A STUDY OF THE INTERACTIONS OF VITAMIN B_{12} AND DESOXYCORTICOSTERONE ACETATE IN UNILATERALLY NEPHRECTOMIZED RATS

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By

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INTRODUCTION

In a recent review of the relationship of nutrition and stress Goodhart and Jolliffe (1952) stated, "it can be said without fear of contradiction that optimal nutrition with vitamins and other necessary nutrients helps to maintain the organism's resistance to stress at its maximum". Ascorbic acid and the B vitamins, especially pantothenic acid and vitamin B_{12}, were mentioned by these authors as being of great importance in protecting the organism against stress.

Many of the metabolic diseases increase the dietary requirement of the afflicted organism. Stresses such as fever, drugs, toxins, pregnancy, lactation, hyperthyroidism, hyperfunction of the adrenal cortex and related conditions may increase the requirement for certain dietary elements. In hyperthyroidism, diabetus insipidus, fever, cardio-renal disease and similar conditions, increases in basal metabolic rate accompany the increased requirement for many substances. This indicates that these changes in requirement are brought about through some alteration in the rate of certain metabolic processes. Strenuous physical activity, aside from increasing the caloric needs of the individual, also elevate the requirement for vitamins of the B complex. When the role of the B vitamins in carbohydrate metabolism is considered, it seems within the realm of possibility that such a change should occur.

Various hormone imbalances often result in an altered vitamin requirement. Hyperthyroidism in both man and experimental animals is accompanied by a need for greater quantities of vitamins, including vitamin B_{12}. The administration of large doses of estrogens (Meites and Shay 1951) and cortisone (Meites 1952) produce elevated needs for vitamin B_{12}.

Although much interest has been shown in the vitamin requirements as they are altered by stresses such as endocrine hyperfunction, many studies have been conducted in which the effects of the vitamins on endocrine function have also been studied. Morgan (1951) has reviewed this relationship as it pertains to the adrenal cortex. The changes in form and function of the arenal cortex in pantothenic acid deficiency indicate a depletion of the hormone-forming capacity of this gland in the absence of this particular vitamin. There is no evidence at present to indicate whether the impaired metabolism seen in this condition is a result of a primary effect on the adrenal or is secondary to an effect produced in the altered metabolic processes of the tissues.

The interrelationship of hormones and vitamins is an interesting one. The recent use of desoxycorticosterone acetate (DCA) in producing pathological lesions and the questions regarding the etiology of this pathology led to the undertaking of this problem. Since increased levels of vitamin B_{12} will protect the animal from thyrotoxicosis

and will prevent the growth depressions seen when large quantities of cortisone or estrogen are given, it seemed logical to determine whether or not vitamin B_{12} would exert a protective influence against DCA pathogenesis.

REVIEW OF DESOXYCORTICOSTERONE

Desoxycorticosterone was first synthesized by Steiger and Reichstein (1937) and was later found in very minute quantities in adrenal cortical extract by Reichstein and von Euw (1938). There is still some question however, whether desoxycorticosterone is a true adrenal cortical hormone (Hartman and Brownell, 1949). Because of its ease of synthesis and relative abundance, desoxycorticosterone has been studied in great detail. The results of these studies have shown this 11-desoxy steroid to have such important biological qualities as partial replacement for the adrenal cortex, as a powerful regulator of salt and water metabolism and as a factor which can produce hypertension and many types of pathology.

Replacement for the Adrenal Cortex. Remington, Parkins, Swingle and Drill (1941) were able to maintain adrenalectomized dogs on DCA (desoxycorticosterone acetate) for long periods of time. Hartman and Brownell (1949) maintained an adrenalectomized dog on DCA for the greater part of four years. However, there were times during this period when it was necessary to administer whole adrenal cortical extract because of a loss of appetite and the appearance of other cortical insufficiency symptoms. Thorn and Firor (1940) have used DCA successfully in the clinical treatment of

Addisonian patients. Although DCA will suffice to maintain adrenalectomized animals, and has been used successfully in the treatment of Addisonians, the indications are that it is not a complete replacement for the adrenal cortex.

Britton and Cline (1941) have compared the effects of whole adrenal cortical extract and DCA in the treatment of adrenalectomized cats and rats, and have found the whole extract to be superior therapy. The animals in these experiments which were maintained on whole cortical extract maintained higher blood sugar levels and liver glycogen contents than the animals receiving DCA. These findings led the authors to postulate that DCA probably has no direct effect on carbohydrate metabolism. The work of Ingle and Thorn (1941) in which they compared the diabetogenic properties of cortisone and DCA, also indicate that DCA is practically inert with respect to carbohydrate metabolism. Ingle (1941) has also shown that DCA is relatively inactive in its effect on the work performance of adrenalectomized rats.

Electrolyte and Water Metabolism. Although little or no carbohydrate activity can be attributed to DCA, this steriod has been shown to be a very effective factor in salt and water metabolism. Electrolyte and water metabolism are so intimately related and interdependent that it is extremely difficult to discuss them separately or devise experiments which will eliminate one of these factors as a variable. For

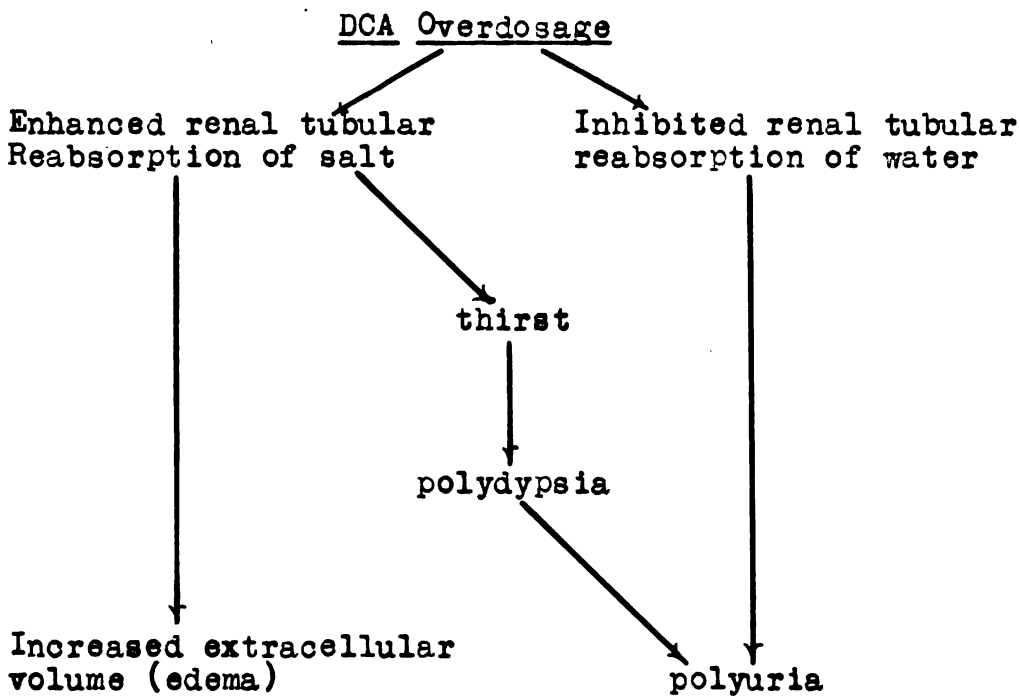
this reason this discussion will consider them simultaneously.

Ragan, Ferrebee, Phyfe, Atchley and Loeb (1940) conducted experiments in which they studied the effect of DCA in a normal female dog. Prior to the administration of DCA this dog drank 400 ml. of water and excreted an equal volume of urine per day of specific gravity of 1.045. After six days of DCA administration the dog was drinking 1000 ml. of water per day and excreting 1000 ml. of urine of specific gravity 1.025. This polydypsia was further exaggerated by the administration of sodium chloride in the drinking water. There was no evidence of excessive fluid retention in these animals since the water consumption paralleled the fluid loss by excretion. Also there was no significant gain in weight in any of the animals. The treatment of these animals with DCA was accompanied by an elevation of serum sodium and a decline in the level of serum potassium. It is also of interest to note that the serum protein concentration and blood glucose levels were not effected by this experimental regime. Kuhlman, Ragan, Ferrebee, Atchley and Loeb (1939) reported similar findings in other dogs.

Thorn, Howard and Emerson (1939) noted in Addisonians that overdosages of DCA would produce edema if sodium chloride was included in the therapy. Ferrebee, Ragan, Atchley and Loeb (1939) reported similar findings. Although it is not

well understood why DCA should produce an edema in some cases and a diabetes insipidus-like syndrome in others, it is generally believed that the nature of the diet, the quantities of DCA given and the duration of the DCA administration are all important factors.

Gaunt and Birnie (1951) have recently reviewed a portion of the literature dealing with the hormones and salt and water metabolism. These authors discuss some of the physiological factors which may effect the manifestation of DCA overdosage. The conditions resulting from the administration of large quantities of DCA is best summarized by a diagram taken from Gaunt's review:



Desoxycorticosterone in Hypertension. Hartman and Brownell (1949) reported that Loeb, Atchley, Ferrebee and Ragan (1939) were the first investigators to note the effect of a DCA overdosage on the blood pressure of a human. Rodbard and Freed (1942) administered DCA to normotensive, spontaneously hypertensive and Goldblatt hypertensive dogs and produced elevated blood pressures in each of these cases. Grollman, Harrison, Williams (1940) and Friedman, Friedman, and Polley (1948) have produced hypertension in rats also by administering DCA.

Swingle, Parkins and Remington (1941) studied the circulatory dynamics of normal and adrenalectomized dogs following DCA treatment. The administration of DCA was followed by a plasma volume increase and an elevation in blood pressure in both cases. However, the increased blood pressure could not be attributed to the increased plasma volume since no blood pressure elevation was seen when normal animals were transfused with normal serum. Ziegler, Anderson and McQuarrie (1944) have studied the electrolyte and water content of tissues from hypertensive animals and have found an increased level of water and sodium in these tissues.

It is beyond the scope of this literature review to survey the attempts which have been made to determine the etiology of DCA-induced hypertension. However, the sodium retaining property of DCA and the popular use of low sodium

diets by Perera and Blood (1947) and Kempner (1944) in treating hypertension indicates that this quality of DCA may be responsible for its role as a hypertension-producing substance.

Effects on Tissue and Organ Pathology. DCA has been shown to produce many characteristic changes in the various tissues and organs. Selye (1941) reported that in short term experiments in which rats were given DCA, an involution of the thymus, spleen and adrenal cortex resulted, accompanied by renal hypertrophy. The results of long term experiments revealed similar findings except that enlargement of the thymus and spleen occurred. Selye and Pentz (1943) in another experiment noted that cardiac and hepatic enlargement were also produced by DCA and that these effects and the renal hypertrophy were augmented by unilateral nephrectomy. Hall, Finerty, Hall and Hess (1951) likewise reported an adrenal cortical atrophy following treatment with DCA. However, since this atrophy was not accompanied by a reduction in cortical ascorbic acid, these authors postulated that the action of DCA could not be considered as a stress.

Selye, Mintzberg and Rowley (1945) studied the effects of various electrolyted upon DCA-induced pathology, and found sodium chloride to add greatly to DCA-induced pathogenesis. Selye, Stone, Timiras and Schaffenburg (1949) have shown that when DCA is administered to unilaterally nephrectomized

animals maintained on a normal diet and 1 percent saline, pathological lesions similar to those seen in man during hypertension often result. When unilaterally nephrectomized rats were kept on a regime of 6 mg. of DCA daily and 1 percent saline for 27 days, he found nephrosclerosis, periarteritis nodosa in the mesentric vessels, and degenerative changes and Aschoff bodies in the myocardium. Selye, Sylvester, Hall and Leblond (1944) also stated that they produced a hyperemia and swelling in the tarsal joint region of the hind feet of rats maintained under similar experimental conditions. At autopsy, signs of nephrosclerosis, periarteritis nodosa or rheumatic nodules in the hearts of most of these animals were apparent. It is of interest to note that all of these pathological developments were exaggerated by adrenalectomy or thyroidectomy. Timiras, Fairbault and Selye (1949) employing similar experimental procedures but using larger doses of DCA, produced severe brain lesions in rats. These lesions were characterized by an edema of the brain and a consequent volume increase in both cerebral hemispheres, a narrowing and sometimes obliteration of the sulci and a flattening of the gyri. They also noted histological evidence of periarteritis nodosa of the cerebral arterioles accompanied by a pronounced hyalinization of the vessel walls, venous stasis, congestion and multiple small thromboses. Degenerative changes in the nervous tissues were also reported.

Selye and Stone (1943) were able to elicit a nephrosclerosis, generalized tissue edema and cardiac dilatation similar to the cardiovascular changes seen in human hypertensives by simply placing chickens on a 2% saline drink. When DCA was administered, much smaller quantities of NaCl were required to produce these changes. The pathology of the kidneys of these animals were characterized by hypertrophy of the convoluted tubules, cloudy swelling and the development of hyaline casts. The glomeruli were enlarged and some of the glomerular capillaries showed hyalinization and proliferation in the region of the epithelial cells. It is of interest that progesterone produced similar results.

Other workers in trying to reproduce lesions similar to those reported by Selye, Sylvester, Hall and Leblond (1944) have met with varying degrees of success. Rosenberg, Woodbury and Sayers (1952) reported that from the large number of rats which they kept on experimental regimes similar to that utilized by Selye, not one animal developed any articular pathology. Harrison (1946) was likewise unable to produce any signs of arthritis in unilateral nephrectomized rats receiving DCA and saline. This author suggested that a direct or indirect infection may have been the cause of the experimentally produced arthritis reported by Selye, since it was reported that a large number of Selye's animals succumbed because of pneumonia. It was also suggested that low

air temperatues might have been a factor in the etiology of the nephrosclerosis and rheumatic cardiac lesions.

Darrow and Miller (1942), however, were able to demonstrate cardiac lesions in rats rollowing repeated injections of DCA. It was postulated that this effect was due primarily to the low content of potassium in cardiac muscle as a result of DCA administration. Guillemin and Fortier (1951) produced myocardial nodules, periarteritis nodosa and macroscropic signs of nephrosclerosis in unilaterally nephrectomized rats receiving saline and DCA. The kidneys of these animals showed glomerular hyalinosis, intense tubular enlargement, and a cellular vacuolization which was predominant in the proximal segment of the tubule. This pathology was greatly reduced and in some cases prevented by Phenergan, a compound known to alter membrane permeability. Since the sodium content of the diet is known to be critical in DCA-induced pathogenesis, and since sodium chloride alone will produce typical lesions in some species, these authors interpreted their results to indicate that the sodium chloride was responsible for the pathology, and that DCA only facilitated the accumulation of large quantities of sodium chloride in the tissue.

Rosenberg, Woodbury and Sayers (1952) have recently produced such pathology as focal glomerulitis, interstitial myocarditis and periarteritis nodosa in unilaterally nephrectomized rats receiving DCA and saline drink. These workers were

able to reduce or prevent this occurrence of lesions by administering cortisone of ACTH. These authors believe that the probable action of the adrenal cortical steriods in preventing collagen diseases is due to general inhibition of all inflammatory processes regardless of the causative agent. It is also stated that there is no reason to conclude that the collagen diseases of man and DCA-treated rats have common etiology simply because both respond to cortisone and ACTH therapy. Woodbury, Rosenberg and Sayers (1950) have likewise shown that simultaneous administration of ACTH and DCA results in an inhibition of the pathological changes normally induced by DCA. These authors postulate that the exogenous DCA inhibits the production of 11-oxygenated steriods by the adrenal and consequently lowers the resistance of the animals to stress.

Selye (1946) has still another explanation of DCA-induced pathogenesis which is perhaps broader and more inclusive than any of the hypotheses so far mentioned. This theory has been named the "general adaptation syndrome". Selye (1946) defined the "general adaptation syndrome" as the sum of all non-specific systemic reactions of the body which follow a long continued exposure to stress. It is postulated that the etiology of such diseases as hypertension, gastrointestinal ulcers, nephrosclerosis, nephritis, rheumatic fever and periarteritis nodosa may be a result of this

phenomenon. The general adaptation syndrome has three phases: (1) the alarm reaction, (2) a period of resistance and (3) a stage of exhaustion. The alarm reaction represents the response of an organism to any stimuli to which it is not adapted. Factors which may elicit such a response are infections, burns, hemorrhage, exposure to extreme temperatures, trauma, drugs, toxic substances, dietary deficiency, hormonal agents, etc. The period of stress is followed by a phase of resistance during which the animal has acquired adaptation to the alarming stimuli. This period is characterized by hyperactivity of the adrenal cortex. While it is postulated that many of the adrenal steriods serve to protect the tissues during this stage, it is also believed that the increase of some of the endogenous hormones may be the etiological factor in producing certain cardiovascular, renal and joint diseases.

A stage of exhaustion ensues when the organism is no longer capable of maintaining resistance. Functional changes are considered the principal manifestation during the course of the general adaptation syndrome. These include such factors as blood pressure changes, blood clotting time changes, absorption of various substances and metabolic changes involving enzymatic activity. The pathological changes seen include atrophy of the thyroid, gonads, lymph nodes, thymus and spleen, while the pancreas and adrenal cortex hypertrophy.

Characteristic morphological changes are also seen in the liver, kidney, lungs, heart and lens of the eye.

REVIEW OF VITAMIN B_{12}

Effects on Hemopoiesis. Rickes, Brink, Konisuzy, Wood and Folkers (1948a) of the Merck Research Laboratories first announced that a compound had been crystallized from liver extracts which was active in the treatment of Addisonian anemia. E. L. Smith (1948) conducted experiments during this same time and isolated from four tons of ox liver one gram of a red amorphous material which was likewise effective in treatment of pernicious anemia.

Shorb (1947) studied the microbiological requirement for these liver preparations and found that Lactobacillus lactis Dorner required a factor present in the liver extracts. It was also reported that the therapeutic value of the different factors paralleled the potency of the preparations as assayed by this particular strain of bacteria. The suggestion was made that these factors were identical. In a later publication Shorb (1948) stated that crystalline vitamin B_{12} was extremely active when measured by the microbiological assay, and his data also indicated that vitamin B_{12} was partially if not entirely responsible for the activity of the liver preparations in Lactobacillus lactis Dorner.

When vitamin B_{12} became available, many laboratory and clinical studies were conducted to determine its exact therapeutic and physiologic significance. Some of the very early clinical investigations were conducted by West (1948)

in which he elicited favorable responses in all of three pernicious anemia patients treated with vitamin B_{12}. In one patient who received 150 micrograms of vitamin B_{12} in a single intramuscular injection, the red blood cell count rose from 1.5 million to 3.4 million in twenty-three days. The other two patients received much smaller doses and showed a positive but less dramatic response.

The hematological effectiveness of vitamin B_{12} was soon established but a question remained regarding the difference between this substance and other known substances such as folic acid which were also known to stimulate erythropoiesis. Berk, Denny-Brown, Finland and Castle (1948) reported a case in which a victim of pernicious anemia had been treated with pteroylglutamic acid and had responded favorably hematologically but had developed the neurologic complications normally associated with this disease. The subsequent treatment of this patient with vitamin B_{12} resulted in an alleviation of this neurologic syndrome and led these authors to postulate that pteroylglutamic acid was not responsible for vitamin B_{12} activity. Chemical evidence by Rickes, Brink, Konisuzy, Wood and Folkers (1948b) indicated that vitamin B_{12} was a complex containing cobalt and phosphorus and therefore must be a distinct vitamin entirely different from any other known vitamin.

Effects on Growth and Protein Metabolism. Animal studies prior and subsequent to the discovery of vitamin B_{12} have contributed greatly to the understanding of the nature and biological significance of this substance. Hammond (1944) in an attempt to find a substance which would promote growth in chicks maintained on a vegetable diet, found cow manure, rumen contents and sardine fish-meal to be effective. Bird, Rubin and Groschke (1948) were successful in concentrating the growth factor present in cow manure.

Zucker and Zucker (1948) found a growth factor present in animal protein but absent in plant materials which they called "Zoopherin" and which later became known as the "animal protein factor". Nestler, Byerley, Ellis and Titus (1936) showed that the chick growth factor found in cow manure was evidently the same as the factor necessary to maintain the hatchability of eggs when hens were kept on vegetable diets.

Ott, Rickes and Wood (1948) demonstrated that vitamin B_{12} had growth activity in chicks maintained on the soybean protein diets used by Bird, Rubin, Whitson and Haynes (1946) in studying the "animal protein factor". Lillie, Denton and Bird (1948) compared vitamin B_{12}, purified cow manure growth factor and liver extract, using the chick growth as a criterion. Their data indicated that these factors are very similar if not identical.

McGinnis, Hsu and Graham (1948) studied the blood non-protein nitrogen and body growth in chicks maintained on a vegetable diet. When this diet was supplemented with vitamin-free casein or alpha protein, the animals receiving this diet exhibited an elevated blood non-protein nitrogen and a decreased growth rate. An alcoholic extract of liver corrected both of these factors. When the chicks were fed the basal ration supplemented with fish meal, blood non-protein nitrogen was lower and the growth rate higher than when the vegetable ration alone was fed. These authors concluded that an unidentified factor present in liver is necessary for normal protein metabolism.

Charkey, Wilgus, Patton and Gassner (1950) found that vitamin B_{12} depressed the blood level of non-protein nitrogen and at least seven amino acids when administered to vitamin B_{12}-deficient chicks. The increased growth rate and feed utilization efficiency accompanying this depression of circulating levels of amino acids, led these authors to postulate that vitamin B_{12} affects growth by enhancing the utilization of the circulating amino acids.

Rose and Schweigert (1952) determined the desoxyribonucleic acid and ribonucleic acid content of tissues from vitamin B_{12}-sufficient or deficient animals. Their data revealed that the livers of animals deficient in vitamin B_{12} contained less desoxyribonucleic acid and ribonucleic acid

on the basis of percentage of fresh weight, dry weight or total nitrogen than did the livers of the animals receiving vitamin B_{12}. The content of ribonucleic acid and desoxyribonucleic acid per cell was the same for the two experimental groups. These results indicate that the decreased rate of nucleic acid synthesis is accompanied by a decreased rate of cell division in the livers of the vitamin B_{12}-deficient rats.

It is apparent from the literature cited that most authors place great importance on vitamin B_{12} insofar as it effects protein metabolism. It is of interest to note, however, that Chow (1952) has shown that vitamin B_{12}-deficient animals have a low fat, high water but normal protein content and that these abnormalities can be corrected by the administration of vitamin B_{12}. This author and also Bosshardt, Paul and Borns (1950), McCollum and Chow (1950) and Rupp (1951) support the hypothesis that vitamin B_{12} is involved primarily in the utilization of carbohydrate and synthesis of fat from carbohydrate rather than in protein metabolism.

Interactions between Vitamin B_{12} and Hormones. Ershoff (1947) reported that liver counteracted the retardation of body growth and inhibition of ovarian development in immature rats fed toxic amounts of thyroid. This author interpreted these findings as indicating that normal body growth and gonadal development require a substance present in liver. The requirement for this substance is increased by feeding thyroid.

Betheil and Lardy (1949) also found vitamin B_{12} to be active in counteracting retardation of growth in thyroid-treated rats.

Ershoff (1949) conducted experiments in which he studied the relative effectiveness of vitamin B_{12} and liver in inhibiting thyrotoxicity in rats fed a casein-sucrose diet. The results revealed that liver was very active in promoting growth under these conditions while vitamin B_{12} had no effect at all. Robblee, Nichol, Cravens, Elvehjem, and Halpin (1948) demonstrated that induced hyperthyroidism increased the effective range of the chick assay for an unidentified growth factor present in fish solubles, whole liver powder, and other liver fractions.

Meites (1950a) found in rats that thyroid weight and rate of radioactive iodine uptake by the thyroid was not influenced by vitamin B_{12}. These same experiments revealed that vitamin B_{12} was capable of eliciting an increased body weight gain in hyperthyroid animals.

Nichol, Dietrich, Cravens and Elvehjem (1949) showed vitamin B_{12} to be very similar if not identical to factors assayed by Robblee, Nichol, Cravens, Elvehjem and Halpin (1948). It was shown that the inclusion of iodinated casein in the assay diet was beneficial. These authors state that the thyrotoxicity produced by feeding iodinated casein could be completely counteracted by orally or parenterally administered vitamin B_{12}.

Bolene, Ross and MacVicar (1950) found that greater quantities of vitamin B_{12} were needed to counteract the thyrotoxic effects of iodinated casein in female than in male rats. In both sexes greater responses were obtainable from liver than vitamin B_{12}. Frost, Fricke and Spruth (1949) reported that the sex of the animal had no effect on the response of the hyperthyroid rat to vitamin B_{12}.

Watts, Ross and Whitehair and MacVicar (1951) studied the growth response of normal and castrate hyperthyroid rats to vitamin B_{12} and liver. Both substances were found to be more effective than liver. These authors postulated that the male sex hormone may act synergistically with a factor present in the liver which is not vitamin B_{12}. It is also possible that the female sex hormones act antagonistically with such a factor.

Meites and Shay (1951) have shown that vitamin B_{12} will partially inhibit the growth retarding effects of diethylstilbestrol and thyroprotein. While vitamin B_{12} increased the food intake of these animals, it did not reduce the effectiveness of these two hormones in depressing testes weight or the tendency of diethylstilbestrol to increase the weight of the seminal vesicle. It was suggested that one reason for the effectiveness of vitamin B_{12} in these experiments was its ability to increase food consumption.

Monroe and Turner (1949) presented data indicating that vitamin B_{12} may increase the catabolism of thyroxine, which could also explain the observations of Meites and Shay (1951). This seems unlikely, however, since the latter workers found that vitamin B_{12} has no effect on normal thyroid function. Meites (1951b) and Libby and Meites (1952) have shown that vitamin B_{12} counteracts the growth-inhibitory effects of thiouracil in rats and chicks. Vitamin B_{12} was found not to alter thyroid function in these species.

Wells and Kendall (1940) demonstrated that growth in immature rats is inhibited by large doses of cortisone. Ingle, Higgins and Kendall (1938) showed that a negative nitrogen balance can be induced by cortin treatment. Meites (1952) found that hair growth is greatly retarded when animals are given large doses of cortisone. Since all of these disturbances are largely protein in nature, and may well be due to an increase in protein catabolism it seemed logical that the effects of vitamin B_{12} upon cortisone overdosage should be studied. Meites (1952) conducted such a study and found that vitamin B_{12} or aureomycin partially or completely prevented the inhibition of body, hair and thymus growth which results from cortisone treatment. While either substance was effective in counteracting these effects of cortisone, a combination of the two was even more effective. Both vitamin B_{12} and aureomycin elicited an increase in food

consumption and increased the efficiency of converting food into body weight gains. Although it is not well understood how vitamin B_{12} or aureomycin function in producing these changes, the enhancement of food intake and possibly a specific effect on protein metabolism may account for their beneficial effects in hormone-treated animals.

The two experiments which are described in this thesis were carried out to determine the effect of vitamin B_{12} on the cardio-renal manifestations of a DCA overdosage. These experiments were also designed to determine to what extent the saline consumption might influence the actions of DCA and vitamin B_{12} on kidney and heart, and to ascertain if the action of vitamin B_{12} was secondary to an increase in food consumption.

Experiment I. Relation Between Desoxycorticosterone Acetate and Vitamin B_{12} in Unilaterally Nephrectomized Rats.

Procedure:

Forty-seven male rats of the Carworth strain, weighing 51 ± 12 grams each, were used in this experiment. These rats were maintained on the following vitamin B_{12}-deficient diet* for thirty-two days.

Yellow corn meal	35%
Ground wheat	25%
Linseed oil meal	10%
Soybean oil meal	20%
Alfalfa leaf meal	6%
Brewers yeast	3%
Sodium chloride	1%

Immediately following this thirty-two day period the rats were unilaterally nephrectomized and divided into eight groups. Each of these groups was given 1 percent saline to drink and the above mentioned diet supplemented with various quantities of crystalline vitamin B_{12}**. Groups number 2, 5, 6, 7 and 8 received subcutaneous injections of an aqueous suspension of desoxycorticosterone acetate*** twice daily,

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* This diet is a modification of a diet used successfully by Dr. C. A. Hoppert of the Chemistry Dept. at Michigan State College, East Lansing, Michigan.

** Crystalline vitamin B_{12} was kindly furnished by Merck and Company, Inc., Rahway, New Jersey.

*** Desoxycorticosterone acetate was kindly supplied by Dr. W. J. Haines of the Upjohn Company, Kalamazoo, Michigan.

totaling 3 mg. per rat per day. The experimental treatments for each group are summarized in Table I.

Table I

Treatments of Rats in Experiment I.

Group No.	No. of Rats per Group	DCA (mg. per Rat per Day)	Vit. B_{12} (ug. per kg. of diet)
1	10	0	0
2	9	3	0
3	5	0	50
4	5	0	400
5	4	3	50
6	5	3	100
7	5	3	200
8	4	3	400

Each group of rats was kept in a separate cage and the largest groups in two cages, inorder that their daily food and saline consumption could be measured. The rats from each group were weighed every four days to determine their growth rate. At the end of twenty days, all of the animals were sacrificed by severing the spinal cord. These rats were weighed and autopsied. The heart, kidney, adrenals and thymus were removed and weighed on a Roller-Smith balance. The heart, kidney, thymus and duodenum

from two animals of each group were fixed, sectioned, and stained for microscopic examination. Each animal was also examined at autopsy for any gross pathology which might exist.

The data obtained from this experiment and the subsequent experiment were analyzed statistically using the following formula:

$$S.\ E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

RESULTS:

The results of this experiment are summarized in Tables II and III. Table II shows the average body weight gain, saline intake, food consumption and food utilization efficiency of the respective groups. The average kidney, heart, thymus, and adrenal weights are shown in Table III.

It is apparent from the data of Table II that the animals receiving vitamin B_{12} (groups 3-8) grew faster, consumed more food and utilized their food more efficiently than did those rats (groups 1 and 2) receiving a diet which was deficient in this vitamin. These results also indicate that DCA greatly increased the saline consumption, especially in those rats (groups 5-8) which were receiving a diet supplemented with vitamin B_{12}. The percentage increase in saline consumption was considerably greater than the increase in food intake. The DCA-induced polydypsia (group 2) was markedly inhibited by a vitamin B_{12} deficiency.

It can be seen from Table III that DCA (groups 2, 5-8) produced a renal and cardiac hypertrophy. This effect was less pronounced in the absence of vitamin B_{12} (group 2). DCA produced little or no adrenal atrophy (group 2) and had no significant effect on the weight of the thymus gland.

The results of the histological study of the kidney, heart, thymus and duodenum revealed that vitamin B_{12} had no effect on either the thymus or duodenum, but the renal and myocardial hypertrophy seen in the DCA-treated animals was partially inhibited by a vitamin B_{12} deficiency. DCA had no demonstrable effect on the thymus or duodenum, except that some evidence of arteriosclerosis in the arterioles of the latter was noted. DCA produced a myocardial hypertrophy and an arteriosclerosis in the small coronary blood vessels. Nephrosclerosis and hyaline casts in the renal tubules were also seen following DCA treatment. Cloudy swelling was characteristic of the kidneys of all the animals receiving DCA. All of these pathological findings have previously been reported by other authors using similar procedures (Selye and Stone, 1943; Rosenberg, Woodbury and Sayers, 1952).

EFFECT OF VITAMIN B_{12} AND/OR DESOXYCORTICOSTERONE ACETATE ON GROWTH, FOOD AND SALINE CONSUMPTION OF UNILATERALLY NEPHRECTOMIZED RATS

Group no.	Treatment	Avg. body wt. initial gm.	Avg. body wt. final gm.	Avg. saline intake/rat/day ml.	Avg. food intake/rat/day gm.	Avg. wt. gain/100 gm. of diet consumed gm.
1 (10)*	Controls	121 ± 5	152 ± 6	35	10.1	15.3
2 (9)	3mg. DCA/day	123 ± 5	150 ± 6	50	9.4	14.5
3 (5)	50 ug. vit. B_{12}/kg. diet	118 ± 8	186 ± 3	36	14.0	24.3
4 (5)	400 ug. vit. B_{12}/kg. diet	136 ± 17	197 ± 17	45	14.1	21.7
5 (4)	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet	133 ± 13	190 ± 13	100	13.5	26.6
6 (4)	3mg. DCA/day 100 ug. vit. B_{12}/kg. diet	111 ± 9	168 ± 7	78	11.6	25.0
7 (4)	3mg. DCA/day 200 ug. vit. B_{12}/kg. diet	127 ± 6	194 ± 11	90	13.0	26.2
8 (4)	3mg. DCA/day 400 ug. vit. B_{12}/kg. diet	142 ± 17	194 ± 13	83	13.2	19.8

* Values in parenthesis indicate the number of rats per group.
** Standard error of mean.

EFFECT OF VITAMIN B_{12} AND/OR DESOXYCORTICOSTERONE ACETATE ON THE ORGAN WEIGHTS OF UNILATERALLY NEPHRECTOMIZED RATS

Group no.	Treatment	Kidney wt. mg.	Heart wt. mg.	Thymus wt. mg.	Adrenal wt. mg.
1	Controls	*1,085 ± 46***	661 ± 35	283 ± 28	23 ± 2
		** 717 ± 28	436 ± 22	186	13
2	3mg. DCA/day	1,353 ± 49	696 ± 27	295 ± 15	21 ± 1
		910 ± 31	468 ± 19	197	14
3	50 ug. vit. B_{12}/kg. diet	1,205 ± 77	719 ± 26	338 ± 21	27 ± 2
		647 ± 11	393 ± 34	182	15
4	400 ug. vit. B_{12}/kg. diet	1,123 ± 75	784 ± 59	346 ± 49	29 ± 2
		586 ± 47	409 ± 38	176	15
5	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet	2,050 ± 234	1,033 ± 59	350 ± 23	25 ± 2
		1,073 ± 62	549 ± 37	184	13
6	3mg. DCA/day 100 ug. vit. B_{12}/kg. diet	1,842 ± 67	929 ± 59	300 ± 39	23 ± 4
		1,100 ± 46	553 ± 29	179	14
7	3mg. DCA/day 200 ug. vit. B_{12}/kg. diet	2,153 ± 217	995 ± 86	393 ± 36	26 ± 1
		1,110 ± 78	510 ± 19	203	13
8	3mg. DCA/day 400 ug. vit. B_{12}/kg. diet	2,069 ± 241	1,024 ± 81	360 ± 65	35 ± 5
		1,076 ± 30	536 ± 42	186	18

*Upper values represent actual organ weights.
**Lower values represent organ weights in mg. per 100 g. body weight.
***Standard error of mean.

Experiment II. Relation Between Desoxycorticosterone Acetate and Vitamin B_{12} in Unilaterally Nephrectomized Rats.

Procedure:

A second experiment was conducted to determine the reproducibility of the results of experiment I and to further examine some factors not demonstrable in the data of the first experiment.

Forty-five male rats of the Carworth strain, weighing 50 ± 10 grams each, were used in this experiment. These rats were maintained on a vitamin B_{12}-deficient diet identical with that used in the first experiment, for twenty-eight days. At this time the rats were unilaterally nephrectomized and divided into six experimental groups. Each group received the above mentioned diet with or without vitamin B_{12}. Each group was fed ad libitum except group 4 which was pair-fed to group 1. All groups were given 1 percent saline to drink except group 3 which received tap water. Each animal in groups number 2, 3, 4, 5 and 6 received subcutaneous injections of an aqueous suspension of desoxycorticosterone acetate twice daily, totaling 3 mg. per rat per day. The various treatments of the rats are summarized in Table IV.

TABLE IV

Treatments of Rats in Experiment II

Group No.	No. of rats per group	DCA (mg. per rat per day)	Drink	Vit. B_{12} (ug. per kg. diet)
1	8	0	saline	0
2	7	3	saline	0
3	8	3	tap water	0
4	8	3	saline	50
5	7	3	saline	50
6	7	3	saline	20

Each group of rats was kept in a separate cage and their daily consumption of food and drink was noted. These animals were also weighed every four days to determine their growth rate. The animals were maintained under these experimental conditions for twenty days. During the last twenty-four hours of this twenty-day experimental period, four rats from each group were placed in separate metabolism cages and the volume of urine excreted was measured.

At the end of the twentieth day all of the animals were weighed and then sacrificed by severing their spinal cords. The kidney, heart, adrenals and thymus were removed from each rat and weighed on a Roller-Smith balance. The kidney, heart and a sample of muscle and skin from each animal was dried

at 100° C. to constant weight, and the water content was then determined.

RESULTS:

Tables V, VI and VII summarize the results of this experiment. Table V shows the average body weight gain, food and water consumption and food utilization efficiency of each of the six groups. The average kidney, heart, thymus and adrenal weights are summarized in Table VI. Table VII shows the average water content of the kidney, heart, muscle and skin of the rats of each group.

It is apparent from the data of Table V that vitamin B_{12} (groups 4-6) increased growth rate, food consumption and food utilization efficiency above that of the controls (group 2). These increases, however, were not as marked as in the first experiment. It can be seen by comparing group 2 to group 1 that DCA slightly reduced the growth rate and food utilization efficiency, but increased saline consumption. The substitution of tap water (group 3) for saline drink slightly increased food utilization efficiency but greatly decreased water intake. Pair-feeding group 4 with group 1 decreased food utilization-efficiency and growth rate in comparison to groups 5 and 6 which were allowed to eat ad libitum. It can be seen that the groups receiving vitamin B_{12} and DCA (groups 4-6) also consumed much greater quantities of saline than did group 2, which received DCA but no vitamin

B_{12}. Groups 2 and 4 ate similar quantities of food but group 4 drank much greater volumes of saline, indicating that vitamin B_{12} increased saline consumption independent of its effects on food consumption. Comparisons of groups 2 and 4 also indicate that vitamin B_{12} enhances the effects of DCA in increasing saline consumption.

The data in Table VI indicate that DCA caused a renal and cardiac hypertrophy, a reduction in adrenal size and no change in thymus weight. The rats (group 3) given tap water instead of saline (group2) exhibited smaller hearts, kidneys and adrenals than did the animals of the latter group. The rats in group 4 whose food intake was restricted, developed less severe cardiac and renal hypertrophies than were seen in the animals of group 5 which were treated similarly but allowed to eat ad libitum. The animals (groups 5 and 6) which received both vitamin B_{12} and DCA exhibited a more pronounced cardiac hypertrophy than did the rats which received DCA but no vitamin B_{12}. Kidney weight, however, was not noticeably altered by including vitamin B_{12} in the diet of DCA treated rats.

The data in Table VII show that the water content of the kidney, heart, muscle and skin did not change as a result of DCA administration, vitamin B_{12} adequacy or the inclusion of salt in the drinking water. The only possible exception to this is the slightly increased water content of the kidney as a result of DCA administration. This is not conclusively

demonstrated since group 6, which also received DCA, showed a low renal water content.

TABLE 1

EFFECT OF VITAMIN B_{12} AND /OR DESOXYCORTICOSTERONE ACETATE ON GROWTH, FOOD AND SALINE CONSUMPTION OF UNILATERALLY NEPHRECTOMIZED RATS

Group no.	Treatment	Avg. body wt. initial gm.	Avg. body wt. final gm.	Avg. water intake/rat/day ml.	Avg. food intake/rat/day gm.	Avg. wt. gain/100 gm. of diet consumed gm.
1 (8)*	Controls	109 ± 3**	162 ± 8	45	14.9	17.8
2 (7)	3mg. DCA/day	116 ± 5	159 ± 6	67	13.7	15.8
3 (8)	3mg. DCA/day tap water drink	109 ± 6	163 ± 7	24	13.4	19.8
4 (8)	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet pair fed with group no. 1	110 ± 5	161 ± 5	93	14.9	17.2
5 (7)	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet	112 ± 5	177 ± 7	92	16.8	19.3
6 (7)	3mg. DCA/day 20 ug. vit. B_{12}/kg. diet	107 ± 3	174 ± 2	89	18.0	18.7

* Values in parenthesis indicate the number of rats per group.
** Standard error of mean.

TABLE VI

EFFECT OF VITAMIN B_{12} AND /OR DESOXYCORTICOSTERONE ACETATE ON THE ORGAN WEIGHTS OF UNILATERALLY NEPHRECTOMIZED RATS

Group no.	Treatment	Kidney wt. mg.	Heart wt. mg.	Thymus wt. mg.	Adrenal wt. mg.
1	Controls	*1,101 ± 56***	660 ± 40	289 ± 29	25 ± 2
		**686 ± 22	408 ± 11	197	15
2	3mg. DCA/day	1,729 ± 92	791 ± 53	328 ± 16	20 ± 3
		1,087 ± 53	433 ± 71	206	13
3	3mg. DCA/day tap water drink	1,340 ± 91	741 ± 35	294 ± 16	17 ± 1
		826 ± 49	456 ± 11	181	10
4	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet pair fed to group no. 1	1,591 ± 92	810 ± 32	295 ± 33	20 ± 2
		987 ± 55	502 ± 11	183	12
5	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet	1,980 ± 278	1,019 ± 55	345 ± 22	21 ± 3
		1,103 ± 39	576 ± 21	195	12
6	3mg. DCA/day 20 ug. vit. B_{12}/kg. diet	1,586 ± 114	930 ± 29	394 ± 34	19 ± 2
		916 ± 70	534 ± 11	221	11

* Upper values represent actual organ weights.
** Lower values represent organ weights in mg. per 100 gm. body weight.
*** Standard error of mean.

TABLE VII

EFFECT OF VITAMIN B_{12} AND/OR DESOXYCORTICOSTERONE ACETATE ON THE WATER CONTENT OF TISSUES OF UNILATERALLY NEPHRECTOMIZED RATS

Group no.	Treatment	Water content (ml./kg. of tissue)			
		Kidney	Heart	Muscle	Skin
1	Controls	736.9	797.4	775.6	641.8
2	3mg. DCA/day	790.4	801.3	773.3	646.9
3	3mg. DCA/day tap water drink	789.0	802.0	774.3	647.2
4	3mg. DCA/day 50 ug. vit. B12/kg. diet pair fed with group no. 1	796.3	802.7	769.0	636.5
5	3mg. DCA/day 50 ug. vit. B_{12}/kg. diet	775.3	796.9	777.2	645.1
6	3mg. DCA/day 20 ug. vit. B_{12}/kg. diet	745.2	806.0	773.9	666.5

DISCUSSION

These experiments were designed primarily to study the interactions between vitamin B_{12} and desoxycorticosterone in unilaterally nephrectomized rats. It is important in interpreting the data obtained from these experiments that the reader keep in mind that all of the animals, with the exception of those in group 3 of experiment II, were given 1 percent saline to drink. This increased sodium consumption undoubtedly altered the animals' salt and water metabolism. Sodium has also been shown to render unilaterally nephrectomized rats hypersensitive to DCA toxicity (Selye, Mintzberg and Rowley 1945).

The results of both experiments indicate that vitamin B_{12} increased the growth rate of all the groups receiving this substance. This was much more noticeable in the first than in the second experiment. It is possible that the rats in the second experiment had greater initial body reserves of vitamin B_{12} and hence were stimulated less when this vitamin was added to the diet. The increase in growth rate is due in part at least to the increased food consumption which resulted when vitamin B_{12} was included in the diet. When a group of animals (group 4 of experiment II) maintained on a vitamin B_{12}-sufficient diet was pair fed to a group receiving no vitamin B_{12}, the two groups showed identical

growth rates. This would indicate that vitamin B_{12} was ineffective in stimulating growth in the absence of increased food intake, an observation in agreement with the generally reported actions of vitamin B_{12} in pigs and fowl. The data from these two pair-fed groups is not absolutely conclusive, however, since the group receiving vitamin B_{12} also received desoxycorticosterone.

A second factor contributing to the increase in growth rate of the animals receiving vitamin B_{12} is the increased food-utilization efficiency. All of the groups receiving vitamin B_{12} exhibited greater body weight gains per one hundred grams of diet consumed than did any of the groups not receiving this essential dietary factory. This seems to be a very likely possibility when the role of vitamin B_{12} in protein synthesis is considered (Rose and Schweigert, 1952; Charkey, Wilgus, Patton and Gassner, 1950). Chow (1952) and Rupp (1951), however, have reported findings which indicate that vitamin B_{12} may be even more effective in influencing fat and carbohydrate metabolism than in stimulating protein synthesis. The increased food-utilization efficiency accompanying vitamin B_{12} administration can perhaps be partially attributed to the facilitation of certain transmethylation reactions in which this vitamin is known to act as a co-enzyme (Schaffer, Knowles and Salmon, 1951). Therefore, vitamin B_{12}-sufficient animals are capable of

utilizing substances for the synthesis of metabolites which animals deficient in this vitamin cannot use. It thus seems reasonable that an increased feeding efficiency should result from vitamin B_{12} administration.

Animals receiving quantities of vitamin B_{12} greater than 50 micrograms per kilogram of diet did not consume more food or grow at a faster rate. This indicates that 50 micrograms of vitamin B_{12} was sufficient for optimal growth under these experimental conditions.

When a group of animals (group 3 of experiment II) was given tap water to drink, they did not develop the severe polydypsia and polyuria seen in the rats given saline to drink. The food consumption of the group receiving the tap water was slightly less than that of the group receiving the saline, but the food utilization efficiency of the animals getting tap water was somewhat greater than that of the rats given saline. The same is true for the controls (group 1 of experiment II) which were given saline but no DCA. This group developed a less severe polydypsia than the animals receiving both DCA and saline.

The over-all effect of DCA alone or DCA and saline on the organ weights was to produce a renal and cardiac hypertrophy and a slight adrenal atrophy with no discernable effect on the thymus. These results are in agreement with those of Friedman, Friedman and Nakashima (1950) and also

Guillemin and Fortier (1951). The increased kidney weight may have been due to DCA-induced hypertension or to a direct renal injury (Grollman, Harrison and Williams, 1940). The greatly increased load of the kidney in these experiments may also account for the renal hypertrophy seen here. In these experiments the kidney weight did parallel the volume of urine being excreted. The fact that the kidney is capable of responding to an increased load in this manner is evidenced by the compensatory renal hypertrophy which normally follows unilateral nephrectomy.

The cardiac hypertrophy is probably a response to DCA-induced hypertension. Since the procedure employed in this experiment is very similar to that used in producing experimental hypertension, it is quite possible that an elevated blood pressure was present in these rats. A cardiac hypertrophy is a normal and sensitive response to hypertension and is preferred by some authors as an index of blood pressure.

Although Ingle (1940) has reported a slight thymus atrophy following the administration of DCA, the results of this experiment are inadequate to confirm or contradict this finding. The error inherent in these experiments because of the small number of animals employed, made impossible the demonstration of such small differences.

Vitamin B_{12} seemed to act synergistically with DCA in producing renal and cardiac hypertrophy. If the cardiac and

renal hypertrophy is a growth process involving the metabolic pathways employed in normal body growth, it is possible that vitamin B_{12} may have facilitated this hypertrophy while a vitamin B_{12} deficiency retarded the growth of these organs. However, another possibility must be considered. The animals receiving vitamin B_{12} also consumed and utilized more food. Handler and Bernheim (1950), using hypertensive rats, have shown that the blood pressures of such animals can be greatly reduced by decreasing the caloric intake, and can be even more dramatically reduced by decreasing the dietary protein of these animals. The error in protein metabolism or the decreased food consumption brought about by a vitamin B_{12} deficiency may be capable of reducing the blood pressure and consequently the renal and cardiac hypertrophy normally seen in hypertension. Since group 4 of experiment II ate the same amount of food as did group 2 of this same experiment, the increased saline consumption of group 4 can be attributed to an effect of vitamin B_{12} which is independent of food consumption. This hypothesis is further substantiated by the fact that group 5 of this experiment ate more food than group 4 but consumed the same quantity of saline.

The pathological lesions seen in the animals of this experiment were similar to but less frequent and severe than those reported by Selye and Stone (1943). This is not surprising since these workers used much larger doses of DCA

and ran experiments of longer duration than reported here. It is conceivable that the rats of the Carworth strain are less sensitive to DCA pathogenesis than the strains of rats used by Selye and Stone (1943), but Rosenberg, Woodbury and Sayers (1952) also reported only little pathogenesis in unilaterally nephrectomized rats treated with DCA. The errors inherent in quantitating pathological data with the small number of animals used in these experiments make difficult any exact interpretations of the effects of vitamin B_{12} upon DCA-induced histological changes. However, the data from this study point to the probability that vitamin B_{12} enhanced rather than prevented the deleterious effects of desoxycorticosterone and saline drink on kidney and heart size. These data indicate, therefore, that a deficiency of vitamin B_{12} may be beneficial for preventing the effects of DCA and saline in enlarging heart and kidney size.

SUMMARY

1. Two experiments were conducted to study the interactions between vitamin B_{12} and desoxycorticosterone acetate in unilaterally nephrectomized rats. The animals used in the first experiment were maintained on a vitamin B_{12} deficient diet for thirty-two days and in the second experiment for twenty-eight days prior to unilateral nephrectomy. Following the removal of the left kidney the animals of each experiment were grouped and given rations containing various levels of vitamin B_{12}. Some of the groups of each experiment received subcutaneous injections of 1.5 mg. of DCA twice daily. All of the animals in these experiments, with the exception of one group in experiment II, received 1 percent saline instead of tap water to drink during the period of the experiment following unilateral nephrectomy. The food intake, saline consumption, and growth rates of each group was followed for twenty days. At the end of this twenty-day period the animals were sacrificed and the kidney, heart, thymus and adrenal weights determined. In the first experiment microscopic sections of heart, kidney, thymus and duodenum from two rats of each group were prepared for histologic study. The heart, kidney and a sample of skin and muscle were removed from each rat of the second experiment and dried at 100° C. to determine the water content of these tissues.

2. In the first experiment vitamin B_{12} increased food consumption, food-utilization efficiency and growth rate. DCA produced a polydypsia and polyuria which was made more severe by vitamin B_{12} administration. DCA also induced a renal and cardiac hypertrophy which was also more pronounced when vitamin B_{12} was included in the diet. Although DCA produced a nephrosclerosis and a myocardial hypertrophy, no gross articular pathology such as swelling of the tarsal joints was seen following DCA treatment. There was no evidence of rheumatic heart lesions following DCA administration. With the exception of renal and cardiac hypertrophy the effects of DCA on the tissues were not effected by the vitamin B_{12} adequacy of the diet.
3. In the second experiment, vitamin B_{12} again increased food consumption, food utilization efficiency and growth rate. These effects were less marked than in the first experiment. This can be at least partially attributed to the animals of the first experiment being maintained on the vitamin B_{12}-deficient diet longer prior to beginning the experiment than were the rats used in the second experiment. As in the first experiment, DCA induced a polydypsia and polyuria which was made more severe by supplementing the diet with vitamin B_{12}. The increased fluid consumption accompanying DCA treatment was greatly

reduced but not completely prevented by giving the animals tap water instead of saline to drink. DCA produced a renal and cardiac hypertrophy. This effect was also less marked in the animals receiving tap water instead of saline to drink. The restriction of food intake had a similar but less marked effect in reducing this cardio-renal manifestation of DCA treatment. As in the first experiment DCA produced a greater increase in heart weight in the animals receiving vitamin B_{12} than in the animals which were deficient in this vitamin. The DCA-induced renal hypertrophy was not greatly effected by the vitamin B_{12} level of the diet. This may have been due to more adequate body stores of vitamin B_{12} in the animals of the second experiment. The water content of the kidney, heart, skin and muscle of the animals of this experiment was not altered appreciably by DCA treatment, by 1% saline or by the vitamin B_{12} adequacy of the animals.

4. In these experiments DCA was more effective in producing a polydypsia and cardio-renal hypertrophy in animals receiving vitamin B_{12} than in the animals which were deficient in this vitamin. One reason for this may have been the increased food consumption resulting from vitamin B_{12} administration. The increase in saline consumption resulting from including vitamin B_{12} in the diet of DCA-treated animals, was much greater than the increase in food consumption. In the second experiment two DCA-treated groups

were given equal quantities of food, while only one of the groups was given vitamin B_{12}. The group receiving vitamin B_{12} drank more saline and exhibited larger kidneys and hearts than did the group receiving the vitamin B_{12}-deficient ration. These findings indicate that vitamin B_{12} augments the effect of DCA by increasing saline consumption and this may occur independently of an increase in food consumption.

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