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E.C.G. AND CoA CONTENT OF THE HEART AND LIVER IN PANTOTHENIC ACID, IN COMBINED PANTOTHENIC ACID AND BIOTIN, AND BIOTIN DEFICIENT RATS

A THESIS

By

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HISTORICAL SURVEY

The history of biotin and pantothenic acid will be treated individually in this synopsis. Part I will consist of the history of biotin and Part II of pantothenic acid.

PART I - BIOTIN

After Wildiers' discovery of 'bios', a substance which he found was indispensable for normal development of yeast (1), work was continued along those lines. M.A. Clark (2) measured the rate of reproduction of yeast cells. Clark's work led to a convenient method of determining quantitatively 'bios'.

Again G.H. Lucas (3) found that by a certain treatment with baryta, Wildier's 'bios' could be seperated into two constituents. The one precipitated down by baryta he gave the name of Bios I, and that in the filtrate, the name Bios II. The isolation and crystallization of Bios I was made by Edna Eastcott, University of Toronto (4) and the identification of Bios I as Inosite by F.S. Allan (ibid). It wasn't long before the second factor, Bios II was identified as a mixture

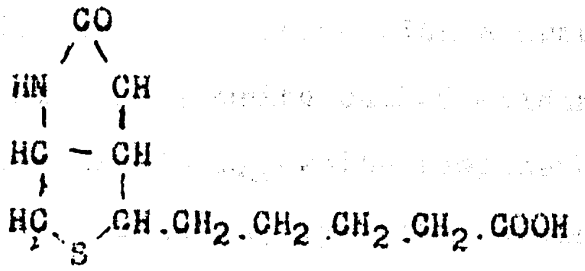
of pantothenic acid and biotin.

The first clue to the existence of a new vitamin, which was later to be called vitamin H, co-enzyme R, came in 1927, when Miss Boas, of the Lister Institute in London, found that rats became ill if fed on a special diet containing much uncoagulated egg-white (5). The symptoms could be cured, if the eggs were cooked or if appropriate components such as raw potato, potato starch, arrowroot, dried yeast, caseinogen, egg yolk, milk, cabbage and spinach leaves, banana or dried horse serum were added to the diet. Four years later, P. Gyorgy (6) found that a certain factor which he called vitamin H, protected rats from egg-white injury and other animals from skin troubles, especially that of a certain scaly condition of the skin, which was invariably found to occur when an egg-white diet was fed to rats. It was soon realized that the anti-egg white factor of Miss Boas was the same as his own vitamin H (11).

In 1935, F. Kogl (7) gave the offshoot name of biotin to the Bios IIB designation. During that time, Allison et al. (8) in 1933, had discovered a factor

which they called co-enzyme R, and which was defined to be a growth and respiratory substance for many strains of the legume nodule organisms Rhizobium. Two years prior to Gyorgy's paper on vitamin H, two different groups of workers, Wilson and West (9) and Nilsson and co-workers in Sweden (10), proclaimed similarity in properties between Biotin and co-enzyme R. This factor gave strong suspicions to Gyorgy that his vitamin H was identical with the other two. Further proof was brought by Kogl and Tonnis (11).

The structure of biotin was soon investigated and final proof was obtained by exhaustive methylation studies on the diaminocarboxylic acid from biotin (12). Finally, S.A. Harris and his group of workers synthesized biotin in the Merck Laboratories, (13) (14) (15) (16), and thus proved its structure to be



Biotin

The search for any role which biotin could play in coenzyme and enzymatic compounds was undertaken without delay. The first suggestion regarding the enzymatic action of biotin related it to the synthesis of aspartic acid (17) (18) (19) and also to the oxidation of pyruvic acid (20).

Biotin participates also in many other enzymatic reactions such as the decarboxilation of oxaloacetate and succinic acid (21) (22), in the deamination of serine and threonine (23) and in the dehydrogenation of succinic acid (24).

Biotin is a water soluble dialysable compound. It appears that biotin in tissues,and in other metabolic functions, occurs in a bound form that is linked to proteins or to peptides or to other similar high molecular compounds. However, the most interesting bound form of biotin is its combination with a special protein like constituent of egg-white called avidin. Avidin is a toxic factor in raw egg-white responsible for the production of egg-white injury. The avidin-biotin complex is easily split by steaming for a short time at 100°. Purified avidin produces effects in rats

similar to those caused by dried unheated egg-white (25).

PART II.- PANTOTHENIC ACID

Phase I (1901-1935)

As soon as Funk in 1912 formulated his theory that each of the then known deficiency diseases was caused by the absence from the diet of a separate definite substance, and gave the name of vitamine to these specific chemical substances, McCollum and associates approximately at the same time discovered that certain fats such as butter and cod liver oil contained a substance that in small amounts was capable of promoting the growth of young rats and preventing xerophthalmia. Since the beri-beri preventing factor was soluble in water, McCollum concluded that there were two accessory dietary factors notably, a fat soluble factor and a water soluble factor. He designated the fat soluble factor as 'A', and the water soluble factor as 'B'. Later, Holst and Frolich in Norway, showed that the anti-scurvy preventing

factor was different from the anti-beriberi factor and was called water soluble C.

In 1920, when it had become evident that there were at least three factors, two of which were not related to amines, Drummond suggested that the terms of Casimir Funk and McCollum be combined, by giving the term vitamine of Funk and adding the letter proposed by McCollum to the factors. This was how the name and lettering of the vitamins originated. As new vitamins have been discovered they have been designated by letters added to the class name or by use of subscripts to the letter.

In 1924, G.W. Lucas (3), observed that 'bios' was not composed of a single factor but could be treated with baryta, as described in the history of Biotin, so as to produce two distinct factors: one present in the precipitate he designated as 'bios I' and the one factor which was left in the filtrate as 'bios II'. Soon 'bios II' was to be subdivided in 'bios IIA' and 'bios IIB'. Bios IIA was then to be identified as pantothenic acid.

Numerous attempts were made to elucidate the nature of 'bios IIA'. Finally Williams and his co-workers isolated a crystalline product to which they gave the name pantothenic acid and later suggested pantothen to indicate its universal distribution (26) (27).

In the meantime other groups of workers were interested in an extract prepared from liver variously designated as filtrate factor, chick anti-dermatitis factor or also referred to as Factor II. This also helped to stimulate progress towards the identification of the unknown vitamin.

A third synonym 'filtrate factor', was given to the unknown factor when dealing in a different line of inquiry connected with nutritional research on rats. At this stage of research, more interest was manifested for determining the nature, properties, and value of pantothenic acid in nutrition. Therefore this history of pantothenic acid is divided into a second part, and a more detailed expose of the vitamin will be given, laying stress on the effects of nutritional deficiency of the 'filtrate factor' in the rat and other animals.

Phase II (1935-1956)

The following sketch illustrates how the B group was divided at the time:

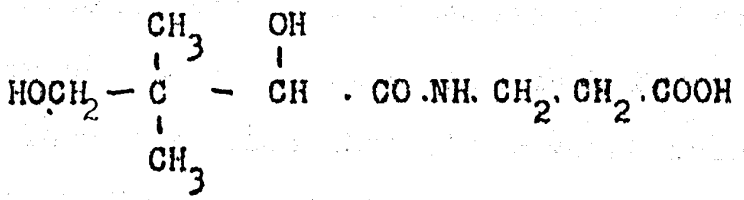
A - Heat labile factor B_1.

Adsorbate { nicotinamide, riboflavin, vitamin B_6

B - Heat stable factor B_2:

Filtrate { pantothenic acid and other vitamins

It was not till 1940, that the Merck group (28) of investigators crystallized pantothenic acid, found its structure and finally reported its synthesis. The structure of pantothenic acid was reported to be the following:



Lepkovsky, Jukes and Krause (29) were probably the first to show the need in rats of a factor distinct from thiamine, B_6 and riboflavin. Since this factor remained in the filtrate after the removal of riboflavin and B_6 by absorption, it was given the name 'filtrate factor'. They had noted that concentrates which were free from vitamin B_6 (then called factor I) were active both for the prevention of dermatitis in chicks and the growth in rats. The properties of the 'filtrate factor' were studied further by Halliday and Evans (30), Edgar and Macrae (31) and by Schultz and Mattill (32).

Halliday and Evans stated that their experiments confirmed the results obtained by Lepkovsky and Jukes in regard to the growth stimulating action of the 'filtrate factor'. Fractions prepared from whole wheat and from liver stimulated growth without protecting the rats from dermatitis. Edgar and Macrae investigated physical and chemical properties of the 'filtrate factor', and an attempt was made to purify it. A comparison was given of its properties with that found by other workers. They suggested that their yeast extract factor was distinct from vitamin B_6 but perhaps identical

with the 'filtrate factor' from the liver extract obtained by Lepkvosky et al., Schultz and Mattill demonstrated that 2 additional factors besides riboflavin and thiamine are necessary for the growth and well being in the rat.

The first factor is vitamin B_6 (factor I) of Gyorgy (33) (34), and is necessary for growth and prevention of dermatitis. The second is 'factor II' which is probably the precipitate factor of Elvehjem, Koehn and Oleson (35) and is essential for growth. Vitamin B_2 (filtrate factor of Elvehjem and Koehn) is not necessary for growth in the rat. A concentrate of 'factor II' probably contains it.

Widespread interest in the problem from a nutritional point of view, gained momentum when Morgan, Cook and Davison (36), and Lunde and Krinstad (37), in 1938 reported graying in rats maintained on synthetic diets deficient only in the 'filtrate factors' of the B-complex that is factors other than thiamin, riboflavin, and pyridoxine not adsorbed on fullers earth. Synthetic diets which contained casein, sucrose, salts, corn oil or other unsaturated fat with cod liver oil,

thiamine, pyridoxine and riboflavin, induced graying in piebald and black rats in approximately 6 to 7 weeks. All the animals showed various gross deficiency symptoms which could be cured by the addition of crude filtrate fractions to the diets.

Oleson, Bird, Elvehjem and Hart (38) indicated that if thiamine, riboflavin, choline, nicotinic acid were supplemented in their diets still four main symptoms were noted: acrodynia, hemorrhagic diseases, paralysis and the spectacled eye condition. They stated that their filtrate carried the factor necessary for the prevention of the spectacled eye condition in this case, whilst the filtrate fraction which had been obtained by Lepkovsky and Jukes (39) and that of Edgar and Macrae (31) carry the growth promoting factors.

Pantothenic acid was shown to be identical with the chick antidermatitis factor by Wooley et al. (40) (41) and Jukes (42). Gyorgy, Poling and Subbarow (43) observed that 3 different types of lesions occurred when their rats had been fed a diet containing the antidermatitis component of the filtrate factor. The lesions were described as beginning first with sores around the mouth and a scaly dermatitis, followed by alopecia and

finally by a generalized scaliness. The salt of Zinc Pantothenate was found to be active in the cure of the above symptoms. Their diet had been supplemented with nicotinic acid amide, uracil, unidentified organic acids, vitamins B_1 and B_6, and riboflavin. Gyorgy and Eckart (44) in the same year were soon to relate vitamin B_6 to skin lesions. They indicated that B_6 alone did not permit growth in rats. Symptoms previously noted by Olson et al. (38) were also apparent. They concluded therefore that both B_6 and the filtrate factor were necessary for normal growth and development in rats.

Hitchings, Subbarow (45) in the same year, fed albino rats a basal diet essentially free from B-vitamins but supplemented with pure thiamine, riboflavin and by Fullers earth adsorbate from liver extracts. Casein, sucrose, salts, peanut oil and wood flour made up the remainder of the bulk of the diet. They suggested that pantothenic acid was responsible for a part of the growth promoting activity of liver extracts. This assertion was confirmed by them (46) in the same year when they found that the calcium salt of pantothenic acid actively stimulated rat growth.

Lunde and Kringstad (47) and Morgan and Simms (48) made the pertinent observation that yeast and yeast extracts always promoted growth, but did not always cure graying, whilst rice bran and liver filtrate had less effect on growth, but brought about a prompt darkening of the fur. Oleson, Elvehjem and Hart (49) confirmed the results obtained by Lunde and Kringstad in the same year, that the rusting of fur of albino rats in correlation with the growth of the animals fed a diet consisting of casein, sucrose, salts, riboflavin, pyridoxine, thiamine, cod liver oil and butter fat, could not be associated with the latter and that the dietary factor which prevents nutritional achromotrichia is distinct from all factors of the B- complex which have thus far been identified. The factor does not seem to be involved in the growth of the rat.

Chick, Macray and Worden (50) studied the relation of skin lesions in the rat to deficiency in the diet of different B_2 vitamins. Their work pointed out that if rats were deprived of the filtrate fraction in their diets, the animals showed slow subnormal growth up to a maximum weight of about 100-130 gm. weight: they

developed poor coats with matted and stringy fur which often became gray in the pigmented areas over the head and shoulders and was frequently stained with a reddish exudate containing protoporphyrin on the forearms and the abdomen. On the other hand if the rats were deprived of vitamin B_6 (eluate fraction), a characteristic dermatitis developed which was promptly cured when B_6 was given. Later in the same year, Lythgoe et al. (51) showed that the liver filtrate factor was identical with pantothenic acid.

So many symptoms were recorded in this field that we thought it proper to discuss each general symptom under one heading.

A. Growth

One of the main topics of research on pantothenic acid deficiency diets was the effect which this vitamin could have on the growth of the rat and other animals.

Subbarow and Hitchings (46) in 1939, indicated that pantothenic acid was necessary for rat growth. After feeding their rats with the calcium salt of pantothenic acid, they found it actively stimulated growth in their animals. They concluded that pantothenic acid was

necessary for the growth of the rat. Their diet had been supplemented with thiamine and riboflavin. This question was also answered by the work of Olson, Wooley and Elvehjem (52). Their diet consisted of casein, sucrose, corn oil, supplemented with choline, niacin, riboflavin, pyridoxine, thiamine, cod liver oil and salts.

When inactivated pantothenic acid prepared by treating the concentrate with NaOH, and heating to 95' on a steam bath for one hour and neutralizing, was fed to the rats, the animals were found to fail to grow, and their weights plateaued at 40-60 gm weight. Morgan and Simms (53), obtained subnormal growths after maintaining their rats on their pantothenic acid deficient diet composed of an adequate amount of fat soluble vitamins, fed as cod liver oil, crystalline B_1 (Merck), B_6, and riboflavin.

They indicated that the administration of pantothenic acid brought rapid healing to their animals. Administration of vitamins B_1, B_6, riboflavin, niacin or minerals such as Cu, Fe or epinephrine, were all ineffective in relieving the deficiency symptoms.

While studying the effects of synthetic pantothenic acid on adrenal hemorrhage, atrophy and necrosis in the rat, Daft and Sebrell (54), noted that rats on a pantothenic acid deficient diet lost weight; other vitamins such as riboflavin, thiamine HCl, choline, pyridoxine and nicotinic acid were added. There was an immediate and rapid gain in weight which was sustained only for 2-3 weeks. Again the diet was supplemented with synthetic pantothenic acid and administered to a group of their rats. The symptoms of nose bleeding, ocular exudate and loss of weight disappeared entirely or decreased in severity. R.R. Williams (55) in his experiments on graying of fur noted that growth was greatly enhanced by giving pantothenic acid concentrates and pure pantothenic acid to his animals.

Ashburn (56) stated that the effect of the administration of pantothenic acid to animals deficient in the filtrate factor moiety indicated that the adrenal lesions, abnormal testicular function and cartilage hypoplasia, occurring in the rats were arrested by the supplementation of the vitamin in a daily dose of 100γ.

The effect of pantothenic acid on the growth and reproduction of rats placed on a synthetic diet was studied by Unna in 1940 (57). The diet consisted of casein, dextrose, crisco, salts, cod liver oil, supplemented with thiamine, riboflavin, nicotinic acid, amide, choline chloride and a-tocopherol. His results showed that the incorporation of adequate amounts of thiamine, riboflavin, nicotinic amide, B_6, pantothenic acid, into a synthetic diet free from water soluble vitamins assures continuous growth until maturity and reproduction. Unna again studied the requirement of pantothenic acid by the rat. The rats maintained on a pantothenic acid deficient diet ceased to grow after 3-4 weeks (58). The diet in this case had been supplemented with thiaminem riboflavin, B_6, choline chloride and nicotinic acid amide. This was also noted by Morgan and Simms in the same year. (48). In this case the diet had been supplemented with thiamine chloride, riboflavin, wheat germ autolysate. The relationship between cystine and pantothenic acid as concerned with the growth of the rat was studied by Pavcek and Baum (59). Response in growth were seen to be more evident when both pantothenic acid and cystine were present

than when only pantothenic acid had been supplemented.

G. C. Supplee, R.C. Bender and O.J. Kahlenberg (60) studying the interrelated vitamin requirements, noted that pantothenic acid influences growth rate but seemingly not in a direct arithmetical relationship. Twelve micrograms or less of calcium pantothenate per rat per day retarded growth and led to numerous sudden deaths within 8-10 weeks. Twenty micrograms and above permitted normal growth when fed in conjunction with other synthetic vitamin supplements, thiamine, riboflavin, and B_6.

In 1945, Carter et al. (61) noted that on their diet deficient in pantothenic acid, the growth response in rats was much inferior and weight became stabilized at about 100g. Their deficient diet was not uni-deficient however since folic acid, biotin, inositol and B_{12} were also absent.

Changes in the endochondral ossification of the tibia accompanying acute pantothenic acid deficiency in young rats was studied by Nelson et al. (62). Their experimental animals experienced decreased growth of the tibia by impairment in chondrogenesis, in osteogenesis and hematopoiesis. They stated that pantothenic acid is a vital factor for the stimulation and maintenance of

normal chondrogenesis, osteogenesis and hematopoiesis in the rat; their diet was incomplete however. It had only been supplemented with thiamine, pyridoxine, riboflavin, PAB, nicotinic acid, inositol and choline. The response to growth hormone by the adult rat as related to pantothenic acid deficiency was closely investigated by Lotspeich (63). A high fat diet and a high carbohydrate diet was fed to the rats. Loss of weight and the usual symptoms attributed to pantothenic acid deficiency were all noted, such as porphyrin incrustration of whiskers and fur, red painful feet, matted fur and loss of luster. In the following 16 days, anterior pituitary growth hormone was administered. Both groups showed a definite gain in weight in response to the hormone. In the second week after the administration of the hormone, loss of weight was noted in the pantothenic acid deficient animals. The rats were then fed 1.0 mg. of calcium pantothenate. A dramatic increase in weight resulted. Their conclusion was that adult female rats whose growth cruve has plateaued, can be made to develop acute pantothenic acid deficiency, when stimulated to grow rapidly by the combined injection of anterior pituitary growth hormone and pantothenic acid. This deficiency is more acute in high fat deficiency diets.

The effects of varying the intake of calcium pantothenate during pregnancy was studied by Everson et al. (64). They found that if the maternal diet was supplied no pantothenic acid from the initiation of pregnancy, small litters of undersized young were produced, pointing to a diminution of growth on pantothenic acid deficiency.

Experiments described by Beare, Beaton and McHenry (65) in 1954, in which the pituitary growth hormone was administered to pantothenic acid deficient rats, showed that rats not provided with either riboflavin or pantothenic acid were not able to respond anabolically to growth hormone as evidenced by the failure to increase their body weight.

Classic signs of pantothenic acid deficiency, such as slowing in weight gain, alopecia, and porphyrin caked whiskers appeared on the animals of Smith and Mefferd (66) when they were investigating the cholesterol biosynthesis of rats fed a pantothenic acid deficient diet. Their diet was incomplete in that it lacked vitamin B_{12} and ascorbic acid.

B- DERMATITIS

Skin lesions were known to occur when some of the B-Complex factors were absent from the diets long before pantothenic acid had been identified. At the time, the 'filtrate factor' was one of the main topics of research, and, therefore was given close scrutiny as to its effects on growth, achromotrichia and acrodynia.

Paul Gyorgy in 1938 (67), after administering concentrate of vitamin B_6 to his rats, indicated that, even then, skin lesions were not regularly made to disappear unless a further supplement corresponding to the filtrate factor was added. In 1939, Gyorgy et al. (68) declared that a crude zinc salt of pantothenic acid proved to be active in the cure of specific skin lesions in the rat.

Morgan and Simms (48) fed their rats a diet containing riboflavin, thiamine and B_6 as water soluble vitamins. A group of small lesions behind the shoulders and extending partially down the sides of the animals was noted. They found that yeast and rice bran filtrates cured all types of dermatitis produced by the deficiency.

Soon pure pantothenic acid could be supplemented in the diets of the rats and skin lesions could be more closely associated with pantothenic acid deficient diets.

Curative concentrates for dermatitis were administered to rats by Morgan and Simms (53) in 1939. They studied the effects of filtrate factor deficient diets and found that, after several months on the diet, a peculiar sloughing of spots and patches of skin sometimes with ulcers resulted when the animals were kept on the diet and that the administration of concentrates of the filtrate factor brought about rapid healing. On the other hand, Richards and Hogan (69) reported that under their experimental conditions both pantothenic acid and vitamin B_6 are required to cure the rat dermatitis which they had obtained on their animals. Their diet consisted of thiamine, riboflavin, cod liver oil, B_6, or a combination of pantothenic acid and B_6.

The effect of the filtrate fraction upon dermatitis was also studied by Chick, Macrae and Worden (70) when they fed rats having florid dermatitis, roughly symmetrical with swelling of the digits of the paws and of the ears, spreading over the body with cracking and desquamation of the skin. They indicated that complete cure occurred only

if the eluate fraction was also supplemented.

Gyorgy, Pöling and Subbarow (71) noted that when pantothenic acid in amounts of 40-50 µg was supplemented in their diet which contained vitamin B_1, B_6, the cutaneous lesions produced by the deficient diet regressed. On a diet consisting of casein, egg white, sucrose, melted butter fat, cod liver oil and salts, supplemented with thiamine chloride, pyridoxine and riboflavin, the experimental animals of Gyorgy and Pöling (72) developed skin lesions and despite the administration of 50-100 µg. of calcium pantothenate, after a period of 6 months, cutaneous lesions still appeared in the rats, these lesions being a predominantly scaly dermatitis and thinning of the pelt.

The pantothenic acid requirement of the rat was also determined by Unna (58). His diet had been supplemented with choline, niacin, riboflavin, pyridoxine, thiamine. Rats maintained on this diet developed the usual symptoms of pantothenic acid deficiency with hemorrhages in the skin and adrenals. Unna stated that 50 µg. daily of pantothenic acid fed to his animals produced marked weight response but the other deficiency syndromes disappeared only after prolonged administration of the vitamin.

Dimick and Lepp (73) indicated that pantothenic acid is an important part of the rat filtrate factor (factor 2). It promotes growth of the animals, cures the reddening and the swelling of the eyelids which develops on a factor-2 deficient diet, improves the skin condition and decreases the greying of the fur. In the chick they stated that synthetic pantothenic acid will cure or prevent nutritional dermatitis. The diet had been supplemented with thiamine, riboflavin, and pyridoxine.

Unna and Sampson (74) found that if their rats were fed a diet supplemented with thiamine, riboflavin, nicotinamide and B_6, the animals developed generalized scaly dermatitis, inflammation of the nasal mucosa and other symptoms related to a pantothenic acid deficiency. When, however, graded amounts of calcium pantothenate were added to the diet in doses of from 80-100 µg calcium pantothenate, this supplementation prevented all deficiency symptoms.

Unna and Richards (75) in 1941, experimented with rats fed a B-complex free diet supplemented with thiamine, riboflavin, nicotinic acid pyridoxine and choline. The

usual symptoms of pantothenic acid deficiency developed such as scant coarse fur, inflammation of the nose. They indicated that 100 µg. of calcium pantothenate prevented the development of all deficiency lesions. The same authors in 1942 indicated, when investigating the relationship between pantothenic acid requirements and age in the rat, that the maintenance dose of pantothenic acid for the prevention of deficiency lesions were of 100 µg. per day at 3 weeks and 25 µg per day when the rats were 10 weeks old.

C. ACHROMOTRICHIA

In the early work on the effect of pantothenic acid deficiency on greying, different laboratories reported contradictory results.

Morgan and co-workers (36) and Lunde and Krinstad (37) had reported that achromotrichia could be prevented or corrected by supplementing filtrate factor concentrates in their diets.

Concentrates of pantothenic acid were soon to be investigated for their role in the curing of greying. Oleson et al. (49) concluded that nutritional achromotrichia could not be prevented by the addition of pantothenic acid concentrates. Chick et al (50) reported that

some of their rats deprived of their pantothenic acid concentrate, developed graying especially over their heads and shoulders. Crude concentrates of pantothenic acid were reported to have a definitely curative effect on achromotrichia in rats by Gyorgy, Poling and Subbarow (71). Their pantothenic acid concentrates had a degree of purification varying up to 50%.

Incomplete prevention or cure of achromotrichia was reported by many authors in 1940 and '41, by using the pure compound.

Gyorgy and Poling (76), reported that with the administration of 75-100 micrograms of pantothenic acid, curing of graying, in the pantothenic acid deficient rats, was practically complete. Their diet had been supplemented with thiamine, riboflavin and vitamin B_6. Gyorgy and Goldblat (77) stated that if the diet was devoid of vitamin B_6, this syndrome is less frequently observed, owing probably to the phenomenon of competition of vitamin deficiency diseases. Later Gyorgy and Poling (72) showed that Biotin had some effect for curing greying, but stated that even when pantothenic acid and biotin had been added in their diets, pigmentation was not restored entirely to normal.

Dimick and Lepp (73) stated that additional factors were required by the rat in rice bran extract and that pantothenic acid does not completely restore hair color nor does it completely prevent greying.

The curative capacity of liver and yeast extracts was shown not to be entirely due to their pantothenic acid content by Frost, Dann and Moore (78). They indicated that certain samples such as liver fraction, brewers yeast, had much higher anti-greying potency than the relative pantothenic acid content warranted.

Ansbacker (79) pointed out that para-amino-benzoic-acid played a role in the greying of the rat and stated that this symptom appeared even if the diet contained sufficient pantothenic acid. Chicks were investigated by Groody and Groody (80) to determine whether pantothenic acid had any effect on greying of the feathers. They concluded that the depigmentation occurring could be due entirely to pantothenic acid deficiency or that some other factor could be involved.

Pavcek and Baum (59) stated that the blackening of fur of their rats began 4-5 weeks after the administration of 200 micrograms of calcium pantothenate per day,

and reached a maximum intensity only after 12-14 weeks. The regeneration of the black fur was incomplete and a stippling effect of the coat resulted. Emerson and Evans (81) in compiling their results noted much the same results.

The inefficacy of pantothenic acid as a curing agent against greying of the rat was indicated by William (55). His diet had been supplemented with B_1, B_2, B_6, nicotinic acid and choline. The diet differed from that of Gyorgy and Poling (76) in that 8% butter fat had been substituted for 2% corn oil. Owens et al. (82) stated that if choline was omitted from the diet, rustiness developed in 6 weeks regardless of the high amount of pantothenic acid added in the diet.

Finally complete cure or prevention was reported by Unna et al. (72) and Sampson (83) and by Henderson et al. (84). Unna et al. stated that their black rats maintained on a B-complex free diet, supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine and choline, developed greying within 3-7 weeks. The addition of 100 µg. of calcium pantothenate per day, prevented the development of achromotrichia and restored the black pigmentation of the fur in rats which had been fed the pantothenic acid deficient diet. Henderson et al. stated that rats fed a pan-

tothenic acid deficient diet became grey in 4-6 weeks and that if levels of 40 µg of calcium pantothenate were fed the animals per day, it prevented or cured this condition. Their diet had been supplemented with thiamine, pyridoxine, riboflavin, nicotinic acid and choline.

Other factors concerned in the greying of the rats were also investigated. Froe (85) demonstrated that the factors of the B-complex were inefficient in curing achromotrichia due to copper deficiency. He also pointed out that copper was inefficient in curing achromotrichia induced by a dificiency of the B-complex. J.M. Hundley (86) fed his rats a diet supplemented with thiamine, riboflavin, pyridoxine, calcium pantothenate (2 mg), choline, inositol, nicotinic acid, menadione, biotin and folic acid and different salts. He observed that black rats fed a purified diet containing 14.6 µg. of copper per gram, grew well but developed marked achromotrichia. The achromotrichia could only be cured by the addition of copper sulfate to the diet in amounts of 20 µg of copper per gram.

Hundley and Ing (87) noted grey hair in their rats maintained on a copper deficient diet. Adrenalectomy or hypophysectomy caused partial or complete return of blackening in the coats of their animals. According

to them, all necessary vitamins required by the rats had been supplemented in their diets. The same authors reported that pantothenic acid deficiency may produce grey hair by blocking the utilization of copper in hair growth and melanin formation (88).

Other symptoms of greying have been reported by folic acid deficiency produced by the administration of sulfonamides in the rat diet. (89) (90).

Deficiency of cystine (59), biotin (72), sodium chloride (91) and choline (92) have all been reported as having effects on rat greying.

The relation of para-amino-benzoic acid with pantothenic acid in its role to greying was indicated by Martin (93). He stated that the ratio of pantothenic acid to that of para-amino-benzoic acid is the important factor in rat greying. These results, however, were not confirmed by Dann, Moore and Frost (94). They concluded that PAB had no effect on greying alone or slight if any with calcium pantothenate.

D. ADRENALS

The effect of pantothenic acid deficiency on rats, in relation to the adrenals was also given prime consideration in earlier years and a complete histological picture

was depicted by many authors.

In 1939, Daft and Sebrell (95) reported hemorrhagic necrosis of the adrenal glands on deficient diets which they claimed was due to some unidentified dietary factor. They also noted that if the rats received adequate amounts of B_6, flavin, thiamine, nicotinic acid, besides the basal diet composed of leached and alcohol extracted casein, cod liver oil, wesson oil, salts and sucrose without adding the filtrate factor, adrenal necrosis was much more apparent. If, however, the animals were given crude fullers earth from liver or rice polishings, plus flavin, thiamine, nicotinic acid and the basal diet, adrenal necrosis did not appear. In the same year Morgan and Simms (53) made histological studies of the adrenals when their animals had been fed filtrate factor deficient diets and noted consistent atrophy of the adrenal glands. They stated that the function of the adrenal cortex was apparently particularly affected in every case. Their curative concentrates had been made from yeast, rice bran and liver. The effect of synthetic pantothenic acid on adrenal hemorrhage, atrophy and necrosis in the rats was studied in 1940 by Daft et al. (54). Their animals had received a basal diet supplemented with thiamine, flavin, pyridoxine,

nicotinic acid and choline. Graded amounts of pantothenic acid were administered. It was found that the rats which were lacking pantothenic acid in their diets had hemorrhage, necrosis or atrophy of the adrenals or a combination of these lesions. They concluded that for the repair or the prevention of adrenal hemorrhage, necrosis and atrophy, a daily dose of 100 micrograms of synthetic pantothenic acid was required.

Ashburn (56) published a report on the effect of the administration of pantothenic acid on the histopathology of the "filtrate factor" deficiency state in the rats. This work was based largely on previous data obtained by Daft et al. (54); Ashburn used undiscussed data from Daft experiments, and confirmed them on other large series of animals fed the same deficient diet. His report dealt only with the pathologic aspects of the problem.

Complete investigation of the adrenals revealed that congestion, hemorrhagic foci, atrophy, necrosis, scarring, fibrosis, hemosiderin deposition and cortical fat depletion, occurred as independent or combined lesions in all of the deficient animals. Other organs such as the spleen, testes, bone and pancreas, were all examined in both the deficient and pantothenic acid treated animals.

It was observed that the adrenal lesions, abnormal testicular function and cartilage hypoplasia occurring in the rats fed the diet deficient in the filtrate factor, were markedly affected by the supplementation in the diet of 100 micrograms of pantothenic acid daily. He stated that arrest and repair of adrenal lesions occur, testicular function is improved and skeletal growth is accelerated with pantothenic acid supplementation.

Unna (58) also studied the effect of synthetic pantothenic acid on rats whose diets had been supplemented with choline, niacin, riboflavin, pyridoxine and thiamine. Adrenal hemorrhages were found to be occurring in his animals on his pantothenic acid deficient diet. He stated that daily feeding of 50 micrograms of pantothenic acid to his rats, brought about a relief of these symptoms, only after prooonged administration.

Severe damage to the tissues of the adrenal glands were noted by Morgan and Simms (48) when rats had been subjected to their basal diet, composed of the following: washed casein, salt mix, crisco and sucrose, and supplemented with thiamine chloride, riboflavin, a wheat germ preparation rich in B_6 and cod liver oil.

Filtrates from Fuller's earth-treated extracts of rice bran, yeast, liver, crude cane molasses and alfafa in every case cured the symptoms but restored normal growth only in varying degrees.

Supplee, Bender and Kahlenberg (60) studied the effect of pantothenic acid deficiency in correlation with adrenal hemorrhage and other symptoms in pantothenic acid deficient rats. Adrenal hemorrhage was noted with the above deficient diet. They indicated that animals deprived of pantothenic acid following a low intake of pantothenic acid during development, consumed about twice as much Na as NaCl when allowed a free choice as did animals of the same weight not deprived of pantothenic acid. They indicated that this evidence was indicative of adrenal impairment resulting from pantothenic acid impoverishment. The basal ration consisted of the following: casein, sucrose, hydrogenated vegetable oil, salts, powdered agar-agar, and cod liver oil. The basal diet was supplemented with thiamin, riboflavin and B_6. The variants consisted of synthetic pantothenic acid and "rice polish factor II", unheated or autoclaved.

The supposition of Supplee et al (60) that sodium depletion in rats fed a deficient pantothenic acid diet

was due to adrenal impairment was not confirmed by McQueeney et al (96). They stated that sodium metabolism studied on pantothenic acid deficient diets on their rats showed that no defect in the conservation of sodium appeared, even when prominent histopathologic changes in the adrenals had occurred on the deficient diet. Their pantothenic acid deficient diet had been supplemented with thiamine, pyridoxine, choline, folic acid, nicotinic acid, biotin, inositol and riboflavin. The measurement of the sodium was made with a flame photometer.

Hurley and Morgan (97) studied carbohydrate metabolism and adrenal cortical function in pantothenic acid deficiency on rats. Their findings were interpreted as demonstrating that pantothenic acid deficiency imposed a stress on the adrenal cortex, resulting in the exhaustion of the gland and adrenal hypofunction. Cholesterol metabolism in pantothenic acid deficiency was investigated by Guering and Hurkey (98) in 1952. They indicated that a specific relationship existed between pantothenic acid and the metabolism of cholesterol fats. Furthermore, they stated that their findings could result from the role which pantothenic acid plays in the CoA structure in the regulation of acetate metabolism and the synthesis of

cholesterol and its analogues.

Winters et al (99) studied the role of the adrenal cortex in pantothenic acid deficient rats as related to their eosinophile and lymphocyte responses. To determine this, they used epinephrine, ACTH, and cortisone. These compounds were administered to the rats. Counts were made for eosinophile and lymphocytes. The deficient animals were seen to show practically no changes either to epinephrine or to ACTH, but changes occurred in the deficient animals receiving cortisone. They, therefore, stated that their findings indicated that one site of functional insufficiency of the pantothenic acid deficient rats was the adrenal cortex. They suggested that pantothenic acid being part of the CoA structure is important for the biosynthesis of steroid hormones by the adrenals probably by acting in some metabolic process involving the utilization of active acetate. Following the same trend of research these authors (100) using again ACTH and cortisone treatment, stated that adrenal damage to pantothenic acid deficient rats could be much magnified by the administration of ACTH. Cortisone on the other hand prevented characteristic development of adrenal lesions.

Lipogenetic studies in certain B-vitamin deficiencies were undertaken by Guggenheim and Olson (101). Their experimental animals were given radio active carboxyl-labeled acetate in their food for a determined period after the rats had been subjected to the deficient diets. The adrenals of pantothenic acid deficient animals were analysed for cholesterol. The C^{14} in cholesterol was determined. The specific activity of adrenal cholesterol was found to be decreased in pantothenic acid deficiency and the pair fed animals contained more cholesterol than those of the normal rats.

Adrenal function in pantothenic acid deficiency was investigated by Perry et al. (102) in 1953. These authors correlated the adrenal cortex and pantothenic acid using the adrenal cholesterol and ascorbic content as means of comparison. Their basal diet was supplemented with nicotinamide, PAB, pyridoxine, thiamine, riboflavin, biotin and folic acid. All of the above were injected daily in the animals. They summarized their findings by indicating that the deficient pantothenic acid animals as compared to the controls, had a progressive decline in adrenal cholesterol concentration: increase in succinic dehydrogenase activity of the adrenal up to the 50th day

with a decline thereafter. Finally no loss in the ability to react to stress as judged by the discharge of ascorbic acid.

Pituitary adrenal function was investigated by Ershoff et al. (103). After using as index of activity the measurement of the peripheral lymphocyte count, adrenal ascorbic acid depletion and reduction in cholesterol, it was concluded by comparing both the deficient and normal animals that no significant impairment in pituitary-adrenal function was observed in pantothenic acid depleted animals. The basal ration had been supplemented with thiamine hydrochloride, riboflavin, pyridoxine HCl, nicotinic acid, biotin, 2-methyl-naphtoquinone, folic acid, ascorbic acid, PAB, inositol B_{12}, choline chloride.

The synthesis of adrenal cholesterol following stress on animals deficient in pantothenic acid was studied by Dumm et al. (104). Their findings indicated that the adrenal cholesterol was significantly depressed compared to that of control animals. The normal animals following stress regained their initial value of serum cholesterol in 24 hours, whilst that of the deficient animals was not brought back to normal up to 7 days after the stress had been applied. The data was interpreted as

evidence that the synthesis of adrenal cholesterol is decreased in pantothenic acid deficiency.

Morgan and Lewis (105) fed three different diets varying in hypolipotropic quality to young rats, with complete choline deficient, choline and pantothenic acid deficient and pantothenic acid deficient vitamin supplements. In all cases it was noted that the pantothenic acid deficient animals failed to develop fatty livers. They concluded that in pantothenic acid deficiency the rat adrenal hormone production is depressed and fat metabolism is seriously deranged.

Hurley (106) studied levels of adrenal cholesterol in pantothenic acid deficiency and compared them to normal rats. The levels were compared before fasting, after 24 hours of fasting, at atmospheric pressure and after 24 hours fasting at 20,000 feet. Liver glycogen blood sugar and adrenal ascorbic acid levels were determined. It was found that the level of adrenal cholesterol before fasting was lower in pantothenic acid deficiency than in normal animals: after fasting the adrenal cholesterol level rose to 3.9%. No rise had been shown by the normal animals. Following exposure to anoxic anoxia with fasting

the adrenal cholesterol content fell in both normal and deficient animals. At the same time, Hurley stated that the normal animals exhibited a 20 fold increase in liver glycogen while the deficient rats showed no changes in this respect.

These results were interpreted as to indicate that the level of adrenal cholesterol itself is not an invariable index of adrenal activation.

Hurley and MacKenzie (107) observed extreme variability in the levels of adrenal cholesterol and ascorbic acid in both normal and deficient rats examined at different seasons. Liver glycogen and blood sugar levels, however, were fairly constant. The deficient, untreated animals, were observed to have lower adrenal cholesterol, liver glycogen and blood sugar levels and high adrenal weights and ascorbic acid levels than comparable normal animals. On exposure to a stress period, the normal rats showed an increase in liver glycogen and a significant rise in blood sugar as compared to their fasted controls. Pantothenic acid deficient animals showed no change in these constituents. A decrease in adrenal cholesterol levels following anoxia was observed in both normal and deficient rats.

E. BLOODY WHISKERS

Many workers had noted red staining of whiskers of their animals when working with filtrate factor deficient diets. In 1940, Chick et al. (50) identified the red material as being protoporphyrin. They stated that this pigment seemed to be characteristic of filtrate fraction deprivation. Unna (58) working on the pantothenic acid requirement of the rat noted also that his rats acquired blood caked whiskers on his deficiency diet. His diet had been supplemented with choline, niacin, riboflavin, pyridoxine and thiamine.

Figge and Atkinson (108) studied the relation of water metabolism to porphyrin incrustrations in pantothenic acid deficient rats. By limiting the amount of water given to each rat, his animals developed fluorescent porphyrin around the nose, forepaws, or eyelids. If water was reinstated in the diet to a normal measure, the symptom disappeared. He therefore concluded that pantothenic acid could be involved in the regulation of water metabolism in the rats. Carter et al (61) also noted a reddish brown staining of the hair of the rats on the abdomen and nose. Their diet was deficient in ascorbic acid, inositol, menadione, PAB,

niacin, biotin and folic acid.

McQueeney et al. (96) observed porphyrin incrustrations about the nostrils of their animals, when these were fed a pantothenic acid deficient diet. The diet was deficient in ascorbic acid, menadione, PAB, and B_{12}. McElroy et al. (109), investigated the nature of fluorescent porphyrin of the blood caked whiskers of pantothenic acid deficient rats. They concluded that the red material deposited around the nostrils was not blood but coproporphyrin and that it was derived from the Harderian glands.

F. OTHER PATHOLOGICAL CHANGES

Besides the above symptoms most commonly found in pantothenic acid deficiency, as described by different workers, many described other pathological signs. Lesions of the kidney and liver were reported by Nelson (110), Dean and McKibbin (111) and Supplee et al. (60). Signs of abnormal testicular function were reported by Nelson (110) and Ashburn (56).

Studies were made on the effect of pantothenic acid deficiency on inhibition of skeletal growth and changes in bone marrow of rats by Ashburn (56), McQueeney et al. (96), Gyorgy et al. (112), Daft et al. (113) and

Carter et al. (61).

Acetylation reactions in this vitamin deficiency were carried out by Riggs and Hegsted (114) who concluded that rats on the deficient diet acetylated only 50% of a 1 mg. dose of PAB, whilst normal rats acetylated 70%. Also they stated that if a simultaneous injection of 1 mg. Ca pantothenate was administered, the animals immediately returned this acetylation to normal. The diet had been supplemented with thiamine, riboflavin, pyridoxine hydrochloride, nicotinic acid and choline. The same authors reported in another paper (115) that weanling animals showed a decreased acetylation of PAB (1 mg. dose) almost immediately after the removal of pantothenic acid from the diet, whilst older animals continued to acetylate PAB to a normal extent after 2 months or so. Further investigation of acetate metabolism was carried out by Smith and Mefferd (116) using the C^{14} isotope. CO_2 expiration and lipogenesis studies were carried out. Pantothenic acid deficient rats were found to expire CO_2 following a single intraperitoneal injection of acetate-1-C^{14}, slightly more rapidly than do pair fed controls not PA deficient. They stated that maxima in specific activity are attained

within 5-10 minutes after injection in the deficient, within 10-20 minutes in control animals. It was also observed that maxima in "Relative Isotope Content" are attained after 5-10 minutes in deficient rats and after 10-20 minutes in non-deficient animals. The total amount of CO_2 over the first 90 minutes following injection is the same for both groups. Finally maxima in specific activities of fatty acids of pantothenic acid deficient rats and non-deficient rats are reached about 30 minutes following injection of acetate.

The blood sugar, the liver, heart and muscle glycogen and the total lipids in the albino rats after the administration of pantothenic acid were given close scrutiny by Cascio et al. (117). The glycogen content of the muscles decreased slightly after the injection of 100 mg pantothenic acid, that of the heart was unaffected and that of the liver was considerably increased after 90 minutes and had fallen again after 240 minutes. The total lipids in the liver increased considerably and progressively.

The effect of the administration of calcium pantothenate, in varying amounts during the pregnancy period of pantothenic acid deficient rats, was investigated by

Everson et al. (64). If no pantothenic acid was given to the rats from the initiation of pregnancy, small litters of undersized young rats were produced, pyruvic acid accumulation was encountered, blood concentrations for pantothenic acid were reduced. The diet was deficient in ascorbic acid.

Another line of investigation was followed by Zucker and Zucker (118), who fed pantothenic acid deficient diets to their rats and correlated this deficiency to a loss of natural resistance to bacterial infection in the rat. They concluded that pantothenic acid deficient rats may lose the innate species determined resistance to infection with a type of corinebacterium so far known to be pathogenic only to mice.

A relation to thyroid control of lipid metabolism in pantothenic acid deficiency was investigated by Harold et al. (119). It was observed that pantothenic acid deficient rats fed a diet containing 1% cholesterol did not develop fatty liver or cholesterol rich livers but had slightly increased blood cholesterol levels. If pantothenic acid was added along with this same diet, the animals developed fatty livers in 3 weeks. It was noted also that the deficient rats, after excision of their pituitary or

thyroid, showed a fat metamorphosis of the liver within 21 days following the incision. The blood cholesterol concentration was increased enormously and the increase confined to the esterified fraction. The administration of 5-10 ug. of crystalline thyroxine/day resulted in a decrease of liver lipid but not of liver cholesterol in the hypophysectomized pantothenic acid deficient rats and also a diminution of both liver lipid and cholesterol in the thyroidectomized deficient rats. Blood cholesterol concentration was decreased by thyroxine to the level of that of intact deficient rats in both the hypophysectomized and thyroidectomized deficient rats. They stated therefore that the disturbance of lipid metabolism in PA deficiency appears to be concerned principally with the thyroid.

Axelrod and Pruzansky (120) noted a marked inhibition in the antibody response in rats fed a pantothenic acid deficient diet. The diet in this case was not stated.

Another important aspect of pantothenic acid deficiency in the rat was investigated in 1939 by Nelson (110). He studied various symptoms among which pathological changes in the heart. He reported that of 54 hearts examined, 3 showed myocardial lesions: one rat heart had a

fatty change, another a focal polymorphonuclear infiltration, and the other a focal coagulation necrosis. No mention of controls was made however. Supplee et al. (60) noted heart damage, characterized by enlargement and/or extensive hemorrhage in the auricular region, which was present among animals which had died suddenly on the low pantothenic acid diets, and was frequently concurrent with severe adrenal hemorrhage. The diet had only been supplemented with thiamine, riboflavin and vitamin B_6 and was therefore multi-deficient.

A few years later, Olson and Kaplan (121) measured the COA content in the heart of pantothenic acid deficient rats. Their results proved that the COA content of the rats was diminished.

Beznák and van Alphen (122) correlated cardiac automatism and choline acetylation with thiamine and pantothenic acid deficiency in rats. The results obtained were not in agreement with most workers so far as the most regularly appearing pantothenic acid symptoms were concerned. They stated that the rats on their pantothenic acid deficient diets grew as the normals did and e.c.g.'s taken of the heart did not show any abnormalities. They attributed the lack of symptoms to a difference in experimental conditions of workers feeding a multi-deficient

diet to their animals whilst they had fed their rats a uni-deficient diet.

G. INTERRELATIONSHIP OF DIFFERENT VITAMINS AND OTHER COMPONENTS IN PANTOTHENIC ACID DEFICIENCY

The role played by different vitamins when subtracted from an already pantothenic acid deficient diet was studied by many authors. Vitamin B_6 was investigated by Black et al. (123) for its relation with pantothenic acid, to factor W. They reported that pantothenic acid was contained in factor W and that significant growth responses were had when both B_6 and the factor had been added to the diet.

In greying, different vitamins were administered for its cure, such as nicotinic acid, and pyridoxine. These two vitamins were found to be ineffective in curing and preventing greyness by Morgan and Simms (46). On the other hand liver filtrates, yeast extracts, rice bran filtrate, all cured the symptoms.

Cystine in pantothenic acid deficiency was investigated by Pavcek and Baum (59) who indicated that by the supplementation of 75 mg. cystine in their basal diet containing 18% casein in addition to 200 migrograms of pantothenic acid per rat per day, markedly decreased the time

required for the replacement of the grey hair in achromotrichia and produced better growth results. These findings confirmed results obtained by Melford and Griffith (124) that 18% casein did not provide enough cystine for maximum growth of the rat.

The effects of linoleic acid, pyridoxine and pantothenic acid on rat dermatitis was studied by Quackenbush et al. (125). It was found that if the three compounds were added to the diet, the rat was relieved of acrodynia; however, a scaly condition still persisted on the tail and hind paws. They stated that another factor was involved for complete cure.

Anemia and granulocytopenia in relation to pantothenic acid and folic acid was investigated by Daft et al. (126). They stated that anemia was not directly due to a pantothenic acid deficiency but rather developed indirectly due to the deficiency of an unknown factor which became apparent in the absence of pantothenic acid. Folic acid did not have any effect alone either. The anemias however responded well to both folic and pantothenic acids.

Water metabolism was investigated by Gaunt et al. (127), who studied the effect of riboflavin and pantothenic

acid for their role in the above. The basis of these tests was the well-established finding that marked deficiencies in water diuresis and in resistance to water intoxication, resulted from hypofunction of the adrenal cortex in animals and man. To gain evidence on this point, a study was made of the response of animals on a pantothenic acid deficient diet to water administered by stomach tube. It was observed that rats on a deficient pantothenic acid diet showed a lethargic diuresis when the water was given by stomach tube and a decreased resistance to water intoxication. Defects noted were remedied by the administration of either calcium pantothenate or adrenal cortical hormone. The diet consisted of sucrose, casein, salts, crisco and cod liver oil supplemented with thiamine, pyridoxine, riboflavin, nicotinamide, choline chloride and inositol. In riboflavin deficiency, effects on water metabolism was produced only if the riboflavin symptoms were at an advanced stage.

Corneal vascularization in pantothenic acid and pyridoxine deficiency was studied by Bowles et al. (128). Rats on this diet developed corneal vascularization; however, corneal vascularization on only pyridoxine deficient diets was not as extensive as vascularization observed in rats fed on the pantothenic acid deficient diet. The diet was incomplete in that it lacked folic acid, niacin and B_{12}.

Vitamin C as related to pantothenic acis in a deficiency diet for rats was investigated for its effects by Daft (129). He reported that if 2% ascorbic acid was introduced in a pantothenic acid deficient diet, all the usual symptoms attributed to pantothenic acid deficiency, such as porphyrin staining of the fur and whiskers, growth retardation and decreased life expectancy, were greatly delayed or modified and the life of the animal was lengthened. Vitamin C administration to pantothenic acid deficient rats during pregnancy revealed that where a definite sign of pantothenic acid deficiency was established: lower concentrations of blood pantothenic acid, low pantothenic acid content in the total tissues, including hepatic tissue, such as smaller litters composed of lighter youngs, ascorbic acid lessened the severity of the deficiency. In several instances they noted that the protection afforded by ascorbic acid was equal to that of 100 micrograms of calcium pantothenate (130).

Folic acid and biotin in the utilization of pantothenic acid by the rat was investigated by Wright and Welch (131) who fed their rats succinyl-sulfathiazole. It was found that the utilization of pantothenic acid by the

rat appeared to depend on the availability of folic acid and biotin. Vitamin B_{12} and ascorbic acid were absent from the diet.

The interrelationship between biotin and pantothenic acid in rats fed succinyl-sulfathiazole was investigated by Emerson (132). She concluded that a biotin deficiency induced in weanling rats by feeding the sulfa drug may be aggravated by superimposing a deficiency of pantothenic acid.

H. COENZYME A.

The identification of pantothenic acid as part of COA brought about many workers to investigate the role played by the vitamin in the amount of the coenzyme which was present in different parts of the body.

Olson and Kaplan (121) studied the effect of pantothenic acid deficiency upon the COA content and pyruvate utilization of the rat and duck. The rat livers showed a decreased ability to utilize pyruvate. This was accompanied by a low COA content. The diet in this case, however, was incomplete, lacking in many vitamins. These were inositol, menadione, PAB, biotin, folic acid, B_{12}, a-tocopherol, and ascorbic acid.

All living material was found to contain the coenzyme in variable amounts (133) (134). The pantothenic acid content in the tissues was found to be present entirely in the form of coenzyme A (134) (135).

Liver coenzyme A was also determined in the rat by Boyd (136) who stated that the liver COA in the rat on a pantothenic acid deficient diet was reduced to 1/2. His diet lacked ascorbic acid and B_{12}.

The relationship between coenzyme A and lipid synthesis was also investigated by Klein and Lipmann (137). The pantothenic acid deficient rat livers were analysed for their coenzyme A content. A parallel was found to exist between coenzyme A levels and the synthesis of cholesterol as well as total lipids. This diet was also incomplete.

The influence of dietary pantothenic acid and methionine on rat liver COA levels was investigated by Dinning et al. (138). Rats receiving the deficient diet in both the above exhibited a low level of coenzyme A. Both were required to restore the coenzyme A to normal values. They concluded that most probably, dietary methionine served as a precursor to the thioethylamine moiety of coenzyme.A. The diet in this case was not stated.

H. MISCELLANEOUS

As research work was progressing with diets deficient in pantothenic acid, other workers were investigating symptoms brought about under different conditions, symptoms which were described as being found in pantothenic acid deficiency only, but which were unrelated to this specific deficiency.

Plateauing of weight and alopecia were noted in the deficient riboflavin diet of Chick et al. (70). Growth symptoms and dermatitis were indicated by Gyorgy and Eckardt (44) on rats deficient in B_6. The anti-dermatitis effect of B_6 was also studied by Unna (139) who indicated that when 100 micrograms of the vitamin was administered to his animals a 100% cure was observed.

Para-amino-benzoic acid was found to be a chromotrichial agent for rats by Ansbacker (79). Again this specific vitamin was investigated for its role in rat greying by Martin (93). He stated that the ratio of calcium pantothenate to that of PAB, was the important factor concerned in achromotrichia.

Inositol was found to prevent the onset of alopecia by Nielsen and Black (140). They stated that the

animals receiving this vitamin grew better and had a more tidy appearance.

Biotin deficiency was studied by Emerson and Keresztesy (141) for its role in rat alopecia, greying and the spectacled eye condition. All of the above symptoms were observed.

B_{12} was investigated for its role in the building up of the COA levels in rat liver. Higher levels of COA were found to occur when this vitamin was made deficient to rats (142).

Other nutritional components were studied for their effects on greying and other related symptoms to a pantothenic acid deficiency.

Potassium and Magnesium (143), manganese, iron and copper (144) were all given consideration. Hundley (86) fed a diet deficient in copper to his animals. Within 5-6 weeks most of his animals had developed a marked degree of greying. Alopecia, dull hair and thinning of the fur were all noted conditions. He stated that the diet had to contain 2.5 micrograms of $CuSO_4$ per gram of basal diet, so as to prevent greying. His final conclusion was that the activity of liver, yeast, alfafa to cure achromotrichia was due solely to their Cu content.

His diet was deficient in ascorbic acid, p-amino-benzoic acid and B_{12}. It had been supplemented with pantothenic acid.

GENERAL INTRODUCTION

A great number of contradictory reports were published concerning pantothenic acid deficiency in the rat by workers using multi-deficient diets, semi-synthetic diets and synthetic diets. The fact that most so-called pantothenic acid deficient diets lacked one or many vitamins led us to believe that the symptoms attributed to pantothenic deficient diets noted in the past experimental diets, could not be attributed to the absence of pantothenic acid from the diet but as already stated by Beznák and van Alphen (122), to a multi-deficiency in dietary components.

Coenzyme A has been shown to be a pantothenic acid derivative by Lipmann et al. (145). The effect of dietary components on the CoA concentration in liver and heart of different experimental animals was studied by different authors.

Olson and Kaplan (121) stated that rats maintained on a pantothenic acid deficient diet, for a period of 9 weeks, maintained normal levels of CoA in their tissues for 2-3 weeks and then showed a gradual depletion to a level 35-40% of normal, and that ducklings subjected to

a somewhat similar diet, showed still more rapid depletion in their tissues to 40% of the normal animals. The rat diet of these workers lacked besides pantothenic acid, the following vitamins: inositol menadione, PAb, biotin, folic acid, a-tocopherol, ascorbic acid and vitamin B_{12}; the diet of the ducklings lacked PAB, B_{12}, ascorbic acid, inositol besides pantothenic acid. Klein and Lipmann (137), in investigating the relationship of coenzyme A to lipid synthesis, fed their animals the diet of Riggs and Hegsted (114), to obtain pantothenic acid deficiency in their animals. This diet was also multideficient since it lacked besides pantothenic acid, the same vitamins as did the diet of Olson and Kaplan (121). These authors observed a decrease in coenzyme A concentration of the liver after analysis was made using the method of Kaplan and Lipmann (133).

Olson and Stare (146) studied the metabolism in vitro of cardiac muscle in pantothenic acid deficiency. The animals used were ducklings, which were fed the diet of Olson and Kaplan, less pantothenic acid. Coenzyme A was analysed for its concentration in the cardiac auricle and ventricle, and found to be reduced by 40 to 80%. They

also pointed out that in both the normal and deficient animals, the ventricle contained twice as much COA as did the auricle.

Boyd (136) studied the possible role of coenzyme A in the biosynthesis of cholesterol in the rat. His basal diet was supplemented with thiamine hydrochloride, riboflavin, nicotinamide pyridoxine hydrochloride, biotin, folic acid, menadione, inositol, PAB and choline chloride: his supplemented diet lacked ascorbic acid, and vitamin B_{12}. Their conclusions were that when their animals were maintained on a fat free diet, the liver ester cholesterol concentration and the plasma ester cholesterol concentration appeared to be dependent on dietary pantothenate and hence on the coenzyme A content of the tissues.

The influence of dietary pantothenic acid and methionine on rat liver coenzyme A was studied by Dinning et al. (138). These authors fed graded levels of methionine with and without pantothenic acid. Rats receiving the diet deficient in both pantothenic acid and methionine exhibited very low levels of liver coenzyme A. Supplementation of the diet with either pantothenic acid or methionine resulted in an increase in coenzyme A. Both were

required to restore the coenzyme A level to normal. In this case no diet was stated. Their conclusions were that it seemed probable that dietary methionine served as a precursor of the thioethylamine moiety of coenzyme A.

It has been observed by R.E. Olson (147) that dietary restriction of the sulfur amino acids may depress hepatic coenzyme A levels in the rat as much as the dietary restriction of pantothenic acid. The diet fed animals was that of Himsworth and Glynn (Clinical Science 5: 1944) (148), which was also multi-deficient.

Within 5-7 weeks, 75-90% of the rats fed the basal diet developed typical hepatic lesions which could be prevented by a-tocopherol, methionine, but not entirely by cystine in the absence of vitamin E. Since coenzyme A contains thioethylamine, they stated that according to their results, a reduced sulphur amino acid intake may be a limiting factor in coenzyme A synthesis.

Hoagland and Novelli (149) found that for the biosynthesis of coenzyme A, both pantothenate and cysteine are required, but that cystine itself is inactive. The following compounds failed to replace cysteine: homocysteine, thioacetate thioglycolate, mercaptosuccinate, b-mercaptoethylamine and H_2S.

The effect of coenzyme A precursors, e.g. pantetheine, panthetine, N(d-pantothenyl-l-cysteine), N(d-pantothenyl-cystine), and pantothenate on hepatic COA levels in pantothenic acid deficiency in organic sulfur deficient rats was studied recently by Ching-sing Yang et al. (150). They indicated that dietary restriction of the sulfur-amino-acids may depress the hepatic coenzyme A levels. The pantothenic acid deficient animals responded to all compounds with increases in hepatic coenzyme A although pantothenate was the most effective. In the animals deprived of organic sulfur, however, none of the above compounds was effective. They, therefore indicated that the synthesis of hepatic coenzyme A occurs again from nutrients and other small molecules and that the capacity for this synthesis is impaired in sulfur amino acid deficiency in the rat.

Lipmann's coenzyme A plays an important role in the acceptor system of choline acetylation (151); on the other hand cocarboxylase, a thiamine derivative is responsible for the donor system. This last factor was studied by Beznák and van Alphen (122), who had correlated cardiac automatism and choline acetylation in rats on thiamine and pantothenic acid diets, as well as on a diet

deficient in both thiamine and pantothenic acid. Changes in cardiac automatism (e.c.g.) were found only when the diet was deficient in thiamine either alone or in combination with pantothenic acid. On a uni-deficient pantothenic acid diet, no e.c.g. discrepancies were observed. The choline acetylation of the acetone powders of the hearts did not change in any of the groups.

In 1939, Nelson (110) studied various symptoms occurring in pantothenic acid deficiency including pathological changes in the heart. Myocardial lesions, focal polymorphonuclear infiltration and focal coagulation necrosis were all noted. In this case the diet fed the animals was far from being complete. The basal diet consisted of the following ingredients: casein, cod liver oil, Wesson oil, salts and sucrose, and was supplemented with riboflavin and thiamine chloride; nicotinic acid was added in the above diet for another group of animals. A few years later, Supplee et al. (60) described sudden death and cardiac hypertrophy with auricular hemorrhage in the rat. Their basal diet had been supplemented with thiamine, riboflavin and pyridoxine only. Schaefer et al. (153) experimenting with dogs fed a pantothenic acid deficient diet found that the hearts of their animals

developed usually a rapid heart rate, besides other symptoms such as sudden prostration, convulsions and gastro-intestinal injury.

Egg white injury as a clinical manifestation of biotin deficiency was produced in rats as early as 1927, when Miss Boas of the Lister institute of London found that rats fed a special diet containing much uncoagulated egg-white developed peculiar and impressive skin changes, which finally led to a fatal outcome.

The most characteristic symptom, as already stated in the history of biotin, seems to be a progressive dermatitis. Emerson and Wurtz (132) indicated that symptoms of biotin deficiency induced in young rats by the administration of succinylsulfathiazole, may be aggravated by simultaneous pantothenic acid deficiency. Symptoms attributed to a pantothenic acid deficiency were reported, by Wright and Welch (154), when rats were fed a highly purified diet which included thiamine, riboflavin and pantothenic acid and was supplemented with succinylfulfathiazole. A marked reduction of pantothenic acid was observed in the liver of the above rats. Biotin and folic acid relieved the symptoms.

Heart changes in biotin deficiency were described by Goddard and Olson (155). Decreased oxygen uptake of myocardial slices from biotin-deficient ducks in the presence of substrates such as succinate or pyruvate, was reported by Olson et al. (156).

Wong and Schweiger (142) recently stated that when B_{12} was deficient in the diet, COA was found to be 2-3 folds more concentrated. That B_{12} was left out of most so-called pantothenic acid deficient diets is seen by giving close scrutiny to the past experimental diets. The question as to whether the presence of B_{12} in a completely uni-deficient pantothenic acid diet would change the concentration of the latter in the liver is still doubtful.

The participation of biotin in coenzyme and enzymatic compounds was indicated by different workers relating it to the synthesis of aspartic (17) (18) (19) and also to the oxidation of pyruvic acid (20).

Survey of the literature shows clearly that pantothenic acid deficiency symptoms, especially cardiac ones and a diminution in COA content of organs, were only described on multi-deficient diets. It remained still to be determined whether or not a uni-deficient pantothenic

acid diet would produce symptoms and lower coenzyme A level in the rat and heart and whether or not e.c.g. patterns would be affected by a singular pantothenic acid deficiency.

For at least two reasons it seemed interesting to extend our investigation on biotin. In the earlier works in which cardiac involvements were mentioned, biotin and pantothenic acid were both missing from the diet, thus the cardiac involvements might be the consequence of the combined deficiencies only. And as there are no investigations into the effects of a singular biotin deficiency on the cardiac automatism i.e. on e.c.g., it is not known whether a singular biotin deficiency would not have an effect.

The other reason is a certain amount of similarity between the molecules of biotin and pantothenic acid. This renders possible that the two compounds might mutually provide a part of their molecules to one another. Thus the thioethyl moiety of pantothenic acid might be derived from biotin and vice versa, this part of the pantothenic acid molecule might provide groups to the sulphur containing ring structure of biotin.

To obtain some information on these questions, the following experiments were carried out:

1. Olson and Kaplan's experiments, with a multi-deficient diet, were repeated in order to see whether the diminution in the COA content of the liver and heart described could be repeated in our strain of rats and experimental conditions. E.c.g. records were also made in order to see whether cardiac automatism was affected more by this multi-deficient diet than by our singularly deficient one.

2. Growth rate, dermal symptoms and e.c.g. were recorded in 4 groups of rats and the COA content of their liver and heart determined at different phases of development.

The diet was completely synthetic in all the groups which were as follows:

(a) normal

(b) no pantothenic acid (-PA)

(c) no biotin (-B)

(d) no biotin and no pantothenic acid (-PAB).

EXPERIMENTAL

The following precautions were taken to prevent refection:

a) The animals lived in single ½" mesh wire bottomed metal cages.

b) All food containers, water bottles, walls of cages were checked and cleaned twice weekly.

c) The synthetic diet when containing pantothenic acid and biotin ensured good growth, freedom from dermal symptoms and very few e.c.g. "abnormalities".

REPETITION OF PART OF OLSON AND KAPLAN'S EXPERIMENTAL

INTRODUCTION

As already indicated by Olson and Kaplan (121), one of their prime objectives was to determine the effect of dietary restriction of pantothenic acid upon the coenzyme A content of tissues from rats and ducks. Only rats were used in this experiment and analyses of the rat livers and hearts were made for coenzyme A, besides studying e.c.g. patterns of the rat hearts during the experimental period.

CARE AND MAINTENANCE OF ANIMALS

A group of 20 animals was used for this experiment: all animals were of the Albino Wistar strain, male rats of an average weight of about 60 gms. at start.

On arrival from the dealer the 20 rats were put into a large cage, fed purina fox chow for $3\frac{1}{2}$ days. There was crowding in the cage and it is possible that the rats did not have optimum feeding conditions.

After this period the e.c.g.'s were recorded in each rat, these data are referred to as 0 time in the graphs. The normal 10 rats were placed in the same large cage and were given our complete synthetic diet in 3 pots. The other 10 rats were caged individually and were given the Olson Kaplan

PA multideficient diet. Three weanling rats were sacrificed at the beginning of the experiment in order to determine the normal e.c.g. and COA values of the heart and liver of the animals.

DIET FED AND DURATION OF EXPERIMENT

The diet fed the control animals contained the following ingredients:

Vitamin test casein	- 18%
Sucrose	- 68%
Vegetable oil	- 10%
U.S.P. salt mix. No.2	- 4%

	grams/100 lbs. diet
Vitamin A concentrate (200,000 units per gram)	4.5
Vitamin D concentrate (400,000 units per gram)	0.25
Alpha Tocopherol	5.0
Ascorbic acid	45.0
Inositol	5.0
Choline Chloride	75.0
Menadione	2.25
P-Aminobenzoic Acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine HCl	1.0

Thiamine HCl	1.0
Calcium Pantothenate	3.0

	mgms/100 lbs. diet
Biotin	20
Folic acid	90
Vitamin B_{12}	1.35

The experimental group was fed the diet of Olson and Kaplan which was composed of the following ingredients:

Casein	- 18%
Glucose	- 73%
Corn Oil	- 3%
Cod liver oil	- 2%
Salts[1]	- 4%

B- complex factors in crystalline form were supplemented in the following quantities:

Thiamine	- 400 γ per 100 gm.
Riboflavin	- 800 γ " " "
Pyridoxine	- 400 γ " " "
Niacin	-2000 γ " " "
Choline Cl.	- 100 mg. " " "

The duration of the experiment was of 9 weeks. COA determinations were made at 3, 5 and 9 weeks. All animals were fasted 24 hours before the determination.

[1] (Hegsted, D.M., Mills, R.C. and Elvehjem, C.A. -1941- J. Biol. Chem. 138: 459.)

METHOD USED FOR COENZYME A DETERMINATION AND E.C.G. RECORDING

The same method as previously employed by the above authors, that of Kaplan and Lipmann (121), was used to measure the coenzyme A content of the heart and liver of the rats. A Hilger-Spekker absorptiometer using a wave length of 570 mμ and 1/4 cm. cells were used.

The electrocardiograms were taken once a week as described by Beznák and van Alphen (122). R-R time, P-wave, R-voltage and T-wave were studied.

RESULTS AND DISCUSSION

A. COA determinations.

The results obtained from the above experiments are shown in Figs. 1 and 2. Figure 1 shows the COA content of the liver, Figure 2 that of the heart of normal and deficient animals. As can be seen from Figs. 1 and 2, of both the liver and heart, the COA content for the normal animals is increased from the beginning of the experiment to the third week to a plateau. After 3 weeks, the COA content remains constant to the 9th week. The stationary value of the liver is 135 L.U., the high asymptotic 220 L.U. In the heart less COA was found. At the beginning of the experiment 30 L.U., after the third week, 60 L.U.

In the case of the rats on the multi-deficient diet of Olson and Kaplan, a steady drop in liver COA was observed from the start of the experiment to the 9th week reaching a minimum concentration of 40 L.U. The COA

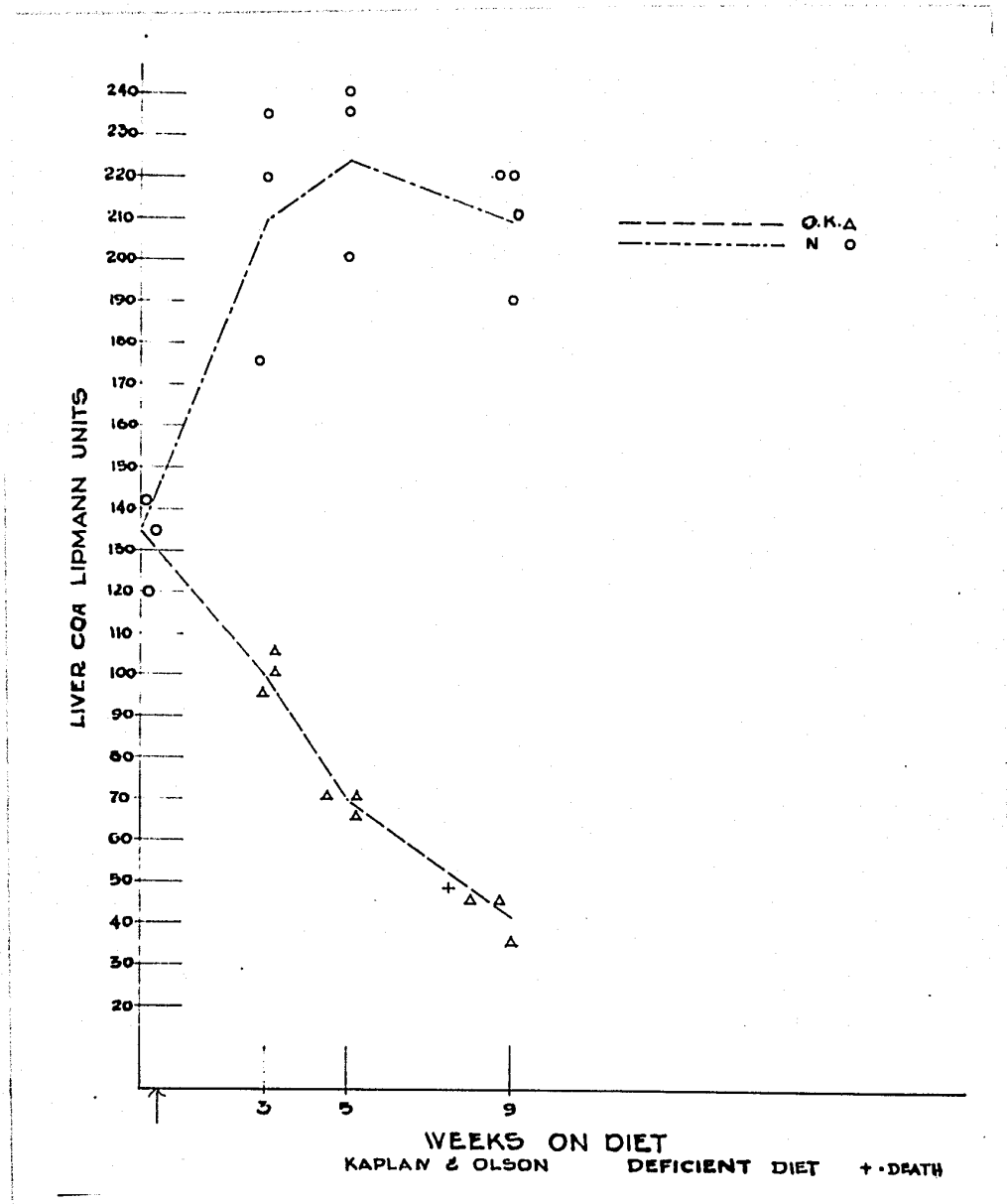


Figure 1. Changes in the COA content of the liver of normal and Olson and Kaplan deficient animals.

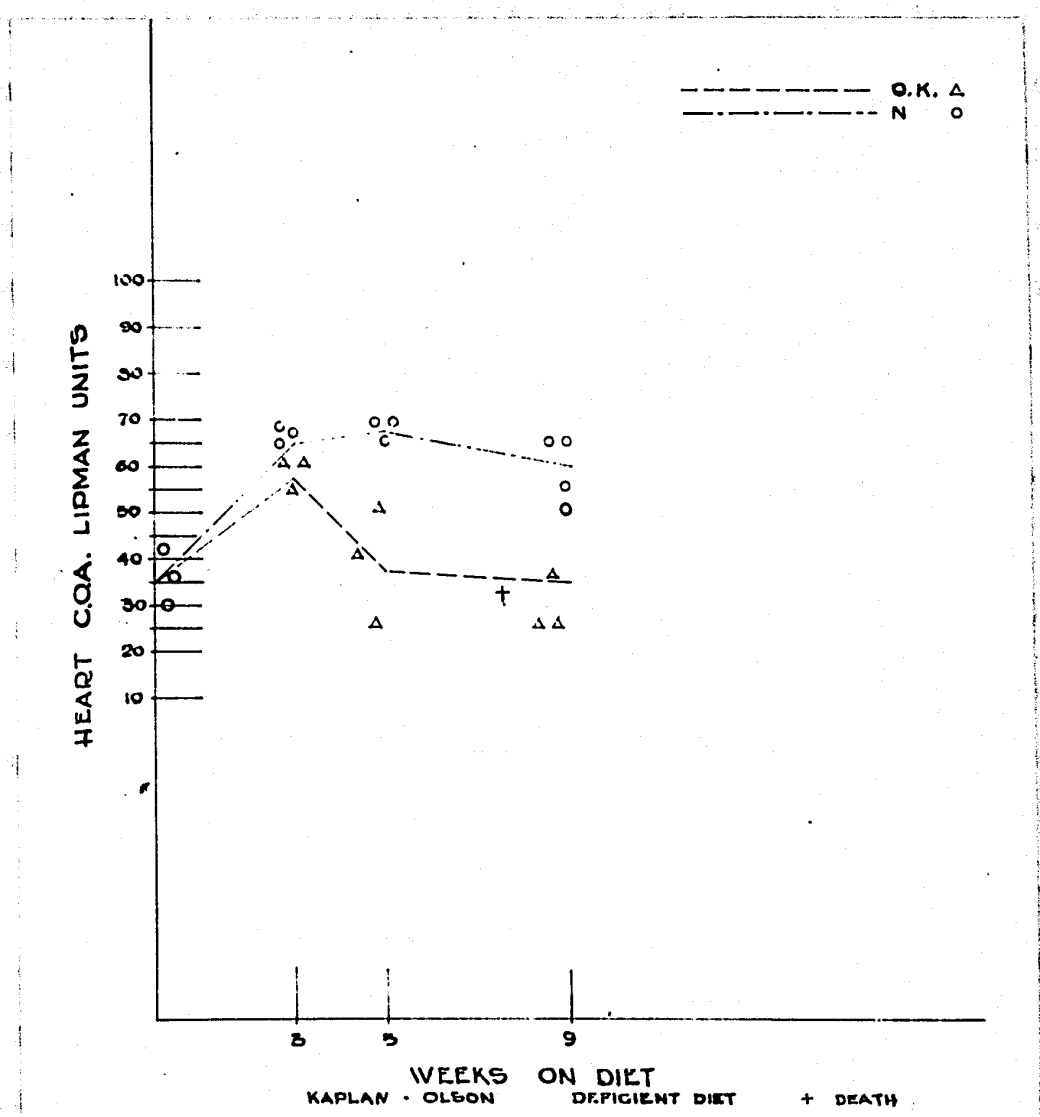


Figure 2. Changes in the COA content of the heart of normal and Olson and Kaplan deficient animals.

concentration dropped to 4/5th of the normal value. The heart COA did not drop from the beginning to the end of the third week, rather it increased as the normal ones did, though perhaps somewhat less. At the end of the 5th week and in the 9th week, however, significantly less COA was found in the deficient hearts than in the normal ones, instead of 60 L.U., only 30 L.U. were observed.

The growing heart,then in the early stages of the deficiency, is capable of increasing its COA content on a pantothenic acid multi-deficient diet whereas the liver is not. Also while in 8 weeks on a deficient diet the liver COA content falls to 1/5th of the normal value, that of the heart is only 1/2 of it.

The frequency distribution histograms for the liver and heart COA content are shown in figures 3 and 4 respectively. It will be seen that while there is no overlap between the COA content of the liver of normal and deficiently fed rats, the values of the heart COA overlap considerably.

We have already seen that the heart COA in rats on the deficient diet begins to diminish only after a longer period than does that of the liver and that diminution reaches its asymptot sooner and is smaller than in the liver. Added to these is the overlap between the two

Figure 3. Histogram of the liver COA for both the normal and Olson and Kaplan deficient animals.

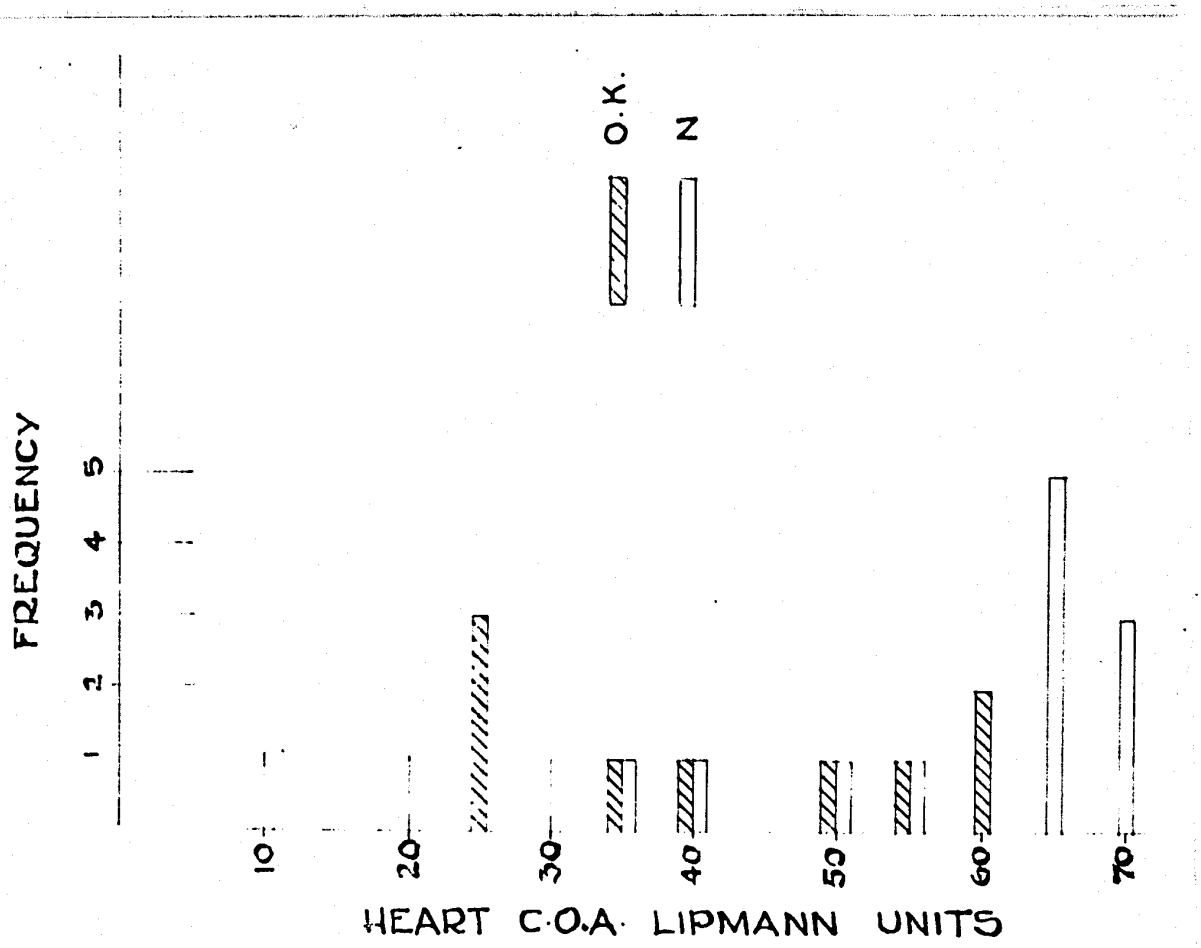


Figure 4. Histogram of the heart COA for both the normal and Olson and Kaplan deficient animals.

populations. The conclusion then is warranted that the heart COA is much more resistant to nutritional deficiencies than is liver COA. It may be that the liver is a supply store of pantothenic acid or COA.

The statistical significance of the difference in the COA content was tested with the 't - test'. In this test all the normal rats (including the 3 weanling ones) and all the deficient ones were included to form their respective groups.

Table I. Liver COA in rats 3 to 9 weeks on the Olson and Kaplan diet and in normal rats.

Group	n	Mean	Degrees Freedom	't'	P
N	13	195.7			
			20	8.97	< 0.001
O.-K.	9	70.0			

Table II. Heart COA in rats 3 to 9 weeks on the Olson-Kaplan diet and in normal rats.

Group	n	Mean	Degrees Freedom	't'	P
N	13	57.3			
			20	4.54	< 0.0001
O.-K.	9	41.6			

As can be seen from these tables, the diminution in the COA content of both the liver and heart is statistically highly significant.

A number of symptoms were recorded in this experiment and are as follows:

B. DERMAL SYMPTOMS

a) Alopecia.- In all animals, mild alopecia appeared between the end of the second and third weeks.

We observed that loss of fur was a general symptom in all the deficiency groups. It followed a general pattern. The alopecia started to appear on the hind legs, proceeded to the front limbs, then it affected the posterior abdominal region spreading towards the anterior abdominal part of the animal, finally reaching the neck region. We measured the degree of alopecia on the basis of the progressive pattern produced by the loss of fur during the deficiency feeding period.(Plate I.).

1st stage: loss of fur on hind limbs only.

2nd stage: loss of fur on all four limbs and slight spreading on the posterior part of the abdomen.

3rd stage: loss of fur on all four limbs and complete alopecia of abdominal region.

4th stage: Alopecia was observed on all four limbs, abdomen, neck region, and in come cases on the rump of the animals.

In order to gain information about the behaviour of alopecia during the experimental feeding the averages of the stages were calculated each week and plotted against time. (Fig. 5)

As will be seen in this figure the loss of hair was very conspicuous from the beginning of the feeding of the deficient diet and it developed very rapidly reaching the 3rd stage after 3 weeks on the diet.

b) Ruffled fur.- The ruffling of fur became distinct at the end of the third week generally. Some animals, however, were not affected by this symptom.

C. Porphyrin staining

b) Porphyrin staining had its start at the end of the third week. The bloody whiskers, as often called in the past, appeared first on both sides of the head in the ear region, reaching the neck and other parts of the body later, indicating perhaps that the substance ejected from the hardevian lachrymal glands was being secreted in

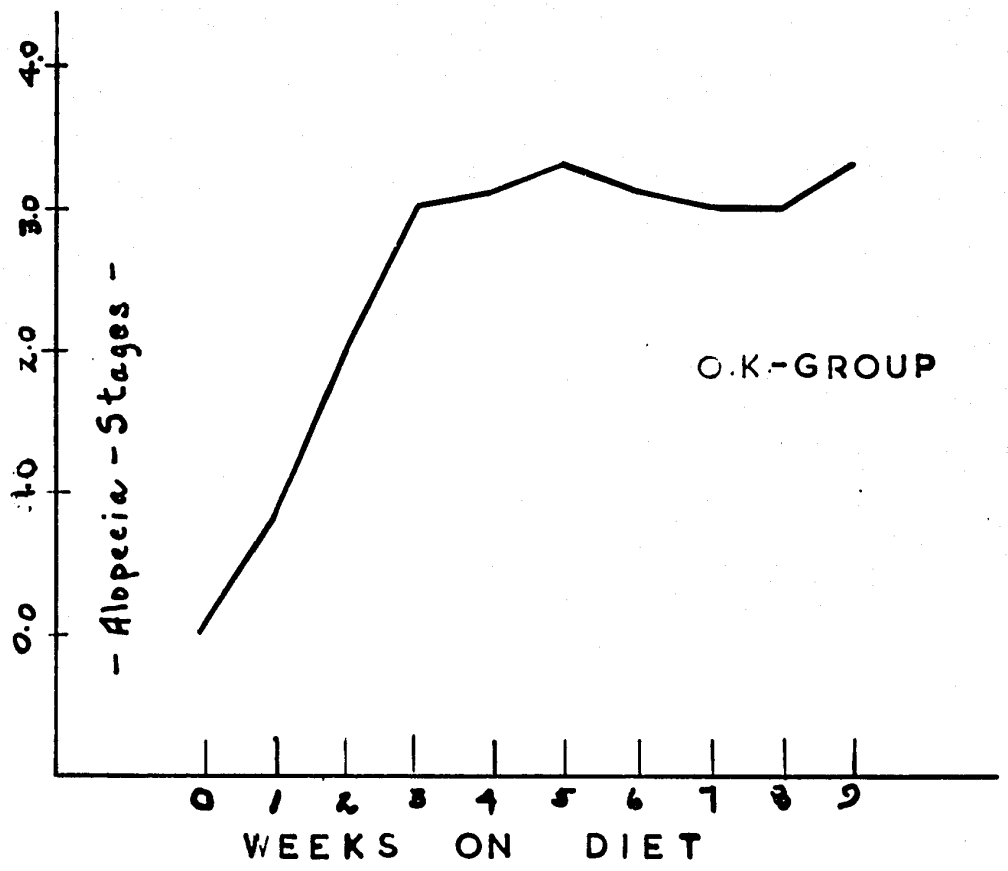


Figure 5: Stages of alopecia in the O.-K. deficient rats plotted against time.

greater amounts and thus was more available for spreading.

D. WEIGHT.

In Figure 6 can be seen the growth curve obtained for normal and deficient Kaplan and Olson animals. At the start of the experiment the animals averaged 66 gms. At the end of the 9th week, the normal animals averaged 284 gms. and the deficient rats 144 gms. From this figure it is seen that deficient animals kept growing but at a very reduced rate as compared to that of the normal ones.

The deficiency produced a stunting effect on growth.

E. E.C.G.

Beznák and van Alphen showed that the properties of the e.c.g. may show considerable variations in a population of normal rats. The question of e.c.g. changes on a diet must, therefore, be approached statistically. R-R time, voltage of the P, R and T waves were measured in the two groups of rats from the end of the 1st week at fortnightly intervals, till the end of the 9th week in each rat alive at the time.

The mean was calculated for each group and plotted against time (Fig. 7).

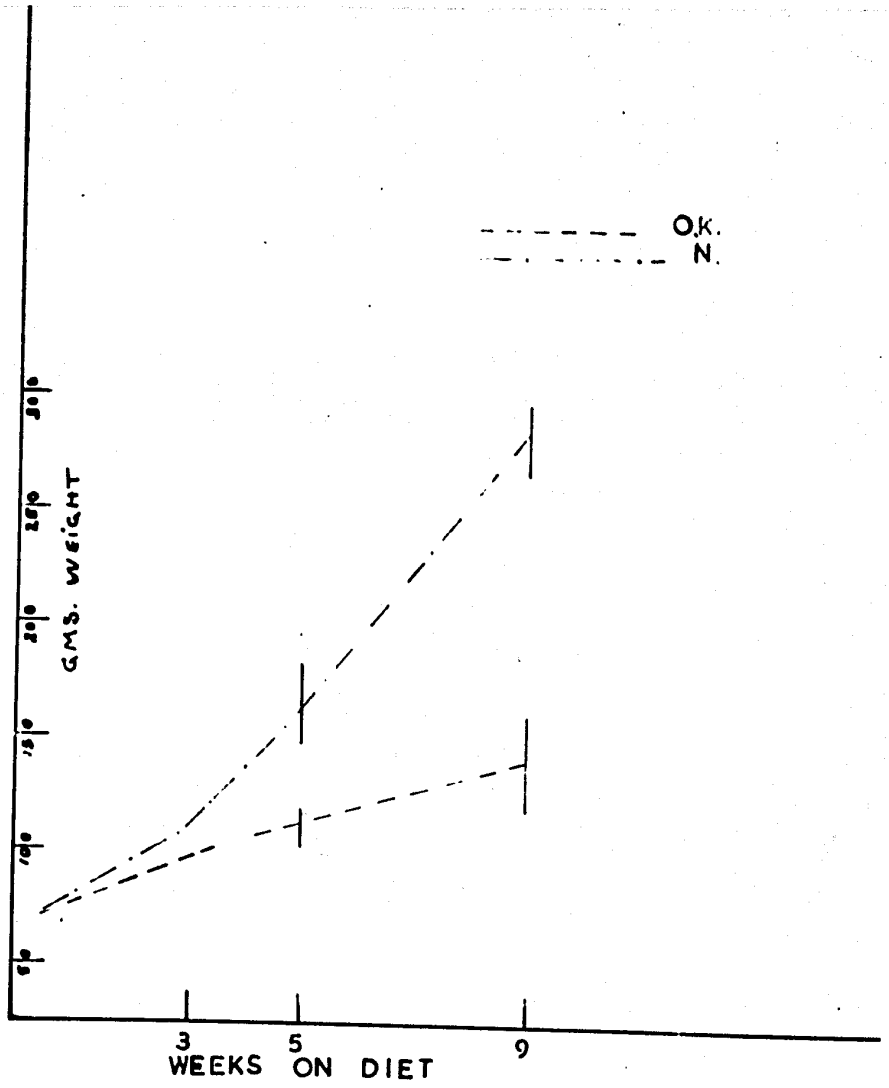


Figure 6. Growth curve for the normal and Olson-Kaplan rats, with twice standard deviation at 3rd, 5th and 9th weeks.

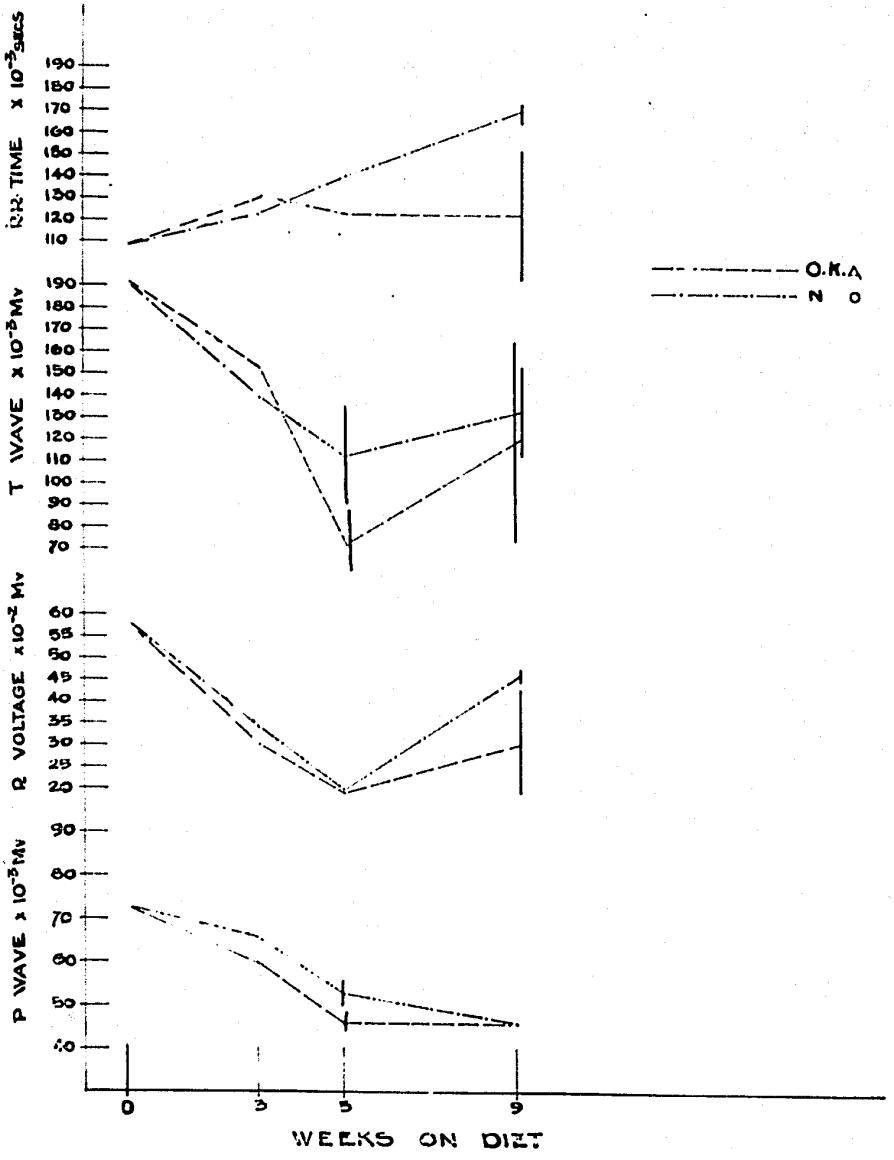


Figure 7. E.C.G. records*(R-R time, T-voltage, R-voltage and P-voltage) in rats on a complete (N) and Olson-Kaplan diet.

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* In all e.c.g. tables, mm.'s are given.
1 sec. = 23.5 mm.
1 mV. = 15 mm.

In the normal animal during the 9 weeks the R-R time increased steadily i.e. the heart rate diminished. To see whether this difference was due to the process of aging, 't' test was performed between the normal R-R time data at the start of the experiment and at the end of the 9th week. These results are given in Table III.

Table III. The difference between 1st and 9th week R-R times in normal rats

Group	weeks	n	Mean	Degrees Freedom	't'	P
N	1st	10	2.7			
				12	17.1	< 0.001
N	9th	4	4.1			

From the above data, we found that as the animal progresses in age the R-R time increases. In the case of the normal animals a great significance was found to be present between the 1st and 9th weeks.

Since the diet fed the animals was complete, the difference or acceleration in R-R time can only be due to the process of aging in the normal group.

As in the normal group, so in the deficient group the significance of the difference between the R-R times

at the beginning and at the end of the experiment was tested. As it can be seen in Table IV. the small slowing of the heart from 110 to 124 msec. has a P value between 0.02 and 0.010.

Table IV. The difference in R-R time between the 1st and 9th weeks in rats on the Olson-Kaplan diet.

Group	weeks	n	Mean	Degrees Freedom	't'	P
O.-K.	1st	10	2.7			
				11	2.80	0.02>0.01
O.-K.	9th	3	2.9			

Although there was a slowing of the heart rate in the rats on the Olson-Kaplan diet, the slowing was much less than that in the normal rats. In this latter group the R-R time was near to 170 msec. as against the 124 msec. in the deficiency fed rats. The difference has a very low P, see Table V.

Table V. The difference in R-R times between the normal and Olson-Kaplan animals at the end of the 9th week.

Group	weeks	n	Mean	Degrees Freedom	't'	P
N	9th	4	4.0			
				5	7.8	<0.001
).-K.	9th	3	2.9			

P, R and T voltages behaved principally the same in the deficient group as they did in the normal. There was, however, an apparent tendency in all the three waves to a greater diminution in the deficient animals than in the normals. Also the increase in the voltage during the second phase tended to be smaller in the deficient groups but none of these apparent differences received a statistical support. At the point of maximum differences, 't' test gave the following results: In the T-wave, the maximum of the differences occurred on an average at the 5th week but its P was greater than 0.1 (Table 6).

Table VI. 't' test on the difference in T-wave between normal and O.-K. groups at the 5th week.

Group	weeks	n	Mean	Degrees Freedom	't'	P
N	5th	3	1.77			
				4	1.73	>0.1
O.-K.	5th	3	1.1			

The difference in R- voltage was greatest at the end of the feeding period and its P value was also greater than 0.1 (Table VII).

Table VII. 't' test on the difference in R-voltage between normal and Olson-Kaplan groups at the 9th week.

Group	weeks	n	Mean	Degrees Freedom	't'	P
N	9th	4	6.9			
				5	1.5	>0.1
O.-K.	9th	3	4.6			

Similarly the maximum difference in the P-wave at the 5th week was proved to be due to chance (Table VIII).

Table VIII. 't' test on the difference in P-voltage between normal and Olson-Kaplan groups at the 5th week.

Group	weeks	n	Mean	Degrees Freedom	't'	P
N	5th	3	0.8			
				4	0.47	>0.1
O.-K.	5th	3	0.7			

We conclude that e.c.g. changes take place in rats on the Olson and Kaplan diet. The heart rate does not show the same slowing observed in normal rats with aging. Figuratively speaking, the deficient diet keeps the heart young in this respect. On the other hand as regards voltages of the P, R and T waves, while they all diminish in the normal rat with aging they apparently do more so

in the deficient animals, but only with a P around 0.1.

In this respect then the deficiency diet quickens or accentuates the aging process.

IRREGULARITIES OF E.C.G. PATTERN

The e.c.g. often shows irregularities in its pattern. Beznák and van Alphen obtained a quantitative comparison of the frequencies of these irregularities in their different rat groups by ascribing different weights to the different irregularities. Then they calculated the averages of the weights of irregularities for each group at different progressing times of the experimental period and plotted the results against time. They found with this method that the frequency of e.c.g. pattern irregularities increased in thiamine and thiamine plus pantothenic acid deficient rats from the 36th day of feeding onwards. This is the 91st day of extra-uterine life and is in the 3b phase of growth.* In their pantothenic acid deficient group a small increase in the irregularities was found but the difference between it and the normal group was statistically not significant.

We ascertained the frequencies of e.c.g. pattern irregularities with the method used by Beznák and van Alphen.

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* For explanation of the term 3b phase of growth see later parts.

The arbitrary units assigned to the irregularities in the pattern of the e.c.g. were as follows:

Normal pattern of the entire e.c.g. = 1.

Uncertain pattern of the entire e.c.g. = 2.

P - irregularities:

slightly wavy or absent = 3.

wavy = 5.

very wavy = 7.

QRS - irregularities

QRS deviations = 7 - 11.

T- changes

absent or negative = 13.

An inspection of Fig. 8 reveals the presence of irregularities of pattern in the e.c.g.-s of N rats as pointed out by Beznák and van Alphen (l.c.). The frequency of irregularities increases till the end of the 5th week of experimental feeding, therefrom it diminishes with great oscillation till the end of the experimental period. In the Olson Kaplan group it increases from the 1st to the last day on the experimental diet. The increase takes place on an exponential curve, at a fast rate up to the end of the

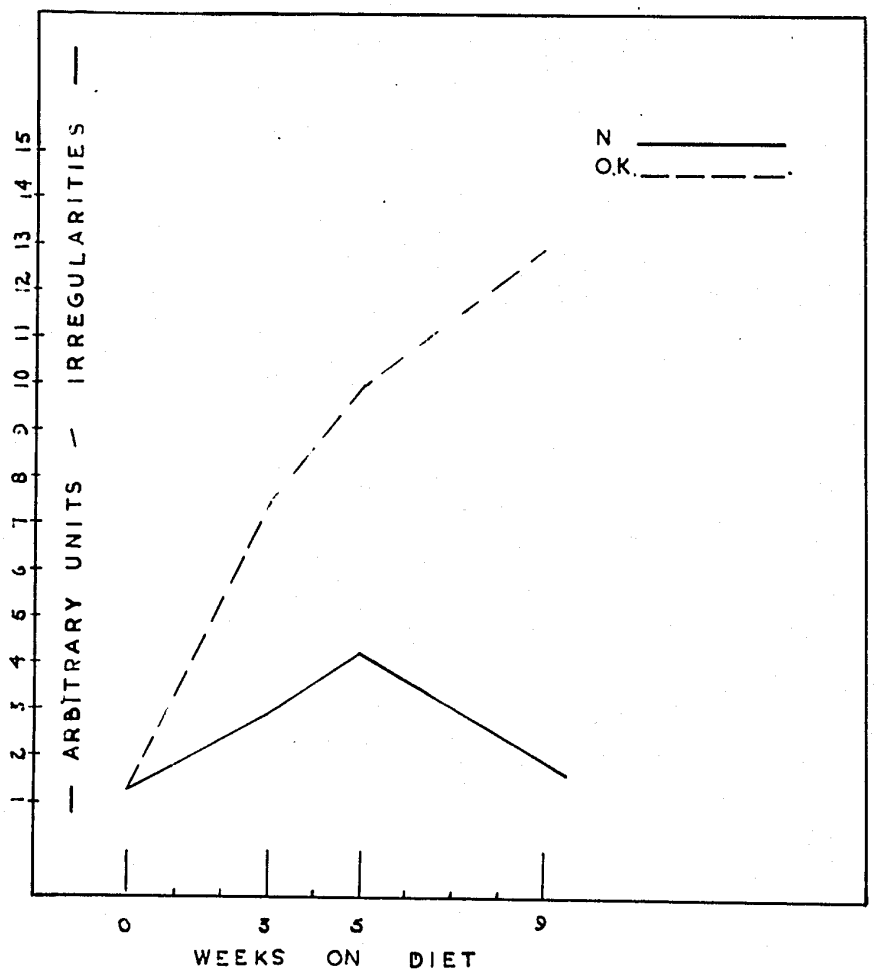


Fig. 8 shows the frequencies of irregularities on the e.c.g. pattern in rats on a complete synthetic diet and on the Olson-Kaplan PA-multi deficient diet, in progressing time.

3rd week, therefrom at a reduced rate but showing no tendency to reach an asymptote at the end of the experiment in the 9th week.

SUMMARY

1. On the Olson and Kaplan diet, deficient in pantothenic acid, inositol menadione, p-amino-benzoic acid, biotin, folic acid, B_{12}, -tocopherol and ascorbic acid, the following deviations from the normal development of rats were noted:

a. Growth was stunted.

b. Alopecia.

c. Porphyrin staining of the fur developed.

2. Olson and Kaplan's finding about the changes in the COA content of the liver and the heart were confirmed and extended. Liver COA steadily diminished throughout the feeding time of the deficient diet and it reached a very low level. Heart COA started to diminish later than liver COA, the diminution reached an asymptot and was smaller than that in the liver.

3. Changes took place in the e.c.g. in the course of the experimental feeding, these were:

a. An absence of the bradycardia which developed with age in the normal rats;

b. P. R and T waves tended to become smaller with age then they did in normal rats, but these latter changes had a high 0.2 P 0.1 value.

c. The frequency of irregularities as measured by the standards used by Beznák and van Alphen increased in the rats on the Olson Kaplan diets from the first to the last day of experimental feeding. The rate of increase was fast up to the end of the 3rd week, thence somewhat slower.

The e.c.g. and the CoA content of the liver and heart in rats on diets deficient only in pantothenic acid, biotin and pantothenic acid plus biotin.

1.) Introduction:

In the general introduction of this thesis, we pointed out that in most previous experiments on pantothenic acid deficiency, other vitamins were also lacking. Olson and Kaplan (121) described certain symptoms on such a diet and showed that the CoA content of the liver and heart of these rats is diminished. We could confirm the findings of these authors and made additional observations. It was found that heart CoA was more resistant than liver CoA, it was reduced to a smaller degree and more slowly. A change in the e.c.g. was also observed. The slowing of the heart rate present in normal rats was less pronounced in rats on the Olson and Kaplan diet.

In the following part of this thesis, experiments are described with diets from which pantothenic acid or biotin or biotin and pantothenic acid alone were missing. They answered the question whether these two uni-deficiencies and their combined deficiency cause changes in the growth, pelt, porphyrin staining, e.c.g. and CoA content of the liver and heart as do multi-deficient diets from which besides pantothenic acid, other factors are also missing.

2.) Care and Maintenance of Animals:

All animals used in this experiment were of albino Wistar strain. Only male rats were used, which at the start were at a weanling stage. The total number of animals used numbered 40, and were divided in 4 groups of ten.

The average weight of the rats was 50 gms. at the beginning of the experiment. All animals were placed in individual wire screen-bottom cages. The type of food containers were those designed by K.W. Franke. The same precautions against refection were taken for these experiments as were taken with the animals on the Olson-Kaplan diet.

All the animals both normal and deficient were allowed to eat their food and drink distilled water ad libitum throughout the experimental stage.

3.) Grouping of Animals; Diets:

The four groups of rats, 10 animals in each group were fed the following normal and deficient diets:

a.) Normal Group: /N/.

This group of animals was used for control and were fed a diet known to be adequate for good normal growth, containing all necessary ingredients. The diet had the following composition:

Vitamin test casein	.... 18%
Sucrose	.... 68%
Vegetable oil	.... 10%
U.S.P. salt mix. No.2	.... 4%

The basal diet was supplemented with the following ingredients:

	gms./100 lbs. diet		gms./100
Vitamin A concentrate (200,000 units/gm.)	4.5	p-amino-benzoic acid	5.0
Vitamin D concentrate (400,000 units/gram)	0.25	niacin	4.5
-tocopherol	5.0	Riboflavin	1.0
Ascorbic acid	45.0	Pyridoxine HCl	1.0
Inositol	5.0		
Choline chloride	75.0		
Menadione	2.25		
Thiamine HCl	1.0		
Calcium pantothenate	3.0		

	mgms./100 lb diet
Biotin	20.0
Folic acid	90.0
Vitamin B_{12}	1.35

b.) Pantothenic Acid Deficient Group: /Pa/.

The animals in this group were fed a uni-deficient, lacking in only pantothenic acid. All other ingredients found in the normal control diet were present.

c.) Biotin Deficient Group: /B/.

This group was fed a uni-deficient diet, deficient in biotin. The animals were fed this diet at the start of the experiment and 30 days before the end of the experiment, egg white flakes containing avidin were added in a ratio of 15 gms./100 gms. basal diet.

d.) Pantothenic Acid and Biotin Deficient Group: /PaB/.

This diet lacked two vitamins, pantothenic acid and biotin. All other necessary ingredients found in the normal diet were present.

4.) Duration of Experiment:

The experiment lasted 13 weeks. Liver and heart CoA determinations were carried out in small groups of animals at the following intervals: 8th, 11th and 13th week respectively.

5.) Method used for CoA determinations and e.c.g. recordings:

The coenzyme A content of the liver and heart of the animals was determined by the procedure of Kaplan and Lipmann (133), based on the enzymatic acetylation of sulfonamide by aged extracts of pigeon liver. Aging destroys the CoA of the pigeon liver extract, but leaves the apo-enzyme active.

Acetylation by the pigeon liver extract will then be the function of the CoA content of the unknown tissue extract added to it. The activity of the unknown extract is matched against that of a standard CoA solution.

A number of pigeons were decapitated and bled as completely as possible, the livers quickly removed and chilled until used. A volume of acetone dried with $CaCl_2$, 20 times the weight of the pigeon liver, was used for homogenization in a Waring mixer. Three quick washings were made on the acetone powder residue with anhydrous Merck ether.

The acetone powder was dried over phosphorous pentoxide and paraffin oil in a vaccum desiccator and kept in the refrigerator.

The preparation and aging of extracts was prepared strictly according to the method of Kaplan and Lipmann. A Hilger-Spekker Absorptiometer with a set wave length of 570 μμ and 1/4 cm. glass cells, was used for all determinations.

The electrocardiograms were taken once a week, as described by Beznák and van Alphen (122). R-R time, P-wave, R-voltage and T-wave were studied.

6.) Results.

a.) Body weight changes:

Figure 1 shows the average growth curves of the 4 groups of rats. The vertical lines are twice the standard deviation of the average at different periods of the experimental stage. It will be seen from the figure that all the 4 groups, normally and deficiently fed run at the same rate till the 15th day of experimental feeding, or 65th to 70th day of life. From this day on the N rats grew faster while the 3 deficient groups grew at the same slower rate till the 35th day. At this time the biotin uni-deficient group leaves the other 2 deficient groups, its growth is speeded up, henceforward grows as fast, later slightly faster, than the N does and finally reaches the normal asymptote. It is to be noted that feeding of egg white flakes from the 65th day on did not have an effect on the growth of these rats. At the 35th day, the growth of both the uni-deficient pantothenic acid group and of the combined pantothenic acid and biotin deficient group come to a stand still and remain so till the 84th day of the experiment. From then on a slight increase in growth resulted till the end of the feeding period.

Beznák, Beznák and Gaspár-Rády (157) divided the life cycle of the. . . . . .

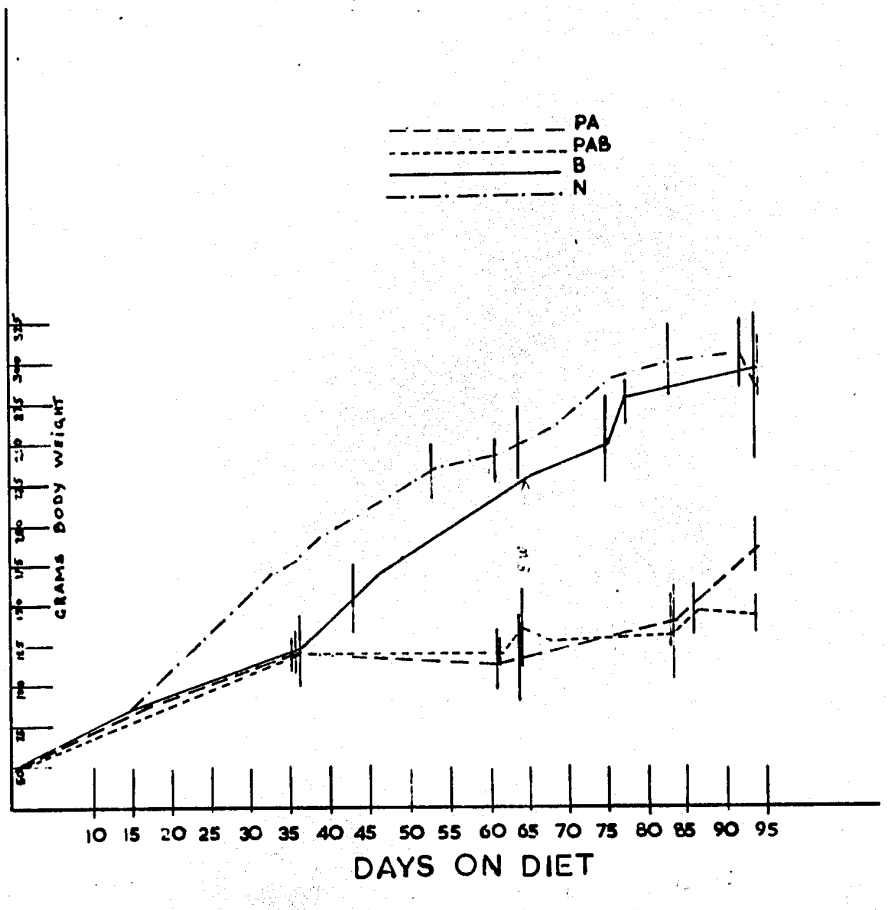


Figure 1: Growth curves for normal, pantothenic acid deficient, biotin deficient and pantothenic acid-biotin deficient animals. Body weight in gms. is plotted against time.

rat into 5 phases using different characteristics of the biology of growth. During the 1st phase of extrauterine life, which lasts from birth to the 30th day (b.w. 28 gm.), the growth velocity of Sachs is increasing; during the 2nd phase the increment of growth velocity rapidly diminishes, it reaches a plateau, then the growth velocity itself diminishes, 30-40th days (b.w. 28 to 38 gm.); during the 3rd phase which is divided into 2 phases (a) in which the diminution of growth velocity is great and (b) during which the diminution slows down and gradually reaches its lowest asymptote, 3a from 40th to 70th day (b.w. 38 to 110 gm.), 3b to the 160th day (b.w. to 240 gm.).

The weanling time falls into the beginning of the 3a phase. Most of the feeding experiments (our own equally) are generally conducted during the 3a and 3b phases.

b.) Other symptoms:

I. Alopecia developed in the same stages as described in the Olson Kaplan diet. Its development was measured in the same way with results as follows:

From the 2nd week on up to the 4th week a small but distinct loss of hair took place in all the deficiency groups. (Fig. 2). This was most marked in the biotin deficient rats. From this time on the PA and PAB curves

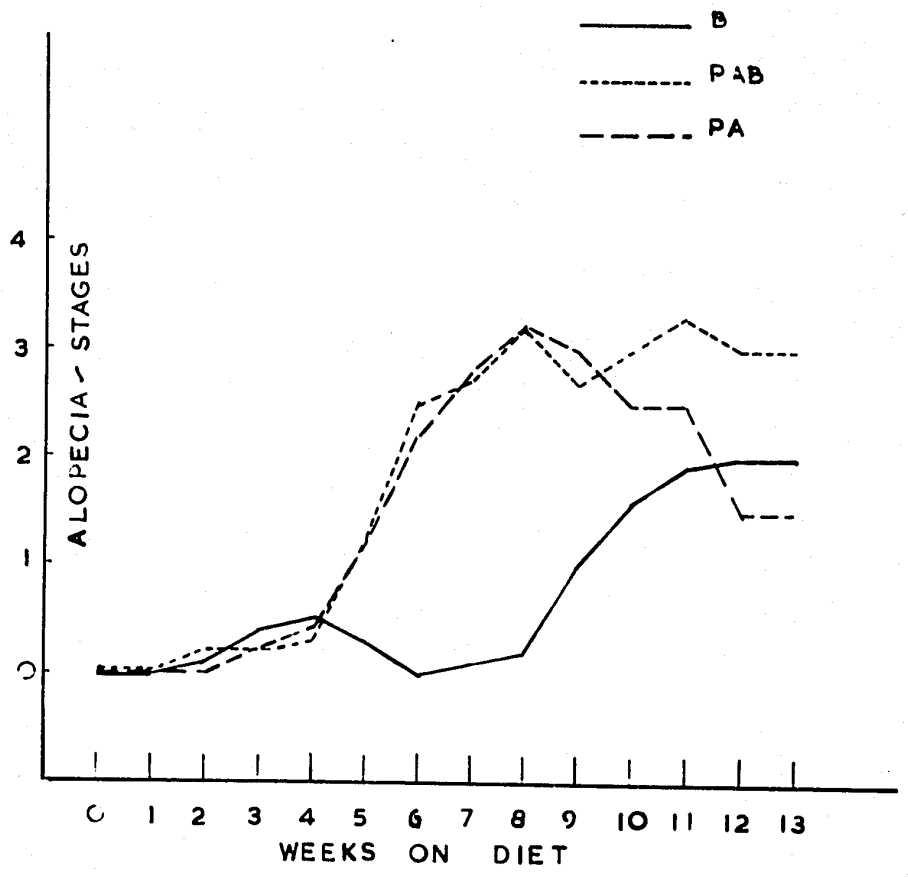


Figure 2: Alopecia stages in the pantothenic acid deficient, biotin deficient and pantothenic acid-biotin deficient groups plotted against time.

parted from the B curve. The former 2 rose sharply till the 6th week, whereas the latter one dropped to nothing. From the 6th to the 8th week the 2 PA curves rose slowly, the B curve remained low. From the 8th to the 13th week the PA curve fell, the PAB curve remained at the level it had already attained while the B curve rose very sharply till the 11th week then slowly.

The fall in the average of the alopecia curve of the PA rats was not due to a regrowth of hair but to the mode by which rats were killed. The selection of rats for killing at a given time was made according to the severity of the alopecia symptoms. As at the 8th week, when the 1st killing took place, and also at subsequent killings, the rats with alopecia stages 4 were eliminated and those with stages 1, 2, or 3 were allowed to survive, the average fell. The drop in the curve therefore means that the individual sensitivity of the fur to PA deficiency is very great in the rats.

In the PAB rats this drop in the severity of the symptoms from the 9th week did not take place. The explanation of this, bearing in mind what was said in the preceding paragraphs, is that the individual resistance of the hair to PA plus B deficiency is much smaller than to PA deficiency alone.

In the B group the stoppage of hair loss at the 4th week coincide with the resumption of growth, it is then most likely due to a supply of biotin by bacterial synthesis. It is interesting to note that the resumption of hair loss and its rapid increase took place when egg white was also fed, although the growth of the rats was not affected. It will be seen below that the frequency of abnormalities in the pattern of e.c.g. and the irritability of the rats also increased in this phase. It is justified to conclude that these life processes are more sensitive to biotin deficiency than growth.

II. Pantothenic acid deficient group.

α.) Edema - Various degrees of edema resulted from the beginning of the appearance of alopecia to the 4th stage of the symptom. The edema encountered however never reached severe proportions and was usually of a more or less mild nature.

β.) Ruffling of fur - Ruffled fur appeared usually before any signs of alopecia. The back of the neck and top of head of the animal were usually affected.

γ.) General weakness: As the animals progressed on the deficiency diet they became more slow

to react if stimulated and were weaker in general.

III. Biotin deficient group:

During the first stage of the feeding period, no egg white flakes had been added to the diet. After 4 weeks on the deficient diet, a slight ruffling of fur did appear, but disappeared in a week's time.

After the incorporation of egg white flakes in the biotin deficient diet, 30 days before the termination of the feeding period, certain changes did occur in the deficient animals.

α.) Alopecia - A week after adding the egg white flakes, to the basal diet, the trays below the wire screen bottom cages became covered with a thin layer of fur. As the animals progressed on the deficient diet, more hair accumulated in the pans. Not patching or complete loss of fur occurred however.

β.) Edema did develop in the nose region of some animals but never attained serious proportions.

γ.) Irritation - Towards the end of the feeding period, the biotin deficient animals

became very irritative and hard to manipulate.

IV. Pantothenic Acid Biotin Deficient Group:

In general it may be said that the symptoms encountered in this deficiency group followed much the same pattern as did those of the pantothenic acid deficient rats.

α.) Alopecia.- The loss of fur in this case appeared to be more severe than in the pantothenic acid deficient animals since alopecia was not confined generally to the abdomen as was the case in the former group, but appeared frequently in patches on the flanks and back of the rats as well at the end of the 3rd stage and during the 4th stage.

β.) Edema.- Edema was of a mild nature in all animals, during the entire deficiency period.

γ.) Ruffled fur.- As in the case of the pantothenic acid deficient rats ruffleness appeared usually before any alopecia was observed.

δ.) General weakness.- As the animals progressed on the deficient pantothenic acid-biotin diet, they became less active in response to stimuli, and showed a general

weakness.

V. Normal group:

No symptoms were ever noticed during the experimental stage in the normal animals. All animals grew well and remained healthy.

C.) Electrocardiographic Observations:

Since considerable variations in a population of normal rats do occur in e.c.g. patterns (122), statistical analyses of the R-R time, P-wave, R-voltage, and T-wave were made in all four groups

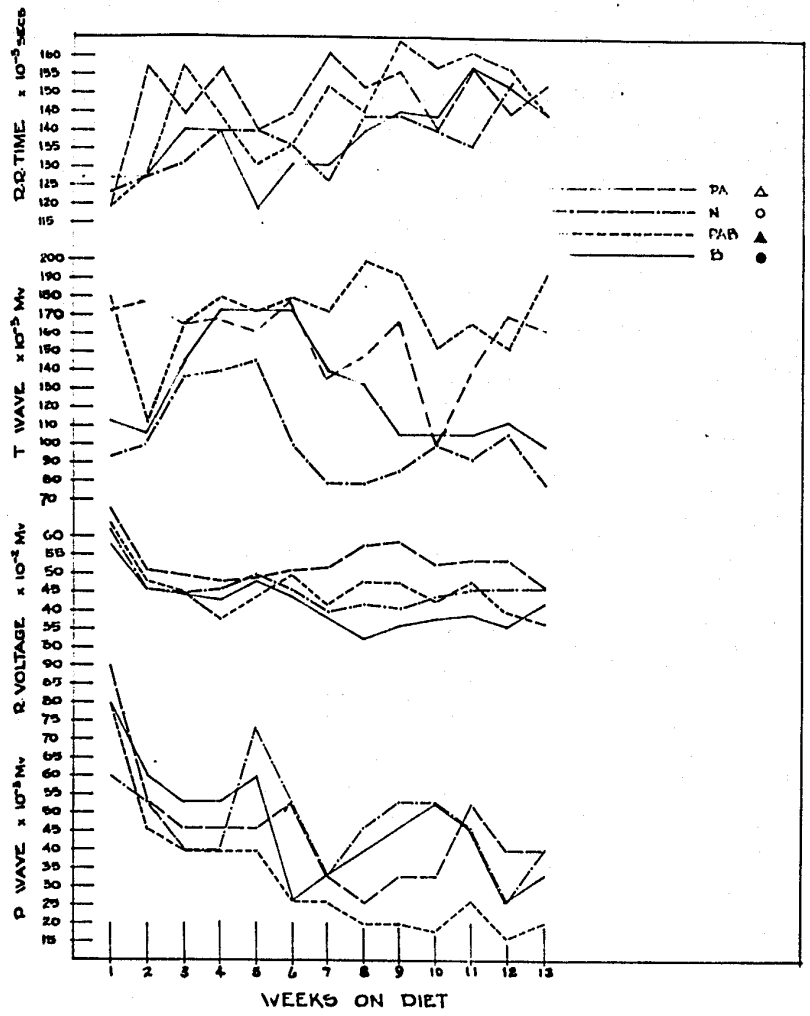
In figure 3, the mean of each groups was calculated and plotted against time.

1.) R-R time.

I. Variabilityin R-R time was measured at the beginning and end of the experiment to see whether differences existed between the normal and deficient groups.

Table I: Analysis of variance of R-R time in the 4 groups of rats for the 1st week of the feeding period.

Group	Source of variance	Degrees Freedom	Mean Squares	Variance ratio	F
N	Between groups	3	1130.6		
PA				1.09	>0.20
PAB	Within groups	36	942.0		
B					



GROUP	NUMBER OF ANIMALS												
	1	2	3	4	5	6	7	8	9	10	11	12	13
PA	10	10	10	10	10	9	9	9	4	4	4	2	2
B	10	10	10	10	10	10	10	10	10	9	9	5	5
PAB	10	10	10	10	10	9	9	9	6	6	6	4	4
N	10	10	10	10	10	10	10	10	8	8	8	6	6

Figure 3: e.c.g. records (R-R time, T-wave, R-voltage, and P-wave in rats on normal, pantothenic acid, biotin and pantothenic acid plus biotin, ... deficient diets.

Table II: Analysis of variance for the four groups of rats at the end of the feeding period.

Group	Source of variance	Degrees freedom	Mean squares	Variance ratio	F
N	Between groups	3	25.95		>0.01
PA				3.8	<0.05
	Within groups	12	6.71		
PAB					
B					

From the above data, it is seen that at the beginning of the experiment, when all the animals were still normal no statistical significance was found, between the variability of normal and deficient R-R times. The individual variability was randomly distributed in the 4 groups. At the end of the experimental period, the F value fell between 0.05 and 0.01, indicating a significant statistical difference in the variability between the normal and deficient groups.

II. Aging.

It was shown in Part I that the R-R time is prolonged during aging and that the aging prolongation is much smaller in rats on the Olson-Kaplan multi-deficient diet. 't' tests were performed for the three deficient groups of animals between the beginning and end of the experimental period.

a.) Table IIM shows that the difference in R-R time in the biotin deficient group between the 1st and last week, has a P value greater than 0.02 but less than 0.0i.

Table IIM: The difference between the R-R times in Biotin deficient rats at the 1st and last weeks of the experiment.

Group	n	Mean	Degrees Freedom	't'	P
B (1st)	10	2.9			
			12	2.7	0.02 > P > 0.01
B (14th)	4	3.3			

Here again we find that for the biotin deficient rats the difference in R-R time from the beginning of the experiment to the last week is statistically significant, and compares well with the results obtained with the normal animals.

Table IV: The difference between R-R times in pantothenic acid-biotin deficient rats at the 1st and last weeks of the experiment.

Group	N	Mean	Degrees freedom	't'	P
PAB (1st)	10	2.8			
PAB(13th)	4	3.4	12	3.3	0.0i > P > 0.00i

Table V: The difference between R-R times in pantothenic acid deficient animals at the 1st and last weeks of the experiment.

Group	n	Mean	Degrees Freedom	't'	P
PA (1st)	10	2.8			
			10	3.5	0.001>0.01
PA (last)	2	3.6			

Here again we found that the P value for both PAB and PA R-R times was greater than 0.01 which is statiscally significant.

All animals both deficient and normal experienced a slowing of the heart throughout the experiment. The three deficient groups had a P between 0.001-0.02 as compared to that of 0.05 for the normal ones. A test of significance of the differences between the variances was made, pooling the three variances of the deficient groups together to form one population and comparing these against the variance of the normal group. No significant statistical difference exists between the two groups.

Inspection of the curves of R-R time reveals that both the PA and PAB curves run closely parallel throughout the experiment. On the other hand the normal and biotin deficient curves follow much the same path. Two groups

were formed, PA and PAB as one and N and B as the other. Averaging the results of the R-R times obtained for the two different groups, we found that at the 2nd and 9th weeks, a difference existed between the two groups.(Fig. 4) 't' tests were done at the 2nd and 9th weeks and P was found to be between 0.02 and 0.05, for the 2nd week and 0.01 and 0.001 at the 9th week. It can be concluded that in pantothenic acid deficiency (whether singular or combined with biotin deficiency) the R-R time increases with age, on this general trend cyclic variations are superimposed, the increasing cycles start early and are greater in these animals than in normal or biotin deficient rats.

2. The T wave.

I.) Variability.- An analysis of variance in the 4 groups of rats 5 days on the experimental diets showed

TABLE VII: Analysis of variance of the T voltage of the 4 groups of animals at the 1st week of the experiment.

Group	Source of variance	Degrees Freedom	Mean squares	Variance ratio	F
N					
PA	Between groups	3	3.5	4.3	0.01
PAB	Within groups	34	0.8		
B					

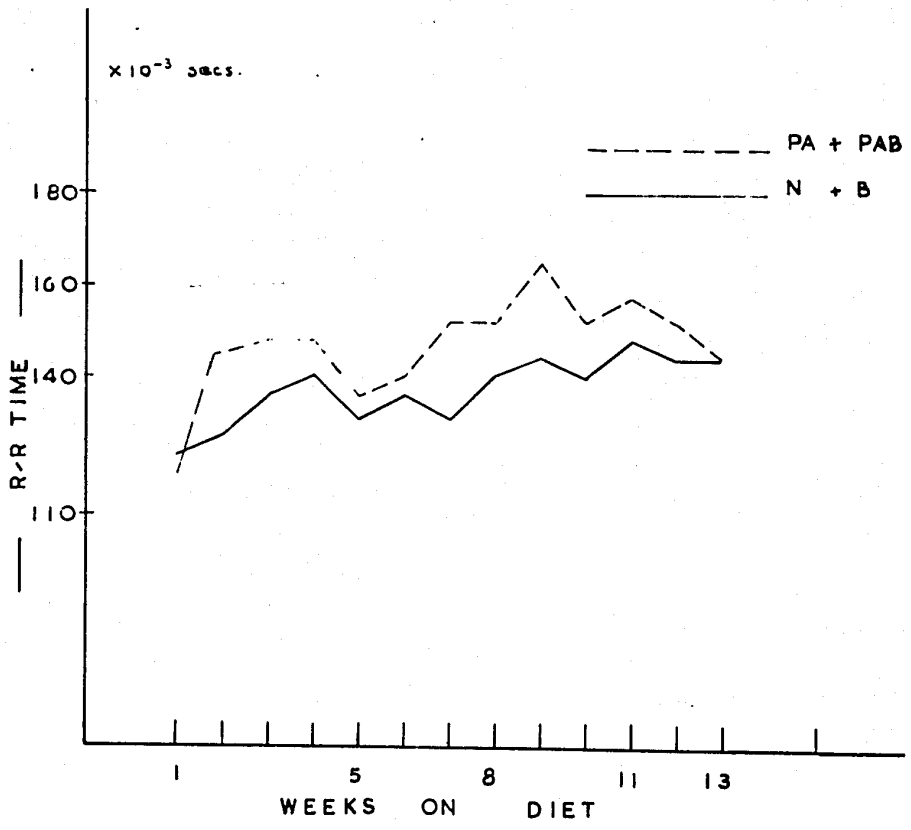


Figure 4: R-R time of the averages of the PA and PAB deficient groups as compared to that of the averages of the normal and Biotin deficient groups plotted against time.

that grouping by the different diets already unrandomized the total population of rats because the between group variance was significantly greater than the within group variance.

An analysis of variance on the values 13 weeks on the experimental diets still showed a significant difference of the between group variations over the within group variance indicating that dietary composition affects T voltage. (Table VII .)

Table VII : Analysis of variance of the T voltage on the 4 groups of animals at the 13th week of the experiment.

Groups	Source of variance	Degrees Freedom	Mean Squares	Variance ratio	F
N					
PA	Between groups	3	2.42	5.4	0.05>0.01
PAB					
	Within groups	11	0.445		
B					

II. The effect of the diets on T voltage.

The 1st e.c.g. records were taken on the 5th day of experimental feeding. At that time the 4 groups of rats formed two groups, in one the average T voltage was low in the other one it was high. The former group was

made up by the N and B, the latter by the PA and PA plus B rats. (Fig. 5). While there was no significant difference between the averages of the N and B rats, or PA and PA plus B rats the difference between the averages of the low voltage and high voltage groups was highly significant 0.001 P 0.0001. The conclusion from this is that PA deficiency (uni or combined with biotin) in the young rat iccreases T voltage even after 5 days on the diet.

The T wave voltage curves continue to retain their combination, PA and PA + B on the one hand and N and B on the other, in the type of their behaviours during the subsequent phases of the experimental feeding. There is one common feature in all the 4 dietary groups i.e. the presence of very great and small waves. T voltage rose in the N and B groups till the 4th week to a high level, it remained there for 2 - 3 weeks, thence it gradually descended to the original low value. To decide the question whether this rise was due to chance t tests were made on the differences between the averages of the 1st week and 5th week (i.e. the maximum point of the elevation). In the N group 0.2 P 0.1, in the B group 0.02 P 0.01, and in the pooled N + B group 0.02 P 0.01 were obtained. In the B deficient group then this was a real rise while in the N group there was a tendency only to a rise. In

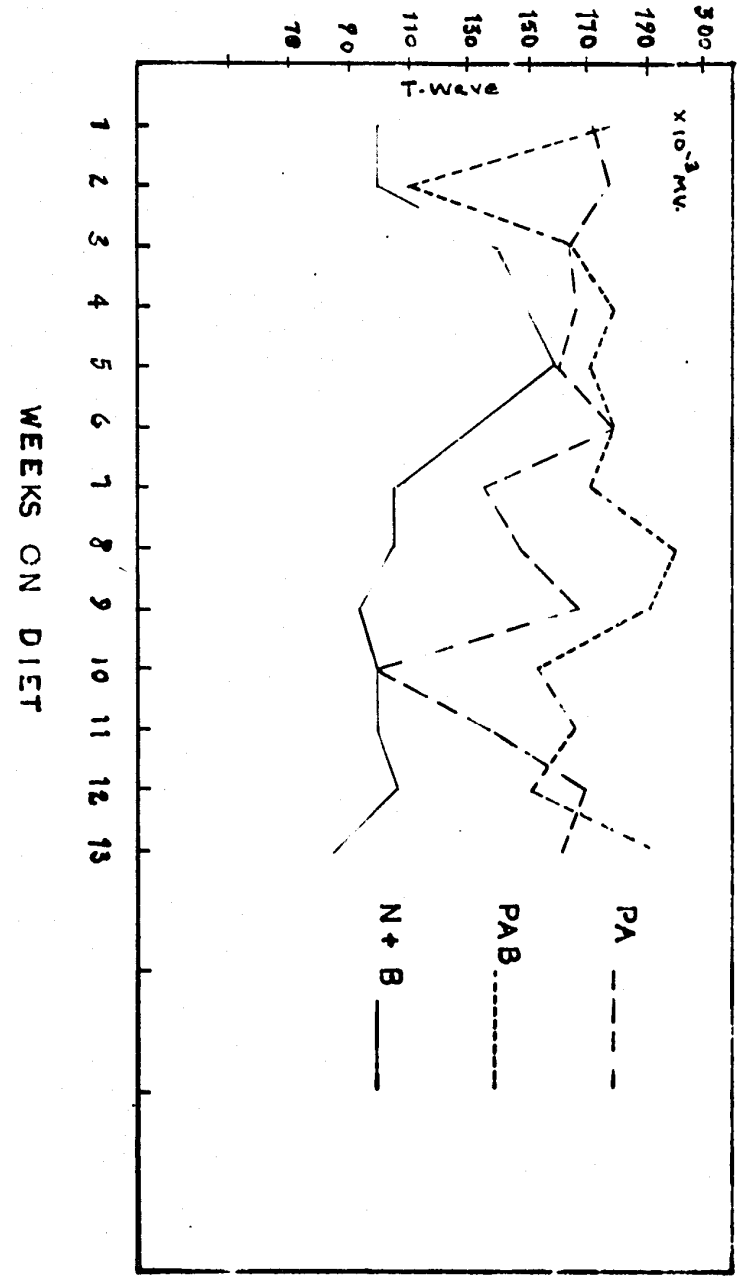


Figure 5: T-voltages of the average of the PA and PAB groups and of the combined N and B groups plotted against time.

the PAB group but for a sharp and deep fall in the 2nd week (0.017 P 0.001) it remained high through the experiment. In the PA rats it remained high during the 1st 6 weeks thence it showed great oscillations around a diminishing trend.

The mechanism by which the great oscillations in the averages in T voltage were caused during the late parts of the experimental feeding,(when at intervals a fraction of the rats was killed) was analysed as follows: It may be produced either by all individual rats, making up the averages at the several times showing simultaneously troughs or crests; or by killing those animals before a trough which have had high T voltages. To decide this question the T voltages against time curves for each rat in the PA group were plotted. (Fig. 6). A study of this graph shows that a combination of both factors brings about the great oscillations. At the 8th week 5 rats were killed 4 of them had T voltages above 150 mV, 1 rat had 100 mV. The surviving 4 rats had relatively low T voltages 3 of them below 150 and one 165 mV. The voltages then gradually, with individually varying phases, rose, so that when they were killed at the 11th and 13th week they all had voltages above 150 mV.

It is noteworthy that the selection of the animals for killing was made by the severity of the dermal (including alopecia), growth and general debility symptoms and not

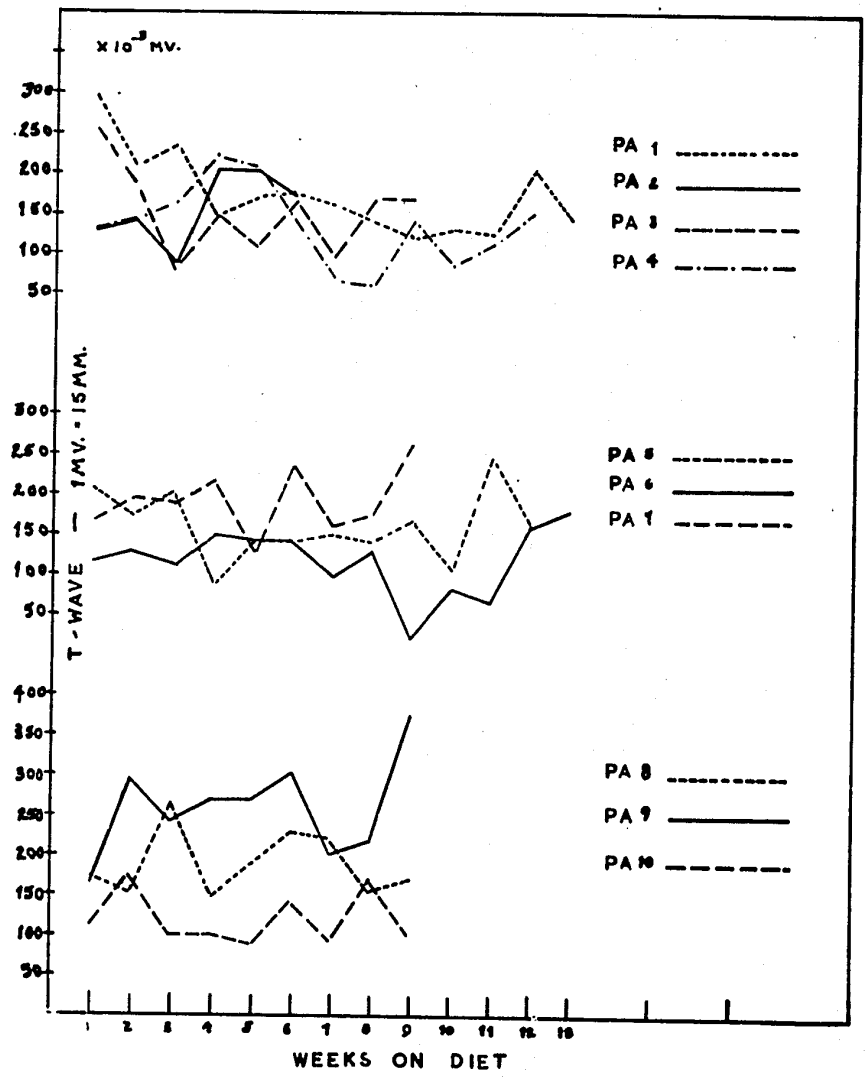


Figure 6: T-voltage of individual rats plotted against time for the entire experimental period.

by the e.c.g. changes. Since in both those animals which developed the symptoms early and in those which developed them later a relatively high T voltage was present the conclusion is justified that the elevation of T voltage is a consequence of PA deficiency.

III. Aging.

As regards aging of T voltage the N and B groups differed from the PA and PAB groups. T voltage in the N and B groups tended to be smaller at the 13th week than was at the end of the 1st week. The difference of the pooled results is small and its P is between $0.3 > P > 0.2$.

Table VIII: 't' test on the difference in T-wave between the 4 groups of rats at the 1st and 14th week respectively.

Group	n	Mean	Degrees Freedom	't'	P
1st week	38	2.03			
			51	1.14	>0.10
14th week	15	1.9			

In the PA and PAB groups T voltage, compared to groups N and B, is elevated already at the end of the 1st week and remained (with considerable oscillations in the case of the PAB group) high throughout.

From these results one can conclude that aging has a much smaller effect on T voltage than it has on the other 3 parameters, R-R time, R and P voltages. Aging does not alter the T voltage increasing effect of PA deficiency.

3.) R voltage.

I. In Fig. 4 R voltage was proved by an analysis of variance to have a variability unaffected by the different diets.

II. As regards the effect of aging on it and the effect of the different diets on the aging of R voltage it will be seen in Fig. 3 that R voltage diminishes with age in all the 4 groups. A series of t tests was carried out on the differences between the first and 13th week averages. In the N and PAB groups P was between 0.01 and 0.001, in the B and PA groups between 0.05 and 0.1. We may conclude justifiably that with progressing age R voltage diminishes. Pa, B and PA + B deficiencies did not modify in any way the effect of aging.

4.) Similar calculations were carried out on P wave voltage. The results of these were identical with that obtained for R voltage. P wave diminished with age and PA, B and PA + B deficiencies have no effect on the aging process.

IV. Irregularities in the pattern of the e.c.g.

These were measured with the Beznák van Alphen scale and plotted against time as was done in the repetition of the Olson Kaplan diet. (Fig. 7.)

The frequency of irregularities was found somewhat elevated in the normal rats at the end of the 2nd week; it showed a downward trend with great oscillations till the end of the experiment.

The phase of increase in the frequency coincides with the beginning of the 3a phase of growth. The gradual diminution of the frequency runs roughly parallel with the diminution in the growth velocity during the later phase of 3a and phase 3b. The biochemical basis of the greater frequency of irregularities is probably the degree of metabolic activity.

The graph also clearly shows that the frequency of irregularities is much greater in the 3 deficiency fed groups than is in the normal one. In all the 3 groups it rises from the 2nd week on, reaching its maximum at about the 4th week. From this time on the 3 deficiencies behave differently.

In the B deficient group the frequency of irregularities drops down to the normal level by the 9th week; therefrom it rises somewhat above the normal level till the

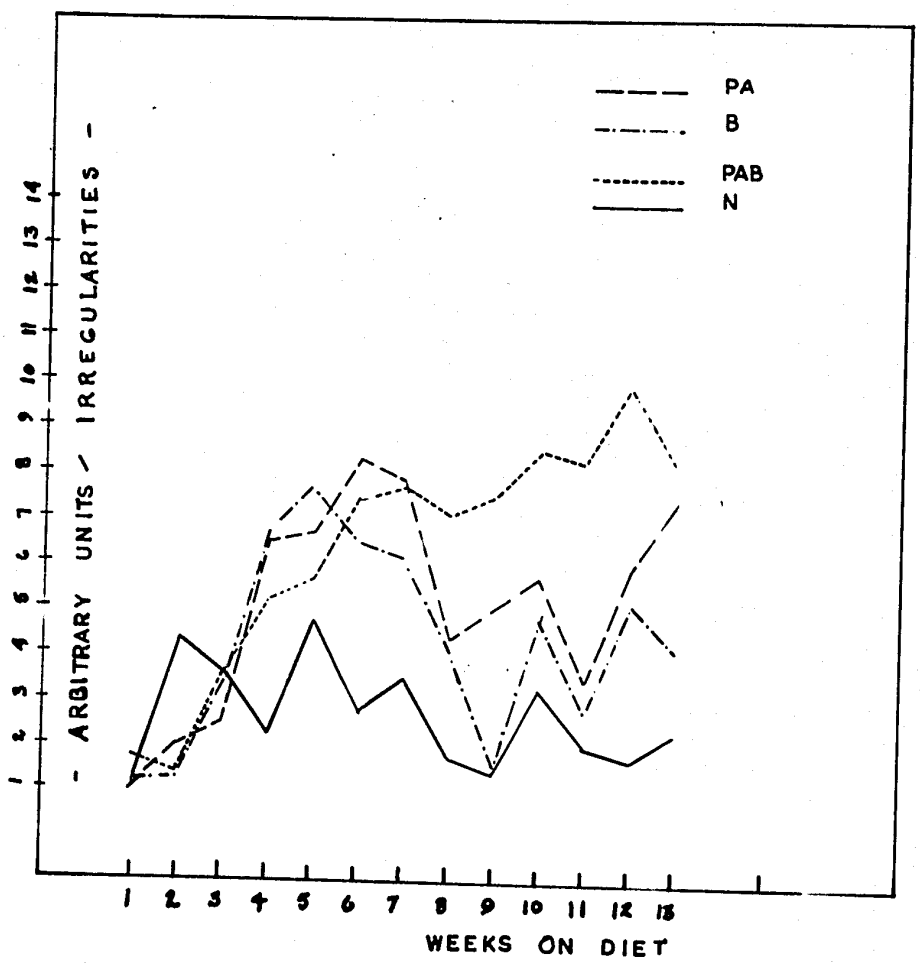


Figure 7: Frequency of irregularities in the pattern of e.c.g. in the normal, PA deficient, B deficient, PAB deficient rats.

end of the experiment. It is significant that the fall in the frequency of irregularities coincides with the resumption of growth in this group. It is legitimate to assume that both have similar biochemical bases. The likeliest is a supply of biotin by bacterial synthesis. Now the increase at the last phase of the experiment coincides again with the feeding of egg white. Though this did not cause a retardation of growth, it caused falling out of hair and a "nervousness" of the rats. It is reasonable to assume that all these symptoms were caused by the reduction in the biotin supply and that growth at this late phase of the growth cycle is less sensitive to biotin deficiency than are the other observed life functions.

d.) An inspection of the time-e.c.g. changes curves shows that all the curves fall into 2 phases: the great change phase from the beginning of the experiment to the end of the 2nd - 3rd week, the small change phase from that time to the end of the experimental period.

e.) A comparison of the growth curves and the e.c.g. change curves

This shows that till the end of the 2nd and 3rd weeks growth rates were the same in the 4 groups. It was from this time on that N animals continued to grow with the

normal rate while the 3 deficiently fed groups grew more slowly.

The phase of great e.c.g. changes coincides with the phase of growth in which these dietary deficiencies have had no effect.

Both those e.c.g. changes which were affected by the diet (R-R time and T voltage) as well as those which were not (R and P voltage) showed the greatest changes in this phase (2nd to 3rd weeks). In this phase of developmental growth the physiological changes of cardiac automatism are the greatest and they are most sensitive to dietary deficiencies.

The rate of increase of the R-R time and of the T-voltage, and the rate of decrease in the R and P voltages in the N and B groups run closely parallel with the rate of decrease in the velocity of body growth throughout the 3a and 3b phases. (Beznák et al.155). It is likely that the 5 phenomena have the same metabolic cause i.e. a slowing of the cata-ana-bolic activity. And it is noteworthy that the sensitivity to PA deficiency of the 2 affected e.c.g. parameters R-R time and T voltage is the greatest just during the first part of the 3a phase when the decrease in growth velocity is the greatest. The development of the biochemical basis of cardiac automatism seems to be more

dependent on an abundant supply of PA at this phase than in later ones.

The effect of PA deficiency on growth becomes apparent only during the later part of the 3a and during the 3b phase i.e. when the cata-anabolic activity is slowing down more rapidly. Perhaps the cause of this phenomenon would be just the circumstance that the slowing of the anabolic activity begins not to supply enough PA for the requirement of mass growth and as it is not supplemented from the diet growth velocity diminishes more rapidly.

The curve of the PA uni-deficient rats is similar to that of the B rats, though the frequency of irregularities remains well above the normal level even after a reduction took place after the 7th week. As the frequency of irregularities diminishes also in the normal during this phase of life and as we assumed that this was the consequence of a reduction in the metabolic activity, a characteristic of this life phase, we can justifiably assume that the same cause was operating here.

In the Pa + B deficient group the frequency of irregularities increases rapidly till the 6th week therefrom it increases at a much slower rate. It may be assumed that the absence of the drop in the frequency of irregularities (seen in the uni-deficient PA and B groups) was due

to the dual deficiency, and the slowing in the increase in frequencies again to a diminution in the metabolic activity of the rat.

To sum up the results of e.c.g. observations, it can be stated that:

1.) A singular PAdeficient and PA plus B deficient diet cause an increase in the R-R time and T voltage during the first 2-3 weeks of feeding.

2.) The aging of R-R time (a prolongation), of P and R voltages (a diminution) is not altered by these diets.

3.) The T wave voltage is affected by the PA and PAB deficient diets but not to a smaller degree and less lastingly by the B deficient ration. On the former diets T voltage increases. There appeared to be a certain parallelism between dermal and growth symptoms and increase in T voltage on these 2 diets.

4.) The frequency of irregularities of the e.c.g. increased in all the 3 deficiently fed groups. In the B group the frequency dropped from the 4th week on the deficient diet. This drop was parallel to the resumption of growth. The frequency of irregularities increased rapidly during the 3 weeks thence slowly in the PA + B groups. In the PA deficient group it dropped somewhat after the 7th week.

5.) There are 2 phases in e.c.g. changes. The 1st phase lasts till the 2-3rd week of feeding. This phase is the 40 to 60-70 days of the extra-uterine life cycle of the rat. In this phase growth velocity (Sachs) diminishes rapidly (3a phase of life cycle), the aging of the e.c.g. takes place at a fast rate. Although growth is the same in the normal and deficiently fed groups and no dermal symptoms appear, nor are the aging processes of P and R changed, the increase in R-R time and T wave is already present in the PA and PA + B diets. In the 2nd phase growth in the PA and PA + B deficient groups slows down, dermal symptoms appear, likewise to a smaller extent in the B group; the e.c.g. aging processes take place more slowly, T wave remain high or starts to increase in rats in which it did not previously. The frequency of irregularities of e.c.g. pattern in the PA and PA + B deficient groups increases rapidly during both the 3a phase as well as during the first part of 3b phase of the rat's life cycle.

f.) The CoA content of the liver and heart.

1.) Normal diet.

As seen from figures 8 and 9 the CoA content of the liver and heart of the normal animals increases from the weanling stage to the 8th week where a plateau is reached. From the 8th week onwards the CoA content of both liver and

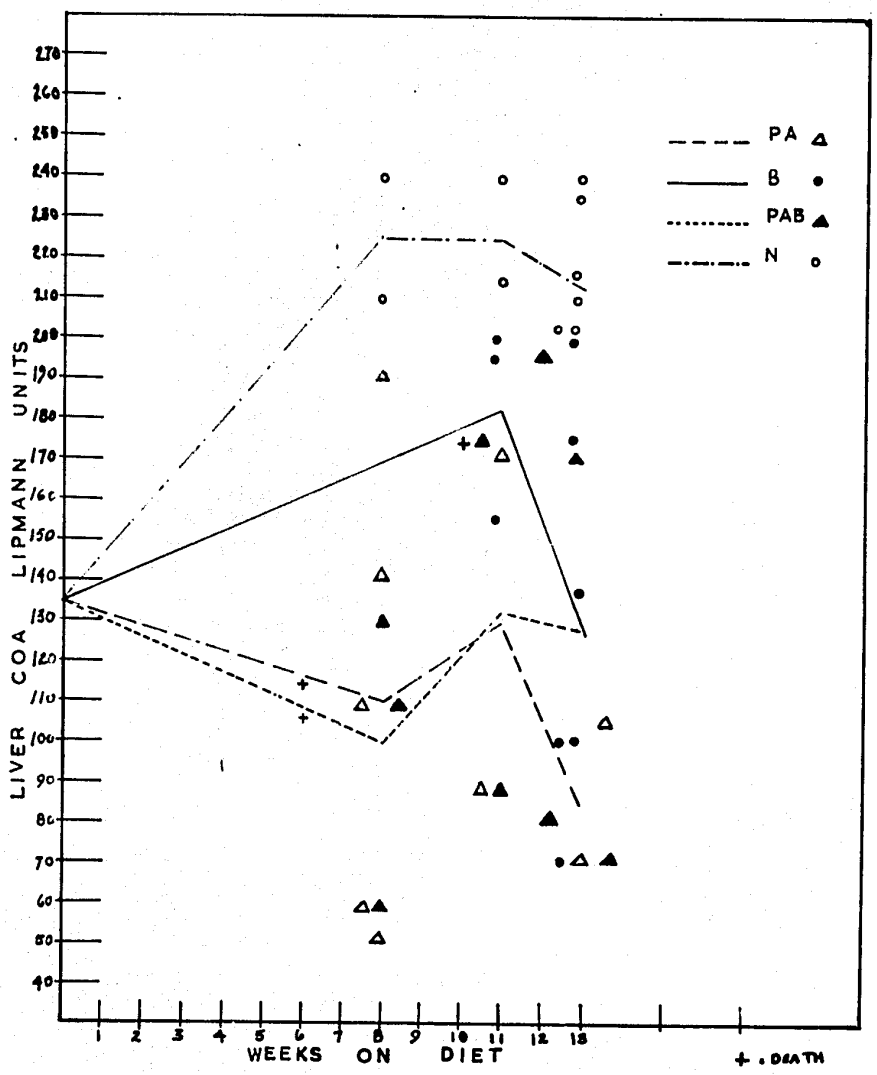


Figure 8: Liver CoA in Lipmann units of all 3 deficient groups and normal group plotted against time.

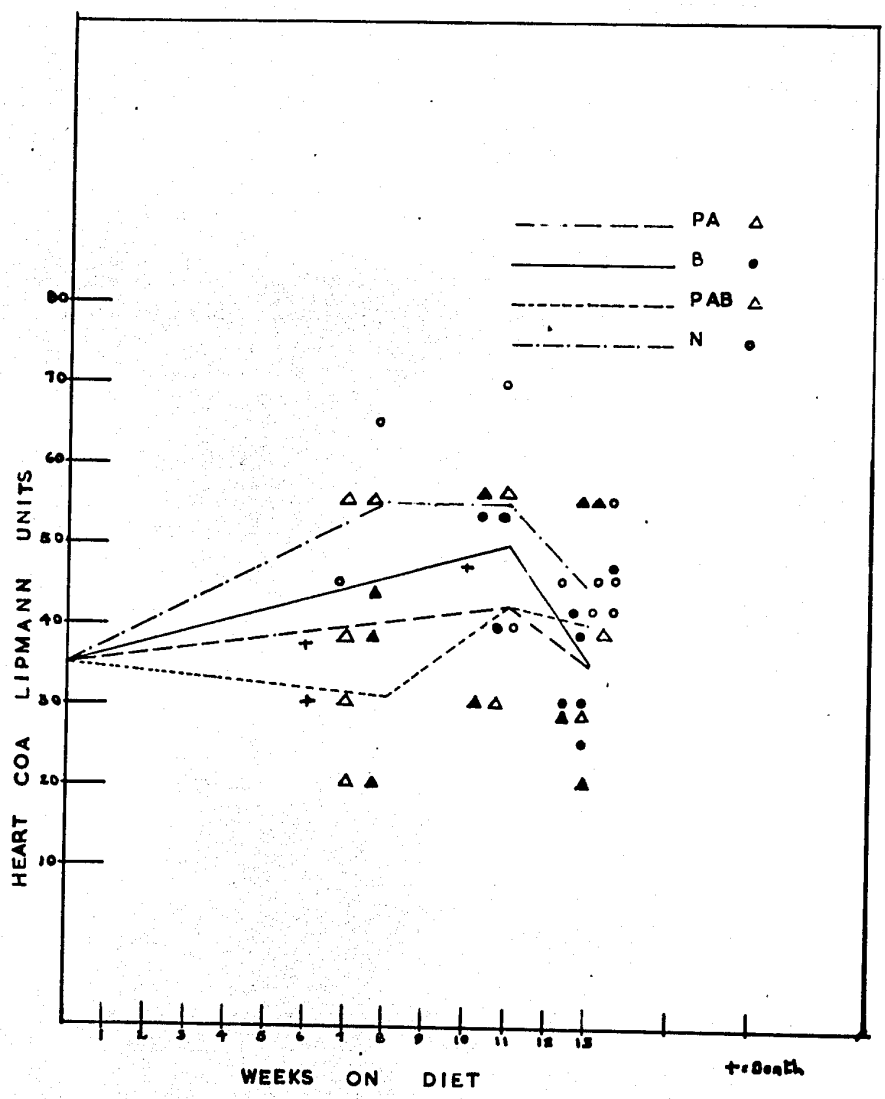


Figure 9: Heart CoA in Lipmann Units of all 3 deficient groups and normal group plotted against time.

heart remains fairly constant. In the liver (Fig.8) the weanling value is 135 L.U., the high asymptote, reached by the 8th week is 225 L.U. The heart contained less CoA, 35 L.U. at weanling and 55 L.U. after the 8th week (fig. 9) of the experiment.

2.) Biotin deficient diet.

The animals deficient in biotin only increased their liver CoA from a weanling value of 135 L.U. to an average of 185 L.U. at the end of the 11th week. From the 12th week on till the 13th and a half week the average of CoA content of the liver decreased to a minimum value of 124 L.U. In the heart the CoA content reached a high asymptote of 55 L.U. at the end of the 11th week and a slight decrease was noted in CoA content at the 13th and a half week.

3.) Pantothenic acid deficient diet.

In the case of the rats on the uni-deficient pantothenic acid diet liver CoA dropped up to the 18th week to 110 L.U., then to the 13th week more rapidly to 85 L.U. In the heart a slight drop of CoA, not statistically significant, was observed only at the 13.5 week.

4.) Pantothenic acid and Pantothenic acid plus biotin deficient diets.

The liver CoA of the pantothenic acid and biotin deficient animals was about 100 L.U. at the 8th week. At

At the 11th and 13.5 weeks the liver CoA concentration was 125 L.U. In the heart as in the liver, the CoA concentration remained at the weanling value through the 13.5 week of the experiment.

From both figures 8 and 9 it is seen that both liver and heart CoA are increased at the 11th week on a biotin deficient diet though to a lower value than in the normal animals. 't' test was applied between the 11th week and 13.5 week of deficiency to see whether or not the effect of egg-white flakes affected the CoA content in the livers. No significance was found to exist between the two groups.

Frequency distribution histograms were made for both the liver and heart CoA and are shown in figures 10 and 11.

From figure 10 it is seen that the liver CoA content in all the groups is greatly scattered. There is an overlap in all curves most pronounced between the normal and Biotin group.

From the above it is concluded that in all three deficient groups, the liver CoA is more sensitive to nutritional deficiencies than is the heart CoA.

To answer the question whether the apparent differences between the averages of the liver CoA in the different groups is due to the differences in the diets or to chance sampling, 't' tests were made as shown in the subsequent tables.

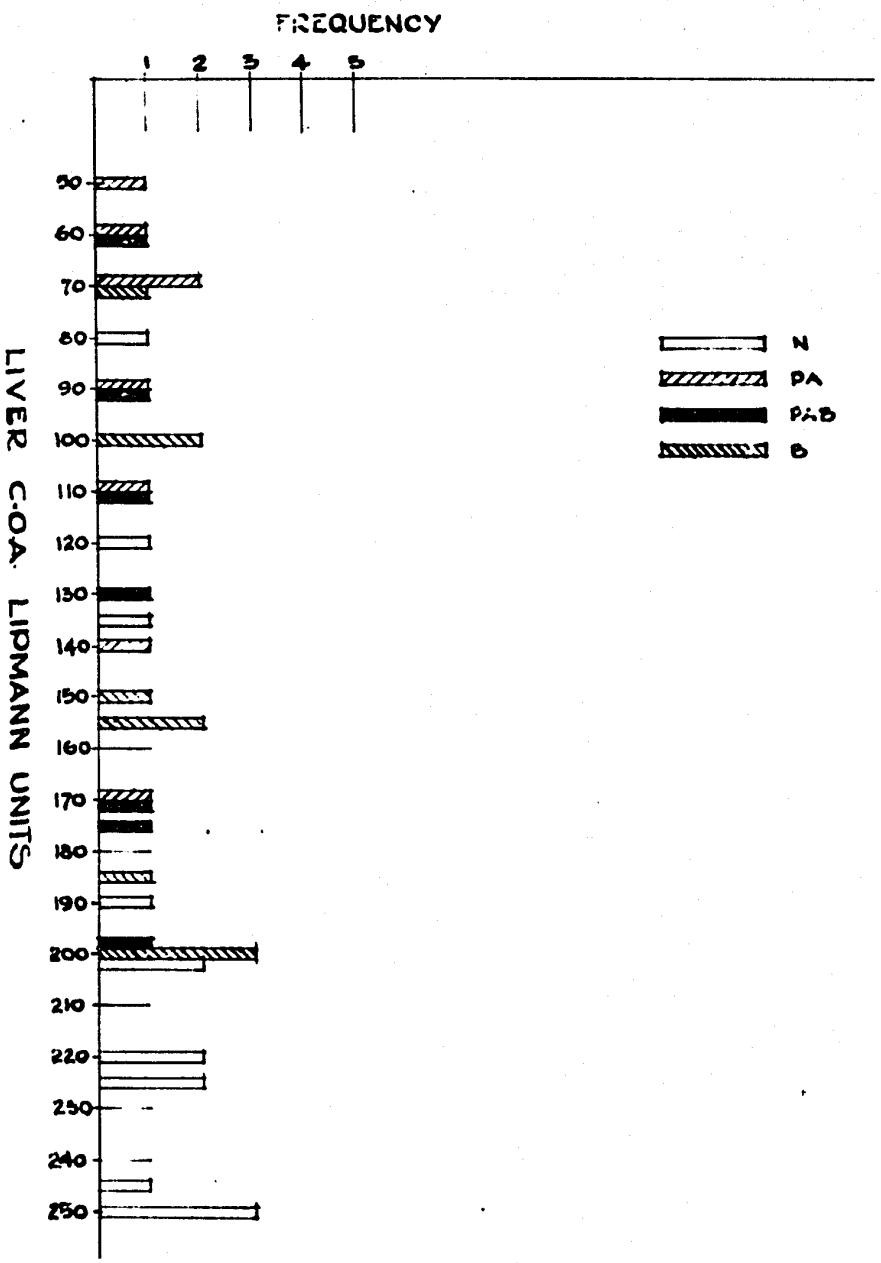


Figure 10: Histogram of the liver CoA for the normal and three deficient groups.

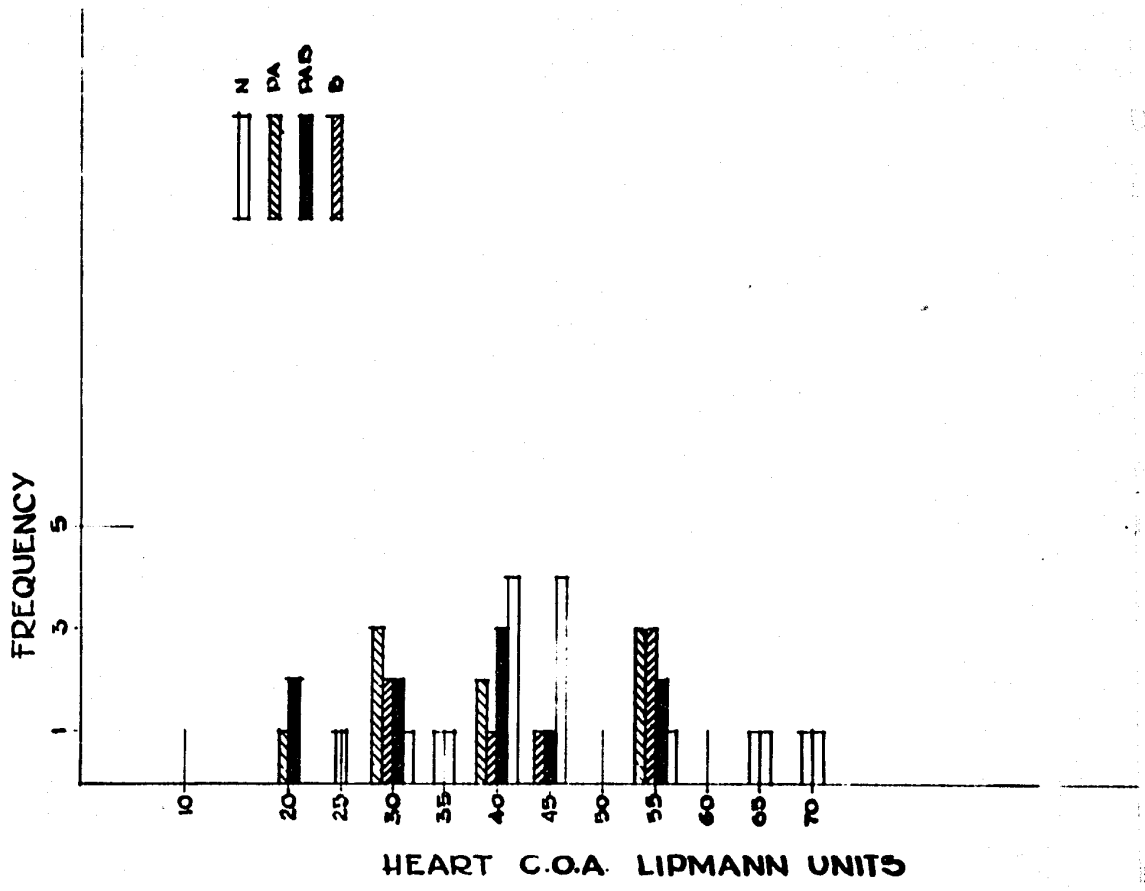


Figure 11: Histogram for the heart CoA of both normal and three deficient groups.

Table I: Liver CoA in rats 8 to 13 weeks on the three deficient diets.

Group	n	Mean	Degrees Freedom	't'	P
PA	9	105.5		PA- PAB : 0.61	>0.10
			16	PA- B : 1.9	0.10>P>0.05
PAB	9	120.0		PAB- B : 1.3	0.20>P>0.10
B	9	151.0			

As can be seen from this table, the difference in liver CoA between the two PA deficient groups is not significant. The difference between the PA and B group is barely significant. Therefore it was decided to treat the three deficient groups as one population and to compare them with the normal group. To compare the variabilities of the two groups, an analysis of variance was done. The data is seen in Table II.

Table II: Analysis of variance for the normal and three deficient groups taken as one population during the experimental period.

Group	Source of Variance	Degrees Freedom	Mean Squares	Variance Ratio	F
N	Between groups	1	49,630	20.06	<0.001
Deficient groups	Within groups	36	2473.8		

From the above table, it is seen that the difference in the variances of CoA content of the liver of the 3 deficient groups (treated as one population) as compared to the normal liver CoA, is statistically highly significant.

To see if any statistically significant difference existed between the normal and individual deficient groups, a 't' test was done, from the 11th and 13th weeks. The data are given in table III-IV and V for all deficient groups.

Table III: Liver CoA in rats 11-13 weeks on the normal and pantothenic acid-biotin deficient diet.

Group	n	Mean	Degrees Freedom	't'	P
N	8	218.7			
			12	4.2	0.001<0.01
PAB	6	130			

Table IV: Liver CoA in rats 11 to 13 weeks on the normal and pantothenic acid deficient diet.

Group	n	Mean	Degrees Freedom	't'	P
N	8	218.7			
			11	7.1	< 0.001
PA	5	102.0			

Table V: Liver CoA in rats 12-14 weeks on the normal and biotin deficient diet.

Group	n	Mean	Degrees Freedom	't'	P
N	8	218.7			
			15	3.6	0.001 < 0.01
B	9	151.1			

The order of significance in the above 't' tests, indicate that the animals which were subjected to a pantothenic acid deficient diet, had a P value much greater than 0.001 and 0.01 and therefore proved to be highly statistically significant. The pantothenic acid-biotin deficient group had a P value less than 0.001 but greater than 0.01, which made it therefore statistically significant. Finally the biotin deficient group had a P value slightly lower than that of the PAB group but significant at 0.01.

Before any final conclusion as to whether the lowering of CoA in the liver of the deficient animals was really due to the diets in question, the process of aging must be given consideration. Whether or not the liver reduces its CoA content on maturation was investigated in all groups.

The statistical significance of the difference in CoA content between the normal and deficient groups, at the start and end of the feeding period, was tested with the 't'

test. These results are found in Table VI, VII, VIII and IX.

Table VI: 't' test on the difference in liver CoA in the 1st and 13th week in the normal group.

Group	n	Mean	Degrees Freedom	't'	P
N	3	135.0			
			7	6.9	<0.001
N	6	216.5			

Table VII: 't' test on the difference in liver CoA between 1st week in normal and 13th week PA deficient group.

Group	n	Mean	Degrees Freedom	't'	P
N	3	135.0			
			4	3.2	0.05 > 0.02
PA	3	8.33			

Table VIII: 't' test on the difference in liver CoA between the 1st week normal and 13th week PAB deficient group.

Group	n	Mean	Degrees Freedom	't'	P
N	3	135.0			
			5	0.16	>0.10
PAB	4	128.7			

Table IX: 't' test on the difference in liver CoA between the 1st week normal and 13th week deficient B group.

Group	n	Mean	Degrees Freedom	't'	P
N	3	135.0	7		
				0.0	>0.10
B	6	135.0			

From the above data we conclude that as the animal progresses in age the concentration of the liver CoA increases in normal animals fed our complete diet. All other deficient groups did show a reduced liver CoA storage in the liver, PA deficient animals having been mostly affected. No changes between the 1st week and 13th week of liver CoA were noted in either the pantothenic acid-biotin or biotin groups, indicating thus a stationary liver CoA content through the experimental period, or incapability of the liver to synthesize the coenzyme, at a greater rate than at the weanling stage.

Figure 2 illustrates the results obtained on analysis of the heart CoA. In this case there was less scatter in individual CoA determinations. To see whether the variability differed in the 4 groups, an analysis of variance was performed. All determinations were pooled together to form one population.

Table X: Analysis of variance on heart CoA, all groups treated as one population.

Group	Source of Variance	Degrees Freedom	Mean Square	Variance ratio	F
N	Between groups	3	339.07		
PA				0.18	>0.20
PAB	Within groups	36	1990.60		
B					

No statistically significant difference in heart CoA could be found between all 4 groups. Furthermore a 't' test proved that no sifnigicant difference exists between the normal and the 3 deficient groups taken as one population to the end of the feeding period. The P in this case was less than 0.001.

Table XI: "t" test on the difference in heart CoA between the normal and 3 deficient groups treated as one population.

Group	n	Mean	Degrees Freedom	't'	P	
N	13	45				
			38	1.81	0.1	0.05
Deficient groups	27	38.7				

As can be seen from this table, the heart CoA content of both the normal and deficient groups treated as 1 population is not statistically significant. The conclusion is warranted therefore that the heart CoA rises in a pantothenic acid uni-deficient diet, pantothenic acid-biotin deficient diet and a biotin uni-deficient diet, to the same level as it does in normal rats. Heart CoA then is more resistant to CoA depletion than is liver CoA under the same experimental conditions.

We conclude that liver CoA differs in biotin deficient rats from the normal rats in showing a smaller increase to the 11th week and therefrom to the 13.5 week an appreciable reduction.

The CoA content of the pantothenic acid and pantothenic acid-biotin deficient animals is not able to increase from the weanling stage to the 18th week. From then on there is a drop in CoA to the end of the experiment.

At the end of the 13.5 week on both these deficient diets the rat liver CoA is only 1/5th of the normal in the pantothenic acid deficient animals, and 1/3rd of the normal in the pantothenic acid-biotin deficient rats.

The growing hearts of rats on a pantothenic acid, pantothenic acid and biotin, and biotin deficient diets, do not differ appreciably from that of the normal animals in

their CoA content. All the deficient hearts are able to increase their CoA content from a weanling value of 35 L.U. to 55 L.U. at the 11th week of deficiency. From then on only slight reductions are observed.

g. A comparison of the effects of the Olson Kaplan diet and the PA, PA plus B and B deficient diets.

α.) Body weight increased in the multi-deficient and in the uni- and PA plus B deficient diets at similar rates. Up to about the 15th day growth rate was the same in all the 5 groups (including the normal group), though there was a suggestion that growth rate on the O. & K. diet was below all other groups. From about the 15th day on growth rate on the 4 deficient diets was much less than on the normal diet and the multi-deficient group lagged behind from now on in a more pronounced degree. In unideficiency on biotin growth was retarded between the 15th to 35th days of feeding. After this day growth rate became equal to that of normal rats. The difference in weight due to the delayed resumption of normal growth rate was eliminated only by the 95th day of experimental feeding. Adding egg white to the diet during the last 30 days did not influence growth.

β.) Alopecia as measured by our standards developed in a greater number of animals, faster and to a more severe

degree in the multideficient rats than in the uni- and bi-deficient ones. Edema, ruffled fur and general weakness behaved similarly to alopecia.

In the rats on a biotin unideficient diet alopecia and the other dermal symptoms appeared in 2 phases. The 1st phase coincided with the first 15 to 35 days slow growth the 2nd with the feeding of egg-white. During this latter phase the rats were aggressive and excitable.

γ.) Porphysin staining of the fur was seen only in the multideficient diets.

δ.) The following effects were noted in the e.c.g. R-R time is increasing in rats on a complete diet due to aging. In the P uni- and PA + B deficient diets there was a sudden and great increase in R-R time during the first 2-3 weeks wherefrom with oscillations the R-R time increased with aging. In the multideficient rats R-R time increased during the 1st 2-3 weeks at the same rate as it did in normal rats (perhaps slightly faster) but from that time on the increase was much below the normal rate. The B unideficient R-R curve was close to the N curve.

T voltage was most responsive to differences in the composition of the diet and the responsivity is greater during the early phase of life straight after weaning than later. Pantothenic acid, PA + B deficiency, food restriction,

multideficiency (as in the normal controls of the O.-K.group at the start) all cause in this life phase a substantial and statistically highly significant rise in T voltage. Rats on PA uni- or PAB bideficient diets maintain the high voltage with a tendency to diminution in the later phases. Rats which had an originally high T voltage value on a multideficient diet lose this high voltage gradually and they do it somewhat faster than do rats on a complete, synthetic diet, which had originally also a high T value. Biotin unideficient rats fall between the N and PA deficient ones but closer to the N's.

R and P voltages behaved similarly in the normal, PA and PAB groups. They diminished rapidly during the first 2-3 weeks thence more slowly. This is an aging process which is not affected by anyone of these diets. The The PA multideficient diet seems to accelerate this aging process.

Irregularities in the pattern of the e.c.g. as measured by our arbitrary units increased up to the 5th week in all the five groups, but in the normal the highest level was much below of all the deficiently fed groups. The level was highest in the multideficient group. From the 5th week on e.c.g. abnormalities became much less frequent in the N and B rats, somewhat less frequent in the

PA rats, slowly on the PA plus B deficient diets and rapidly on the multideficient diet.

.) CoA content of the liver increases from the weanling phase to the adult phase in normal rats. It increases to a small degree or perhaps not at all in the uni- B deficient rats, it does not increase in the uni- P and bi- PA plus B deficient rats, it diminishes rapidly and to a low value in the multideficient rats.

.) CoA content of the hearts is only about 1/4th of that of the liver. It increases from weanling to adult stage in N, P, B, and P + B deficient rats though perhaps somewhat less than it does in N rats, in the multideficient rats it increases somewhat in the first 3 weeks but thence falls back to the weanling low level.

.) An inverse correlation was established between liver CoA content and severity of alopecia, and e.c.g. pattern irregularities, and a direct correlation between growth and liver CoA content.

.) Heart CoA and frequency of irregularities in the e.c.g. pattern ($r = -0.14$, P 0.1) or aging symptoms of the R-R time are not correlated.

DISCUSSION.

A unideficient pantothenic acid diet causes stunted growth, alopecia, accelerated increase in R-R time and irregularities in e.c.g. pattern.

One of the questions we had sought an answer to was whether omission of PA alone from a complete, synthetic, well balanced diet will bring about changes in the growing rat, in particular in its e.c.g. This question was of importance because most of the diets used in previous researches on PA deficiency were either deficient in other dietary constituents as well or they did not deal with the question of cardiac automatism. Two corrective additions must be made to this statement. Some of the earlier works dealt with the heart but in lose terms only. Beznák and van Alphen attempted to answer this same question in apparently the same way we approached it but they obtained negative results. They found on an average a slightly stunted growth, a small increase in the frequency of irregularities, but none of the differences were statistically significant. These authors kept their rats in a single large cage. Under this condition refection is not prevented satisfactorily. The assumption - also made by these authors - is justified that their rats were supplied with enough PA by bacterial synthesis to maintain a close to normal level in the life

processes observed by them.

PA synthesis was probably also going on in our PA unideficient rats as follows from the following considerations:

The weight of the liver was found to be 4% of the body weight. At the beginning of the experiment a normal rat had a liver of 2.6 gm. with a CoA concentration of 135 L.U. The total CoA content of the liver was 350 L.U. At the end of the experiment liver weight averaged 12.0 gm., CoA concentration 215 L.U., total liver CoA 2580 L.U. The newly accumulated CoA amounted to 2230 L.U. In the PA deficient rat at the end of the experiment liver weight averaged 7.2 gm. liver CoA concentration 80 L.U., total CoA 576 L.U. The difference between the value at the start and at the end is 220 L.U. Considering the great scatter of the determinations and the turnover rate of CoA we can only conclude that some small synthesis of CoA and presumably PA did take place in the PA unideficient rat. Whether this newly formed CoA was synthesized by the rat cells or by intestinal bacteria can not be decided.

As a positive correlation was established between liver CoA and body weight and an inverse one between irregularities of the e.c.g. pattern and liver CoA it is justified to assume that in the experiments of Beznák and

van Alphen the statistically not significant slowing in growth and the increase in irregularities of e.c.g. pattern were due to PA synthesis (probably by the intestinal flora) falling somewhat short of the optimum requirement.

The concentration of heart CoA reached on maturation in the PA uni-deficient hearts the same level as it did in the normal ones. Since, however, heart weight was smaller by about 1/3 of the normal, the total CoA content present in the heart was also less.

Abnormalities of the e.c.g. pattern developed in the PA unideficient rat in spite of the unchanged CoA concentration of the heart. The frequency of e.c.g. abnormalities was correlated inversely to liver CoA concentration when all the groups were treated as one population.

As regards the mode of production of these e.c.g. abnormalities there is only one information given by the above data: an insufficient CoA level leads to such changes in the complex nervous and chemical processes of the organism which lead to these changes in e.c.g.

Our other question was whether a combined dietary deficiency in biotin and pantothenic acid leads to more severe symptoms than does a unideficiency on either of the 2 substances. Biotin was selected because of its partial structural similarity to pantothenic acid.

We obtained only a suggestive reply to this question. Biotin unideficiency did cause similar abnormalities in growth, alopecia, e.c.g. and CoA content as did PA deficiency. The changes, however, were statistically barely significant and they were transient. These facts suggest that the biotin requirement may be satisfied more easily by intestinal synthesis than that of PA. The symptoms of the rats on a pantothenic acid and biotin deficient diet were qualitatively the same as those on the PA unideficient diet and they were not more severe. This again suggests that the biotin requirement was met by bacterial synthesis.

It is noteworthy that a satisfactory synthesis (probably by the bacterial flora) of biotin took place even in the absence of pantothenic acid from the diet. The sulphur and other atomic requirements of biotin synthesis can therefore be met without recourse to dietary pantothenic acid.

It is to be noted that the B deficient group developed severe alopecia and irritability when during the last 3 weeks they were fed egg-white flakes. As these symptoms were not present in the pantothenic acid plus biotin deficient rats they are to be attributed to the combined effect of biotin deficiency (owing to avidin binding of biotin) and perhaps to a direct poisonous effect of the

egg-white flakes.

Beznák and van Alphen (l.c.) recorded the effects on growth and e.c.g. of a pantothenic acid plus thiamine deficient diet. They found that the symptoms developed on this bideficiency were those of thiamine deficiency - but somewhat attenuated. The thiamine and pantothenic acid deficient rats also lived in one large cage, supply of the 2 omitted vitamines by bacterial synthesis and refection was the same for these rats as for their pantothenic acid unideficient group. In this latter group PA synthesis did take place as witnessed by the almost normal state of the rats. Thiamine deficiency symptoms, however, developed both in the thiamine unideficient as well as in the thiamine plus pantothenic acid deficient groups. Beznák and van Alphen concluded that thiamine is synthesized by the intestinal flora and/or rat cells to a much smaller extent than is pantothenic acid. We found that biotin is synthesized more readily than pantothenic acid. The ease with which lack of these 3 vitamins is made up can be arranged in the following increasing order: thiamine --- pantothenic acid --- biotin.

As regards the symptom complexes produced by combined bideficiencies of these 3 vitamins one may arrive at a generalization: <u>In combined dietary deficiencies of 2</u>

of the vitamins thiamine, pantothenic acid and biotin, the symptoms of that missing substance will dominate the picture the synthesis of which by the bacterial flora and/or the cells of the organism is the slowest. The effect of the other constituents will be a modification of the basic symptom complex.

This generalization is born out by our experiments with Olson Kaplan pantothenic acid multideficient, unbalanced diet. There again the symptoms were clearly those of pantothenic acid unideficiency but they were somewhat modified. Growth was retarded more, alopecia was more severe and developed faster, the abnormalities of the e.c.g. pattern were more frequent, the diminution in CoA content of the liver was greater and faster. While in these symptoms the absence of other factors only accentuated those seen in PA unideficiencies, some changes in the e.c.g. were different in the PA unideficient than in the PA multideficient diets. Thus on a PA uni- and PA + B deficient diet R-R time increased rapidly during the 1st days of feeding, whereas on the PA multideficient diet this increase did not take place. This diet prevented the development of aging bradycardia.

A comparison of the e.c.g. changes seen in thiamine deficiency (single or combined with pantothenic acid) with those seen in pantothenic acid deficiency (single or combined

or multiple with biotin) reveals that the former ones are much more severe. The thiamine deficient rat dies in heart stoppage. The pantothenic acid deficient rat does not die in the same time as do thiamine deficient ones and their e.c.g. abnormalities do not suggest lethal change in cardiac automatism. It is suggested that the heart failures described by earlier authors and attributed to pantothenic acid deficiency were actually due to some combined deficiency.

The biochemical cause of this difference seems to be the difference in the ability of the heart to maintain on a deficient diet its cocarboxylase content on the one hand and its CoA content on the other. While cardiac cocarboxylase content diminishes (cf. 158), parallel with the severity of the e.c.g. symptoms in thiamine deficiency (122), cardiac CoA content remains on a high level in dietary PA deficiency, yet the e.c.g. changes appear. On the Olson Kaplan PA multideficient diet the CoA content was lower than on the PA uni- or PA + B bideficient diets. It seems, therefore, likely that only a PA multideficient diet fed for a long time might cause such a depletion of cardiac CoA which could have a lethal effect on cardiac automatism.

While the heart is relatively resistant to dietary PA deficiency the hair seems to be rather sensitive to it.

The same sensitivity was seen in biotin deficiency, and a lack of thiamine (159). It follows from these that it is not the specific property of anyone of these substances to prevent alopecia but rather it is the consequence of the great sensitivity of the hair roots to any dietary deficiency.

R.J. Williams (160) presented evidence that living organisms possess individually differing patterns of metabolic traits which are genetically controlled. There is no doubt that from the biochemical point of view the different organs have different patterns of metabolic traits genetically controlled. The pattern of metabolic traits of hair production then is such that it makes it extremely sensitive to many dietary deficiencies. The fact that the number of genes controlling hair growth is rather great bears out this assumption.

We found that the maturation of e.c.g., i.e. prolongation of R-R time, and diminution of the P, R and T voltages, takes place at a greater rate during the early phase of life. The same applied to the rate of development of the deficiency symptoms.

Beznák, Beznák and Gaspár-Rády (157) divided the life cycle of the rat into 5 phases. This division was based on simultaneous, consecutive changes in the absolute

growth velocity (3achs), in food, salt solution and water consumption. Placing our findings on this 5 phase curve we found that the greatest rate of e.c.g. (maturation) and development of deficiency symptoms falls into the 3a phase. In this phase the absolute growth velocity is still very high though rapidly decreasing. Maturation of the e.c.g. and the deficiency symptoms are almost fully developed by the 3b phase i.e. when the absolute growth velocity is very small. In phase 3a the volume of total metabolic activity is still increasing and a large part (though steadily diminishing) of it is taken up by the building up of new tissues. In phase 3b the amount of newly produced tissue as a fraction of the old tissue is very small, while the total volume of metabolic activity has nearly reached its maximum value but most of the energy is dissipated and most of the material taken in with the food is used only for replacement of old material. It is understandable that consequences of dietary deficiencies will be most pronounced in that phase of metabolic activity in which most of the new material is being built up. This conclusion is fully born out by the experiment of earlier workers that the earlier after birth a deficiency diet is fed the greater is the regularity, strength and rate of development of the deficiency symptoms.

SUMMARY.

1. Changes in body weight, in alopecia, edema, porphyrin staining, e.c.g., CoA content of the liver and heart were recorded in rats on a complete synthetic well balanced diet, on a pantothenic acid unideficient, biotin unideficient, pantothenic acid plus biotin bideficient and pantothenic acid plus multideficient , fat poor diet (Olson Kaplan).

2. The changes observed were essentially the same in all the 3 pantothenic acid deficient diets. These were retardation of growth, alopecia developing on a regular pattern. Facial and paw edema. These symptoms were the same in the PA uni- and PA plus B bideficient diets but they were more pronounced and developed more rapidly on the PA multideficient Olson Kaplan diet. Porphyrin staining was only seen on the Olson Kaplan diet.

The e.c.g. changes were different on the PA uni and PA plus B deficient diets than on the Olson Kaplan diet. On the former diets the R-R time increased in the 1st 5 days of feeding as compared to normal and remained high. The normal R-R time reached this high level at the end of the experiment by a steady increase (maturation or aging of the e.c.g.). T voltage also rose quickly on these

two diets and remained (with oscillations) high. P and R voltages diminish during the maturation in normal rats, and this process was not influenced by these diets. Irregularities in the pattern of the e.c.g. increased in frequency on this diet. On the Olson Kaplan diet the increase in R-R time with maturation did not take place. T voltage was high in these rats (owing to previous underfeeding) and it dropped during the early part of feeding somewhat faster than in the normal rats. P and R voltages became smaller as in normal rats, though there was a tendency to a faster aging. Frequency of irregularities in the pattern of the e,c,g, increased more and more rapidly on the Olson Kaplan diet than on the other 2 PA deficient diets.

3.) Rats fed a biotin unideficient diet grew more slowly during the 1st 1/3 of the feeding period and there was alopecia developing. Growth was then resumed at normal rate and alopecia development stopped. During the last 3 weeks of feeding egg white flakes were fed to these rats, growth was not altered, but alopecia developed strongly and the rats became very excitable, and aggressive. The e.c.g. alterations were similar to those seen in PA deficiency but the degree and frequency of the changes were not great enough to make the average of the differences between normal

and biotin unideficient groups statistically significant.

4.) The CoA content of the heart in normal rats was found to be about 1/5th of that of the liver. It increases in both organs from the weanling to the mature phase of the extrauterine life cycle. Liver CoA changes differently from heart CoA on the 4 deficient diets. Liver CoA diminishes on the 3 PA deficient diets below the weanling level, whereas heart CoA - affected only by the multideficient diet - does not rise above the weanling level. The changes are greatest on the PA multideficient diet. The effect of PA uni- and PA plus B bideficient diets is about the same.

5.) Biotin unideficiency also tends to diminish liver CoA and reduces somewhat the maturation rise of heart CoA.

6.) A statistically significant positive correlation was found between body weight (growth) and liver CoA. A statistically significant inverse correlation was found between degrees of alopecia, frequency of irregularities of e.c.g. pattern and liver CoA.

7.) No correlation was found between heart CoA and frequency of irregularities of e.c.g. pattern.

8.) By way of discussion it is pointed out that the sensitivity to dietary deficiencies is greatest in the early phases of the extra-uterine life cycle. In these phases (denoted by Beznák et al. 1, 2, 3a) the absolute growth velocity of Sachs is still great, synthesising activity of the genes is high. It is also pointed out that the life processes of hair growth are specially sensitive to dietary deficiencies because their pattern of metabolic traits controlled by genes is very complex.

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