THE EFFECTS OF NIACIN DEFICIENCY ON PHAGOCYTOSIS AND SUSCEPTIBILITY TO INFECTION

by

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INTRODUCTION

Nicotinic acid (niacin) has been known since 1867 as a product obtained by oxidation of nicotine. It was not until 1912 that niacin was isolated from a natural source. Suzuki and Funk, while searching for an antiberiberi factor, isolated it from yeast and rice polishings.

Pellagra is a serious disease which has for centuries occurred in epidemic and endemic forms in various regions throughout the world and was quite common in the southern United States. It was clearly demonstrated by Goldberger (1912) that pellagra originated from a dietary deficiency, the exact mechanism of which was not clear. In 1917 it was shown that a condition in dogs called "canine blacktongue" had much in common with pellagra of humans. Elvehjem (1937) isolated nicotinic acid and its amide form, nicotinamide, from liver extract and showed that they were highly effective in curing canine blacktongue. Niacin was later shown to have a curative effect on clinical pellagra as well (Cantarow and Schepartz, 1957). Warburg and Christian (1935) demonstrated that nicotinamide participated in cellular oxidative systems as a hydrogen transport agent. This observation along with what was known about riboflavin indicated that certain vitamins were essential components of important intracellular enzyme systems.

As early as 1800, man was attempting to correlate the influence of diet upon the incidence, course, and termination of infection and disease. The presumption that alterations in the diet may have a direct or indirect effect on the susceptibility of the host to an infection was well worth investigating. Since the 1930's an extensive amount of study has been done to find the correlation between the diet of man and animals and the incidence of infection in these two defined groups (Watson, 1934; Clausen, 1934; Wilson and Topley, 1938; Perla and Marmorstom, 1941; Aycock and Lutman, 1944; Leitel, 1945; Schneider, 1946; Wilson and Miles, 1946; and Clark et al., 1949).

Many environmental conditions can cause a nutritional unbalance: i.e., war, famine, floods, hurricanes, insect damage to crops, etc. (and source of food for diet). Human beings are reservoirs for potential pathogens that are capable of causing disease if the normal host-parasite relationship is upset. Much of the disease in our society is caused by pathogenic agents normally present which manifest their presence only under adverse conditions that reduce the resistance of man.

In the last 20 years many investigators have studied the effects of vitamins upon susceptibility to infectious disease. A lack of these extrinsic factors may alter the host-parasite balance by interference with the defense mechanism, or altering metabolic processes, or by

modifying the environment so that conditions tend to favor the parasite in its attempt to survive.

Evidence is available which indicates a marked increase in susceptibility of a host maintained on deficient diets when challenged with certain bacterial and rickettsial agents. Werkman (1923a) reported that rats deficient in "B vitamins" were more susceptible to infection by pneumococci and the anthrax bacillus than were the non-deficient animals.

Dogs sustained on diets void of "B vitamins" exhibited positive bacteremia cultures after injection with Bacillus welchii, while dogs in the control group showed no bacteremia (Rose, 1928). Barlow (1930) was able to produce bacteremia in normally non-susceptible pigeons which were deficient in "B vitamins" in a similar study using Bacillus welchii.

A reduction in the resistance of rats to infection by Salmonella muriotitus after being fed a diet deficient in the "B vitamins" was reported by Rose and Robertson (1932). Rose and Rose (1936), employing a Salmonella organism, introduced a paired weight control group and reported that increased fatality in the vitamin deficient animals was not due to the state of inanition resulting from deficiency.

Members of the vitamin B-complex group were isolated and purified after 1930. The possibility of obtaining

these extrinsic factors in the pure state opened the door to single-vitamin deficiency studies.

There was much evidence available to show that animals maintained on specific vitamin deficient diets exhibited an increase in susceptibility to specified bacterial and rickettsial infections. Rats maintained on riboflavin deficient diets (Pinkerton and Bessey, 1939) and thiamine deficient diets (Guggenheim and Buechler, 1946) showed an increased susceptibility to typhus rickettsiae. Wertman and Sypherd (1960) reported that rats deficient in riboflavin were quite susceptible when challenged with Type I Diplococcus pneumoniae.

Thiamine deficiency in the rat greatly reduced the resistance of the rat to rat leprosy (Badger et al., 1940). Pigeons deficient in thiamine demonstrated a marked reduction in resistance to psittacosis (Pinkerton and Swank, 1940). White rats of the Sprague-Dawley strain were more susceptible to Type I pneumococcal infection when maintained on diets deficient in thiamine (Wooley et al., 1942; Wertman and Groh, 1958).

Experimental diets deficient in other members of the vitamin B-complex group have also been shown to lower the resistance in animals subjected to bacterial and rickettsial infection. Animals deficient in pantothenic acid (West et al., 1944; Fitzpatrick, 1948; Seronde, 1954), pyridoxine (Robinson and Siegel, 1944), and biotin

(Kligler et al., 1946) showed decreased resistance to infection. Animals subjected to a folic acid deficient diet exhibited a marked increase in bacteremia over that of non-deficient animals when challenged with Type I Diplococcus pneumoniae (Wertman and Pathak, 1960).

Evidence is at hand which indicates that a lack of a specific vitamin may induce increased resistance to viral agents. The replication of virus in the host depends on nutritional factors present. If critical nutrients are absent in the host, viral multiplication may be greatly impaired. Rats maintained on thiamine deficient diets exhibited a greater resistance to poliomyelitis virus than those animals continued on a well balanced diet (Foster et al., 1942). An increased resistance to poliomyelitis in Swiss mice maintained on diets deficient in biotin, inositol, pyridoxine or thiamine was reported by Rasmussen et al. (1944) and Lichstein et al. (1945).

An increase in resistance to several bacterial infections has also been observed with experimental deficient diets. Mutant organisms of Salmonella typhi are dependent upon a source of para-aminobenzoic acid (PABA). When these mutants were injected in mice they were unable to manifest their pathogenicity unless sufficient quantities of PABA were added to the diet of these mice (Bacon et al., 1951).

In some cases vitamin deficiency has little or no

effect on the resistance of deficient animals to infection. It was observed by Higgins and Feldman (1943) that rats fed diets low in thiamine and riboflavin had no effect on the resistance of the animal to avian tuberculosis. Fitzpatrick (1948) observed no alteration in the resistance of pyridoxine deficient rats when challenged with Rickettsia mooseri. No change in the resistance of rats deficient in pantothenic acid was observed when these animals were injected with pneumococcus (Day and McClung, 1945).

These many investigations have established a correlation between diet variation and resistance of the host to bacterial, rickettsial, and viral infections. The means by which the vitamins alter the susceptibility of the host has only recently been examined. The theory that alterations in cellular metabolism may cause a shift in host-parasite relationship in favor of the parasite was reported by Dubos (1956).

The mechanisms involved in the alteration of resistance to disease have not been sufficiently studied. In order for clear-cut observations to be reported there must be a better understanding of the physiological defense mechanisms "in vivo". Such things as the inflammatory response, peripheral blood analysis, phagocytosis, complement activity, and antibody formation must be better understood. The part these nonspecific defense mechanisms employ during dietary inadequacies may determine to a

large extent as to whether a potential pathogen will produce disease or remain as a part of the normal flora.

Several of these nonspecific factors were studied in connection with diets deficient in specific vitamins. Wertman and Sarandria (1951) observed a decrease in antibody production in pyridoxine deficient rats. Inhibition of antibody production and also a decreased number of granulocytes and leukocytes was observed in folic acid deficient rats (Wertman et al., 1952 and 1956).

Complement, the thermolabile component of the blood which is necessary for the dissolution of bacteria *in vivo*, is a nonspecific physiological defense factor that has not been exhaustively studied. Complement activity in the sera of animals deficient in the "B vitamins" was not altered to any great extent (Prozansky and Axelrod, 1955). Wertman et al. studied the complement activity of animals deficient in niacin-tryptophan (1953), pyridoxine (1955), folic acid (1956), riboflavin (1957), and thiamine (1958).

Mudd et al. (1934) in observing the factors which induce phagocytosis, postulated that nutrition may have an influence on the activity of leukocytes. Werkman (1923b) studied the effect of B-complex avitaminosis on the ability of the host to elicit opsonins and thus maintain normal phagocytic ability. Results of *in vivo* studies did not compliment the results obtained *in vitro*. The results of the *in vivo* study had indicated a definite decrease in the

phagocytic activity of the vitamin deficient animals. However results from the *in vitro* studies exhibited no difference in the activity of deficient and *ad libitum* leukocytes. The conclusion was made that the impairment in phagocytic activity was not due to the inability of the deficient animals to elicit opsonin.

Upon purification and isolation of members in the vitamin B-complex group it was shown that rats deficient in thiamine, riboflavin, pyridoxine, pantothenic acid, choline, and ascorbic acid demonstrated a decreased phagocytic activity *in vitro* against *Micrococcus albus* (Cottingham and Mills, 1943). Gellhorn and Dunn (1937) made further observations and reported that starvation did not affect the phagocytic rate until there was a loss of over 35% of the body weight. They included an inanition control which Cottingham and Mills (1943) had omitted.

Berry et al. (1945) reported a reduction of 60-65% in phagocytic activity in the vitamin B-complex deficient rats.

It was observed by Wertman et al. (1953) that animals fed a diet deficient in niacin and tryptophan had no significant change in total number of leukocytes and erythrocytes per mm^3 of blood, as compared to inanition and *ad libitum* controls. They also reported that no complement activity could be demonstrated in the sera of niacin-tryptophan deficient animals even though amounts sixteen

times greater than those of the ad libitum controls were employed. Cellular migration to an inflammed area was considerably reduced in niacin-tryptophan deficient animals as compared with ad libitum controls.

A disease in rats termed Panmyelophthisis, a general wasting away or atrophy of the bone marrow, was manifested in animals maintained on prolonged niacin deficient diets (Gyorgy et al., 1935). Once the rats contracted this condition they did not recover with niacin therapy alone. Gyorgy et al. (1937) reported that slow recovery could be obtained if folic acid was fed in excess along with niacin therapy.

Nicotinamide in tissue is largely in the form of dinucleotide coenzymes with the pyridine N linked to a ribose residue. Two of these dinucleotides are: (1) diphosphopyridine nucleotide (DPN) and (2) triphosphopyridine nucleotide (TPN). Diphosphopyridine nucleotide and TPN function as coenzymes for a large number of apoenzymes called dehydrogenases. The apoenzyme generally exhibits a distinct preference, if not an absolute requirement, for the coenzyme involved, either DPN or TPN. These dehydrogenases function quite actively in the catabolism and anabolism of carbohydrates, lipids and proteins. Little is known concerning the metabolic degradation of the niacin coenzymes. Rabbit brain tissues can split nicotinamide from DPN by a nucleosidase action. Rabbit kidney

contain a pyrophosphatase which splits DPN into niacin mononucleotide and adenylic acid. Inasmuch as neither coenzyme nor their component ribosides are excreted in the urine, enzymatic cleavage of the nicotinamide-ribose bond apparently occurs as a general process of degradation. Normal adults excrete both nicotinic acid and nicotinamide in the urine (Cantarow and Schepartz, 1957). In the process of niacin-tryptophan deficiency it would seem plausible that there could be a diminution in both DPN and TPN. The lack of these coenzymes would have multiple effects on host metabolism.

Many conflicting results have been reported from studies concerning the influence of diet deficiencies on host resistance to infection. Experimental data observed prior to 1940 indicated little or no association between host susceptibility and diet. More recent work gives good indication to the concept that certain diet deficiencies subsequently lower host resistance to infection.

The lack of agreement between the earlier nutritional susceptibility studies and the more recent observations may be due to several factors. Guerrant et al.(1935, 1937) showed that starch, when employed as a carbohydrate source in synthetic diets, increases the rate of intestinal synthesis of various members of the vitamin B-complex group. Krehl et al. (1946) reported that in preparing a synthetic diet free from niacin, corn should be used in

place of caesine, because the latter contains large amounts of the amino acid tryptophan which the host can quite readily transform into niacin. Many of the immunological procedures have been subjected to modification and refinement resulting in greater sensitivity and specificity.

STATEMENT OF PROBLEM

The purpose of this investigation was to study the defense mechanisms of rats maintained on a niacin deficient diet with special reference to:

(1) The susceptibility of the vitamin deficient rat to infection when challenged intraperitoneally with strain of Type I Diplococcus pneumoniae.

(2) Determining the degree of bacteremia developing at specified time intervals during the course of infection.

(3) The phagocytic activity of leukocytes.

MATERIALS AND METHODS

Animals and housing. The animals employed in this experiment were male weanling albino rats of the Sprague-Dawley strain. The animals were 20 to 30 days old and weighed approximately 45 grams at the onset of the experiment. All animals were placed in individual mesh-bottom cages and were maintained in an air-conditioned room with a constant temperature of 25 C. Each animal was provided with a water bottle and a food dish which were replenished daily.

The animals for each study were divided into three groups: Ad libitum, niacin deficient and inanition.

Experimental diet and feeding. The basal vitamin free diet which was employed successfully by Wertman et al. (1953) was used for all groups of rats in this niacin study. The ingredients[1,2] used in preparation of the basal diet were of the highest purity available. The basal diet consisted of the following: Corn, medium cracked, 40.00%; caesine (vitamin free), 9.00%; salt mixture #2 (U.S.P.), 4.00%; choline chloride, .20%; i-inositol, .03%;

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[1] The Nutrional Biochemical Company, Cleveland, Ohio

[2] The California Biochemical Corporation, Los Angeles, California

l-cystine, .15%; corn oil[3], 2.00%; sucrose, 44.00%; vitamin K, .001%; p-aminobenzoic acid, .001%; d-alpha-tocopherol, .01%; and 1,4-naphthoquinone, .001%.

Each animal was fed, in addition to the above daily basal diet, one vitamin tablet. Vitamin tablets were prepared with the following vitamin composition (in micrograms): thiamine, 40; riboflavin, 60; pyridoxine, 50; pantothenic acid, 150; niacin, 150; biotin, 1; and folic acid, 1. Niacin was omitted from the tablets fed to the deficient group.

Vitamin tablets were compounded using lactose as a binder. The vitamin-lactose mixture was granulated by wetting with 50% Karo white syrup and passing through a #8 seive. After drying, the granulated material was passed through a #16 sieve and a lubricant, 2% sodium stearate, was added by weight to improve the efficiency of the tablet punching machine (Remington, 1956). In addition to the basal diet and vitamin tablet supplement, each animal received 300 U.S.P. units of vitamin A and 30 U.S.P. units of vitamin D once each week orally through three drops of cod liver oil[4].

After arrival at the laboratory, all animals were maintained on the basal diet and the complete vitamin

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[3] Mazola Oil

[4] Squibbs

supplement for a stabilization period of one week. This procedure was intended to acclimate the animals to their new diet and living arrangements (Wertman et al., 1954). Following the stabilization period, the animals in the ad libitum and deficient groups were fed the basal diet ad libitum, while the inanition group received the basal diet in amount sufficient to maintain their weights equal to that of the animals in the niacin deficient group. The animals were maintained on this feeding schedule for eight weeks. At this time the animals in the niacin deficient group showed the typical symptoms of niacin deficiency.

At the end of the eighth week of experimental feeding, the ad libitum and inanition control groups were divided into two sub-groups. One sub-group was challenged with Diplococcus pneumoniae at the end of the eighth week and the second sub-group was challenged at the end of the ninth week. The niacin deficient group of animals was divided into three sub-groups. Sub-group #1 was challenged at the end of the eighth week along with the first sub-groups of the ad libitum and inanition controls. The #2 niacin deficient sub-group was given 150 µg of niacin daily, for the seven day period following the first challenge. This sub-group was then challenged at the end of the ninth week with the second sub-groups of the ad libitum and inanition controls. The #3 sub-group was maintained on the basal diet and niacin deficient tablet

throughout the entire experiment to determine if any deaths resulted from the vitamin deficiency. Initial and final mean weights for each group appear on Table I.

Bacterial cultures. The bacterial organism employed in this experiment was a strain of Type I Diplococcus pneumoniae. The organism was transferred daily in beef "hormone" broth (beef infusion broth with 2% glucose and 0.5% gelatine). Virulence was obtained by daily passages of 0.5 ml of an 18 hour culture injected intraperitoneally into mice. At the end of five to six hours the mouse was sacrificed, the viscera swabbed, and the exudate inoculated into heart infusion broth, which was employed as the inoculum for the next passage. After ten passages the virulence of the organism was raised to the point that it killed mice in six to eight hours. The virulence of the organism was maintained by daily passages through heart infusion broth and a weekly passage through mice. To prepare the organism for use in the experiment, an 18 hour culture was washed and resuspended in sterile broth. It was adjusted to a reading of 0.08 optical density (O.D.) in a Lumetron, at a wave length on 650 mu. The bacterial count equivalent to 0.08 O.D. was found to be 2×10^5 organisms/cu mm.

Susceptibility and bacteremia studies. A challenge study was performed to determine the susceptibility of niacin deficient rats to infection. The results of this study were compared with those of the ad libitum and

inanition control animals under identical conditions.

At the end of the eighth week of experimental feeding, deficiency signs were observed. At this time the first challenge was conducted using 2 ml of virulent Type I Diplococcus pneumoniae. The bacterial suspensions were prepared as was described previously. After injection the animals were observed closely for a few hours to watch for deaths due to trauma, so as not to include these deaths in the final results.

It was observed by Loughlin et al. (1945) that normal albino rats are susceptible with a high mortality rate to lobar pneumonia, when exposed to Diplococcus pneumoniae through the respiratory tract, but Wertman and Groh (1959), Wertman and Sypherd (1960), and Wertman and Pathak (1960) reported that normal albino rats are not susceptible via the intraperitoneal route. In view of this last information the intraperitoneal route was used in the challenge study.

Also at this time preparations were made for a bacteremia study, at intervals of 12, 24, and 48 hours. At the end of each selected time period one third of the animals included in the first challenge were bled by cardiac puncture. Immediately after drawing approximately 1.5 ml, a portion of this, 1.0 ml, was inoculated into 9.0 ml heart infusion broth. Proper dilution techniques were employed to get 1:100 and 1:1000 dilution of blood from each animal.

Each tube was shaken ~~vi~~gorously and 1.0 ml of each dilution was placed in a petri dish and covered with liquid agar. After 36 hour incubation at 37 C the colonies of Diplococcus pneumoniae on each plate were counted.

After the first challenge (sub-group #2), niacin deficient animals received 150 µg of niacin every day for a period of seven days while the ad libitum and inanition control rats continued on the experimental diet. A second challenge and bacteremia study was performed at the end of this seven day period (end of the ninth week).

Sub-group #3 of the niacin deficient group was maintained on the deficient diet for the entire experiment. This was done in order to determine deaths which could be attributed solely to vitamin deficiency. This group was not included in either challenge study. No deaths occurred in this group.

Phagocytosis. This study was conducted in order to determine white blood cell activity, particularly that of the neutrophils in the niacin deficient group as compared to the rats in the ad libitum and inanition controls.

The phagocytic studies were performed according to Cottingham and Mills, modified by Wertman and Groh (1959) and Wertman and Sypherd (1960). The phagocytic studies were conducted at the time when deficiencies typical of niacin were established in the rats. At the end of the fifth week the rats were bled by means of the cardiac

puncture, using syringes ~~that~~ had been rinsed in heparin. From each rat approximately 1.0 ml was withdrawn and exactly 0.5 ml of this sample was placed in the side arm of a Warburg flask. To this flask 0.2 ml of 2 X 10^5 bacterial suspension was added. The blood bacteria suspension was immediately placed into a 38 C water bath and slowly agitated in lateral motion of 240 reversals per minute for four minutes. This agitation thoroughly mixed the Diplococcus pneumoniae with the blood. After four minutes the flasks were removed from the water bath and five smears were prepared from each sample. The slide preparations were air dried and stained with Wright's stain. The cells were counted and the percentage of active phagocytizing neutrophils was recorded. In addition, the number of bacterial organisms phagocytized by neutrophils in 100 unclumped and unruptured cells was counted. The average number of organisms per cell was then determined.

At the end of the seventh week a second phagocytic study was performed involving the same techniques as that in the first. This study was conducted to determine if the niacin deficient rats showed a decreased neutrophil activity as a result of prolonged niacin deficiency.

Following the second phagocytic study a group of the niacin deficient rats were fed 150 µg of niacin each day for a seven day period. At the end of this period a third phagocytic study was performed on these animals and

representatives of the ad libitum and inanition controls who were maintained on the experimental diet.

A consideration was made of the time involved in bleeding and making smears. Cottingham and Mills (1943) reported that no alteration in the phagocytic activity of leukocytes held for as long as five hours at 37 C could be observed.

RESULTS

Typical symptoms of niacin deficiency were observed at the end of the sixth week of experimental feeding. The *ad libitum* animals appeared healthy and had smooth, even coats and were gaining weight. The inanition group appeared healthy even though they were restricted as to diet intake. They also had white, smooth, even coats. The deficient animals gained little or no weight in the last two weeks, their skin was rough and becoming discolored and alopecia was apparent. The distribution and initial and final mean weights of each group appear in Table I.

Three separate, but related studies, were completed: (1) the susceptibility of the groups to infection by *Diplococcus pneumoniae*, (2) the degree of bacteremia produced by this organism in vitamin deficient and control animals, and (3) the *in vitro* phagocytosis of virulent Type I *Diplococcus pneumoniae* by neutrophils of rats.

The infectivity studies implied that niacin deficient rats were more susceptible to *Diplococcus pneumoniae* infection, while the *ad libitum* and inanition animals were not. Of the 18 animals in the *ad libitum* group that were included in the first challenge study only one animal died, one out of 17 animals in the inanition group died, and 29 animals out of 52 in the niacin deficient group died as a

result of infection. The fatality rates for the three groups were as follows: ad libitum 5.5%; inanition 6.0%; and deficient 55.0%. The group of deficient animals that received 150 µg of niacin daily for one week after deficiency had been established were not as susceptible to infection as the first group. All the animals in this second group survived the challenge dose. Seven animals that had been in the inanition group for eight weeks were fed ad libitum for one week and challenged with the standardized Diplococcus pneumoniae inoculum. All animals of this group survived the infection. There were no fatalities in the ad libitum group as a result of the second challenge dose.

Five animals from the niacin deficient group were maintained on the deficient diet for the entire experimental period of nine weeks. These animals were unchallenged and were included to indicate any deaths that may have been a result of niacin deficiency. All the animals in this group survived the nine week extended deficiency. The data obtained from the challenge studies are presented in Table II.

The bacteremia study was conducted because earlier pilot challenge studies had indicated a small percentage of deaths in the niacin deficient group. The bacteremia study indicated that niacin deficient animals developed a severe bacteremia. The inanition animals showed bacteremia to a

greater extent than ad libitum rats but to a much lesser extent than the deficient group. The bacteremia study performed in conjunction with the seventh week challenge gave numbers of organisms per ml of blood 12 hours after injection: ad libitum 1,335, inanition 6,460, and deficient 280,000. After 24 hours the counts were: ad libitum 740, inanition 1,750, and deficient 94,000; and after 48 hours: ad libitum 200, inanition 882, and deficient 2,870 organisms per ml of blood.

The niacin deficient group that had received 150 µg of niacin daily for one week showed a marked reduction in bacteremia after receiving a challenge dose. The results of this second bacteremia study, which was conducted at the same time as the second challenge, are as follows: at the end of 12 hours: ad libitum 875, inanition 6,700, niacin deficient 6,375; after 24 hours: ad libitum 300, inanition 1,040, and niacin deficient 407. The data obtained from bacteremia study appears in Table III.

Wertman et al. (1953) reported that there was no significant difference in total leukocyte count between niacin-tryptophan deficient animals and ad libitum control animals. It was observed that cellular migration to the inflammed area was considerably reduced in the niacin-tryptophan deficient animals. Complement activity could not be demonstrated in the sera of niacin-tryptophan deficient animals as compared with the exudates from ad libitum

control rats. The phagocytic study conducted here was to determine the differences in phagocytic activity of leukocytes (neutrophils) of animals in the niacin deficient group as compared to ad libitum and paired weight animals of the inanition group. This was accomplished by noting (1) the percent of neutrophils active in phagocytosis, and (2) the degree of activity shown by these cells as indicated by the average number of organisms engulfed per phagocyte. A definite difference was observed both in the percentage of active neutrophils and in the degree of activity. The study was performed at the end of the seventh week and again at the end of the eighth week when a group of niacin deficient animals had received 150 µg of niacin for seven days. The results obtained from the seventh week phagocytic study were 79.2% of the ad libitum leukocytes, 50.1% of the inanition, and 13.6% of the niacin deficient leukocytes were active in phagocytosis of the Diplococcus pneumoniae organism. The average number of bacteria phagocytized per cell was: ad libitum neutrophils 11.9; inanition neutrophils 5.3; and niacin deficient neutrophils 2.1. This study indicates that niacin deficiency does decrease the phagocytic ability and efficiency of the neutrophil in removing foreign materials.

A second phagocytic study, similar to that of the first, was conducted on a second group of niacin deficient animals which had been maintained on a niacin free diet for

seven weeks and then were fed 150 µg of niacin daily for one week. The differences in leukocyte activity and efficiency between the deficient and ad libitum groups at the end of the eighth week were not as severe as those observed at the end of the seventh week. The niacin deficient animals that received 150 µg in their diet every day had an increase in phagocytic activity (24.2%) as compared to the animals that did not receive the vitamin (13.6%). There was also an increase in the average number of organisms engulfed per cell in the rats fed the vitamin. At the end of this seven day period the number of organisms per leukocyte was 3.4 as compared to 2.1 organisms engulfed per leukocyte at the end of the seventh week. The results of the phagocytic study appear on Table IV. Only results of the seventh and eighth week phagocytic studies are included because the results of fifth week phagocytosis were in comparison the same as results of the seventh week. Animals involved in fifth week phagocytic study were used again for the seventh week experiment.

By comparing the active leukocytes within each experiment it was observed that the number of active leukocytes of the deficient rats was 17.2% that of the active leukocytes in the ad libitum at the end of the seventh week. This percentage was increased to 39.3% after the deficient animals received 150 µg of niacin daily during the eighth week. The average number of bacteria engulfed also showed

an increase in percentage at the eighth week (35.6%) to (17.6%) observed at the seventh week.

DISCUSSION

The results of the phagocytic study indicate that extended absence of niacin in the diet of rats contributes to the reduction of phagocytic activity in the leukocytes in these niacin deficient animals. The inanition animals also demonstrated a decrease in leukocyte activity, however, not of the same order as that of the niacin deficient animals. The exact mechanism for reduced phagocytic activity in the niacin deficient rats has not been determined at this time. However, certain facts are at hand which may account for the reduction. Wertman et al. (1954) reported no significant difference in the total number of erythrocytes and leukocytes per mm^3 of blood in niacin-tryptophan deficient, inanition, and ad libitum control animals. Cellular migration to an inflammed area was considerably reduced in niacin-tryptophan deficient animals. Complement activity could not be detected in the animals maintained on a niacin-tryptophan deficient diet. Several investigators have shown the importance of complement in antigen-antibody reactions and in enhancing phagocytic activity. Skarnes and Watson (1957) report that complement in conjunction with normal antibody produce bactericidal effects and promote increased phagocytic activity. Ward and Enders (1933) reported an increased phagocytic rate by leukocytes due to

complement and antibodies making the microorganism more adhesive to the leukocyte. Wright and Douglas (1903) observed a specific thermolabile antibody, opsonin, which combined with surface components of microorganisms so that they were more readily phagocytized. Complement is thought to participate with opsonin in its activity. The source of complement is still not exactly known, however it does enhance antigen-antibody reactions and phagocytic activity. Suter (1956) reported that complement and calcium ions are responsible for increased motility of leukocytes. This observation gives a good explanation as to why there was reduced leukocyte migration to infection sites in niacin deficient animals in which no complement activity could be demonstrated. It is feasible to expect greatly reduced phagocytic activity in the niacin deficient animals due to the absence of detectable complement which enhances body defense mechanisms. The fact that there was a significant reduction in total leukocytes recovered from inflammatory exudate of deficient animals over that of the ad libitum and inanition control animals confirms the present results of reduced phagocytic activity in the niacin deficient animals.

It is conceivable that a variation in phagocytic activity is due to a change in the leukocyte during vitamin deficiency (Cottingham and Mills, 1945). Cellular composition might be altered due to modification or hindrance of

the metabolic processes in which niacin is involved.

Niacin exists in the rat in its amide form as nicotinamide. The rat is able to synthesize nicotinamide from nicotinic acid as it appears in the diet or from the amino acid tryptophan (Snell, 1953). The nicotinamide in combination with adenylic acid is found in the form of the coenzymes diphosphopyridine nucleotide (DPN or coenzyme I) and as triphosphopyridine nucleotide (TPN or coenzyme II). Diphosphopyridine nucleotide and TPN function as prosthetic groups for a large number of apoenzymes called dehydrogenases. These dehydrogenases function quite actively in glycolysis, the hexose monophosphate pathway, the citric acid cycle, and in fatty acid synthesis and degradation (Cantarow and Schepartz, 1957). The apoenzyme generally exhibits a distinct preference, if not an absolute requirement, for the coenzyme involved, either DPN or TPN. Although there is not a great turnover of DPN(TPN), the exclusion of niacin and tryptophan from the diet will affect the metabolism as the two coenzymes are catabolized. The first modification will be that of generally slowing down the catabolic processes in which DPN(TPN) are involved. A key reaction is the action of pyruvic acid with coenzyme A to form acetyl CoA. Diphosphopyridine nucleotide is involved here as the coenzyme. A slowing up of this reaction due to insufficient quantities of DPN can affect the cell in a number of ways. First of all acetyl CoA leads directly

into the citric acid cycle where oxidation takes place, leading to ATP synthesis. This operation relies fully on DPN and TPN which act as hydrogen carriers for their specific apoenzymes upon dehydrogenation of the citric acid cycle intermediates. The lack of adequate quantities of DPN and TPN can reduce the rate at which the citric acid cycle proceeds and as a result hamper ATP synthesis. It is possible that the cycle may completely stop.

Sbarra and Karnovsky (1959) observed an increased lipid synthesis in the leukocyte during phagocytosis, which the authors suggested was required to maintain the cell wall membrane. It was reported by Milbrandt (1930) and Monaghan (1932) that animals suffering B-avitaminosis had a decrease in amount of blood and tissue phospholipids. Snell (1953) observed that DPN and TPN were involved in fatty acid synthesis and fatty acid oxidation. Acetyl CoA leads into fatty acid synthesis and is the product of fatty acid oxidation. The quantity of acetyl CoA present can alter the rate of fatty acid synthesis. Diminishing quantities of DPN will slow down acetyl CoA formation and therefore slow down or stop fatty acid synthesis.

It is possible that reduced amounts of DPN(TPN) due to niacin-tryptophan deficiency, and diminution of phospholipids would reduce leukocyte lipid synthesis, especially since phospholipids are thought to be responsible for transporting fatty acids for synthesis into fats

(Fruton and Simmonds, 1960). Sinclair (1934) reported an increase in blood phospholipids during high fever and infection, indicating a greater demand for fat metabolic intermediates during infection. An observation can be made at this time in regard to acetyl CoA and DPN(TPN) and their roles in fatty acid synthesis. It appears reasonable that if these two constituents appear only in limited quantities that fatty acid synthesis would be sufficiently reduced so as to be unable to meet the demand by the leukocyte for increased fat synthesis.

Diphosphopyridine nucleotide and TPN function in amino acid metabolism to some extent also. Diphosphopyridine nucleotide functions as a cofactor in the deamination of glutamic acid (Fruton and Simmonds, 1960). Both TPN and DPN are involved in various degradation and synthesis processes of the amino acids. It is conceivable that protein synthesis may be either hindered or modified to some extent. This may also have an effect on the phagocytic activity and general nonspecific resistance of the host.

Diminishing amounts of DPN and TPN would in effect slow down respiration. In most instances reduced forms of DPN and TPN initiate a series of oxidation reduction reactions involving the transfer of two electrons to a final acceptor. In the process ATP is synthesized. Decreasing amounts of the reduced coenzyme would slow up respiration

and ATP synthesis. Lack of a sufficient energy source would help to impair metabolism processes. The last observation can be shown from the weight differences exhibited by the niacin deficient animals as compared to the ad libitum control animals (Table I).

A two week lag in the recovery of normal phagocytic activity when deficient animals were fed the proper vitamins was reported by Cottingham and Mills (1945). This lag was demonstrated after one week of including 150 µg of niacin in the diet. Results show that complete recovery was not obtained after one week of the niacin feeding. The lag observed during niacin feeding may correspond to regeneration in the nicotinamide nucleotide coenzymes, increase in respiration and ATP synthesis, and increase in phospholipid synthesis (Cottingham and Mills, 1945). After one week of niacin feeding, phagocytic activity increased 100%.

Loughlin et al. (1945) showed that normal albino rats are susceptible to death from lobar pneumonia, but not from bacteremia after intraperitoneal injection (Wertman and Groh, 1959). The death rate among the niacin deficient rats might be expected due to the reduced phagocytic activity (Table IV), the reduction in complement activity, reduced leukocyte migration to inflammation site, and decreased metabolic processes.

SUMMARY

Male albino rats of the Sprague-Dawley strain were maintained on well defined diets deficient in niacin in order to study the effect of deficiency on (1) the susceptibility to infection by virulent Type I Diplococcus pneumoniae, (2) the determination of the degree of bacteremia, and (3) the phagocytic activity of leukocytes. Adequate numbers of inanition and ad libitum control animals were included. The following observations were made.

1. Animals deficient in niacin were very susceptible to infection by Diplococcus pneumoniae. The rats in the inanition and ad libitum groups were not very susceptible. No fatalities occurred in the niacin deficient group when given the niacin daily requirement for one week prior to challenge.

2. The niacin deficient animals exhibited a high degree of bacteremia in comparison to the ad libitum. Inanition rats showed a much lesser degree of bacteremia than the deficient but a larger degree than the ad libitum. The deficient animals placed on the niacin daily requirement for one week prior to challenge showed a marked reduction of bacteremia.

3. The leukocytes of niacin deficient rats were greatly reduced in their phagocytic activity on Diplococcus

pneumoniae. The percentage of neutrophils actively engaged in phagocytosis and the number of bacteria each neutrophil had engulfed were considerably lower than those of the ad libitum rats. The leukocytes from the inanition animals exhibited a reduction in activity over that of ad libitum cells. The inanition neutrophils were considerably more active than the deficient neutrophils.

TABLE I

Distribution and Initial and Final Mean Weights of Rats

Group	Total No. rats	Mean Weight in Grams		
A. Susceptibility and Bacteremia Study				
		Initial	8th week	9th week
Ad libitum control	34	66	199(34)†	233(15)
Niacin deficient	74	71		
Sub-group #1*			111(56)	- - -
Sub-group #2**			114(13)	125(13)
Sub-group #3***			108 (5)	110 (5)
Inanition control	31	70	119(31)	129(14)
B. Phagocytic Study				
		Initial	7th week	8th week
Ad libitum control	37	65	188(37)	195(23)
Niacin deficient	61	62		
Sub-group #1			92(25)	- - -
Sub-group #2			90(21)	121(21)
Sub-group #3			93(15)	91(15)
Inanition control	19	65	91(19)	125(13)

*Rats maintained 8 weeks on deficient diet.

**Rats maintained 8 weeks on deficient diet, followed by 1 week on complete diet including 150 µg niacin daily.

***Rats maintained 9 weeks on deficient diet.

† Number of animals in experiment at end of indicated time period, in parentheses.

TABLE II

Susceptibility of Niacin Deficient and Control Animals to Diplococcus pneumoniae

Group	No. of rats	No. survived	Fatality %
8th Week Challenge			
Ad libitum control	18	17	5.5
Niacin deficient	52	23	55.0
Inanition control	17	16	6.0
9th Week Challenge			
Ad libitum control	7	7	0.0
Niacin deficient*	11	11	0.0
Niacin deficient**	5	5	0.0
Inanition control	7	7	0.0

*Rats in this group were fed 150 µg niacin daily for 1 week following 8 weeks of deficiency.

**Rats in this group were on an extended deficiency and remained unchallenged.

TABLE III

Number of Bacteria Cultured from the Blood of Deficient and Control Rats at Specific Time Intervals after Injection with Diplococcus pneumoniae.

Group	Total No. Rats	No. of Bacteria /ml of Blood 12hr*	24hr	48hr
		8th Week Challenge		
Ad libitum control	17	1,335	740	300
Niacin deficient	35	280,000	94,000	2,870
Inanition control	16	6,460	1,750	882
		9th Week Challenge		
Ad libitum control	7	875	300	300
Niacin deficient**	11	6,375	1,190	407
Niacin deficient***	5			
Inanition control	7	6,700	2,100	1,040

*Time after injection of virulent Diplococcus pneumoniae.

**Animals were fed 150 µg of niacin daily for 1 week following 8 weeks of deficient diet.

***Animals on extended 9 week deficiency. Some of these animals died as a result of infectivity.

TABLE IV

Phagocytic Activity of Leukocytes from Niacin Deficient and Control Rats

Group	Average % active neutrophils in each group	No. bacteria /cell	
		Average	Range
7th Week Study			
Ad libitum control	79.0(15)*	11.9	9.6 - 16.5
Niacin deficient	13.6(25)	2.1	.6 - 3.9
Inanition control	50.1 (6)	5.3	2.8 - 7.6
8th Week Study			
Ad libitum control	61.0(18)	9.6	6.4 - 12.5
Niacin deficient**	24.0(20)	3.4	2.2 - 4.9
Inanition control	46.8 (6)	4.2	3.5 - 4.9

*Number of animals in experiment at end of indicated time period.

**Niacin deficient group was fed 150 µg of niacin daily during the 8th week.

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