117
483
THS

THE EFFECT OF PENICILLIN AND DICOUMAROL ON BLOOD COAGULATION AND DPN CYTOCHROME-c REDUCTASE ACTIVITY IN THE RAT

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Sister Mary Romana McDermott, S. N. J. M.

1959

MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 01079 1881

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.

DATE DUE	DATE DUE	DATE DUE
049		

MSU Is An Affirmative Action/Equal Opportunity Institution
c:\circ\datedue.pm3-p.1

THE EFFECT OF PENICILLIN AND DICOUMAROL ON BLOOD COAGULATION AND DPN CYTOCHROME-c REDUCTASE ACTIVITY IN THE RAT

by

Sister Mary Romana McDermott, S.N.J.M.

A THESIS

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Foods and Nutrition
College of Home Economics

1959

ACKNOWLEDGEMENT

This worker wishes to express her sincere thanks to Dr. Dorothy Arata for her encouragement, guidance and constructive criticism; and to Catherine Carroll for her assistance in checking data.

TABLE OF CONTENTS

TABLES

INTRODUCTION

INTRODUCTION

Antibiotics have come to play an important role in the lives of the present generation. The medical profession recognized the usefulness of antibiotics in the treatment of disease. The nutritionist discovered their ability to improve the growth and quality of livestock. The antibiotics were widely used before reports began to appear which indicated possible unfavorable reactions to their wanton use.

In the case of penicillin, one of the most widely used of the antibiotics, conflicting reports appeared concerning the effect of this compound on the coagulation time of the blood. Some laboratories reported an observed increase in coagulation time in patients under penicillin therapy, while other workers reported the opposite effect, and still others could measure no effect of penicillin on blood clotting time. Obviously, any effect of penicillin on blood coagulation would seriously restrict the use of this antibiotic in certain clinical conditions.

In contemplating the possible interrelationship between penicillin and blood coagulation, vitamin K is immediately implicated. Vitamin K has long been recognized as an essential factor in the synthesis of prothrombin, a protein necessary for the normal coagulation of blood.

The

ties

dire

port

cerr

form

play

be e

of p

may

lati

resp

In c

redu

acti

sume

for

anim

be c

stud

prac

The fact that the sulfa drugs exert many of their properties by virtue of their action on microflora and thus indirectly on the synthesis of several vitamins, lends support to the association of penicillin with vitamin K.

In recent years several workers have been concerned with functions of vitamin K other than prothrombin formation. It has been suggested that the vitamin may play a role in cell metabolism, and that this role may be equally as important as that of catalyzing the synthesis of prothrombin. Recent findings indicate that vitamin K_3 may function as a hydrogen carrier in the respiratory chain.

This experiment was undertaken to study the relationship, if any, between penicillin and vitamin K with respect to both blood coagulation and cellular metabolism. In order to measure the latter, the enzyme DPN cytochrome-c reductase was chosen for two reasons: (1) it is an enzyme active in electron transport where vitamin K is also presumed to function; and (2) a relatively simple assay method for this system is known.

The albino rat was chosen as the experimental animal for obvious reasons. It was hoped that data would be collected which would provide suggestions for further study in human subjects with ultimate implications for practical clinical application.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Effect of Vitamin K on Blood Coagulation

Prothrombin is a protein which functions as a precursor for thrombin -- a substance necessary for the formation of a clot. The liver is intimately concerned with the production of prothrombin. Two main lines of experimentation have been the basis of this evidence: (1) the effect of liver poisons and liver injuries on the prothrombin level in the plasma; and (2) the influence exerted by vitamin K on the formation of prothrombin (Chargaff 1945). It is probable that prothrombin is present in most of the body fluids, but satisfactory evidence as regards its distribution in tissues is lacking since the material is easily contaminated with blood. From calculations based on the volume of blood, it was shown that vitamin K was not part of the prothrombin molecule but was concerned with the mechanism that produced prothrombin. It was suggested (Kemmerer 1952) that vitamin K serves as the prosthetic group which unites with the apoenzyme to form the active synthesizing enzyme. Bernheim and Bernheim (1940) have shown that naphthoquinone derivatives are able to catalyze the formation of the -S-S- groups in prothrombin from -SH groups of cysteine <u>in vitro</u>.

Hypoprothrombinemia is produced by a number of compounds which act as metabolic antagonists to vitamin K; the best known of these is dicoumarol. The administration of vitamin K counteracts the action of dicoumarol.

Hypoprothrombinemia has been observed clinically, particularly in cases of jaundice and sprue where there is interference with the absorption of fat, and thus of the fat soluble vitamin K. According to Reich et al (1947) the newborn are particularly susceptible to vitamin K deficiency, and hence to hypoprothrombinemia, due to a relatively inactive microflora.

In an effort to isolate the specific role played by vitamin K in the synthesis of prothrombin Dam (1942) observed that when vitamin K is given intravenously it is possible to study the effect at different time intervals from the moment of its introduction into the blood stream. Using this procedure, it was shown that the action of the vitamin was not immediate. Approximately 5 hours were required to increase the prothrombin content of the blood of a K-avitaminous chick following injection of vitamin K_1.[1] The prothrombin level reached a normal value in most cases 24 hours after the injection. Thereafter

1. Several forms of vitamin K are known. In this paper reference will be made to 3 forms of the vitamin: (1) vitamin K_1 which is found in green plants; (2) vitamin K_2 which is synthesized by bacteria; and (3) vitamin K_3 (menadione) which is a synthetic form of the vitamin.

it decreased unless large quantities of vitamin K_1 were given, in which case the prothrombin level remained normal for a few days before a marked decrease was observed. If smaller quantities were injected than were necessary to bring the prothrombin up to the normal value, then the decrease occurred more rapidly. Similar results have been obtained in experiments with oral administration of the vitamin.

Dam (1942) noted further that the prothrombin content of the blood from a K-avitaminous animal does not increase when vitamin K is added in vitro, even if the vitamin remains in contact with the blood for 5 or 6 hours at body temperature. This observation suggests that the action of the vitamin takes place in tissues other than the blood.

SITE OF ACTION OF VITAMIN K

Several workers have studied the various organs in an attempt to define the site of action of vitamin K in the synthesis of prothrombin. Dam (1942) proposed, as a method of examining the importance of a given organ to the action of vitamin K_1, the removal of the organ from a K-avitaminous animal, introduce vitamin K intravenously, and follow the rate at which prothrombin is synthesized.

With this technique it has been shown that the spleen is not essential to the action of vitamin K. The work of Andrus et al (1941) showed that the removal of a portion of the liver of normal dogs resulted in a decrease in the level of prothrombin in the blood. This observation corroborated that made by Warner et al (1938) who reported a decrease in prothrombin after removal of two-thirds of the liver in rats.

More recent studies with labeled vitamin K have supported these observations. Taylor et al (1956) administered vitamin K_1-C^{14} by various routes to rats. The liver showed the greatest amount of radioactivity. In further work by Taylor et al (1957) vitamin K_1-C^{14} was found concentrated in the liver of the rat.

EFFECT OF PENICILLIN ON BLOOD COAGULATION

Reports in the literature of the effect of penicillin on blood coagulation have been contradictory. Moldavsky, Hesselbrook, and Cateno (1945) found that the coagulation time of the blood was decreased following the administration of penicillin. Further work from this same laboratory (Moldavsky et al 1953) showed that the coagulation time in human subjects of apparent good health was consistently decreased after administration of penicillin.

There was a direct relationship between the clotting time and the concentration of penicillin in the blood. Analysis of blood samples obtained at fifteen minute intervals after intramuscular injection of penicillin revealed no change in the hemoglobin, red blood cell count, the sedimentation rate and hematocrit. Those elements associated with coagulation, i.e., plasma fibrinogen and serum calcium and prothrombin also remained unaffected by the presence of penicillin in the blood. However, the number and size of platelets in the circulating blood during this period increased. There was greater platelet fragility, and a more pronounced tendency to aggregate and agglutinate. Since it is known that the spleen is associated with the maintenance of normal platelets (Olef 1936, 1937) it was concluded that this organ was implicated in the effect of penicillin on blood coagulation. Moldavsky et al (1945) proposed that the penicillin must mediate its effect by stimulation of the spleen since this antibiotic had no effect on blood coagulation when the spleen was removed from human subjects. A similarity was noted between the effects of penicillin on the blood platelets as described by Moldavsky et al (1945, 1953) and the effects of adrenaline as described by Olef (1935, 1936, 1937). Adrenaline empties the blood reservoirs in the spleen and produces an increase in the number of platelets. The fact that

penicillin increased blood coagulation was also noted by Frada (1946). He observed an increased incidence of embolism of the large blood vessels occurring in the course of penicillin therapy in acute and subacute endocarditis.

The opposite effect of penicillin on blood coagulation in the human was observed by Lewitus (1948). He observed a prothrombinopenic effect of penicillin. Lewitus has suggested that in the combined dicoumarol-penicillin therapy for infective thrombosis, less dicoumarol would be needed than would be necessary in the absence of penicillin.

In contrast to these reports, Lewis (1946) was unable to demonstrate any effect of penicillin on blood coagulation in normal or hemophiliac subjects. Likewise, Weiner et al (1948) found that when therapeutic quantities of penicillin were administered to humans the coagulability of the blood did not change to a significant degree. Triantaphyllopoulos and Waisbren (1952) carried out experiments in vivo and in vitro on the effect of varying concentrations of crystalline penicillin on the prothrombin time, coagulation time, and clot retraction of normal human blood. Penicillin had no effect on these processes. These data were in confirmation of previous studies which had failed to show an effect of penicillin on these factors, and in conflict with those which indicated a relationship between penicillin and blood coagulation.

may be

observ

ment w

fectiv

cillin

F was

worke

fluen

penic

line

of bl

the a

ing

the

when

an i

hours

after

if ar

penic

agula

A possible explanation of these conflicting data may be found in the work of Macht and Ostro (1947) who observed that the type of penicillin used in the experiment was of marked importance. Penicillin X was most effective for increasing the coagulability of blood. Penicillins K and G ranked second and third, while penicillin F was least effective in increasing coagulability. These workers also found that the purity of the penicillin influenced the results. They observed that the amorphous penicillin increased coagulability, but that the crystalline penicillin caused no increase in the coagulation time of blood. They suggested that the impurities present in the amorphous product were responsible for the conflicting reports in the literature.

Another suggestion has been advanced to explain the contradictory results on coagulation time obtained when penicillin is administered. Lewis (1946) stated that an increase in prothrombin time occurred in the first few hours following the administration of penicillin and thereafter decreased below normal. According to this thesis, if an insufficient period of time is allowed following penicillin treatment, erroneous data regarding blood coagulation time may be obtained.

ROLE OF VITAMIN K IN ELECTRON TRANSPORT

Several workers have noted that vitamin K_1 and vitamin K_3 apparently do not follow the same metabolic pathway. Vitamin K_1 has been found to be more effective than vitamin K_3 in the prevention or treatment of the effects of anticoagulants in humans, rats, and chicks (Boyer 1955). Weber et al (1958) found that Mycobacterium phlei cells exhibited specific dependence on vitamin K_1 for coupled oxidative phosphorylation. This is in agreement with the work of Martius and Nitz-Litzow (1955) who found that vitamin K_1 derivatives, but not menadione, increased the oxidative phosphorylation of mitochondrial preparations for vitamin K deficient chicks. Thus vitamin K_1 appears to be intimately involved with oxidative phosphorylation.

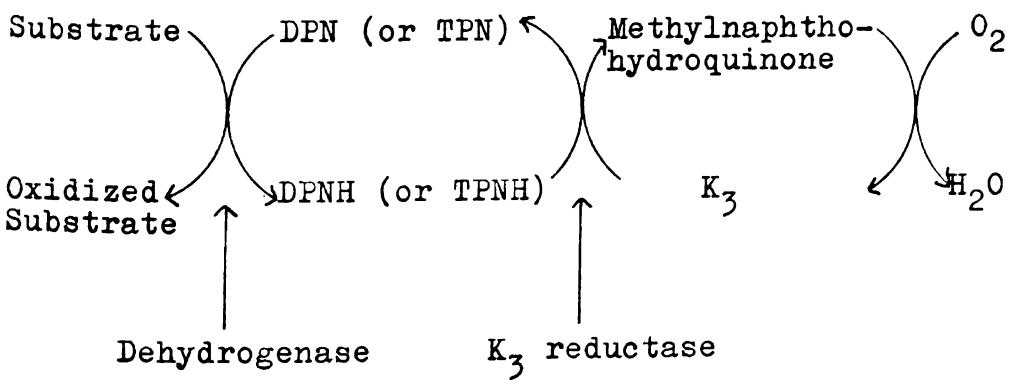
Vitamin K_3 (menadione) has also been shown to be involved in electron transport, though in a different capacity than is vitamin K_1. Schulz and Goss (1956) found that menadione inhibited phosphorylation by promoting electron transport via a pathway which by-passed the phosphorylative mechanism.

Martius and Nitz-Litzow (1954) carried on extensive investigation of the uncoupling effect of oxidative phosphorylation by dicoumarol. According to these workers, this uncoupling action is directly related to its

antagonism to vitamin K. If vitamin K_3 (menadione) is not involved in oxidative phosphorylation, presumably the uncoupling action of dicoumarol must be a function of its antagonism to vitamin K_1.

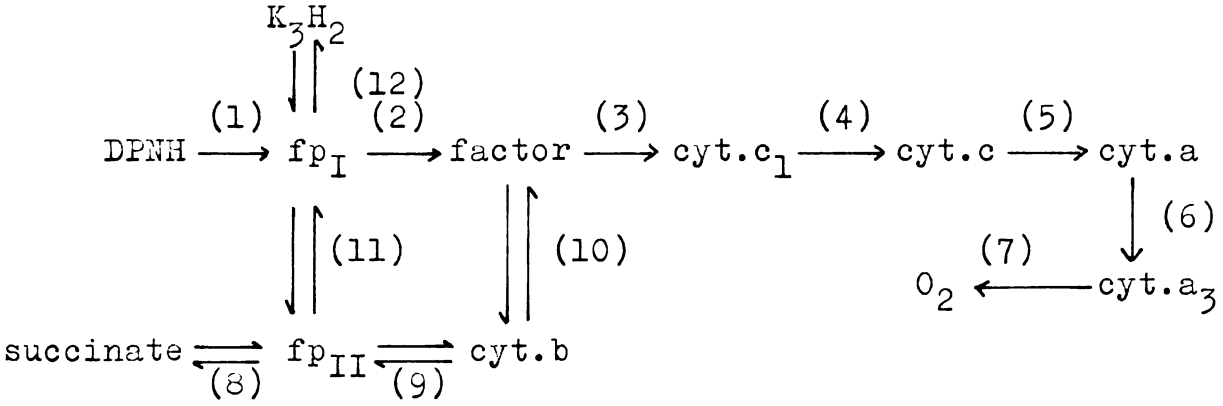
In this study we have been concerned primarily with the role of vitamin K_3 (menadione). The specific role of this form of vitamin K in the respiratory chain has not been fully elucidated. Two hypotheses have been presented.

Uehara et al (1956) showed that the enzymatic oxidation of ethyl alcohol as well as lactate, requiring DPN as cofactor, was promoted by vitamin K_3. According to this theory, vitamin K_3 functions as a hydrogen acceptor from diphosphopyridine nucleotide or triphosphopyridine nucleotide. It was suggested that a yellow enzyme requiring FMN as a coenzyme might participate in the reduction of vitamin K_3 by DPN.



However, further purification of the yellow enzyme is necessary in order to determine whether K_3 reductase is identical with the Warburg yellow enzyme.

Colpa-Boonstra and Slater (1958) have suggested a scheme which fits the data in this report better than that proposed by Uehara *et al*. According to their scheme, vitamin K_3 is an alternate pathway for the transport of hydrogen in the respiratory chain.



(fp_I and fp_{II} represent the flavoproteins, diaphorase and succinate dehydrogenase, respectively)

The oxidase systems are comprised of the following reactions: (A) DPNH oxidase ... (1) to (7); (B) succinate oxidase ... (8), (9), (10) followed by (3) to (7); and (C) K_3H_2 oxidase ... (12) followed by (2) to (7).

This investigation showed that K_3H_2 does not

act between DPNH and cytochrome b in the electron chain as Uehara et al (1956) had proposed. However, Colpa-Boonstra and Slater do suggest that K_3H_2 enters the respiratory chain in the vicinity of the flavoproteins or cytochrome b. Ernster et al (1955) suggest that K_3H_2 enters the respiratory chain by reaction with succinate dehydrogenase rather than with diaphorase.

Thus it is seen that the flavoproteins can be reduced by a) DPNH, b) succinate, or c) K_3H_2. Vitamin K_3, then, can act as an electron shunt around DPNH.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Forty weanling, male, albino rats of the Sprague-Dawley strain were divided into eight groups of five animals each. The average weight per group was 52 grams. The animals were housed in wire mesh, raised bottom cages and allowed food and water ad libitum for a two week experimental period. Food intake and weight records were kept. The basal diet consisted of sucrose 725 gm., vitamin free casein 180 gm., salts W 40 gm., vitamin mix[2] 2.5 gm., choline 1.5 gm., and corn oil[3] 50 gm. per kilogram of diet. The basal diet was supplemented with penicillin G, dicoumarol, and vitamin K_3 singly or in combination (Table I). All supplements were added at the expense of sucrose.

TABLE I

SUPPLEMENTS TO BASAL RATION

GROUP	I	II	III	IV	V	VI	VII	VIII
PENICILLIN G gm/kg	0.5	0.5	-	-	0.5	0.5	-	-
DICOUMAROL mg/kg	3.0	-	3.0	-	3.0	-	3.0	-
VITAMIN K_3 mg/kg	3.8	3.8	3.8	3.8	-	-	-	-

2. Vitamin mix provided thiamine 5 mg, riboflavin 5 mg, niacin 10 mg, pyridoxine HCl 2.5 mg, Ca pantothenate 20 mg, inositol 100 mg, folic acid 0.2 mg, B_{12} 0.02 mg, biotin 0.1 mg, vitamin A 100 mg, vitamin D 1.8 mg, and sucrose 2.25 gm per 2.5 gm of mix.
3. Containing 75 mg α-tocopherol acetate.

At the end of the two week experimental period the rats were killed according to the schedule in Table II. The animals were sacrificed by administering a sharp blow on the head followed by decapitation. The blood samples were collected in screw cap bottles which had been oxalated with 0.1 ml of 0.1 M neutral sodium oxalate. The bottles were swirled to mix the blood with the oxalate. They were capped and stored in the refrigerator until the coagulation time of the blood could be determined.

TABLE II

SCHEDULE FOR SACRIFICING ANIMALS

GROUP	I	II	III	IV	V	VI	VII	VIII
On the 11th day[4]	-	-	2	-	-	-	-	-
On the 14th day	1	1	-	1	1	1	1	1
On the 15th day[5]	-	-	-	-	1	1	1	1
On the 16th day	2	2	-	2	1	1	1	1
On the 17th day	1	1	1	1	1	1	1	1
On the 18th day	1	1	1	1	1	1	1	1

4. Two rats in Group III appeared moribund on the 11th day of the experiment. They were killed before the 14th day and enzyme activity was determined.
5. One rat in Group III was found dead. Autopsy revealed an enlarged cecum filled with gas, and multiple intestinal hemorrhages.

The liver was removed from the carcass as quickly as possible, rinsed with distilled water, chilled for a few seconds in chipped ice, blotted dry with filter paper and weighed. Total liver weight was recorded. One part of liver was homogenized in 19 parts of chilled sodium-potassium phosphate buffer (0.093 M) in a Potter-Elvehjem homogenizer which had been chilled in chipped ice. A measured volume of the chilled homogenate was added to the main compartment of chilled Warburg flasks which had been prepared previously by pipetting the required amounts of each solution into the flasks (Table III).

DPN Cytochrome-c reductase was determined in duplicate for each rat. The flasks were seated on the manometers and immersed in a water bath maintained at 37° C. A ten minute period was allowed for temperature equilibration. The manometers were removed from the water bath, the substrate (0.2 ml of 0.5% DPN) tipped into the main compartment from the side arm, swirled gently and returned to the water bath. The manometers were set at 15 and the stop cocks closed. Readings were taken every 5 minutes for one-half hour. Calculations were based on 20 minute readings; the activity of the enzyme decreased after this interval of time.

The remaining portion of liver was weighed, frozen and stored. When all the enzyme determinations were

completed the frozen liver samples were thawed and homogenized. An aliquot containing 1 gm of liver (wet weight) was taken for nitrogen analysis by the Macro-Kjeldhal method.

TABLE III

REACTION MIXTURE FOR DPN CYTOCHROME-c REDUCTASE

Flasks		1 ml	2 ml	3 ml
KOH 10%	C.W.[6]	0.2	0.2	0.2
H_2O	M.C.[7]	0.2	-	-
Sodium-potassium phosphate buffer 0.1 M pH 7.4	M.C.	0.8	0.8	0.8
Nicotinamide 0.1 M	M.C.	0.3	0.3	0.3
Na Glutamate 0.5 M	M.C.	0.3	0.3	0.3
Cytochrome-c 4×10^{-4} M	M.C.	0.3	0.3	0.3
Crude Malic Dehydrogenase	M.C.	0.6	0.6	0.6
Na Malate 0.5 M	S.A.[8]	0.3	0.3	0.3
DPN 0.5%	S.A.	-	0.2	0.2
Liver Homogenate 5% in sodium-potassium phosphate buffer	M.C.	0.2	0.2	0.2

Crude malic dehydrogenase was isolated from sheep liver according to the method of Potter (1946).

6. C.W.= Center well of Warburg flask.
7. M.C.= Main compartment of Warburg flask.
8. S.A.= Side arm of Warburg flask.

The determination of coagulation time on the oxalated blood samples was accomplished by adding back an excess of Ca Cl_2. The addition of 0.1 ml of 0.1 M Ca Cl_2 to 0.1 ml of oxalated blood on a glass slide proved to be satisfactory. The slide containing the recalcified blood was kept in a moist atmosphere over a water bath in an effort to reduce the error caused by evaporation of moisture from the sample (Todd and Sanford 1943). The coagulation time was taken as the time, in seconds, required for the first appearance of fibrin threads.

RESULTS

RESULTS

Results from this study are summarized in Tables IV and V; data from individual animals are compiled in the appendix.

FOOD CONSUMPTION AND GROWTH RATE (TABLE IV)

The animals receiving menadione + penicillin (Group II) consumed less food than those animals receiving penicillin (Group VI) and penicillin + dicoumarol (Group V). The difference was significant in both instances. Because of the non-specific infection in Group III which necessitated early sacrifice of two rats in this group and the death of a third, growth and food consumption are not considered reliable for this group and will be omitted.

No significant difference in growth rates between groups was observed. This was not unexpected in view of the short feeding trial used in this experiment. (Wostman, Knight, and Reynier 1958).

Liver size was not altered by the composition of the diet; no significant difference was noted among the various groups.

COAGULATION TIME (TABLE V)

Addition of penicillin to the basal ration (Group VI) did not alter coagulation time as compared with the control group (VIII). No significant difference in coagulation time was observed except when dicoumarol was added to the basal ration (Group VII). The addition of 0.3 mg % dicoumarol increased clotting time approximately 45% over the controls. The addition of penicillin (Group V) and/or menadione (Group III) to the ration containing dicoumarol reversed this effect and returned coagulation time to a normal value.

LIVER NITROGEN (TABLE V)

When the experimental groups are compared with respect to the presence or absence of menadione (Group I vs V; II vs VI; III vs VII; and IV vs VIII) it should be noted that in every case the addition of menadione to the ration significantly lowered liver nitrogen. These results cannot be explained on the basis of available data.

The addition of dicoumarol (Group VII) to the basal diet also reduced the nitrogen of the liver below that of the control. This decrease was less marked than that observed with menadione, but the difference was significant.

ENZYME DATA (TABLE V)

Enzyme data are reported as ul O_2/hr/100 mg fresh weight liver tissue. Enzyme activity expressed as ul O_2/hr/10 mg nitrogen may be found in the appendix. The former unit of measure is used in this report because the fluctuations in DPN cytochrome-c reductase activity are independent of the fluctuations in total liver nitrogen.

Group V (penicillin + dicoumarol) and Group VI (penicillin) showed a significantly higher DPN cytochrome-c reductase activity than did any other group; no significant difference was observed between these two groups. The addition of menadione to the ration containing penicillin or penicillin + dicoumarol (Group V vs I, and Group VI vs II) significantly decreased the activity of the enzyme. The addition of dicoumarol to any ration had no significant effect on the activity of DPN cytochrome-c reductase.

DISCUSSION

DISCUSSION

It was noted that the food consumption among the groups varied only slightly. Animals receiving menadione + penicillin (Group II) consumed a significantly lower quantity of food than those animals in any other group. This difference in food intake was not reflected in the growth rate. A greater difference in growth might have been noted had the feeding trial been of longer duration.

The inclusion of penicillin *per se* in the ration had no measurable effect on food intake or growth. Several workers (Phillips and Constant 1954; and Guerrant and Steel 1958) have found that penicillin supplementation increased food intake and growth rate only when animals were fed diets sub-optimum in certain nutrients. In view of the fact that the animals in this experiment were receiving an 18% casein diet in which only vitamin K was deficient, it was not expected that a difference would be observed in food intake or growth rate. The deficiency of vitamin K in the diet for such a short period of time did not impose a marked stress on the animal since the rat is able to synthesize this vitamin.

The effect of penicillin on coagulation time

observed in this study is somewhat puzzling. The fact that penicillin alone was ineffective in altering blood coagulation time from the control group is in agreement with several other workers (Lewis 1946; Weiner et al 1948; Triantaphyllopoulos and Waisbren 1952). The penicillin used in this study was crystalline, thus lending support to the theory of Macht and Ostro (1947) that the impurities present in amorphous preparations were responsible for the recorded effects on coagulation time.

However, although penicillin alone did not affect coagulation time, the antibiotic reversed the anticoagulant action of dicoumarol. In fact, the action of penicillin was identical with that of menadione with respect to counteracting the effect of dicoumarol. The mechanism of this action is obscure. A possible explanation is that penicillin increases the synthesis of vitamin K_2, probably in the intestinal tract.

These data suggest that the clinical use of crystalline penicillin would not present a problem with respect to the coagulation of blood. On the contrary, if penicillin functions at all in the blood coagulation scheme, it functions to maintain a normal coagulation time, even in the presence of the anticoagulant dicoumarol. The use of penicillin may be contra-indicated in cases of embolism if this effect is observed in human subjects.

As suggested earlier, the enzyme data collected in this experiment demand the use of the "shunt hypothesis" for vitamin K_3 (Colpa-Boonstra and Slater 1958). According to this theory, the stream of electrons may be transported *via* DPN or TPN down the electron chain, or as an alternative to this pathway, electrons may be accepted by vitamin K_3 and be introduced into the chain at a point below the pyridine nucleotides. One may assume that the enzyme DPN cytochrome-c reductase would be operative only for those electrons transported *via* DPN, since DPN must serve as substrate for this enzyme.

Under normal conditions, a balance of electrons streaming into the electron chain from these various routes must be attained. The control group (VIII) establishes the measure of this normal balance. As factors operate to shift this balance, such a shift must be reflected in the activity of DPN cytochrome-c reductase. If the supply of electrons normally coming into the chain *via* vitamin K_3 is markedly reduced, and if the total number of electrons streaming through the chain is maintained, then a greater number of electrons must pass through the pyridine nucleotides, with a concomitant increase in the activity of DPN cytochrome-c reductase. Conversely, if factors operate to diminish the number of electrons normally passing through the pyridine nucleotide system, the activity

of DPN cytochrome-c reductase will be reduced. And, still a third possibility, if this electron balance is undisturbed by dietary factors, the DPN cytochrome-c reductase activity will be unchanged as compared with the control.

Since the addition of dicoumarol to any of the diets had no effect on the activity of DPN cytochrome-c reductase the action of this metabolite cannot be centered on the function of vitamin K_3 in the electron transport chain. These data are in agreement with those of Martius and Nitz-Litzow (1954) who postulated the action of dicoumarol to be more specifically mediated through vitamin K_1 rather than vitamin K_3. The blood coagulation data presented in this paper also support this thesis.

The addition of penicillin to the diet markedly increased the activity of DPN cytochrome-c reductase. Therefore, this antibiotic must exert a profound influence on the balance of electron transport along the electron chain. The net result of penicillin administration is an increased flow of electrons through DPN. Based on the assumptions made earlier, penicillin must function either as an inhibitor of the vitamin K_3 shunt into the electron chain, or as a stimulator of the pathway <u>via</u> DPN.

Since the addition of menadione to this penicillin-containing ration reduced the activity of DPN cytochrome-c reductase to that of the control group, the former action

of penicillin is implicated. These data suggest that penicillin exerts a metabolic block in the oxidation-reduction of vitamin K_3 and by including vitamin K_3 in the diet, this inhibition is overcome. Additional work is necessary to support this theory.

The presence of dicoumarol in either of these rations did not alter the pattern. Dicoumarol, as already noted, is inactive in this system.

SUMMARY

SUMMARY

Forty male, weanling, albino rats were fed an 18% casein diet containing penicillin, dicoumarol and menadione either singly or in combination. The control animals were fed the basal diet (18% casein) unsupplemented. Food and water were allowed ad libitum for a period of 2 weeks. Records of food intake and weight gain were kept.

At the end of the experimental feeding period the rats were killed, and the livers were analyzed for nitrogen and DPN cytochrome-c reductase activity. Coagulation time was determined on blood samples taken immediately after the animals were sacrificed.

No significant difference in growth was observed. It was felt that this was due, in part, to the short feeding trial used in this experiment, and in part to the high nutritive quality of the ration.

Dicoumarol was found to have significantly increased the coagulation time of blood. This effect was reversed by menadione. These data are in agreement with many published reports.

Penicillin, when fed alone, had no effect on blood coagulation time. However, when penicillin was fed with dicoumarol, the antibiotic behaved in a manner similar to menadione. Penicillin reversed the anti-coagulant

effect of dicoumarol. Possible clinical implications in penicillin therapy are discussed.

Dicoumarol was found to be ineffective in altering the DPN cytochrome-c reductase activity. Penicillin increased the activity of DPN cytochrome-c reductase 45% over that of the control. Menadione was able to counteract this effect of penicillin. When menadione was added to a diet containing penicillin, the activity of DPN cytochrome-c reductase was identical with the control.

These relationships are discussed. The suggestion was made that penicillin exerts a metabolic block in the oxidation-reduction of vitamin K_3.

Under the conditions of this experiment, dicoumarol appeared to be a more specific antimetabolite for vitamin K_1 than for vitamin K_3. This theory was supported by blood coagulation data and by enzyme data.

TABLE IV

GROUP	SUPPLEMENT	FOOD INTAKE (gm/week)	WEIGHT GAIN (gm/week)	LIVER WEIGHT (gm)
I	menadione penicillin dicoumarol	67±3[9]	33±2[9]	5.9±0.7[9]
II	menadione penicillin	56±4	30±2	5.5±0.1
III	menadione dicoumarol	36±18[10]	20±8[10]	4.7±0.8
IV	menadione	67±2	32±6	5.9±0.4
V	penicillin dicoumarol	74±4	30±9	5.0±0.5
VI	penicillin	74±4	29±2	5.3±0.5
VII	dicoumarol	68±3	26±4	5.6±0.02
VIII	-	68±3	28±1	5.6±0.01

9. Standard error of mean.
10. Due to infection and death in this group, these data are not considered reliable.

TABLE V

GROUP	SUPPLEMENT	COAGULATION TIME (sec)	N/100 mg LIVER (mg)	DPN Cytochrome-c Reductase (ul O_2/hr/ 100 mg LIVER)
I	menadione penicillin dicoumarol	28±3[11]	2.84±0.11[11]	144±16[11]
II	menadione penicillin	27±2	2.71±0.05	136±8
III	menadione dicoumarol	26±3	2.90±0.08	152±8
IV	menadione	23±6	2.98±0.06	133±14
V	penicillin dicoumarol	22±3	3.34±0.01	190±20
VI	penicillin	23±2	3.35±0.03	165±15
VII	dicoumarol	42±9	3.16±0.05	134±13
VIII	-	29±4	3.35±0.16	144±14

11. Standard error of the mean.

LITERATURE CITED

LITERATURE CITED

Andrus, W. D. 1941 The newer knowledge of vitamin K. Bulletin N. Y. Academy of Medicine 17: 116-134

Bernheim, F. and Bernheim, M. 1940 Action of 4-amino-2-methylnaphthol on the oxidation of certain sulfhydryl groups. Journal of Biological Chemistry 134: 457-458

Boyer, P. D. 1955 The Fat Soluble Vitamins. Annual Review of Biochemistry 24: 447-449

Chargaff, Erwin 1946 The coagulation of the blood. Advances in Enzymology 5: 31-65

Colpa-Boonstra, J. P. and Slater, E. C. 1958 The role of vitamin K in the respiratory chain. Biochimica et Biophysica Acta 27: 122-133

Dam, H. 1942 Vitamin K, its chemistry and physiology. Advances in Enzymology 2: 285-324

Ernster, L., Jalling, O., Low, H. and Lindberg, O. 1955 Alternate pathways of mitochondrial reduced diphosphopyridine nucleotide (DPNH) on oxidation studied with amytal. Experimental Cell Research, Supplement 3: 124-135

Frada, G. 1946 Large embolism in bacterial endocarditis in course of penicillin therapy. Giornale di Medicina 3: 95-138 cited from Journal of American Medical Association 133: 354

Guerrant, N. B. and Steel, J. M. 1958 Some effects of aureomycin and penicillin on thiamine and riboflavin metabolism in growing rats. Proceedings Society for Experimental Biology and Medicine 98: 542-545

Kemmerer, A. R. 1952 Fat-soluble vitamins. Annual Review of Biochemistry 21: 333-354

Lewis, J. H. 1946 Effect of penicillin on blood coagulation. Proceedings Society for Experimental Biology and Medicine 63: 538-540

Lewitus, Z. A. 1948 Prothrombinopenic effect of penicillin. Archives of Internal Medicine 82: 625

Macht, D. I. and Ostro, M. 1947 Thromboplastic properties of penicillin and streptomycin. Science 105: 313-314

Martius, C. and Nitz-Litzow, D. 1954 Oxydative phosphorylierung und vitamin K mangel. Biochimica et Biophysica Acta 13: 152-153 Chemical Abstracts 48: 5315i

Martius, C. and Nitz-Litzow, D. 1955 Mechanism of action of vitamin K. Biochemische Zeitschrift 327: 1-5 Chemical Abstracts 50: 5110c

Moldavsky, L. F., Crowley, J. H. and Hesselbrock, Wm. B. 1953 Penicillin effects on blood coagulation. Ohio State Medical Journal 49: 111-112

Moldavsky, L. F., Hesselbrock, Wm. B. and Cateno, C. 1945 Penicillin effects on blood coagulation. Science 102: 38-40

Olef, L. 1935 The enumeration of blood platelets. Journal of Laboratory and Clinical Medicine 20: 416

_____ 1936 The rate of disintegration of platelets. ibid 22: 128

_____ 1937 The determination of platelet volume. ibid 23: 166

Phillips, P. H. and Constant, M. A. 1954 Antibiotics in Nutrition. Annual Review of Biochemistry 23: 333-334

Potter, V. R. 1946 The assay of animal tissues for respiratory enzymes. The Malic dehydrogenase system. Journal of Biological Chemistry 165: 311-324

Reich, C., McCready, R. L., Chaplin, H. and Lipkin, R. 1947 Parenteral vitamin K therapy during antepartum period and its effects on the infants' prothrombin levels. American Journal of Obstetrics and Gynecology 53: 300-302

Schulz, A. R. and Goss, H. 1956 Menadione as a link in non-phosphorylative respiration in mitochondria. Biochimica et Biophysica Acta 21: 578-579

Taylor, J. D., Miller, G. J., Jaques, L. B., Spinks, J. W. T. 1956 The distribution of administered vitamin K_1C^{14} in rats. Canadian Journal of Biochemistry and Physiology 34: 1143-1152

Taylor, J. D., Miller, G. J. and Wood, R. J. 1957 A comparison of the concentration of C^{14} in the tissues of pregnant and non-pregnant female rats following the intravenous administration of vitamin K_1-C^{14} and vitamin K_3-C^{14}. Canadian Journal of Biochemistry and Physiology 35: 691-697

Todd, J. C. and Sanford, A. H. 1943 Clinical diagnosis by laboratory methods. 10th edition W. B. Saunders Co. Philadelphia. p. 198

Triantaphyllopoulos, D. C. and Waisbren, B. A. 1952 Lack of influence of penicillin on blood coagulation. Archives of Internal Medicine 90: 653-659

Uehara, K., Muramatsu, I. and Makita, M. 1956 Role of vitamin K_3 in biological oxidations. Journal of Vitaminology 2: 44-50

Warner, E. D., Brinkhous, K. M. and Smith, H. P. 1938 Bleeding tendency of obstructive jaundice: prothrombin deficiency and dietary factors. Proceedings Society for Experimental Biology and Medicine 37: 628-630

Weber, M. M., Brodie, A. F., and Merselis, J. E. 1958 Possible role for vitamin K in electron transport. Science 128: 896-897

Weiner, M., Zeltmacher, K. and Shapiro, S. 1948 The effect of penicillin upon the coagulation of the blood. Experimental Medicine and Surgery 6: 181-188

Wostman, B. S., Knight, L. and Reynier, J. A. 1958 The influence of orally administered penicillin upon the growth and liver thiamine of growing germfree and normal stock rats fed a thiamine deficient diet. Journal of Nutrition 66: 577-586

APPENDIX

TABLE I

Data for Individual Rats in Group I Fed the Basal Diet (18% Casein) Supplemented with Menadione, Penicillin, and Dicoumarol.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
I_1	52	136	33	61	7.3
I_2	49	106	24	67	4.9
I_3	55	139	37	73	7.6
I_4	56	134	34	75	5.6
I_5	48	120	36	58	4.0
AVERAGE	52	127	33 ± 2	67 ± 3	5.9 ± 0.7

TABLE I (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME	DPN CYTOCHROME-c ul O_2/hr/100 mg liver	REDUCTASE ul O_2/ hr/10 mg NITROGEN
I_1	2.56	25	83	324
I_2	2.82	26	173	613
I_3	2.71	29	152	561
I_4	2.90	28	147	507
I_5	3.22	33	164	509
AVERAGE	2.84±0.11	28±3	144±16	503±48

TABLE II

Data for Individual Rats in Group II Fed the Basal Diet (18% Casein) Supplemented with Menadione and Penicillin.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
II_1	51	133	32	69	5.9
II_2	51	107	23	48	4.8
II_3	57	141	37	63	6.4
II_4	50	112	27	49	5.4
II_5	50	114	32	52	4.8
AVERAGE	52	121	30 ± 2	56 ± 4	5.5 ± 0.1

TABLE II (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr /10 mg NITROGEN
II_1	2.67	24	141	528
II_2	2.71	26	114	421
II_3	2.82	30	122	433
II_4	2.53	25	156	617
II_5	2.81	28	146	520
AVERAGE	2.71±0.05	27±2	136±8	504±36

TABLE III

Data for Individual Rats in Group III Fed the Basal Diet (18% Casein) Supplemented with Menadione and Dicoumarol.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
III_1	51	147	37	60	6.5
III_2	51	122	29	75	5.3
III_3	54[12]	-	-	-	-
III_4	54[13]	64	6	5	3.5
III_5	51[13]	63	8	4	3.3
AVERAGE	52	99	20±8	36±18	4.7±0.8

12. No further data could be collected because of the death of this animal.
13. Animals 4 and 5 of Group III were sacrificed on the 11th day.

TABLE III (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr /10 mg NITROGEN
III_1	2.91	23	141	485
III_2	3.04	23	152	500
III_3	-	-	-	-
III_4	2.96	28	141	476
III_5	2.68	29	174	649
AVERAGE	2.90±0.08	26±3	152±8	528±41

TABLE IV

Data for Individual Rats in Group IV Fed the Basal Diet (18% Casein) Supplemented with Menadione.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
IV_1	51	144	36	76	6.8
IV_2	50	130	33	67	6.9
IV_3	54	128	32	63	5.9
IV_4	54	112	25	64	5.5
IV_5	53	117	32	66	4.5
AVERAGE	52	126	32 ± 6	67 ± 2	5.9 ± 0.4

TABLE IV (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr/ 10 mg NITROGEN
IV_1	2.78	24	148	532
IV_2	3.10	25	180	581
IV_3	3.07	20	125	407
IV_4	2.92	21	102	349
IV_5	3.04	25	110	362
AVERAGE	2.98±0.06	23±6	133±14	446±13

TABLE V

Data for Individual Rats in Group V Fed the Basal Diet (18% Casein) Supplemented with Penicillin and Dicoumarol.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
V_1	50	107	22	60	4.2
V_2	53	121	28	71	5.9
V_3	53	138	37	80	4.8
V_4	53	130	36	83	6.2
V_5	50	106	28	78	3.7
AVERAGE	52	120	30 ± 9	74 ± 4	5.0 ± 0.5

TABLE V (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr/10 mg NITROGEN
V_1	3.60	20	189	525
V_2	2.84	18	143	504
V_3	3.31	25	189	571
V_4	3.41	23	165	484
V_5	3.56	24	264	714
AVERAGE	3.34 ± 0.01	22 ± 3	190 ± 20	560 ± 41

TABLE VI

Data for Individual Rats in Group VI Fed the Basal Diet (18% Casein) Supplemented with Penicillin.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
VI_1	53	126	28	71	5.4
VI_2	54	115	25	63	4.8
VI_3	53	115	27	70	4.0
VI_4	51	120	32	82	6.9
VI_5	49	115	33	85	5.3
AVERAGE	52	118	29 ± 2	74 ± 4	5.3 ± 0.5

TABLE VI (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr/10 mg NITROGEN
VI_1	3.47	22	191	346
VI_2	3.36	21	180	536
VI_3	3.57	25	150	417
VI_4	3.10	20	113	365
VI_5	3.23	25	189	585
AVERAGE	3.35 ± 0.03	23 ± 2	165 ± 15	488 ± 47

TABLE VII

Data for Individual Rats in Group VII Fed the Basal Diet (18% Casein) Supplemented with Dicoumarol.

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
VII_1	52	121	27	74	5.2
VII_2	54	93	16	64	4.6
VII_3	50	140	40	76	6.6
VII_4	54	94	19	60	6.0
VII_5	50	111	31	66	5.5
AVERAGE	52	112	26 ± 4	68 ± 3	5.6 ± 0.01

TABLE VII(Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr/10 mg NITROGEN
VII_1	3.15	35	123	390
VII_2	3.13	34	165	527
VII_3	3.12	57	131	420
VII_4	3.07	45	92	300
VII_5	3.35	40	158	472
AVERAGE	3.16±0.05	42±9	134±13	422±38

TABLE VIII

Data for Individual Rats in Group VIII Fed the Unsupplemented Basal Diet (18% Casein).

RAT #	INITIAL WEIGHT (gm)	FINAL WEIGHT (gm)	WEIGHT GAIN (gm/wk)	FOOD INTAKE (gm/wk)	LIVER WEIGHT (gm)
$VIII_1$	52	124	28	67	5.0
$VIII_2$	53	117	26	57	5.7
$VIII_3$	53	120	29	76	5.4
$VIII_4$	55	113	27	68	6.0
$VIII_5$	49	106	29	73	5.3
AVERAGE	52	116	28 ± 1	68 ± 3	5.5 ± 0.01

TABLE VIII (Continued)

RAT #	N/100 mg LIVER (mg)	COAGULATION TIME (sec)	DPN CYTOCHROME-c ul O_2/hr/100 mg LIVER	REDUCTASE ul O_2/hr/10 mg NITROGEN
$VIII_1$	3.81	25	192	504
$VIII_2$	3.06	30	153	500
$VIII_3$	3.11	32	147	473
$VIII_4$	3.67	35	111	302
$VIII_5$	3.12	25	119	381
AVERAGE	3.35±0.16	29±4	144±14	432±40

THE EFFECT OF PENICILLIN AND DICOUMAROL ON BLOOD COAGULATION AND DPN CYTOCHROME-c REDUCTASE ACTIVITY IN THE RAT

by

Sister Mary Romana McDermott, S.N.J.M.

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Foods and Nutrition
College of Home Economics

1959

Approved _____

ABSTRACT

Forty weanling, male, albino rats were divided into eight groups and fed an 18% casein diet containing penicillin, dicoumarol and menadione either singly or in combination. The control group received no supplement. Food and water were allowed ad libitum throughout the 2 week experimental period.

The animals were sacrificed by decapitation. Livers were analyzed for nitrogen and DPN cytochrome-c reductase activity. Blood samples were collected when the animals were killed and coagulation time was determined.

Dicoumarol increased to a significant degree the coagulation time of the blood. Menadione was found to counteract this effect of dicoumarol. Penicillin alone had no effect on blood coagulation; however, when penicillin was added to rations supplemented with dicoumarol, an effect similar to that of menadione was observed. It was suggested that if penicillin does function in the blood coagulation scheme it operates to maintain a normal coagulation time.

The activity of DPN cytochrome-c reductase was unaffected by dicoumarol. Penicillin was found to increase significantly the activity of this enzyme. The addition

of menadione to the rations containing penicillin returned DPN cytochrome-c reductase activity to normal. From these data it was suggested that penicillin may function as an inhibitor of the vitamin K_3 shunt around DPN in electron transport.

DOCMA USE ONLY

FEB 5

MICHIGAN STATE UNIV. LIBRARIES
31293010791881