free Diet						
58					25	21	26	12
59					34	23	16	10
60	x				30	29	25	15
61					35	26	19	12
62					25	22	19	16
				pH	0.92	0.87	0.87	0.89
63					27	30	23	8
64					30	20	19	10
65					31	28	19	14
66					28	22	20	10
67					19	15	24	13
68					32	18	23	9
69					22	14	23	6
70					32	8	19	11
71					27	17	21	16
72			x		25	9	16	16
73			x		25	17	10	26
74			x		24	16	20	12
75			x		25	14	26	16
76					37	23	23	15
77					20	15	27	15
78	x				37	34	22	15
79					22	11	15	12
80					16	17	35	13
81					31	34	15	12
82					33	20	26	15
				pH	1.00	1.00	0.98	1.30
83					34	27	19	15
84					33	19	29	12
85					20	18	28	11
86					35	25	22	10
87					30	28	25	14
88					25	25	25	12
89					35	17	17	10
90					33	28	20	20
91					16	19	27	12
92					32	28	20	12
93					25	21	22	11
94					30	22	21	5
95					25	12	21	10
96					25	18	15	11
97					32	18	18	7
98					23	21	15	10
				pH	1.06	1.03	1.03	1.09
99	x				38	23	18	10
100	x				35	30	19	13
101	x				40	34	20	11
102	x				14	12	15	9

Figure 50

Table of results.

DOG NO - 3 - SKIPPY

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	3 - 6½ (HOURS)
103	x							
104					37	14	10	5
105					32	23	23	17
106					38	12	18	12
107	x				32	10	8	8
108	x				30	25	18	11
109	x				20	11	14	13
110	x				22	14	11	7
111	x				32	8	16	5
112	x				20	30	10	2
113	x				12	4	4	2
114	x				20	18	16	7
115	x				19	20	30	14
116	x				13	21	30	10
117	x				20	12	24	14
118	x				22	14	17	20
119	x				22	14	18	15
120	x				12	13	13	10
121	x				12	10	19	-
122	x				18	20	18	13
123	x				17	4	20	20
124	x				24	16	10	10
125	x				26	28	14	12
126	x				21	14	21	24
127	x			pH	1.15	0.96	0.93	0.93
					16	12	16	4
		Started on Complete Synthetic Diet Containing Pyridoxine						
128	x				13	16	14	7
129	x				24	25	6	15
130	x				28	28	32	29
131	x				40	36	47	16
132	x				35	20	47	34
133					37	31	49	23
134					36	46	53	27
135					27	45	28	18
136					36	33	40	30
137					36	27	62	8
138					43	38	28	18
139					25	35	58	20
140					30	40	70	15
141					45	29	53	-
				pH	0.90	0.90	0.89	0.89
142					41	45	47	11
143					25	22	24	12
144					37	31	36	26
145					33	32	35	17
146					48	35	45	19
147					49	43	43	13
148					32	19	41	26
149					38	35	56	29
150					50	26	29	13
151					37	37	48	16
152					35	37	54	20
153					25	35	57	16
154					35	32	53	24

Figure 51

Table of results.

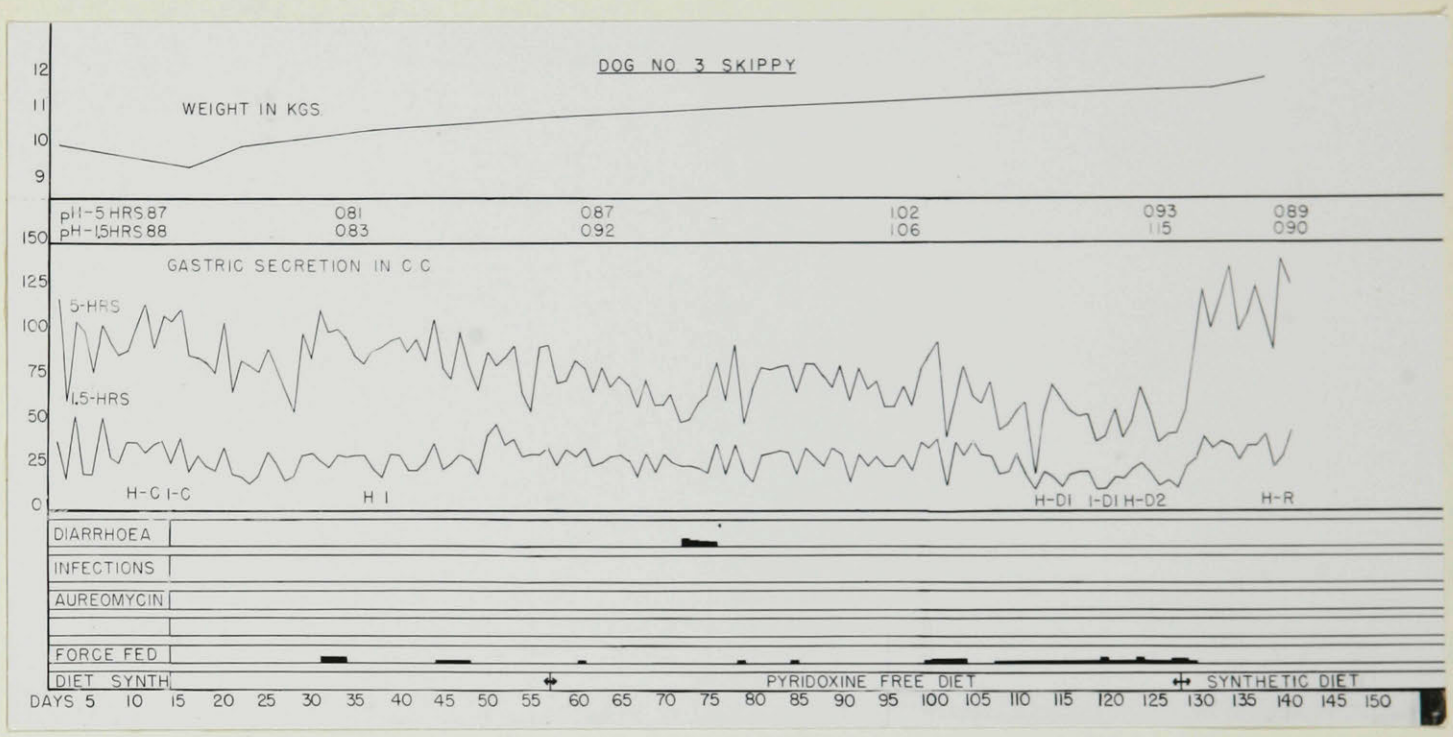


Figure 52

Composite graph of results. Note that the volume and pH of gastric secretion remained constant during control period (1-57 days) but volume dropped gradually and pH rose on pyridoxine free diet (57-128 days). Also the striking return of the volume and pH of gastric secretion on pyridoxine.

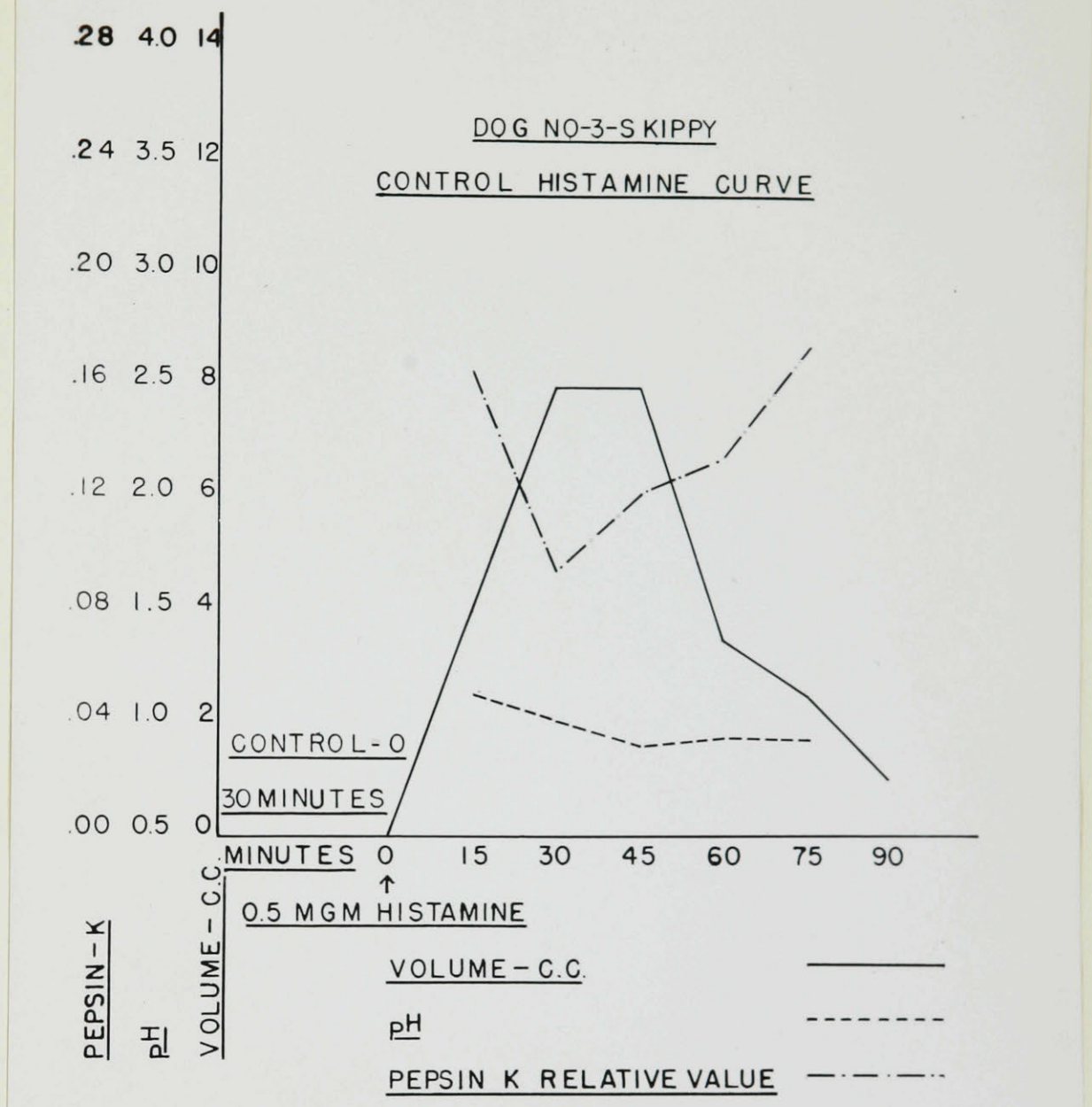


Figure 53

Note large volume (27.0 c.c.) low pH and pepsin of the gastric secretion following injection of 0.5 mgm. histamine.

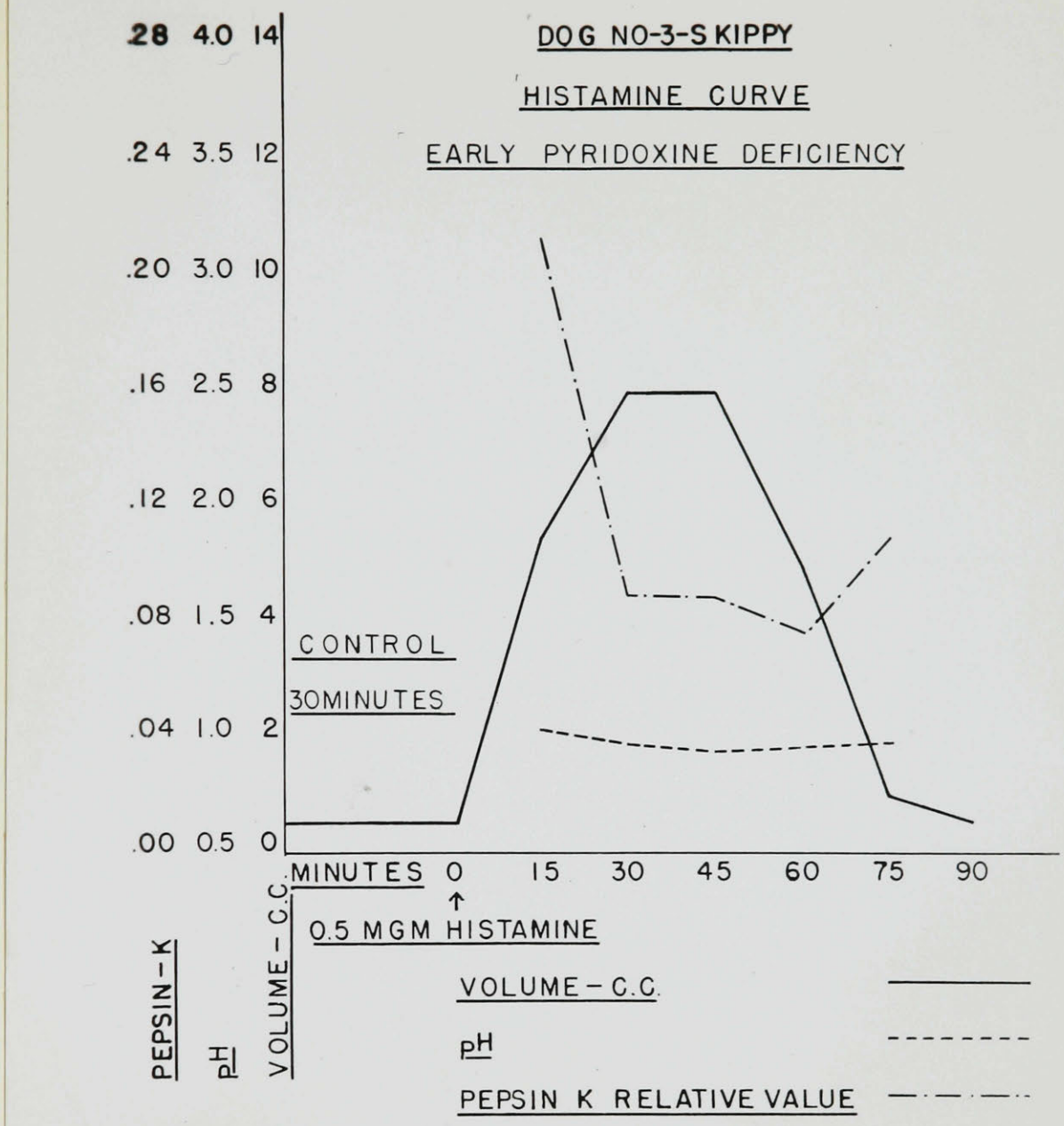


Figure 54.

After a control period of 57 days plus 53 days on a pyridoxine free diet. Note that there is no significant change in the gastric secretion.

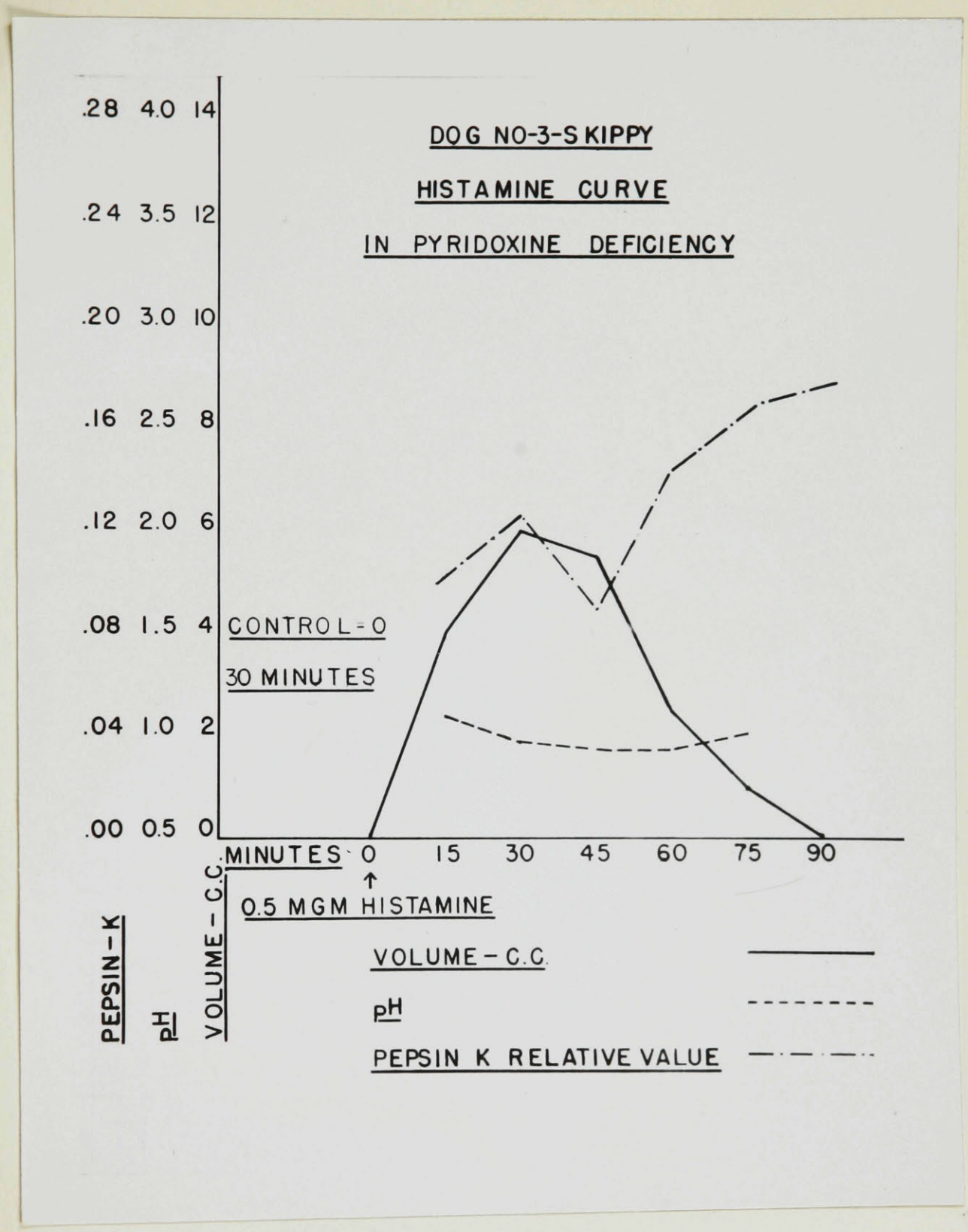


Figure 55

After 17 days on a pyridoxine free diet. Note slight decrease in volume (19.0 c.c.) of gastric secretion.

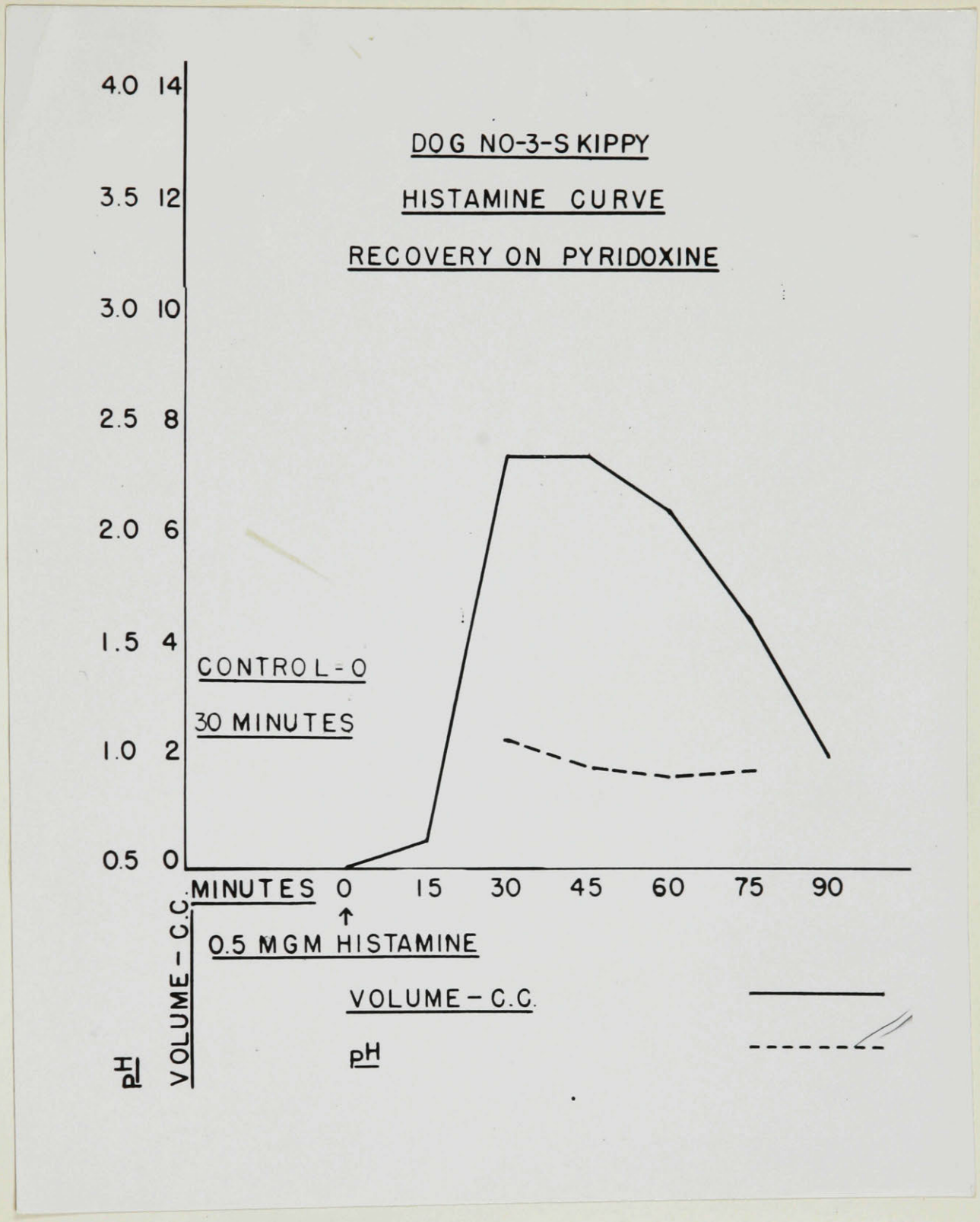


Figure 56

After pyridoxine therapy. Note return of volume of gastric secretion.

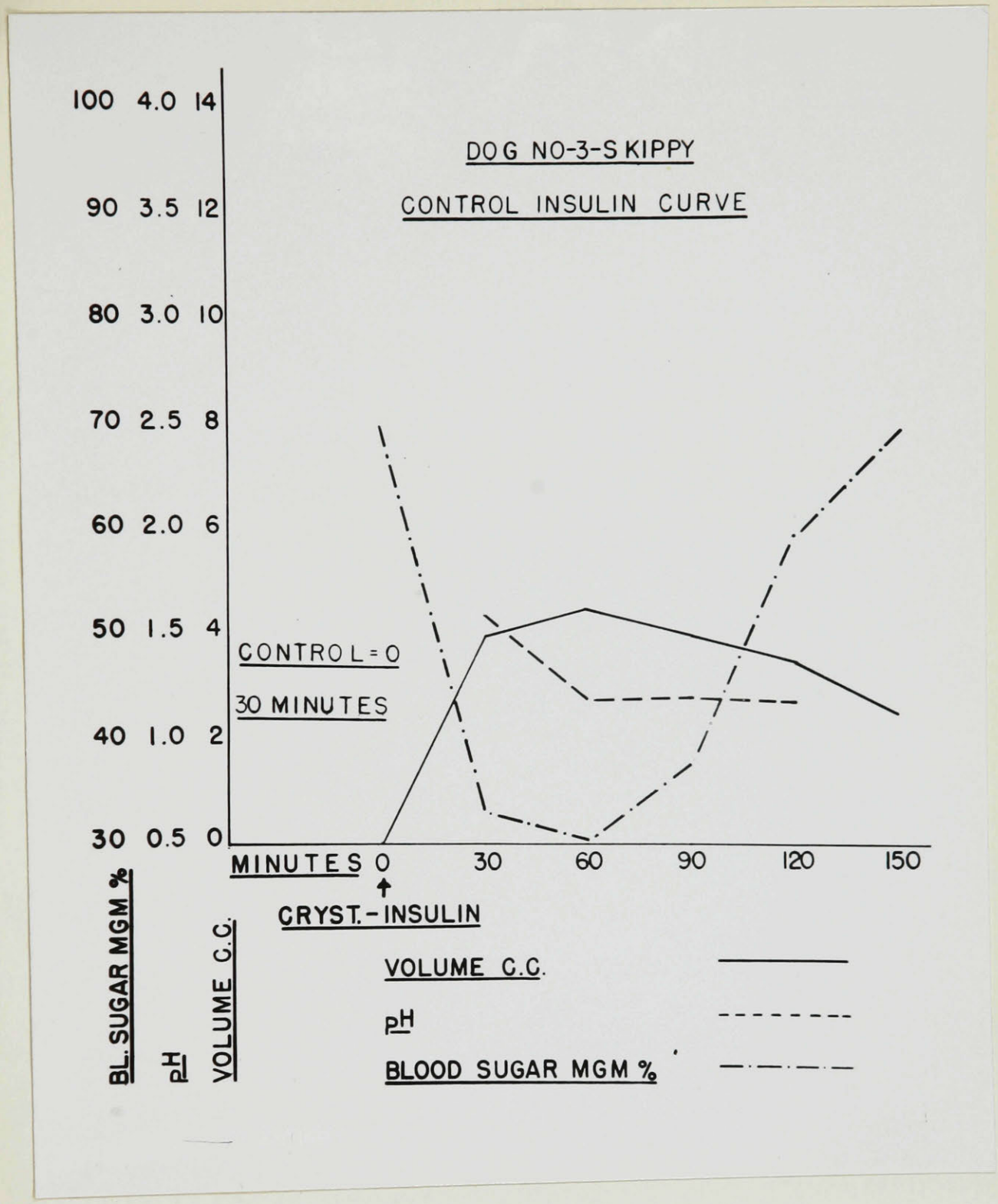


Figure 57_

Note volume (18.5 c.c.) and pH of gastric secretion following injection of 6 units of insulin. Blood sugar fell to 29 mgm. percent.

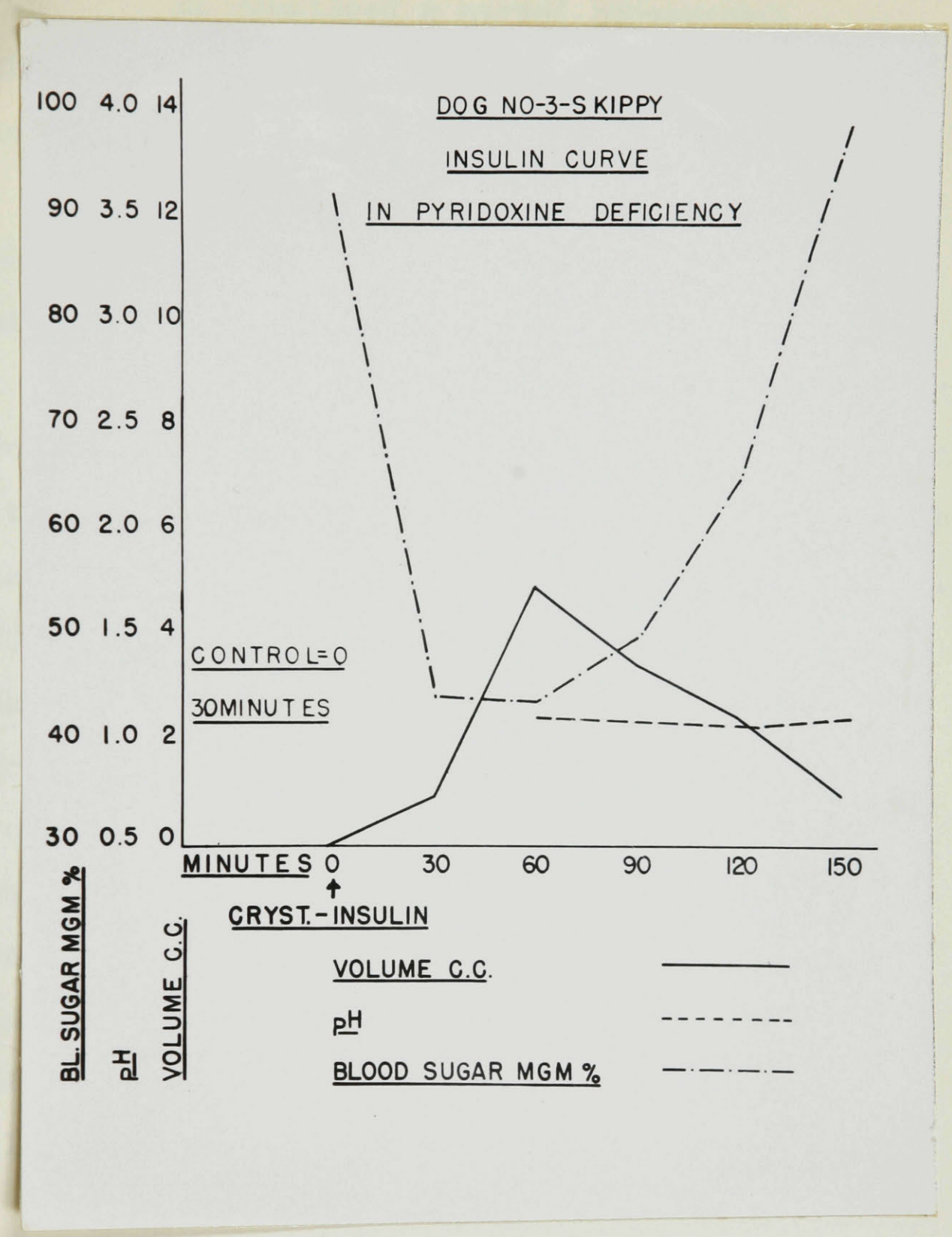


Figure 58

After 63 days on pyridoxine free diet. Note slight reduction in volume (13.0 c.c.) of gastric secretion.

He developed a marked hypochromic microcytic anemia, lucopenia, lymphocytosis and neutropenia (Figure 61).

His blood chemistry was normal except for the proteins. The total proteins was 6.65 gms. % with 2.64 gms. % albumin and 4.01 gms. % globulin.

After demonstrating the marked reduction in volume and elevation of the pH of the gastric secretion, it was decided to start the dog back on pyridoxine before he developed an infection and died. He was given 1.5 mgm. of pyridoxine in his daily diet and 5 mgm. by hypo daily for two days. The dog rapidly returned to normal clinically. There was a rapid rise in the volume and fall in the pH of the gastric secretion to normal. The histamine and insulin curves returned to normal (Figure 56). His blood picture and blood chemistry returned to normal (Figure 61). His total proteins was 7.28 gms. % with 4.99 gms. % albumi, and 2.29 gms. % globulin.

Thus a young dog was kept on a pyridoxine free synthetic diet and allowed to develop pyridoxine deficiency. There was a marked reduction in the volume and elevation of the pH of the gastric secretion. He was then fed pyridoxine and brought

DOG NO. 3 - SKIPPY

HEMATOLOGY

DATE	HEMOGLOBIN	ERYTHROCYTE COUNT	HEMATOCRIT	ERYTHROCYTE SIZE
Jan. 6	81	4,620,000	36	—
Jan.13	Operation - New Gastric Pouch			
Feb. 2	92	5,300,000	46	7.1
Feb. 8	90	5,800,000	45	7.1
Feb.15	86	5,330,000	43	7.1
Feb.21	86	5,350,000	42	7.1
Feb.28	91	5,360,000	43	7.1
Mar. 8	89	5,180,000	41	7.1
Mar.16	93	5,680,000	44	7.1
Mar.20	Started on Pyridoxine Free Diet			
Mar.21	96	5,820,000	44	7.1
Mar.28	92	5,200,000	42	6.88
Apr. 4	79	4,760,000	38	6.62
Apr.11	76	5,200,000	37	6.50
Apr.19	63	4,140,000	32	6.50
Apr.25	61	4,000,000	32	6.50
May 3	50	3,260,000	25	6.50
May 9	45	3,340,000	23	6.50
May 16	41	2,800,000	20	6.50
May 25	33	2,600,000	18	6.50
May 30	28	2,250,000	16	6.50
May 30	Started on Complete Synthetic Diet Containing Pyridoxine			
June 6	62	4,630,000	33	7.1
June 23	80			

Figure 59

Table of results.

DOG NO. - 3 - SKIPPY

HEMATOLOGY

DATE	LEUCOCYTE COUNT	POLYMORPHS	LYMPHOCYTES	EOSINOPHILES	MONOCYTES	BASOPHILES
Jan. 6	15,350	-	-	-	-	-
Jan. 10	11,000	-	-	-	-	-
Jan. 13		Operation - New Gastric Pouch				
Feb. 2	15,600	80	12	8	0	0
Feb. 8	21,250	80	4	14	2	0
Feb. 15	15,400	85	7	7	1	0
Feb. 21	18,750	84	7	9	0	1
Feb. 28	18,600	85	6	9	0	1
Mar. 8	19,500	89	4	7	0	1
Mar. 16	22,350	80	7	12	1	0
Mar. 20		Started on Pyridoxine Free Diet				
Mar. 21	13,750	71	9	19	0	0
Mar. 28	16,350	71	9	18	1	0
Apr. 4	22,850	76	7	16	0	0
Apr. 11	15,000	76	10	14	0	0
Apr. 19	14,750	77	9	14	0	0
Apr. 25	12,550	73	17	9	1	0
May. 3	10,150	74	21	5	0	0
May. 9	10,850	62	21	15	2	0
May. 16	13,300	75	11	14	0	0
May. 25	9,200	69	13	16	0	0
May. 30	11,700	78	15	6	1	0
May. 30		Started on Complete Synthetic Diet Containing Pyridoxine				
June 6	13,700	65	17	8	1	1
June 15	13,050	65	25	10	0	0

Figure 60

Table of results.

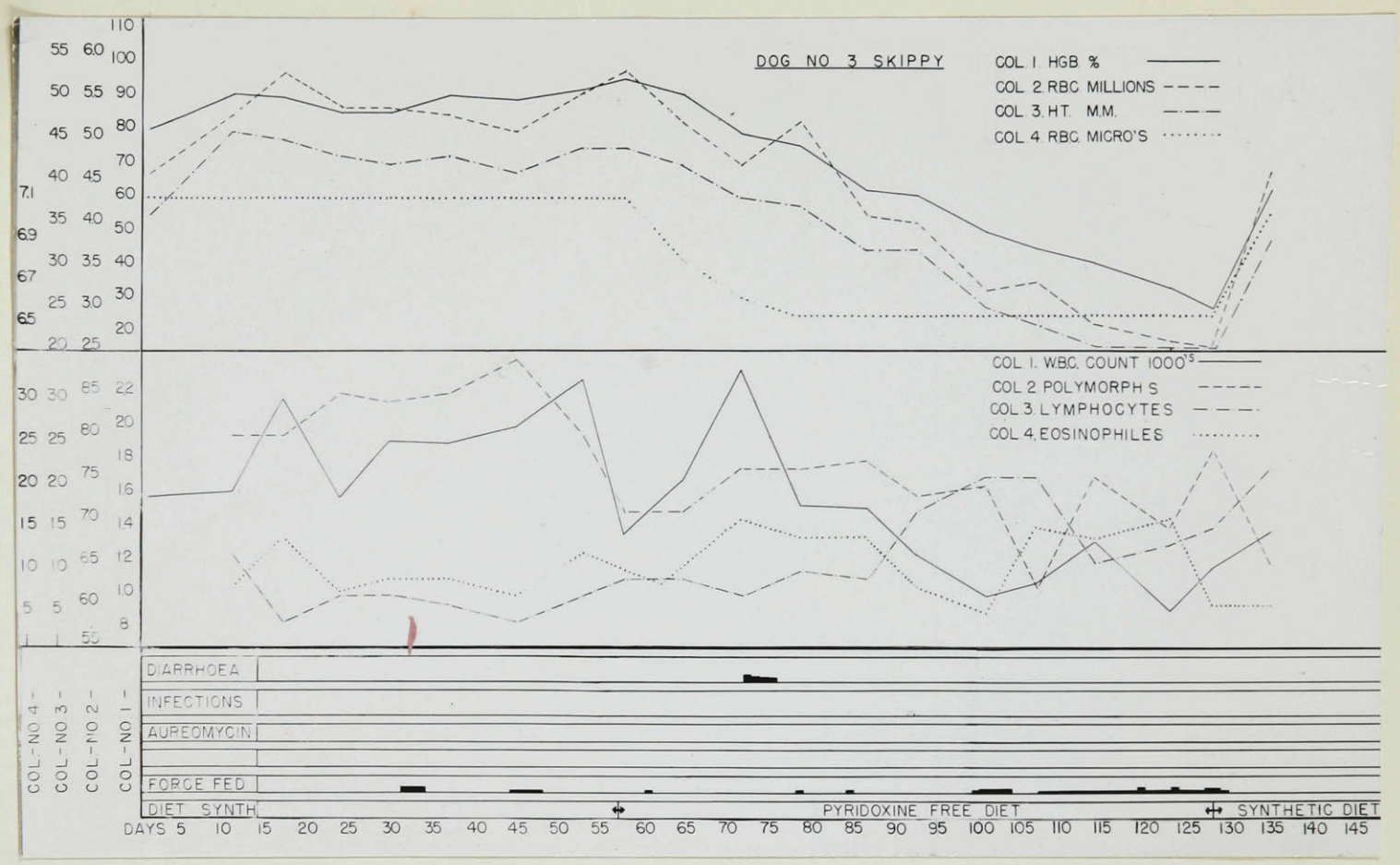


Figure 61

Composite graph of hematological results of Dog No 3 "Skippy". Note maintenance of blood during control period and marked hypochromic microcytic anemia, leucopenia, lymphocytosis and neutropenia on a pyridoxine free diet.

back to normal (Figure 47). The volume and pH of his gastric secretion returned to normal (Figure 52).

Group 4 - The fourth group of dogs consisted of a female mongrel dog approximately one year old (Figure 48). She weighed 13 kg. and was fed 200 gms. of synthetic diet.

She was healthy, ate well and did well clinically. Her pouch was not as large as the other dogs, but it secreted between 50 and 70 c.c. of gastric juice daily. She had a fair innervation to her pouch as was shown by the first 1½ hours daily secretion and the insulin curves (Figure 64). The insulin curves were not included to decrease the volume of this document, but the total volume was 10.5 c.c..

She was then started on 65 mgm. of desoxypyridoxine along with the basal synthetic diet. The dog developed slight anorexia and a marked increase in the volume of gastric secretion. There was also a slight rise in pH (Figure 64). This increase was gradual for 12 days. The desoxypyridoxine was continued until the volume of gastric secretion had stabilized at its new high level. There was no significant change in the histamine curve (Figure 66).

DOG NO. - 4 - CHUBBY

DAYS	FORCE FED	INFECTIONS	DIARRHOEA		GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (Hours)
1					14	26	31	27
2					19	17	31	25
3					12	10	29	25
4					15	15	22	25
5					23	13	31	21
				pH	0.91	0.85	0.86	0.82
6					14	17	21	25
7					17	20	34	29
8					20	14	19	26
9					15	15	20	27
10					16	21	34	23
11					17	13	40	27
			Started on Desoxypyridoxine.					
12					17	14	26	28
13					16	9	40	15
14					13	16	27	30
15	x				6	9	44	16
16	x				11	15	23	32
17	x				29	22	44	35
18	x				19	18	37	32
19	x				15	29	29	22
20	x				15	16	37	24
21	x				17	26	37	22
22	x				20	21	48	34
23	x				28	25	54	32
24					23	23	60	33
25	x				25	25	58	30
				pH	1.10	1.05	1.04	-
26	x				20	25	42	20
27	x				22	13	57	29
28	x				28	21	42	26
29	x				27	23	52	27
			Desoxypyridoxine Discontinued.					
30	x				25	20	55	29
31					16	21	46	18
32	x				24	42	46	15
33					24	38	41	25
34					22	26	43	27
35					21	23	32	20
36	x				22	18	34	15
37					23	33	42	22
38					18	13	40	15
39					20	15	38	20
40					18	15	37	20
				pH	1.0	0.92	0.9	1.02
41					18	22	37	20
42					17	12	39	24
43					17	13	36	16
			Started on Pyridoxine Free Diet and Desoxypyridoxine.					
44					14	14	38	26
45	x				20	13	41	27
46	x				13	12	50	15
47	x				16	10	39	26
48	x				22	16	37	15
49	x				19	13	33	40
50	x				20	10	12	24

Figure 62

Table of results.

DOG NO. - 4 - CHUBBY

DAYS	FORCE FED	INFECTIONS	DIARRHOEA	pH	GASTRIC SECRETIONS (C.C.) 0 - 1½	1½ - 3	3 - 5	5 - 6½ (Hours)
51	x				22	15	23	31
52	x				14	13	14	7
53					18	12	16	17
54	xx				15	11	7	10
55	xx		x		11	7	10	5
56	xx		x		17	5	9	3
57	xx		x		12	8	9	3
58	xx		x		11	11	18	10
59	xx				12	10	12	7
60	xxx				14	12	18	8
61	xxx				13	11	5	5
62	xxxx				11	10	8	6
63	xxx				10	10	6	6
64	xxx				10	8	7	12
65	xxx				13	6	3	5
66	xxx				17	7	10	3
67	xxx				15	7	9	2
68	xxx				12	8	7	7
				pH	1.0	0.98	1.02	-
69	xxxx				5	9	4	3
70	xxx				19	18	15	3
71	xxx				4	14	7	14
			Started on Synthetic Diet Containing Pyridoxine.					
72	xxx				11	8	7	14
73	xxx				18	14	40	27
74	xxx				27	23	40	30
75	xxx				17	21	46	34
76	xxx				25	26	49	20
77	xxx				22	20	35	26
78	xxx				16	23	40	28
79	xxx				20	12	33	30
80	xxx				22	16	51	-
81	xxx				24	13	46	25
82	xxx				--	--	--	--
83	xxx				21	15	30	30
				pH	1.04	--	1.0	--
84	xx				22	14	34	31
85	xx				23	20	30	26
86	xx				20	14	25	33
87	xx				26	12	45	23
88					28	20	35	27
89					25	27	43	34
90	x				25	13	39	35
91	x				32	18	36	31
92					28	25	45	20
93					25	27	55	24
94					32	37	28	23
95					23	23	47	25
96					27	19	42	30
97					29	22	43	24

Figure 63

Table of results.

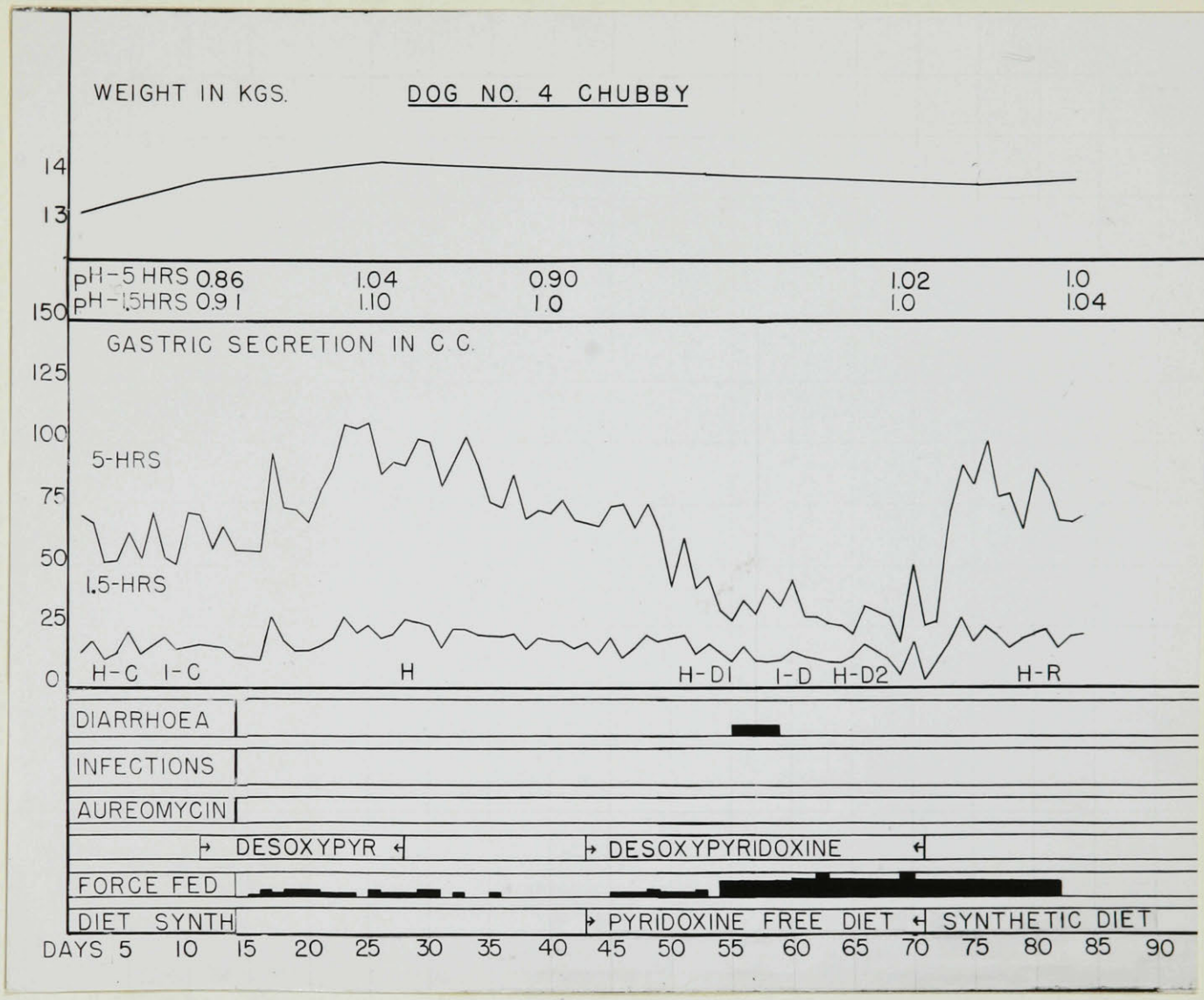


Figure 64

Composite graph of results. Note increase in volume of gastric secretion on desoxypyridoxine alone but decrease in volume on a pyridoxine free diet and desoxypyridoxine.

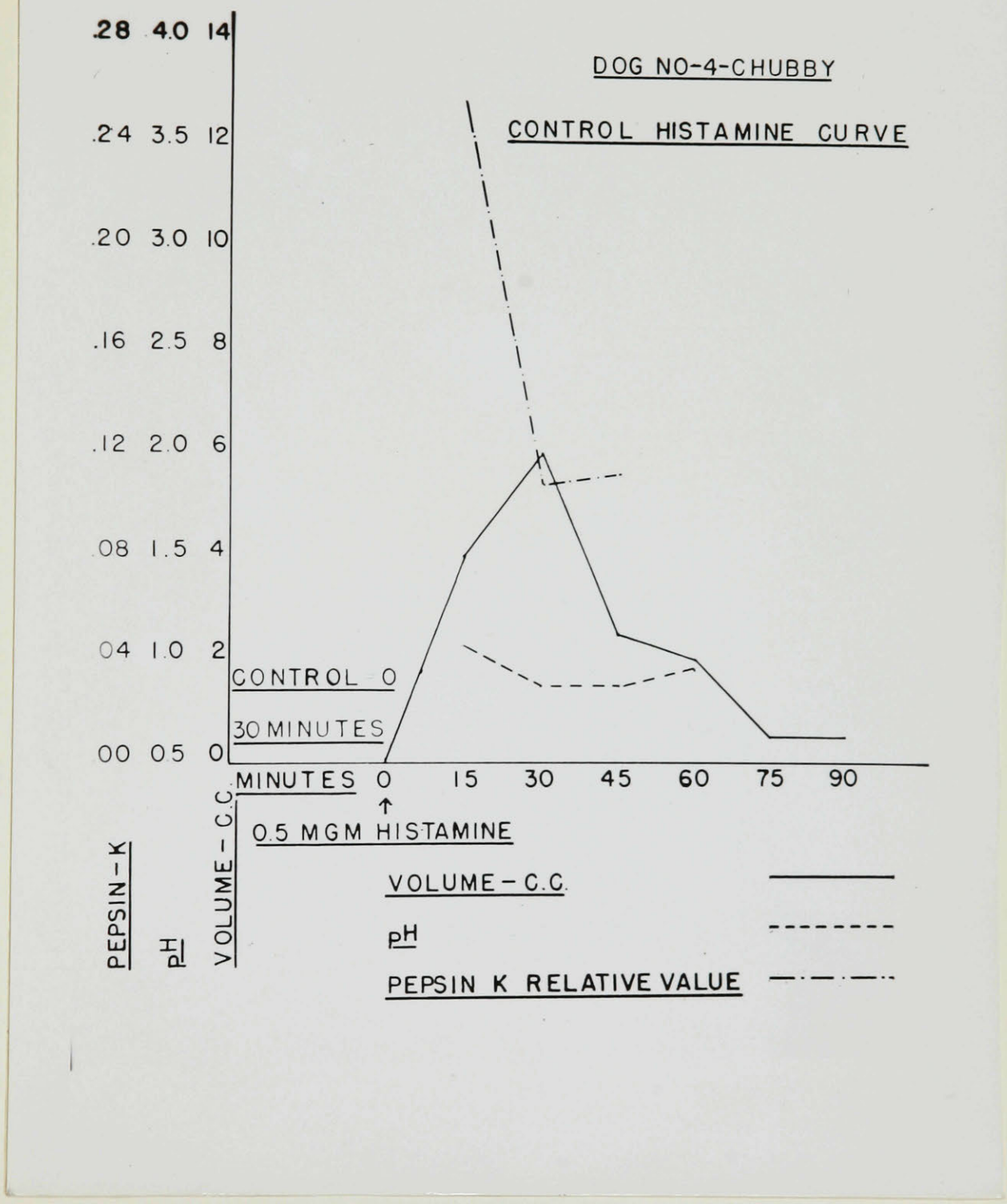


Figure 65

Note volume (15.5 c.c.) and low pH of gastric secretion following injection of 0.5 mgm. histamine.

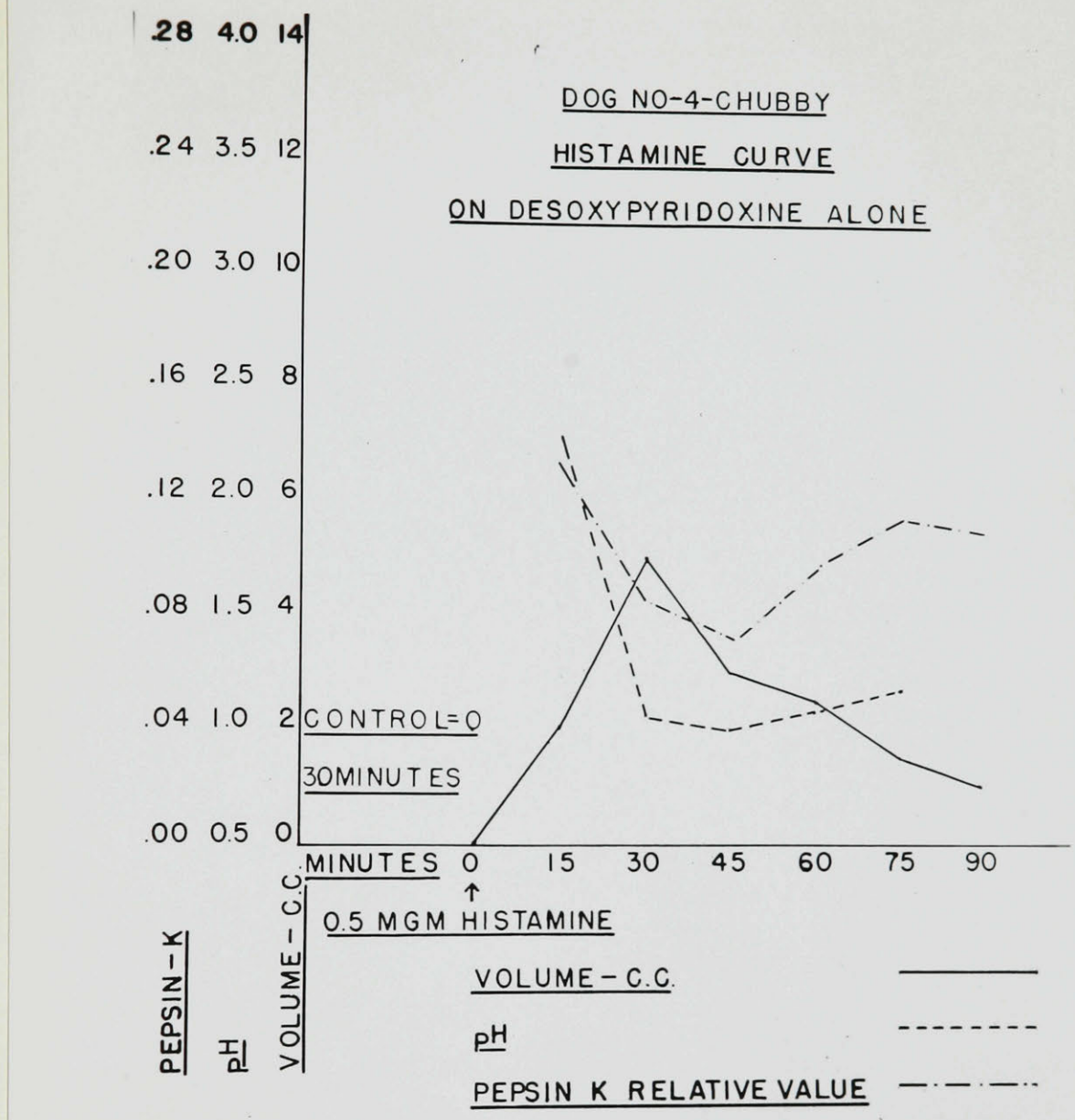


Figure 66

After 14 days on desoxypyridoxine alone.
Note no significant changes in gastric secretion.

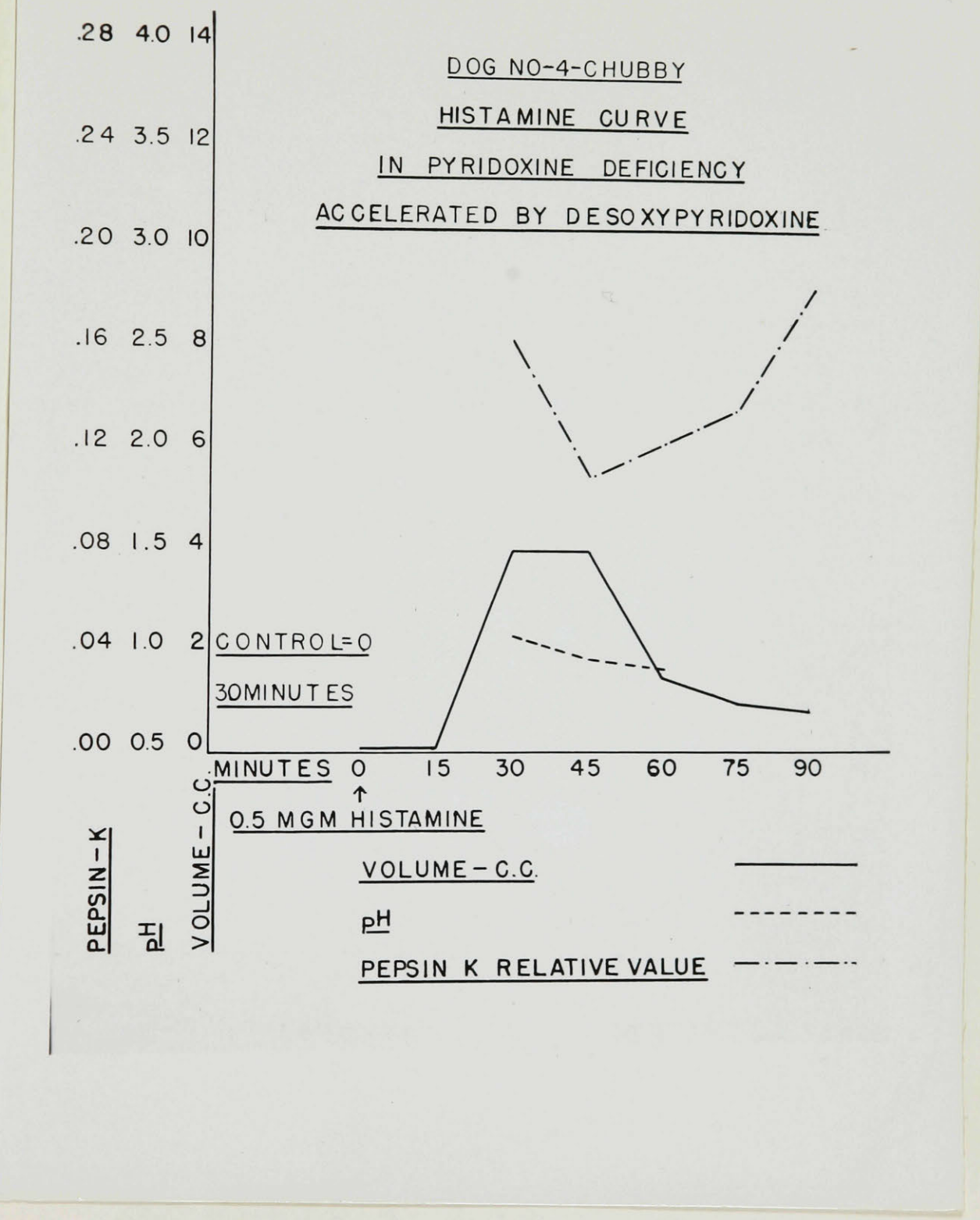


Figure 67

After 10 days on pyridoxine free diet and desoxypyridoxine. Note slight reduction in volume (11.0 c.c.) of gastric secretion.

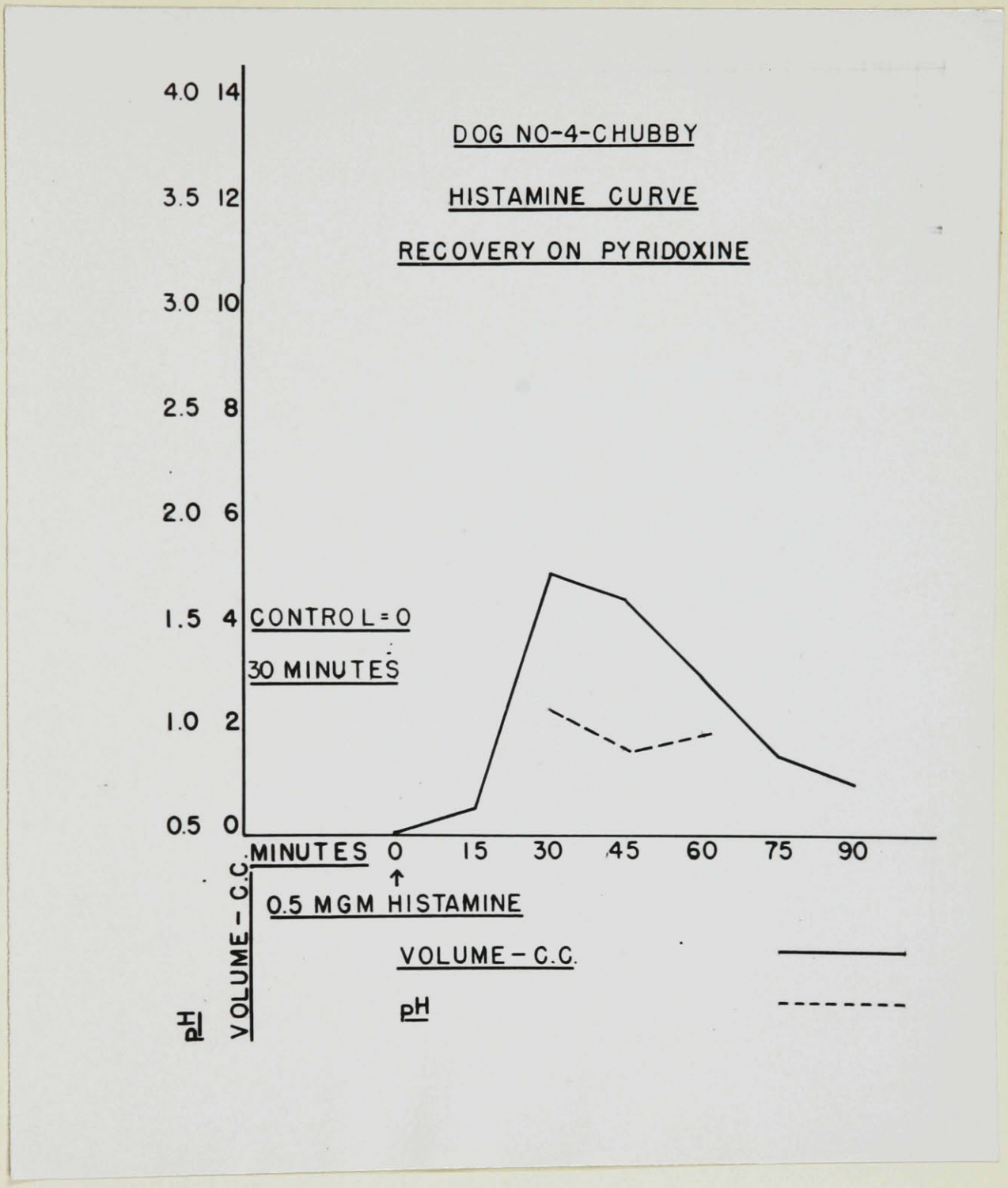


Figure 68

After 15 days on pyridoxine. Note return of volume (15.5 c.c.) of gastric secretion.

DOG NO. - 4 - CHUBBY

HEMATOLOGY

DATE	HEMOGLOBIN	ERYTHROCYTE COUNT	HEMATOCRIT	ERYTHROCYTE SIZE
Mar. 21	100	6,300,000	46	7.1
Mar. 28	102	6,400,000	46	7.1
Mar. 31		Started on Desoxypyridoxine		
Apr. 5	101	5,850,000	44	7.1
Apr. 11	100	6,370,000	46	7.1
Apr. 17		Desoxypyridoxine Discontinued.		
Apr. 19	116	7,100,000	55	7.1
Apr. 26	106	6,280,000	48	7.0
May. 2	107	6,480,000	47	6.95
May. 2		Started on Pyridoxine Free Diet and Desoxypyridoxine.		
May. 10	109	6,580,000	49	6.98
May, 16	99	5,820,000	40	6.82
May. 23	92	5,730,000	40	6.82
May. 30		Started on Complete Synthetic Diet Containing Pyridoxine. Desoxypyridoxine Discontinued.		
May 31.	94	6,090,000	42	6.75
June 6	97	5,600,000	45	7.0
June 15	95	6,020,000	43	7.0

Figure 69

Table of results.

DOG NO. - 4 - CHUBBY

HEMATOLOGY

DATE	LEUCOCYTE COUNT	POLYMORPHS	LYMPHOCYTES	EOSINOPHILES	MONOCYTES	BASOPHILES
Mar. 21	14,150	78	15	7	0	0
Mar. 28	14,800	64	21	14	1	0
Mar. 31	Started on Desoxypyridoxine					
Apr. 5	10,300	69	12	17	1	1
Apr. 11	20,150	88	7	5	0	0
Apr. 17	Desoxypyridoxine Discontinued.					
Apr. 19	10,500	80	12	7	1	0
Apr. 26	10,350	67	22	9	2	0
May. 2	14,300	80	11	9	0	0
May. 2	Started on Pyridoxine Free Diet and Desoxypyridoxine.					
May. 10	14,400	77	10	12	0	1
May. 16	15,300	83	4	9	1	3
May. 23	10,700	78	10	8	1	3
May. 30	Started on Complete Synthetic Diet Containing Pyridoxine. Desoxypyridoxine Discontinued.					
May. 31	16,250	91	3	2	1	3
June 6	23,750	72	20	6	1	1
June 15	12,850	61	22	16	1	0

Figure 70

Table of results.

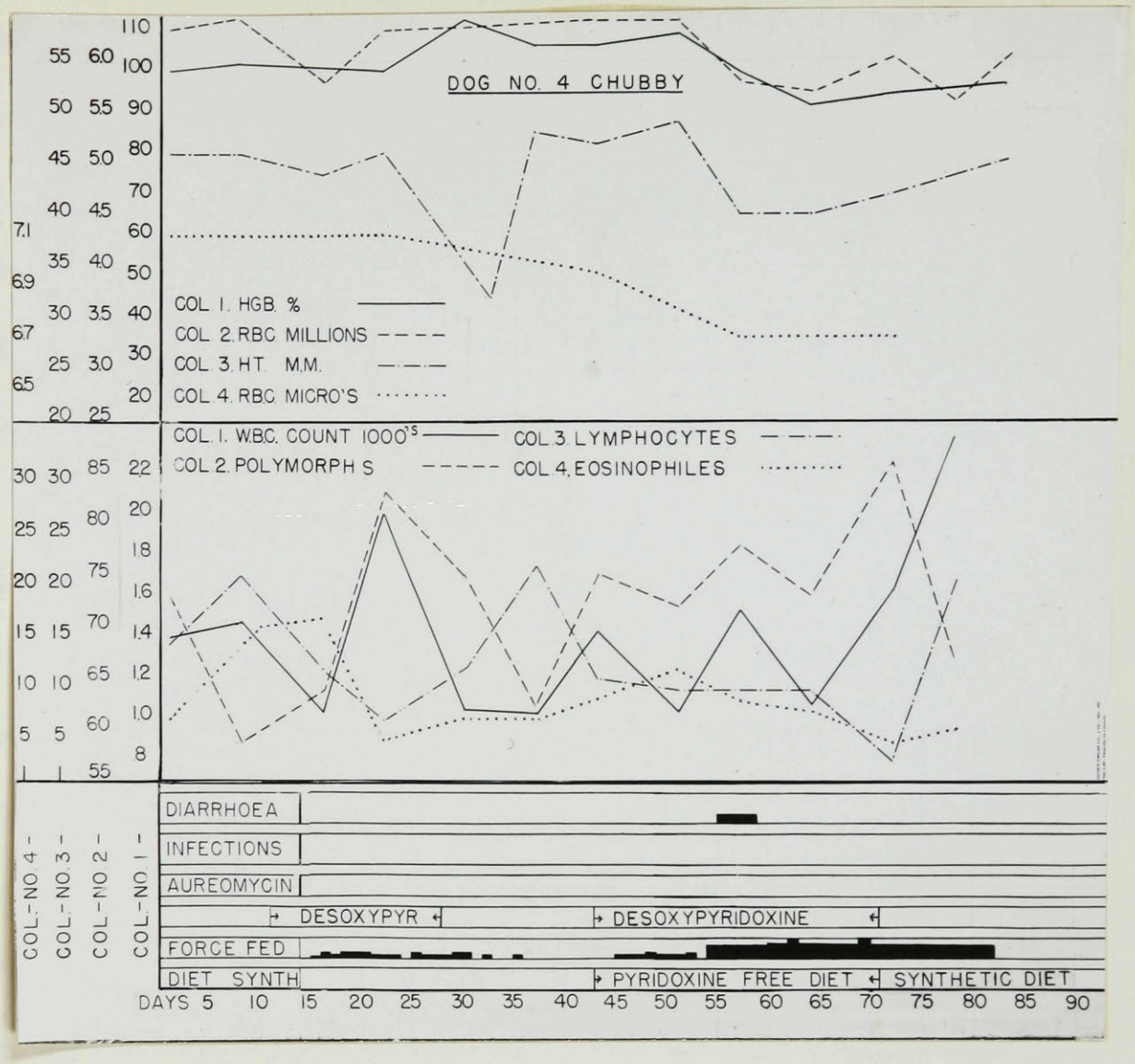


Figure 71

Composite graph of hematological results of Dog No 4 "Chubby". Note developement of slight hypochromic microcytic anemia and leucopenia on a pyridoxine free diet and desoxypyridoxine.

The blood showed a leucocytosis, polynucleosis and lymphopenia.

The desoxypyridoxine was discontinued and the volume and pH of the gastric secretion returned to normal gradually in 12 days. Thus the rise and fall in the volume of gastric secretion took place gradually. The dog was then used to demonstrate a fall in the volume of gastric secretion on a pyridoxine free diet and desoxypyridoxine.

Thus it has been shown that when desoxypyridoxine is given along with a diet containing pyridoxine the dog does not develop pyridoxine deficiency. There is a considerable increase in the volume of the gastric secretion. This is a striking contrast to the marked fall in the volume of the gastric secretion on a pyridoxine free diet alone or with desoxypyridoxine. It must be concluded that the reduction in gastric secretion is due to pyridoxine deficiency.

At the time of writing, three of the dogs with the new gastric pouches "Yapper", "Skippy" and "Chubby" are healthy and gaining weight eight months after starting them on the synthetic diet. They are being used to make further studies on vitamin B12 and the effect of special drugs on gastric secretion.

CHAPTER V

SUMMARY

A historical review of the literature concerning the vitamin B complex and its known role in gastric secretion, the components of the vitamin B complex and their known role in gastric secretion, pyridoxine and the pyridoxine deficiency syndrome, desoxypyridoxine, gastric pouches and vitamin B12, has been made, emphasizing the important discoveries to date. Conclusions pertinent to the present investigation have been drawn.

The materials and methods used in this investigation have been described. The results of the experiments have been illustrated with many tables, graphs and photographs, that have been included as near as possible to the description in the text.

A new synthetic diet was composed to feed gastric pouch dogs. The mineral mixture was that of Jones and Foster and made up 5% of the diet. The vitamin supplement consisted of large amounts of all the known components of the vitamin B complex as well as large amounts of vitamins A, D, E and K.

The first series of dogs with Heidenhein and Cope pouches developed many complications. Three of these dogs died with distemper and then a method was worked out to deal with distemper under existing circumstances. This series showed that denervated pouches must be well attached in the abdominal cavity. They also showed that innervated pouches should be small, easy to construct, have a short mucous membrane bridge that is well protected, have an excellent nerve supply and a ventral stoma so excoriation can be controlled. On the basis of these requirements a new innervated gastric pouch was designed working with Dr. Currie who was also interested in gastric pouches.

The second series of dogs had the new gastric pouches. They all lived and had excellent innervated pouches as shown by the first 1½ hours of daily secretion and the insulin curves. A new collecting apparatus was designed and proven to be useful. However, the Malicott indwelling catheter was found to be the best method of collecting the secretion. This series of dogs was divided into four groups.

The first group consisted of a control dog, with a new gastric pouch, fed the basal synthetic

diet for 57 days. He remained well clinically and was very active. He ate well and gained weight. His red and white blood cell count and hemoglobin remained normal. The range of the volume and pH of his gastric secretion remained constant. Several histamine and insulin curves done throughout this period were constant.

The second group consisting of three dogs was fed the pyridoxine free diet and desoxypyridoxine. All these dogs developed a mild accelerated pyridoxine deficiency characterized by lethargy, varying degrees of anorexia, loss of weight, dermatitis, intermittent diarrhoea, varying degrees of microcytic hydrochromic anemia, leucopenia, lymphopenia and polynucleosis. In all three dogs there was a marked reduction in the volume of gastric secretion with a slight elevation of the pH. The reduction was precipitious in the youngest dog, the volume being reduced from 175 cc. to 41 cc. in twenty-four hours. In the older dogs reductions from 150 cc. to 15 cc. occurred gradually taking one to two weeks. This indicated the reduction was due to accelerated pyridoxine deficiency. The most marked reduction of secretion was in the chemical phase of

gastric secretion. Similarly there was a greater reduction in the volume of gastric secretion in the histamine tests than in the insulin tests, but there was a reduction in both. There was also a significant elevation of the pH of the gastric secretion in the histamine tests. There was a rapid clinical improvement with disappearance of the anorexia in 24-40 hours after the administration of pyridoxine. The volume and pH of the gastric secretion returned to normal in 3-5 days. The blood required up to 3 weeks to return to normal.

The third group consisting of two dogs was fed the pyridoxine free diet. These dogs gradually developed the typical pyridoxine deficiency syndrome characterized by progressive lethargy, anorexia, malnutrition, decrease in weight, arrest of growth, microcytic hypochromic anemia, leucopenia, dermatitis and dryness of hair. The two dogs developed a gradual decrease in the volume of the gastric secretion and an elevation of the pH. There was also a slight reduction in the volume of gastric secretion in the histamine and insulin curves. The reduction was less in these dogs than those receiving desoxypyridoxine. In one dog there was a complete

return to normal of the gastric secretion in 3 days after receiving pyridoxine. In the other there was a marked increase but not quite to normal. The signs and symptoms of pyridoxine deficiency disappeared.

One of the dogs in Group two and one of the dogs in Group three continued to show signs and symptoms of what appeared to be a deficiency syndrome of an unknown component or components of the vitamin B complex. It may be significant that these two dogs were given aureomycin on two occasions for infections.

In the first dog it was characterized by an extensive dermatitis and alopecia on the face and legs, ulcers on the legs, lethargy, intermittent anorexia, intermittent diarrhoea and blood in the stool and a fairly marked reduction in the daily volume of gastric secretion. There was also a reduction in the volume of gastric secretion in the histamine tests. There was little change in this entire syndrome after 40 days of massive liver therapy. There is suggestive evidence that the gastric secretion returned to normal, but this could not be proven definitely as the mucous membrane bridge had broken down.

In the second dog it was characterized by a slight lethargy, dermatitis and marked anorexia. There was also a very slight reduction in

the daily volume of gastric secretion. This was probably due to the anorexia, as it was chiefly in the nervous phase and the histamine and insulin curves were normal. This possible deficiency syndrome responded to vitamin B12 therapy in the dog. There was a marked increase in his weight, despite the fact there was no increase in his food intake. This was an excellent demonstration of the protein sparing function of vitamin B12.

The fourth group consisted of one dog with a new gastric pouch fed the basal diet and desoxypyridoxine. She developed slight anorexia and a marked increase in the volume of gastric secretion with a very slight increase in the pH. This increase was gradual and continued for 12 days before the volume stabilized. When the desoxypyridoxine was discontinued it took 12 days for the volume of gastric secretion to return to normal. This indicates that the desoxypyridoxine itself was not responsible for the reduction of gastric secretion in Group 2, but that the reduction of gastric secretion was due to the accelerated pyridoxine deficiency state.

CHAPTER VI

CONCLUSION

1. A review of the literature of the vitamin B complex indicates that there is still an unknown factor or factors in the vitamin B complex that is essential for the normal nutrition of the dog and that the vitamin B complex is essential for the normal production of gastric secretion. There is suggestive evidence that there is a reduction in gastric secretion in pyridoxine deficiency.
2. Cope pouch dogs developed many complications and are of little experimental value.
3. A new innervated gastric pouch has been designed. It was used in 4 dogs with 100 percent success.
4. A new collecting apparatus has been designed. It was found to be very useful.
5. The Malicott indwelling catheter is the best method of collecting secretions from a gastric pouch.
6. A new synthetic diet has been composed to feed gastric pouch dogs. At the time of writing, three dogs with the new gastric pouches that have been fed this diet for 8 months are well and healthy.

7. A pup 5 months old with a new gastric pouch was fed the synthetic diet for 2 months without developing any signs of a deficiency and without any change in the volume or pH of the gastric secretion. There was no change in the histamine or insulin curves.
8. Feeding dogs with gastric pouches a pyridoxine free diet and desoxypyridoxine results in a marked decrease in the volume of gastric secretions with a slight elevation of the pH. The reduction is more marked in the histamine test and chemical phase of gastric secretion.
9. Feeding dogs with gastric pouches a pyridoxine free diet results in a considerable decrease in the volume of gastric secretion with a slight elevation of the pH. The gastric secretion rapidly returns to normal with administration of pyridoxine.
10. A possible deficiency syndrome in the dog has been shown to respond to vitamin B12.

11. Feeding dogs with gastric pouches the analogue desoxypyridoxine and a normal diet results in a marked increase in the volume of gastric secretion. This returns to normal when desoxypyridoxine is discontinued.

12. Pyridoxine is essential for the normal production of gastric secretion.

BIBLIOGRAPHY

1. Allison, J.R.
   Relation of hydrochloric acid and vitamin B complex deficiency in certain skin diseases.
   South. M.J. 38: 235-241, 1945.

2. Alvarez, W.C.
   Daily variations in concentration of acid and pepsin in three persons observed for two months.
   Am. J. Dig. Dis. & Nut. 3: 762-764, 1936.

3. Antopol, W. and Schotland, C.E.
   The use of vitamin B6 in pseudohypertrophic muscular dystrophy.
   J.A.M.A. 114: 1058, 1940.

4. Armour, J.C.
   A lesser curvature gastroplasty flap exteriorization of ulcer: the formation of a gastric pouch.
   Canad. Med. Ass. J. 23: 756-761, 1930.

5. Axelrod, A.E., Lipton, M.A. and Elvehjem, C.A.
   The production of uncomplicated riboflavin deficiency in the dog.
   Am. J. Physiol. 128: 703, 1940.

6. Axelrod, H.E., Morgan, A.F. and Lepkovsky, S.
   The fate of tryptophane in pyridoxine deficient and normal dogs.
   J. Biol. Chem. 160: 155-164, 1945.

7. Babkin, B.P.
   Nervous control of gastric secretion and effect of vitamin deficiency on its production.
   Canad. M.A.J. 39: 5-9, 1933.

8. Babkin, B.P.
   Secretory mechanism of the digestive glands.
   New York, Hoeber Co., 1944.

9. Baker, A.B.
   Treatment of paralysis agitans with vitamin B6 (pyridoxine hydrochloride)
   J.A.M.A. 116: 2484-2487, 1941.

10. Bellamy, W.D. and Gunsalus, J.C.
Growth requirements of streptococcus fecalis for tyrosine decarboxylation.
J. Bac. 46: 573, 1943.

11. Bellamy, W.D. and Gunsalus, J.C.
Tyrosine decarboxylation by streptococci: growth requirements for active cell production.
J. Bact. 48: 191, 1944.

12. Bellamy, W.D., Umbreit, W.W. and Gunsalus, J.C.
Function of pyridoxine: conversion of members of vitamin B6 group into codecarboxylase.
J. Biol. Chem. 160: 461, 1945.

13. Berk, L., Castle, W.B., Welch, A.D., Heinle, R.W., Anker, R. and Epstein, N.
Observations on the etiological relationship of achylia gastrica to P.A. activity of vitamin B12 as food (extrinsic) factor.
N.E.J. Med. 239: 911, 1948.

14. Berk, L.D., Brown, D., Finland, M. and Castle, W.B.
Effectiveness of B12 in combined systems disease.
N.E.J. Med. 239: 328, 1948.

15. Betheil, T.J. and Lardy, H.A.
Comparative effectiveness of vitamin B12, whole liver substitute and extracts high in A.P.A. activity as growth promoting materials for hyperthyroid animals.
J. Nut. 37: 495-509, 1949.

16. Bethell, F.H., Meyers, M.C., and Neligh, R.B.
Vitamin B12 in P.A. and puerperal macrocytic anemia.
J. Lab. & Cl. Med. 33: 1477, 1948.

17. Birch, T.W., Gyorgy P., and Harris, L.J.
The vitamin B2 complex:-differentiation of the anti black-tongue and the "P.P." factors from lacto flavin and vitamin B6 (so-called "rat pellogra" factor.)
J. Biochem. 29: 2830, 1935.

18. Bird, O.D., Vandenbelt, J.M. and Emmett, A.D.
Adaptation of the Scudi colorimetric method for pyridoxine.
J. Biol. Chem. 142: 317-322, 1942.
Proc. Soc. Am. Chem. 23: 1941.

19. Bischoff, F., Ingraham, L.P. and Rupp, J.J.
Influence of vitamin B6 and pantothenic acid on growth of sarcoma.
Arch. Path. 35: 713-716, 1943.

20. Borson, H.J. and Mettier, S.R.
Relief of hypochromic anemic in dogs with synthetic vitamin B6 influence of filtrate factors.
Proc. Soc. Exp. Biol. & Med. 43: 429, 1940.

21. Bottomley, A.C.
Chemical estimation of vitamin B6 in foodstuffs.
Proc. Soc. Biochem. 38: 5, 1944.

22. Bradford, N.M., Davies, R.E., Ellis, E. and Hughes,D.E.
The concentration of co-zymase and total nicotinic acid in the cat oxyntic cells.
Commun. Biochem. Soc. 266th meeting, 1948.

23. Cantor, M.M. and Scott, J.W.
Agranulocytic angina effectively treated with intravenous pyridoxine (vitamin B6).
Canad. M.A.J. 52: 368-371, 1945.

24. Carpenter, L.E., Elvehjem, C.A. and Strong, F.M.
Effect of pseudopyridoxine on rat and yeast growth.
Proc. Soc. Exp. Biol. & Med. 54: 123, 1943.

25. Carpenter, L.E. and Strong, F.M.
Determination of pyridoxine and pseudopyridoxine.
Arch. Biochem. 3: 375-388, 1944.

26. Cartwright, G.E., Wintrobe, N.N., Lauritson, M. and Humphries, S.
Tryptophane derivatives in the urine of pyridoxine deficient swine.
Bull. Johns Hopkins Hosp. 75: 35, 1944.

27. Cartwright, G.E., Wintrobe, N.N., and Humphries, S.
Studies on anemia in swine due to pyridoxine deficiency together with data on phenylhydrazine anemia.
J. Biol. Chem. 153: 171, 1944.

28. Cartwright, G.E., Wintrobe, N.N., Buschke, W.H., Follis Jr., R.H., Suksta, A. and Humphries, S.
Anemia, hypoproteinemia and cataracts in swine fed casein hydrolysate or zein. Comparison with pyridoxine - deficient anemia.
J. Clin. Investigation 24: 268, 1945.

29. Chick, H. and Roscoe, M.H.
On the composite nature of the water soluble B vitamin.
J. Biochem. 21: 698, 1927.

30. Conger, T.W. and Elvehjem, C.A.
The biological estimation of pyridoxine(vitamin B6)
J. Biol. Chem. 138: 555-561, 1941.

31. Cope, O., MacMahon, C.E., Hagstromer, A. and Thompson, R.H.
A new gastric pouch with a non-leaking stoma and an intact nerve supply: description of a two stage technic used on the dog.
Arch. Surg. 40: 717-732, 1940.

32. Copley, A.L., Stefko, P.L. and Naylor, J.
Comparative hematologic findings in normal, splenectomized and speen transplanted dogs.
Surg. Gyne. Obs. 646-659, 1947.

33. Cowgill, G.R.
A contribution to the study of the relation between vitamin B and the nutrition of the dog.
Am. J. of Physiol. 57: 420, 1921.

34. Cowgill, G.R. and Mendel, L.B.
Studies in the physiology of vitamins: vitamin B and the secretory function of glands.
Am. J. Physiol. 58: 131, 1921.

35. Cowgill, G.R., Deuel, H.J. and Smith, A.H.
Studies in the physiology of vitamins: quantitative aspects of the relation between vitamin B and appetite in the dog.
Am. J. Physiol. 73: 106, 1925.

36. Cowgill, G.R., Deuel, H.J., Plummer, N. and Messer, F.C.
Studies in physiology of vitamins: vitamin B in relation to gastric motility.
Am. J. Physiol. 77: 389, 1926.

37. Cowgill, G.R. and Gilman, A.L.
Effect of lack of the vitamin B complex on the secretion of gastric juice in dogs with gastric pouches.
Arch. Int. Med. 53: 58-70, 1934.

38. Cravens, W.W. and Snell, E.E.
Effects of desoxypyridoxine and vitamin B6 on development of chick embryo.
Proc. Soc. Exp. Biol. & Med. 71: 73-76, 1949.

39. Daft, F.S., Sebrell, W.H., Babcock, S.H. and Jukes, T.H.
Effect of synthetic panthothenic acid on adrenal haemorrhage atrophy and necrosis in rats.
Pub. Health Reports. 55: 1333, 1940.

40. Daniel, E.P., Kline, O.L. and Tolle, C.D.
A convulsive syndrome in young rats associated with pyridoxine deficiency.
J. Nut. 23: 205, 1942.

41. Danysz-Michel and MMe. Koskowski, W.
Etude de quelques fonctions digestives chez les pigeons normaux, nourris au riz poli et en inamition.
Compt. rend. Acad. d. sc. 175: 54-56, 1922.

42. Denko, C.W., Grundy, W.E., Porter, J.W.
Berryman, G.H., Friedemann, T.E. and Youmans, J.B.
The excretion of B complex vitamins in the urine and feces of seven normal adults.
Arch. Biochem. 10: 33-40, 1946.

43. Donnelly, G.L.
Permanent devocalization of dogs by removal of both true and the false vocal cords.
Soc. for Exp. Biol. & Med. 50: 79-80, 1942.

44. Dougherty, T.F. and White, A.
Effect of pituitary adrenotrophic hormone on lymphoid tissue.
Proc. Soc. Exp. Biol. & Med. 53: 132-133,1943.

45. Dougherty, T.F., Chase, J.H. and White, A.
The demonstration of antibodies in lymphocytes.
Proc. Soc. Exp. Biol. & Med. 57: 298, 1944.

46. Dyer, H.M. and Roe, J.H.
Relation of nutrition to gastric function: effect of vitamin B1 deficiency.
Am. J. Dig. Dis. 8: 329-333, 1941.

47. Ehrich, W.F. and Harris, T.N.
The site of antibody formation.
Science 101: 28, 1945.

48. Elvehjem, C.A.
The vitamin B complex.
J.A.M.A. 960, 1948.

49. Emerson, G.A.
The antivitamin B6 activity of desoxypridoxine in the rat.
Fed. Proc. 6: 1, 1947.

50. Emerson, G.A., Wurtz, E. and Zanett, M.C.
Vitamin B12 - a growth factor for young rats.
Fed. Proc. 8: 387, 1949.

51. Emerson, G.
Growth promoting activity of vitamin B12 in rats receiving thyroid substance.
Proc. Soc. Exp. Biol. & Med. 70: 392, 1949.

52. Evans, R.J. and Phillips, P.H.
A new low fluorine diet and its effect upon the rat.
J. Nut. 18: 353, 1939.

53. Farnum, M.B.
Gastric secretion in experimental beri-beri in the dog.
Arch. Int. Med. 37: 212, 1926.

54. Fishberg, E.H. and Vorzimer, J.
Effect of pyridoxine on granulopenia caused by thiouracil.
Proc. Soc. Exp. Biol.& Med. 60: 181-184,1945.

55. Fouts, P.J., Helmer, O.M., Lepkovsky, S. and Jukes, T.H.
Treatment of human pellagra with nicotinic acid.
Proc. Soc. Exp. Biol.& Med. 37: 405, 1937.

56. Fouts, P.J., Helmer, O.M., Lepkovsky, S. and Jukes, T.H.
Production of nicrocytic hypochromic anemia in puppies on synthetic diet deficient in rat antidermatitis factor (vitamin B6)
J. Nut. 16: 197, 1938.

57. Fouts, P.J., Helmer, O.M., Lepkovsky, S. and Jukes, T.H.
Severe anemia in puppies on a synthetic diet in rat antidermatitis factor (vitamin B6)
J.A.M.A. 110: 766, 1938.

58. Fouts, P.J., Helmer, O.M. and Lepkovsky, S.
Cure of microcytic hypochromic anemia in dogs with crystalline factor I.
Proc. Soc. Exp. Biol. & Med. 40: 4, 1939.

59. Fouts, P.J., Helmer, O.M. and Lepkovsky, S.
Factor 11 deficiency in dogs.
J. Nut. 19: 393, 1940.

60. Fouts, P.J. and Lepkovsky, S.
A green pigment producing compound in urine of pyridoxine deficient dogs.
Soc. Exp. Biol. & Med. 50: 221, 1942.

61. Fouts, P.J.
Vitamin B complex studies in dogs: production of cirrhosis of liver.
J. Nut. 25: 217, 1943.

62. Foy, J.R. and Cerecedo, L.R.
The effect of protein on vitamin B6 deficiency.
Proc. Am. Chem. Soc. 24: 1941.

63. Gavin, G. and McHenry, E.W.
The B vitamins and fat metabolism.
J. Biol. Chem. 132: 41, 1940.

64. Gellhorn, A. and Jones, L.O.
Pyridoxine deficient diet and desoxypridoxine in the therapy of lymphosarcoma and acute leucemia in man.
J. Blood. 60: 1949.

65. Gildea, E.F., Kattwinkel, E.E. and Castle, W.B.
Experimental Combined system disease.
New England J. Med. 202: 523, 1930.

66. Gilman, A. and Cowgill, G.R.
The effect of histamine upon the secretion of gastric pepsin.
Am. J. Physiol. 97: 124, 1931.

67. Goldberger, J. and Lillie, R.D.
A note on an experimental pellagra-like condition in the albino rat.
Pub. Health Rep. 41: 1025, 1926.

68. Goldberger, J. and Wheeler, G.A.
A further study of experimental black tongue.
U.S. Pub. Health Reports 43: 657, 1928.

69. Goodman, L., Gilman, A. and Bearg, P.
Catheter device for care of gastric pouch animals.
J. Lab. and Clin. Med. 22: 209-11, 1936.

70. Greenberg, L.D. and Rinehart, J.R.
Xanthurenic acid excretion in pyridoxine deficient Rhesus monkeys.
Fed. Prod. 5: 222, 1946.

71. Gunsalus, J.C. and Bellamy, W.D.
The function of pyridoxine and pyridoxine derivatives in the decarboxylation of tyrosine.
J. Biol. Chem. 155: 157, 1944.

72. Gunsalus, J.C. and Bellamy, W.D.
A function of pyridoxal.
J. Biol. Chem. 155: 357, 1944.

73. Gunsalus, J.C., Bellamy, W.D. and Umbreit, W.W.
A phosphorylated derivative of pyridoxal as the coenzyme of tyrosine decarboxylose.
J. Biol. Chem. 155: 685, 1944.

74. Gunsalus, J.C. and Bellamy, W.D.
The effect of pyridoxine on tyrosine decarboxylation and on fermentation.
J. Bact. 47: 413, 1944.

75. Gyorgy, P.
Vitamin B2 and the pellagra-like dermatitis in rats.
Nature 133: 498, 1934.

76. Hall, B.E. and Campbell, D.C.
Vitamin B12 therapy in P.A. effect on haematoporetic symptoms.
Proc. Staff Meet. Mayo Clinic 23: 584,591,1948.

77. Hall, B.E., Morgan, E.H. and Campbell, D.C.
Oral administration of vitamin B12 in P.A. presence of intrinsic factor in berkefeld filtered pooled human gastric juice.
Mayo Clinic Staff Meet. 24: 4, 1949.

78. Halliday, N. and Evans, H.M.
Dietary production of the syndrome of deficiency in vitamin B6.
J. Nut. 13: 657-667, 1937.

79. Hanson, M.E., Grossman, N.J. and Ivy, A.C.
Doses of histamine producing minimal and maximal gastric secretory in dog and man.
Am. J. Physiol. 153: 242-50, 1948.

80. Harris. L.J.
Flavin and pellagra-preventing factor as separate constituents of complex vitamin B2.
J. Biochem. 29: 776-781, 1935.

81. Harris, R.S.
Antimetabolites of pyridoxine.
Nut. Rev. 6: 2, 1948.

82. Harris, S.A., Stiller, E.T. and Folkers, K.
Structure of vitamin B6.
Am. Chem Soc. J. 61: 1242, 1939.

83. Harris, S.A. and Folkers, K.
Synthesis of B6.
Am. Chem. Soc. J. 61: 1245, 1939.

84. Harris, S.A. and Folkers, K.
Synthetic B6
Science 89: 347, 1939.

85. Harris, S.A., Heyl, D. and Folkers, K.
The structure and synthesis of pyridoxamine and pyridoxal.
J. Biol. Chem. 154: 315, 1944.

86. Harrow, B.
Textbook of Biochemistry.
Philadelphia, W.B. Saunders Co.
4th Ed., 1946.

87. Hartman, A.M. and Cary, C.A.
Basic synthesis in the rat of an unidentified growth-promoting factors.
Fed. Proc. 8: 1949.

88. Hawkins, W.W. and Barsky, J.
An experiment on human vitamin B6 deprivation.
Science 108: 284-286, 1948.

89. Hawkins, W.W.
Some effects observed in dogs of vitamin B6 deprivation and desoxypyridoxin administration.
Proc. Canad. Physiol. Soc. 13: 1949.

90. Hawthorne, G.A.
Nutritional diseases of swine.
North Am. Vet. 22: 147-155, 1941.

91. Heidenhein, R.
Ueber die pepsin bildung in den phlorusdrusen.
Arch. Physiol. 18: 169, 1878.

92. Hesseltine, H.C.
Pyridoxine failure in nausea and vomiting of pregnancy.
Am. J. Obst. & Gynec. 51: 82-86, 1946.

93. Hogan, A.G., Richardson, L.R., Patrick, H., O'Dell, B.L., and Kempster, H.L.
Vitamin B6 and chick nutrition.
Poultry Sc. 20: 180-183, 1941.

94. Hollander, F., and Jemerin, E.E.
Preparation of stomach pouch without interruption of vagal supply.
Proc. Soc. Exp. Biol. N.Y. 39: 87, 1938.

95. Jemerin, E.E., and Hollander, F.
Gastric vagi in the dog, erroneous assumption of uninterrupted vagal innervation in the pavlov.
Proc. Soc. Exp. Biol.& Med. 38: 139, 1938.

96. Jolliffe, N., Rosenblum, L.A., and Sawhill, J.
The effects of pyridoxine (vitamin B6) on persistent adolescent acne.
J. Invest. Dermat. 5: 143, 148, 1942.

97. Jones, J.H. and Foster, C.
A salt mixture for use with basal diets either low or high in phosphate.
J. Nut. 24: 245, 256, 1942.

98. Jukes, T.H.
Vitamin B6 deficiency in chicks.
Proc. Soc. Exp. Biol.& Med. 42: 180, 1939.

99. Karr, W.G.
Some effects of water-soluble vitamin upon nutrition.
J. Biochem. 44: 255-276, 1920.

100. Keresytesy, J.C. and Stephens, J.R.
Crystalline vitamin B6.
Proc. Soc. Exp. Biol. & Med. 38: 64, 1938.

101. Kline, B.E., Rusch, H.P., Baumann, C.A. and Lavik, P.S.
The effect of pyridoxine on tumor growth.
Cancer Res. 3: 825-829, 1943.

102. Lambooy, J.P., and Nasset, E.G.
The inadequacy of 8 synthetic B vitamins for the nutrition of puppies unknown factor (factors) in yeast and probably liver.
J. of Nut. 26: 293, 1943.

103. Landy, M. and Dicken, D.M.
A microbiological assay method for six B vitamins using lactobacillus casei and a medium of essentially known composition.
J. Lab. & Clin. Med. 27: 1086-1092, 1942.

104. Lecoq, R., Chauchard, P., and Nafoue, H.
Antiacidosic effect of vitamins of B group.
Compt. rend. Soc. de Biol. 139:527-528,1945.

105. Lederle Laboratories Division 1947.
Distemper
Lederle Lab., Mont.

106. Lepkovsky, S., Jukes, T.H. and Krause, M.E.
The multiple nature of the third factor of the vitamin B complex.
Jour. Biol. Chem. 115: 557, 1936.

107. Lepkovsky, S.
Crystalline factor I
Science 87: 169, 1938.

108. Lepkovsky, S., Krause, M.E., and Dimick, M.K.
The occurrence of fits in pyridoxine deficient rats.
Science 95: 331-332, 1942.

109. Lepkovsky, S. and Nielsen, E.
A green pigment-producing compound in urine of pyridoxine-deficient rats.
J. Biol. Chem. 144: 135-138, 1942.

110. Lepkovsky, S., Roboz, E., and Haagen-Smit, A.J.
Xanthurenic acid and its role in the tryptophane metabolism of pyridoxine deficient rats.
J. Biol. Chem. 149: 195-201, 1943.

111. Luecke, R.W., McMillan, W.N., Thorp, F., Jr. and Boniece, J.R.
The effect of vitamin B12 concentrated on the growth of weaning pigs fed corn-soybean diets.
Science 110: 139, 1949.

112. Luecke, R.W., McMillen, W.N. and Thopp, F.J.R.
The effect of vitamin B12 animal protein factor and streptomycin on the growth of young pigs.
Arch. Biochem 26: 326-327, 1950.

113. Machella, T.E.
Studies of the B vitamins in the human subject III. The response of cheilosis to vitamin therapy.
Am. J. M. Sc. 203: 114-120, 1942.

114. Martin, G.J., Thompson, M.R. and DeCarvajal Forero, J.
The influence of inositol and other B complex factors upon the motility of the gastro-intestinal tract.
Am. J. Dig. Dis. 8: 290-295, 1941.

115. Maxfield, J.R., Jr., McIlwain, A.J. and Robertson, J.E.
Treatment of radiation sickness with vitamin B6 (pyridoxine hydrochloride)
Radiology 41: 383-388, 1943.

116. Meyer, L.M., Sawitsky, A., Ritz, N.D. and Krim, M.
A. J. Clin. Path. (in press)

117. Miller, E.C. and Baumann, C.A.
Relative effects of casein and tryptophane on the health and xanthurenic acid excretion of pyridoxine deficient mice.
J. Biol. Chem. 157: 551-562, 1945.

118. Miyadere, K.
Uber die funktign der verdaungsdrusen bei avitaminosen.
Biochem. Z. 124: 124, 1921.

119. Morgan, A.F.
The effect of imbalance in the filtrate faction of the vitamin B complex in dogs.
Science 93: 261, 1941.

120. Morgan, A.F., Goody, M. and Axelrod, H.E.
Pyridoxine deficiency as affected by level of dietary protein in dogs.
Am. J. Physiol. 146: 723-738, 1946.

121. Mowat, J.H., Pilgrim, F.J. and Carlson, G.H.
A synthesis of vitamin B6.
J. Am. Chem. Soc. 65: 954-955, 1943.

122. Mueller, J.F. and Vilter, R.W.
Pyridoxine deficiency in human beings induced with desoxypyridoxine.
J. Clin. Inv. 29: 2, 1950.

123. Mushett, C.W., Stebbins, R.B. and Barton, M.N.
Pathological effects produced by 2 analogues of pyridoxine.
Transaction, N.Y. Am.Sc. 9: 291-296, 1947.

124. McCall, K.B., Waisman, H.A., Elvehjem, C.A., and Jones, E.S.
A study of pyridoxine and panthothenic acid deficiency in the monkey.
J. Nut. 31: 685-697, 1946.

125. McCollum, E.V. and Davis, M.
The nature of the dietary deficiencies of rice.
J. Biol. Chem. 23: 181-230, 1912-1915.

126. McCollum, E.V. and Davis, M.
The essential factors in the diet during growth.
J. Biol. Chem. 23: 231, 1912-1915.

127. McGanity, W.J., McHenry, H.B., Van Wyck, and Watt, G.L.
An effect of pyridoxine on blood urea in human subjects.
J. Biol. Chem. 178: 1, 1949.

128. McKibbin, J.M., Madden, R.J., Black, S. and Elvehjem, C.A.
The importance of vitamin B6 and factor W in the nutrition of dogs.
Am. J. Physiol. 128: 102, 1939.

129. McKibbin, J.M., Black, S., and Elvehjem, C.A.
The essential nature of panthothenic acid and another alkali labile factor in the nutrition of the dog.
Am. J. Physiol. 130: 365, 1940.

130. McKibbin, J.M., Schaefer, A.E., Frost, D.V., and Elvehjem, C.A.
Studies on anemia in dogs due to pyridoxine deficiency.
J. Biol. Chem. 142: 77, 1942.

131. Nord, F.F.
Advances in enzymology.
New York, Intersc.Pub.Inc. Vol.6, 1946.

132. Olcese, O., Couch, J.R. and Lyman, C.M.
Vitamin B12 concentrates in the nutrition of the mature domestic fowl.
J. Nut. 41, 1950.

133. Ott, W.H.
Antipyridoxine activity of 2, 4-dimethyl - 3-hydroxy-5-hydroxymethylpyridine in the chick.
Proc. Soc. Exp. Biol. & Med. 61: 125-127, 1946.

134. Ott, W.H., Ricker, E.L. and Wood, T.R.
Activity of crystalline vitamin B12 for chicks growth.
J. Biol. Chem. 174: 1047, 1948.

135. Papp, Von. J.
Effect of vitamin B1 on the gastric secretion.
Deutsche med. Wchnschr. 66: 711-713, 1940.

136. Paterson, W.B. and Stetton, D., Jr.
A study of gastric hydrochloride acid formation.
Science No. 2828: 256-258, 1949.

137. Patton, R.A., Karn, H.W., and Longenecker, H.E.
Studies on the nutritional basis of abnormal behaviour in albino rats. Convulsive seizures associated with pyridoxine deficiency.
J. Biol. Chem. 152: 181-191, 1944.

138. Pavlov, I.P.
The work of the digestive glands.
Translated by Thompson, W.W.
London 2 Ed. 1910.

139. Popper, H., Kochweser, D. and Syanto, P.B.
Protective effect of vitamin B12 upon hepatic injury produced by carbon tetrachloride.
Proc. Soc. Exp. Biol.& Med. 71: 688,690,1949.

140. Porter, C.C., Clark, I. and Silber, R.H.
The effect of pyridoxine analogues on tryptophane metabolisms in the rat.
J. Biol. Chem. 167: 573, 1947.

141. Porter, C.C., Clark, I. and Silber, R.H.
The effect of B vitamin deficiencies on tryptophan metabolism in the rat.
Arch. Biochem. 18: 339-343, 1948.

142. Reedman, E.J., Sampson, W.L., and Unna, K.
Identity of natural and synthetic crystalline vitamin B6.
Proc. Soc. Exp. Biol.& Med. 43: 112, 1940.

143. Rickes, E.L., Brink, N.G., Konivszy, F.B., Wood, T.R., and Folkins, K.
Vitamin B12 - a cobalt complex
Science 108: 134, 1948.

144. Rickes, E.L., Brink, N.G., Konivszy, F.B., and Wood, T.R.
Crystalline vitamin B12.
Science 107: 396, 1948.

145. Riggs, B.C. and Stadie, D.C.
A photoelectric method for the determination of peptic activity in gastric juice.
150: 2, 1943.

146. Rose, W.B., Stuckey, C.J. and Cowgill, G.R.
Studies in the physiology of vitamin B: further contribution to the study of gastric motility in vitamin B deficiency.
Am. J. Physiol. 91: 531-546, 1929-1930.

147. Rose, W.B., Stucky, C.J., and Cowgill, G.R.
Studies in the physiology of vitamin B: relation of gastric motility to anhydraemia in vitamin B and deficient dogs.
Am. J. Physiol. 92: 83, 1930.

148. Rosenblum, L.A. and Jolliffe, N.
The oral manifestations of vitamin deficiencies
J.A.M.A. 117: 2245-2248, 1941.

149. Roth, G.M. and Gabrielson, M.A.
Variation in the concentration of acide of the gastric content in normal subjects before and following immersion of hand and entire body in water at various temperatures.
Am. J. Physiol. 131: 195, 1940-1941.

150. Routh, J.I. and Houchin, O.B.
Some nutritional requirements of the hamster.
Fed. Proc. 1: 191-192, 1942.

151. Ruffin, J.M. and Dick, M.
The significance of gastric acidity after histamine stimulation.
Am. Int. Med. 12: 1940, 1939.

152. Sarma, P.S., Snell, E.E. and Elvehjem, C.A.
Comparison growth and antianaemic potencies of pyridoxal, pyridoxamine and pyridoxine for dogs.
Proc. Soc. Exp. Biol. & Med. 63: 284-286, 1946.

153. Schaefer, A.E., McKibbin, J.M. and Elvehjem, C.A.
Importance of choline in synthetic rations for dogs.
Proc. Soc. Exp. Biol. & Med. 47: 365, 1941.

154. Schaefer, A.E., McKibbin, J.M. and Elvehjem, C.A.
Studies on the vitamin B complex in the nutrition of the dog.
J. of Nut. 23: 491, 1942.

155. Schwartyman, G. and Strauss, L.
Vitamin B6 deficiency in the syrian hampster.
J. Nut. 38: No. 2, 131-153, 1949.

156. Scudi, J.V., Unna, K. and Antopol, W.
A study of the urinary excretion of vitamin B6 by a colorimetric method.
J. Biol. Chem. 135: 371-376, 1940.

157. Scudi, J.V.
On the colorimetric determination of vitamin B6.
J. Biol. Chem. 139: 707-720, 1941.

158. Sebrell, W.H. and Onstoff, R.H.
Riboflavin deficiency in dogs.
U.S. Pub. Health Reports 53: 83, 1938.

159. Seeler, A.O. and Silber, R.H.
Studies on the prolonged maintenance of adult dogs on purified diets.
J. Nut. 30: 2, 1945.

160. Shive, W., Ravel, J.M. and Eakin, R.E.
On interelationship of thymidine and vitamin B12.
J. Am. Chem. Soc. 70: 2614, 1948.

161. Shorb, M.S.
Unidentified growth factors for lactobacillus lactis in refined liver extract.
J. of Biochem. 169: 455, 1947.

162. Shorb, M.L.
Activity of vitamin B12 for the growth of lactobacillus lactis.
Science 107: 397, 1948.

163. Silbernagel, W.M. and Burt, O.P.
Pyridoxine in nausea and vomiting of pregnancy.
Am. J. Obst. & Gynec. 52: 173, 1946.

164. Smith, E.L.
Purification of anti-pernicious anemia factors from liver.
Nature 161: 638, 1948.

165. Smith, E.L.
Presence of cobalt in the A.P.A. factor.
Nature 162: 144, 1948.

166. Smith, S.G., Curry, R. and Hawfield, H.
Pyridoxine deficiency: anemia in dogs.
Science 98: 520-522, 1943.

167. Snell, E.E., Guirard, B.M. and Williams, R.J.
Occurrence in natural products of a physiologically active metabolite of pyridoxine.
J. Biol. Chem. 143: 519, 1942.

168. Snell, E.E.
The vitamin activities of pyridoxal and pyridoxamine.
J. Biol. Chem. 154: 133, 1944.

169. Snell, E.E.
Evidence for the occurrence of pyridoxamine and pyridoxal in natural products.
J. Biol. Chem. 157: 491, 1945.

170. Spies, T.D., Bean, W.B. and Ashe, W.F.
A note on the use of vitamin B6 in human nutrition.
J.A.M.A. 112: 2414, 1939.

171. Spies, T.D., Ladisch, R.K., and Bean, W.B.
Vitamin B6 deficiency in human beings.
J.A.M.A. 115: 1940.

172. Spies, T.D.
Observations on the antianemic properties of vitamin B12.
J. South. Med. 522: 1948.

173. Stern, J.R., Taylor, M.W. and Russell, W.S.
Relation of vitamin B12 to liver basophilia.
Proc. Soc. Exp. Biol. N.Y. 70: 551, 1949.

174. Stiller, E.T., Keresytery, J.C. and Stephens, J.R.
The structure of vitamin B6.
Am. Chem. Soc. J. 61: 1237, 1939.

175. Stoerk, H.C. and Zucker, T.F.
Nutritional effects on the development and atrophy of the thymus.
Proc. Soc. Exp. Biol. & Med. 56: 151, 1944.

176. Stoerk, H.C. and Eisen, H.N.
Supression of circulating antibodies in pyridoxine deficiency.
Proc. Soc. Exp. Biol. & Med. 62: 88, 1946.

177. Stoerk, H.C.
Effects of calcium deficiency and pyridoxine deficiency on thymis atrophy (accidental involution)
Proc. Soc. Exp. Biol. & Med. 62: 90-96, 1946.

178. Stoerk, H.C.
The regression of lymphosarcoma implants in pyridoxine deficient mice.
J. Biol. Chem. 171: 437, 1947.

179. Stone, R.E. and Spies, T.D.
The effect of liver extract and vitamin B12 on the mucous membrane lesions of macrocytic anemia.
J. Lab. & Clin. Med. 33: 1019, 1948.

180. Street, H.R.
Production of canine blacktongue on purified diets.
Proc. Soc. Exp. Biol. & Med. 36: 602-603,1937.

181. Street, H.R., Cowgill, G.R.
The cure of canine blacktongue with nicotinic acid.
Proc. Soc. Exp. Biol. & Chem. 37, 547, 1937.

182. Street, H.R. and Cowgill, G.R.
Acute riboflavin deficiency in the dog.
Am. J. Physiol. 125: 323, 1939.

183. Street, H.R., Cowgill, G.R. and Zimmerman, H.M.
Some observations of vitamin B6 deficiency in the dog.
J. Nut. 21: No. 3, 1941.

184. Sunde, M.L., Cravens, W.H., Elvehjem, C.A. and Halpin, J.G.
An unidentified factor required by chicks fed practical rations.
Poultry Sci. 29: 204-207, 1950.

185. Sure, B.
The present status of vitamin B2 (G)
J.A.M.A. 99: 26, 1932.

186. Thomas, J.E.
A simplified procedure for preparing an improved pavlov pouch.
Proc. Soc. Exp. Biol. & Med. 50: 58, 1942.

187. Umbreit, W.W., Bellamy, W.D., and Gunsalus, J.C.
The function of pyridoxine derivatives: a comparison of natural and synthetic codecarboxylose.
Arch. Biochem. 7: 185, 1945.

188. Umbreit, W.W., O'Kane, D.J., and Gunsalus, J.C.
Mechanism of pyridoxal phosphate function in bacterial transamination.
J. Bact. 576: 1946.

189. Umbreit, W.W. and Waddell, J.W.
Mode of action of desoxypyridoxine.
Proc. Soc. Exp. Biol. & Med. 70: 293, 1949.

190. Unna, K. and Antopol, W.
Toxicity of vitamin B6.
Proc. Exp. Biol. & Med. 43: 116-118, 1940.

191. Vineberg, A.M. and Babkin, B.P.
Histamine and pilocarpine in relation to gastric secretion.
Am. J. of Physiol. 97: 69, 1931.

192. Voegtlin, Carl and Lake, G.C.
Experimental mammalian polyneuritis produced by a deficient diet.
Am. J. Physiol. 47: 558-589, 1918-1919.

193. Voegtlin, C. and Myers, C.N.
A comparison of the influence of secretion and the antineuritic vitamin on pancreatic secretion and bile flow.
J. Pharmacol. 13: 301, 1919.

194. Voit, K. and Arnold, R.
Influence of vitamin B on acidity of stomach.
Klin. Wchnschr. 18: 98-99, 1939.

195. Waisman, H.A. and McCall, K.B.
A study of thiamine deficiency in the monkey.
Arch. Biochem 4: 260, 1944.

196. Waugh, T.R. & Waugh T., Jr.
J. Clin. Invest. In Press.
The measurement of the diameter of red blood cells.

197. Webster, D.R. and Armour, J.C.
Effect of vitamin deficiency on gastric secretion.
Proc. Soc. Exp. Biol. & Med. 30: 1297-1300, 1933.

198. Weinstein, B.B., Mitchell, G.J. and Sustendal, G.F.
Clinical experiences with pyridoxine hydrochloride in treatment of nausea and vomiting of pregnancy.
Am. J. Obst. & Gynec. 46: 283-285, 1943.

199. West, R.
Activity of B12 in addisonian P.A.
Science 107: 398, 1948.

200. Wetyel, N.C., Fargo, W.C., Smith, J.H. and Helikson, J.
Growth failure in school children as associated with vitamin B12 deficiency response to oral therapy.
Science 110: 651, 1949.

201. Wiardi, P.W.
Crystalline vitamin B6 (adermin)
Nature, London 142: 1158, 1938.

202. Wintrobe, N.N., and others.
Pyridoxine deficiency in swine with particular reference to anemia, epileptiform convulsions and fatty liver.
Bull. Johns Hopkins Hosp. 72: 1-25, 1943.

203. Wood, B., Splatt, B. and Maxwell, I.
Effect of administration of vitamin B, upon gastric secretion and motor activity.
M.J. Australia 2: 263-268, 1942.

204. Wright, C.S., Samitz, M. H. and Brown, H.
Vitamin B6 (pyridoxine) in dermatology.
Arch. Dermat. & Syph. 47: 651-653, 1943.

205. Wright, L.D., Skeggs, H.R. and Huff, J.W.
The ability of thymidine to replace vitamin B12 as a growth factor for certain lactobacillus.
J.B.S. 175: 475, 1948.