NUTRITIONAL DEFICIENCIES OF

ALL-PLANT RATIONS

By

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Master of Science

Louisiana State University

Baton Rouge, Louisiana

1941

Submitted to the Faculty of the Graduate School of

the Oklahoma Agricultural and Mechanical College

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

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1950

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ACKNOWLEDGEMENT

The author wishes to gratefully acknowledge the assistance of Dr. O. Burr Ross of the Animal Husbandry Department, Oklahoma Agricultural and Mechanical College, who contributed many valuable suggestions in the planning and execution of these studies.

Also, he wishes to express his appreciation to Dr. C. K. Whitehair of the Animal Husbandry Department, Oklahoma Agricultural and Mechanical College, and to Dr. R. W. MacVicar of the Department of Agricultural Chemistry, Oklahoma Agricultural and Mechanical College, for many helpful suggestions during the course of these investigations.

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INTRODUCTION

During recent years many research workers have been interested in factors causing high mortality in baby pigs. The results of many experiments in which both adult and young swine were fed rations containing only materials of plant origin have suggested that this high mortality might be due, at least in part, to dietary factors. The recent discovery that vitamin B_{12} is one of the factors present in certain materials of animal origin which would increase the growth of microorganisms, laboratory animals, and chicks stimulated much intensive investigation regarding the effect of this vitamin in improving the performance of swine fed an all-plant ration.

It has been established that the nutritional requirement of the rat closely parallels that of swine. Because of the shorter reproductive cycle of rats and the rapid growth of their young, the rat has been used extensively as a laboratory animal in studying the nutritional deficiences of swine rations.

In the studies reported in this thesis, the rat was used as the pilot laboratory animal. The information resulting from these studies will be useful in designing studies with swine relative to the nutritional adequacy of all-plant rations. To simplify the presentation of the findings of the different experiments, this thesis has been divided into four parts. Part I contains the data of the reproduction and lactation performance of rats fed all-plant rations. Part II presents the results of depletion-repletion studies of adult rats fed all-plant rations supplemented with various nutrients. The growth studies of young rats fed an all-plant ration are presented in Part III. Part IV contains data relative to the levels of certain constituents in the blood of young pigs fed such rations but exposed to an infectious gastroenteritis agent.

PART I

REPRODUCTION STUDIES WITH RATS

INTRODUCTION

Extensive studies during recent years have repeatedly demonstrated that rations composed wholly or principally of corn and soybean meal supplemented with minerals and known vitamins were deficient for normal reproduction and lactation of rats. Ross et al (1942), Van Landingham and Lyon (1947), Zucker et al (1948), have all reported varying degrees of failure on such diets. Cary and Hartman (1943-1947) have found a partially purified ration containing highly extracted casein to be inadequate for normal reproduction. Recently they have stated that the supplementation of these diets with vitamin B_{12} permits essentially normal performance. In contrast to these studies, Schultze (1950) has recently reported that he obtained essentially normal growth of young from rats fed purified rations containing isolated soybean protein supplemented with all known vitamins except vitamin B_{12}. Ross, et al (1948) and Watts, et al (1949) have reported essentially normal reproduction and lactation of rats fed rations containing corn and soybean meal supplemented with minerals and all known vitamins except vitamin B_{12}. Gestation failures and high mortality of young have not been consistently observed when rations of this type were employed.

EXPERIMENTAL

A. Assay Techniques:

Female rats of the Sprague-Dawley strain were used during the entire investigation. The rats were housed in large screen-bottom cages. Water and the various rations were supplied ad libitum. At time of breeding, the males were placed with the females and rotated daily to reduce the effect of possible male sterility. At parturition the females were placed in individual cages provided with sugar cane bagasse as litter. Frequent observations permitted a fairly accurate assessment of the number born. Mortality data included both those born dead and those dying during the first 48 hours. After the first 48 hours, litters having more than six young were reduced to six, females being selected preferentially. The young were weaned at 21 days of age. Weanling rats were selected for subsequent reproduction studies on the basis of weaning weight, litter, and ration. The young rats were fed the various experimental rations similar to those supplied the mother during gestation and lactation, and were bred at approximately 90 days of age, when they weighed about 200 grams. In some instances rats were maintained for three successive generations on the same ration.

The rations used in this study appear in Table I. Supplements when added were at the expense of the entire ration. The soybean meal (Staley) used during the entire study was a high quality expeller product. The casein was the alcohol extracted product of General Biochemicals, Inc. The B_{12} Supplement 1 (Merck) was a mixture of crude vitamin B_{12} concentrate, charcoal, and soybean flour, which, according to the manufacturer, had an activity roughly four times that of fish solubles. The B_{12} Supplement 3 (Merck) was a Fuller's earth absorbate, which, according to the manufacturer, had an activity of 12.5 milligrams of vitamin B_{12} per pound by the LLD assay. The APF

Supplement (Lederle) was a by-product of the manufacture of aureomycin. The anti-pernicious anemia liver extract solution (Lederle) contained, by manufacturer's assay, 15 USP injectible units per ml.

B. Fractionation Techniques:

Repeated extraction of 1:20 liver powder (Wilson) with 70 percent ethanol yielded two fractions, the 70 percent ethanol soluble fraction and the residue. The material soluble in 70 percent ethanol was concentrated *in vacuo* to a thick syrup and repeatedly extracted with 95 percent ethanol yielding a soluble fraction and an insoluble fraction. Fractions of a high quality alfalfa leaf meal were prepared in essentially the same manner as liver. The 95 percent ethanol soluble and insoluble fractions of the material which was soluble in 70 percent alcohol were tested.

RESULTS AND DISCUSSION

In the early phases of this study various natural products and fractions were tested for activity in improving reproduction and lactation on corn-soybean meal rations. The results of one typical experiment are presented in Table II. There were no resorptions and toxemias observed, such as had been noted by Ross *et al* (1942). Ration 2 permitted the poorest performance during the first reproductive cycle in the number of viable young at 48 hours, number of young weaned, and weaning weight. Various fractions of liver, APA liver extract (Lederle), and fish solubles were all essentially equally active in supplementing the ration. Analysis of variance (Snedecor, 1946) of the data showed that the differences in weaning weights observed

TABLE I

COMPOSITION OF RATIONS USED IN STUDIES ON REPRODUCTION OF RATS AND GROWTH OF YOUNG TO WEANING

Ration Component	Ration 2	Ration 3	Ration 4	Ration 5
Ground yellow corn	68.85	73.5	83.0	53.0
Expeller soybean oil meal	25.00	25.0		45.0
Vitamin test casein			15.0	
Alfalfa leaf meal	5.00			
NaCl	0.65			
$CaCO_3$	0.50			
Salt mixture*		2.0	2.0	2.0
dl Methionine		0.2	0.2	0.2
Vitamin mixture**		—	—	—

* Hegsted, J. Biol. Chem. 138:450 (1941)

** Basal rations were supplemented with a water soluble vitamin mixture which supplied in crystalline form the following amounts of the various vitamins per kilogram of rations: Thiamin HCl 4 mg., riboflavin 6 mg., pyridoxine HCl 3 mg., calcium pantothenate 20 mg., niacin 20 mg., choline chloride 1 gm., folic acid 1 mg., inositol 20 mg., p-aminobenzoic acid 20 mg. Two drops of a mixture of alpha tocopherol and haliver oil were administered weekly.

TABLE II

EFFECT OF VARIOUS FRACTIONS OF 1:20 LIVER POWDER (WILSON), APA LIVER EXTRACT (LEDERLE) AND OTHER SUPPLEMENTS ON REPRODUCTION OF RATS AND THE GROWTH OF THE YOUNG TO WEANING

Experi-ment	Ration	Number of Litters*	Livability at 48 hrs.	Young weaned of those given to raise	Average Weaning Weight
			percent	percent	grams
	First Reproductive Cycle				
1	Ration 2	6	68	56	27.5
	Ration 2 ≠ 2% 1:20 liver powder	7	97	85	36.5
	Ration 2 ≠ Ethanol insoluble fraction = 2%	7	96	83	36.6
	Ration 2 ≠ Ethanol soluble fraction = 2%	6	94	72	33.0
	Ration 2 ≠ APA Liver extract = 0.4 mls per week	7	96	85	36.5
2	Ration 2 ≠ 2% Fish solubles	5	93	86	37.8
	Second Reproductive Cycle				
1A	Ration 2	5	88	78	34.9
	Ration 2 ≠ 2% 1:20 Liver powder	5	82	96	38.1
	Ration 2 ≠ Ethanol insoluble fraction = 2%	6	94	100	39.7
	Ration 2 ≠ Ethanol soluble fraction = 2%	2	100	100	37.0
	Ration 2 ≠ APA Liver extract = 0.4 ml/wk.	4	88	91	37.8

* Number of litters denotes the number of the females that actually produced litters and does not include those that failed to breed.

were all highly significant when compared to the basal fed lot. Although the females were maintained on the same ration for the second reproductive cycle, the differences were less striking. Statistical significance was not attained, although, in general, there was a tendency for the results to be in the same direction.

Since it has repeatedly been suggested that alfalfa contained a material which was beneficial for pregnant and lactating sows, studies were made with rats to test alfalfa leaf meal and fractions therefrom. Because ration 2 contained alfalfa, another ration (ration 3) was used in this study. This ration permitted normal gestation, good livability of young to 48 hours, and weaning of a high percentage of those given to raise (Table III). The addition of alfalfa leaf meal was definitely detrimental in terms of weaning weight. Fractions of alfalfa had no effect on weaning weight, whereas, as previously observed, 1:20 liver powder gave a significant response.

The failure to experience high early mortality in the young rats in the previous experiment made it seem desirable to use a ration compounded of materials of more definite composition. As a result, ration 4 was formulated using vitamin-test casein as the source of protein. The results of two replications in which various supplements to this basal ration were tested appear in Table IV. The animals used in Experiment 6 were rats from the local stock colony maintained since weaning on the respective rations. The rats used in Experiment 7 were young from Experiment 6 fed from weaning the same rations fed their mothers. Again livability of young to 48 hours was good in all lots and a high percentage of those given to raise was weaned. Liver powder (Wilson) and B_{12} Supplement 1 (Merck) significantly improved the weaning weights of the young on these rations over those of the lot fed

TABLE III

EFFECT OF VARIOUS FRACTIONS OF ALFALFA LEAF MEAL ON REPRODUCTION OF RATS AND THE GROWTH OF THE YOUNG TO WEANING

Ration	Number of Litters*	Livability at 48 hrs.	Young Weaned of those given to raise	Average Weaning Weight
		Percent	Percent	grams
Ration 3	6	94	80	37.0
Ration 3 + 15% Alfalfa leaf meal	4	90	95	27.1
Ration 3 + 95% Ethanol insoluble fraction of the 70% ethanol soluble fraction equivalent to 15% alfalfa leaf meal	4	91	82	39.4
Ration 3 + 95% Ethanol soluble fraction of 70% ethanol soluble fraction equivalent to 15% alfalfa leaf meal	3	97	89	38.1
Ration 3 + 2% 1:20 Liver powder	6	100	100	42.9

* Number of litters denotes the number of the females that actually produced litters and does not include those that failed to breed.

TABLE IV

EFFECT OF VARIOUS SUPPLEMENTS ON THE REPRODUCTION OF RATS AND THE GROWTH OF THE YOUNG TO WEANING

Experiment	Ration	Number of Litters*	Livability at 48 hrs.	Young weaned of those given to raise	Average Weaning Weight
			Percent	Percent	grams
6,7	Ration 4	12	92	100	34.3
	Ration 4 + 0.5% Merck's B_{12} Supplement 1	12	91	93	42.0
	Ration 4 + 2.0% Wilson's 1:20 liver powder	15	97	98	43.2
	Ration 4 + Alfalfa carotene concentrate	8	96	96	38.0
8,9	Ration 3	15	95	95	38.5
	Ration 3 + 0.5% Merck's B_{12} Supplement 3	14	93	99	41.0
	Ration 3 + 2.0% Wilson's 1:20 liver powder	18	95	97	40.4
10,11	Ration 3	11	68	91	37.3
	Ration 3 + 0.5% Merck's B_{12} Supplement 3	10	71	97	40.4
12	Ration 3	7	85	94	37.0
	Ration 3 + 0.5% Lederle's APF Supplement	6	88	100	44.0
	Ration 3 + 50 ppm crystalline B_{12}	7	92	100	38.0
	Ration 3 + 0.5% Lederle's APF + 50 ppm crystalline B_{12}	5	92	100	42.0
13,14	Ration 5	12	75	95	35.0
	Ration 5 + 0.5% Merck's B_{12}Supplement 3	7	86	100	40.2

* Number of litters denotes the number of females that actually produced litters and does not include those that failed to breed.

the basal ration. The lack of reproductive failures in these two experiments may have been due to the presence of an unidentified factor in the incompletely extracted casein (Cary et al 1943-1947).

Since numerous workers have suggested that casein contained the unknown factor and that plant materials such as soybean meal were not active in this respect, further experiments were conducted using ration 3. Table IV summarizes the results of the different experiments using this ration. The animals used in Experiment 8 were the young from Experiment 7 that had been maintained on the experimental rations since weaning. The rats used in Experiment 9 were young from Experiment 8 and received the same ration as their mothers. The results of Experiments 8 and 9 showed that no differences existed among the lots as to viability of the young up to 48 hours or number weaned of those given to raise. As had been noted previously, the rats fed 1:20 liver powder (Wilson) or B_{12} Supplement 3 (Merck) weaned slightly heavier young than did those on the basal ration. These differences did not prove to be statistically significant.

Experiments 10 and 11 were further replications of two of the rations used in Experiments 8 and 9. The rats used in Experiment 10 were the young from Experiment 9 and represented the third successive generation on their respective rations. The rats used in Experiment 11 were young from the stock colony which had been placed on the experimental rations at weaning. The results of these two experiments are essentially the same as those of Experiments 8 and 9. The higher mortality prior to 48 hours in Experiments 10 and 11 was thought to be caused, at least in part, by a high incidence of respiratory infection among the mother rats during these experiments. No differences were found to exist between the supplement fed lots and the basal, and the higher early mortality was not considered to be due to ration. In the four experiments in which B_{12} Supplement 3 was fed, the females receiving this supplement weaned young that averaged 2.1 grams heavier than those from the basal fed lots. This difference was not large enough for significance when tested by analysis of variance (Snedecor, 1946).

A study of the results of Experiment 12 shows that the livability up to 48 hours and number raised to weaning did not differ greatly among the lots. The supplementation of the basal diet with crystalline B_{12} produced only slightly heavier weanling rats. When APF Supplement (Lederle) was added to the basal diet, significant improvement in weaning weights was noted. The addition of both crystalline B_{12} and the APF Supplement to the basal ration did not improve the weaning weights over those of the basal ration supplemented with only the APF supplement.

Since level of protein has been related to vitamin B_{12} requirement, ration 5 was formulated and used in two experiments. The results of Experiments 13 and 14 are found in Table IV. The early mortality was slightly higher in the basal fed lots but the number weaned was essentially the same. The addition of B_{12} Supplement 3 to the basal ration significantly improved the weaning weights of the young when these were compared to the basal fed lots.

At no time during the course of this entire investigation has it been possible to demonstrate a high mortality of young during the first 48 hours or prior to weaning. These results are at variance with those of numerous workers, (Ross, 1942), (Zucker, 1948), (Hartman, 1949). Schultze (1950) has recently reported that he could secure satisfactory weight gains from the first generation of rats fed purified rations containing soybean protein. However, somewhat smaller gains were experienced when the second generation was fed these rations.

It has been our experience that the weaning weight of young from females fed rations presumably low in "animal protein factor" is consistently less than that observed when the ration is fortified with certain natural and fermentation by-product concentrates.

Numerous explanations have been advanced in an attempt to rationalize the differences observed by us during the last three years and those found by Ross (1942) previously and by other workers. There are certain facts that may account for at least some of the differences. First, during these studies generous supplementation with all known dietary nutrients (including choline and folic acid) was practiced. The results of previous studies may have been influenced by a lack of some then-unknown factor. It has recently been shown by Schaefer and others (1950) that the dietary requirements of vitamin B_{12}, choline, and folic acid are interrelated and that one may not be established without considering the concentration of each of the others. Secondly, care was used in all these studies to employ only a high-quality expeller-type soybean meal from a single source (Staley and Co., Decatur, Illinois). The variation that is known to occur in natural feeds with respect to other nutrients probably extends to the factor that is necessary for successful reproduction and lactation. It may be that larger amounts of this factor were consistently present in the high-quality meals used in this study. The fact that intestinal synthesis might, under certain conditions, supply adequate amounts of the factor must be recognized. Hartman and his associates (1949) have proposed that the factor is synthesized in adequate amounts when excessively high levels of riboflavin are fed.

SUMMARY

The reproduction and lactation performance of rats fed diets composed principally of corn and soybean meal supplemented with known minerals and vitamins except vitamin B_{12} has been essentially normal. Resorptions, toxemia deaths, or excessive mortality during the first 48 hours after birth, during the nursing, or after weaning have not been consistently observed. The weaning weights of young from females fed supplements of natural and fermentation by-product concentrates containing vitamin B_{12} and perhaps other unknown factors has been consistently superior to those of young from females fed unsupplemented rations. Liver powder (Wilson's 1:20), concentrates of vitamin B_{12} (Merck No. 1 and 3), a fermentation by-product (Lederle APF Supplement) and an injectable anti-pernicious anemia extract have all been found to stimulate the growth of nursing rats. The factor involved in this stimulation appears to be soluble in 70 percent ethanol and less soluble in 95 percent ethanol.

BIBLIOGRAPHY

Cary, C. A. and A. M. Hartman. 1943-1947. Unidentified nutrients. U.S.D.A. Yearbook of Agriculture, Science in Farming. Page 779.

Hartman, A. M., L. P. Dryden and C. A. Cary. 1949 The role and sources of vitamin B_{12}, Jour. Am. Diet. Assn. 25:929.

Ross, O. B., P. H. Phillips and G. Bohstedt. 1942. The effect of a simplified diet upon the reproduction and lactation in swine and in the rat. Jour. Animal Science 1:86.

Ross, O. B., W. E. Swank, Ray J. Ohman and R. W. MacVicar. 1948. The effect of certain supplements upon reproduction and lactation performance of rats fed natural and partially purified rations. Jour. Animal Sci. 7:531.

Schaefer, A. E., W. D. Salmon, D. R. Strength, and D. H. Copeland. 1950. Interrelationship of folacin, vitamin B_{12}, and choline. Effect of hemorrhagic kidney syndrome in the rat and on growth of the chick. Jour. Nutrition 40:95.

Schultze, M. O. 1950. Nutritional value of plant materials I. Growth of rats on purified rations containing soybean protein. Submitted to Jour. of Nutrition. In Press.

Snedecor, G. W. 1946. Statistical methods applied to experiments in agriculture and biology. Ames: The Iowa State College Press.

Van Landingham, A. H. and P. B. Lyon. 1947. Inadequacy of diets containing whole grain cereals supplemented with soybean oil meal for lactation and growth of young rats. Arch. Biochem. 13:475.

Watts, A. B., O. B. Ross, R. W. MacVicar and C. K. Whitehair. 1949. The effect of certain supplements upon growth, reproduction and lactation of rats fed partially purified swine rations. Jour. Animal Sci. 8:633.

Zucker, L. M. and T. F. Zucker. 1948. Zoopherin: A nutritional factor for rats associated with animal protein sources. Arch. Biochem. 16:115.

PART II

DEPLETION-REPLETION STUDIES WITH RATS

INTRODUCTION

With the isolation and crystallization of vitamin B_{12} and its subsequent identification as part of the 'animal protein factor' complex, many investigators initiated experiments to study the physiological function of this vitamin. The early observations of McGinnis (1948) that chicks maintained on a diet deficient in this vitamin had high non-protein nitrogen blood levels suggested that this vitamin might be functioning in the metabolism of protein. Zucker (1948) also observed that rats maintained on a diet containing isolated plant proteins showed high blood urea values whereas the rats fed the same diet supplemented with a source of vitamin B_{12} had considerably lower values. Charkey et al (1950) in studying the amino acid metabolism of chicks on vitamin B_{12}-deficient diets found that the amino acid levels of the deficient chicks were higher than those of chicks fed the vitamin. These workers concluded that vitamin B_{12} appeared to function in metabolism by enhancing the utilization of circulating amino acids for building fixed tissues.

Studies with rations containing various levels of protein have indicated that the vitamin B_{12} requirement of an animal may be related to the amount of protein in the ration. The results obtained by Hartman and his associated (1949) in studies of the reproductive performance of rats fed increasingly high levels of protein would indicate that, as the level of protein is increased, the need for a factor, which was later identified as vitamin B_{12}, increased. The young of rats fed the high levels of protein (65 percent) showed much more severe symptoms of the deficiency than those from rats fed lower levels of protein (10 percent and 25 percent). Similar observations were made by Rubin and Bird (1947) with chicks fed rations composed primarily of corn and soybean meal. These workers observed that the vitamin B_{12} needs of the chick increased as the level of protein increased.

The recent work of Cunha and others (1950) would indicate that vitamin B_{12} and other unidentified members of the 'animal protein factor complex' function in lowering the protein needs of the pig. These workers concluded that efficient utilization of protein by the pigs in their experiment could be obtained only when a source of the 'animal protein factor complex' was present in the ration. A number of workers have observed increased efficiency of utilization of feed when a source of the 'animal protein factor complex' was added to the rations of pigs (Leucke, 1949) and chicks (Rubin and Bird, 1947).

The experiments to be discussed here were planned with a two-fold purpose in mind: The first objective was to study the effect of vitamin B_{12} on the utilization of the protein from a corn-soybean meal ration by mature depleted rats. If vitamin B_{12} proved to be necessary for proper utilization of the protein, it was planned to develop an assay procedure for vitamin B_{12} using the information obtained from this study.

EXPERIMENTAL PROCEDURE

The technique developed by Wissler and his associates (1946) for studying the regeneration of protein tissue by mature male rats, depleted by effecting a loss of 30 percent of their body weight, was used. These workers have found that rats fed a ration extremely low in protein would lose both carcass and liver protein without any appreciable change in the water or fat content of the carcass (Benditt et al, 1949). This technique has been proposed as a method of study- the quality of protein of various supplements. It appeared that this technique might be suitable to determine the effect of vitamin B_{12} supplementation on the synthesis of body protein.

Ration 1 (depletion) and ration 2 (repletion) fed during this study are shown in Table I. Ration 1 was mixed and stored in an electric refrigerator at approximately 5 degrees Centigrade. Both rations were fed ad libitum. Mature female rats weighing approximately 200 grams were fed ration 1 until they had lost approximately 30 percent of their body weight. At that time they were fed ration 2 and the rate at which lost tissue was replaced was measured. At the end of the depletion phase the rats were allotted on the basis of depleted weight and weight loss so that the differences among groups in this respect was minimal.

The crystalline vitamin B_{12} used in these studies was obtained from Merck and Company. The B_{12} Supplement 3 was a by-product of the manufacture of streptomycin with an activity equivalent to 12.5 milligrams of vitamin B_{12} per pound, according to the manufacturer's assay. The APF Supplement 5 (Lederle) was the residue from the manufacture of aureomycin. The soybean meal used in these studies was a high quality

TABLE I

RATIONS FED IN DEPLETION-REPLETION STUDIES

Ingredient	Ration 1 (Depletion ration)	Ration 2 (Repletion ration)
	percent	percent
Purified wood pulp	5.0	-
Carrots (fresh ground)	30.0	-
Corn oil	4.0	-
Mineral mixture*	4.0	2.0
Vitamin mixture**	+	+
Starch	44.0	-
Soybean meal	-	25.0
Ground yellow corn	-	73.0
dl-methionine	-	0.2

* The mineral mixture was that of Hegsted et al (1941).

** Each kilogram of ration was supplemented with the following amounts of each of the water soluble vitamins: Thiamin HCl 4 mg., riboflavin 6 mg., niacin 20 mg., pyridoxine HCl 3 mg., calcium pantothenate 20 mg., choline chloride 1 gm., folic acid 1 mg., inositol 20 mg., p-aminobenzoic acid 20 mg. In addition the rats received 2 drops weekly of a mixture of haliver oil and mixed tocopherols.

expeller product manufactured by Staley and Company, Decatur, Illinois. The liver concentrate powder used in Experiment 1 was the 1:20 liver concentrate powder produced by Wilson and Company. The supplements were added at the expense of the entire ration. The rats were housed in large colony type cages with four rats per cage during the depletion phase. During the repletion phase the rats were housed in individual cages.

The females used in Experiment 1 were of the Sprague-Dawley strain obtained from the local stock colony. Those used in Experiment 2 were obtained from the Sprague-Dawley Company as 100 gram females and fed a good stock ration until they weighed approximately 200 grams.

In Experiment 2 blood samples were obtained at the end of the depletion period and when the repletion period was concluded. Plasma protein and hemoglobin determinations were made on all the blood samples. Urea determinations were made on one-half of the blood samples at the end of the depletion period and on all the blood samples at the conclusion of the experiment. The procedure of Archibald (1945) was followed in making the urea determinations. The percentage of hemoglobin was determined by a modified acid hematin method. The plasma proteins were determined by the copper sulfate specific gravity method (Hawk et al, 1947). In Experiment 1 the rats were weighed on the sixth, eighth, and tenth days after the beginning of the repletion phase. In Experiment 2 weights were taken on the third, fifth, and seventh days of the repletion phase.

RESULTS AND DISCUSSION

The results of the growth of the depleted rats in Experiment 1 are presented in tabular form in Table II and graphically in Figure 1. It will be noted that the rats fed ration 2 supplemented with B_{12} Supplement 3 and with the liver concentrate powder regained their lost weight at a slightly faster rate than the rats fed the unsupplemented ration 2. These differences were not statistically significant when subjected to the analysis of covariance of Snedecor (1946). It would appear from these data that the supplementation of ration 2 with sources of the animal protein factor complex or with vitamin B_{12} did not significantly increase the rate at which the depleted rats regained their lost weight.

Crystalline vitamin B_{12} was available when Experiment 2 was initiated and was included as one of the supplements. The APF Supplement 5 of Lederle Laboratories was also fed in this experiment.

The results of Experiment 2 are presented in Table III and Figure 2. It will be observed that the supplementation of ration 2 with crystalline vitamin B_{12} failed to improve the rate at which the rats regained their lost weight. The addition of Lederle's APF Supplement 5, the combination of Lederle's APF Supplement 5 and vitamin B_{12}, and B_{12} Supplement 3 to ration 2 improved the rate at which the rats regained the lost weight. Respiratory disorders were observed in the group fed the combination of B_{12} Supplement 3 and vitamin B_{12} which was considered responsible for the poor performance of this group. Covariance analysis of the depleted weight and the three, five, and seven day weight gains showed that no significant differences existed among the different groups as to repletion of weight loss.

TABLE II

A SUMMARY OF THE GROWTH RESULTS OF EXPERIMENT 1

Supplement to ration 2	Number of rats	Average initial weight	Average depleted weight	Percent of loss regained at 6 days	8 days	10 days
		grams	grams			
None	6	247	170	56	74	83
0.5% B_{12} Supplement 3 (Merck)	5	250	173	61	91	101
2.0% Liver concentrate powder (Wilson)	6	263	183	66	84	94

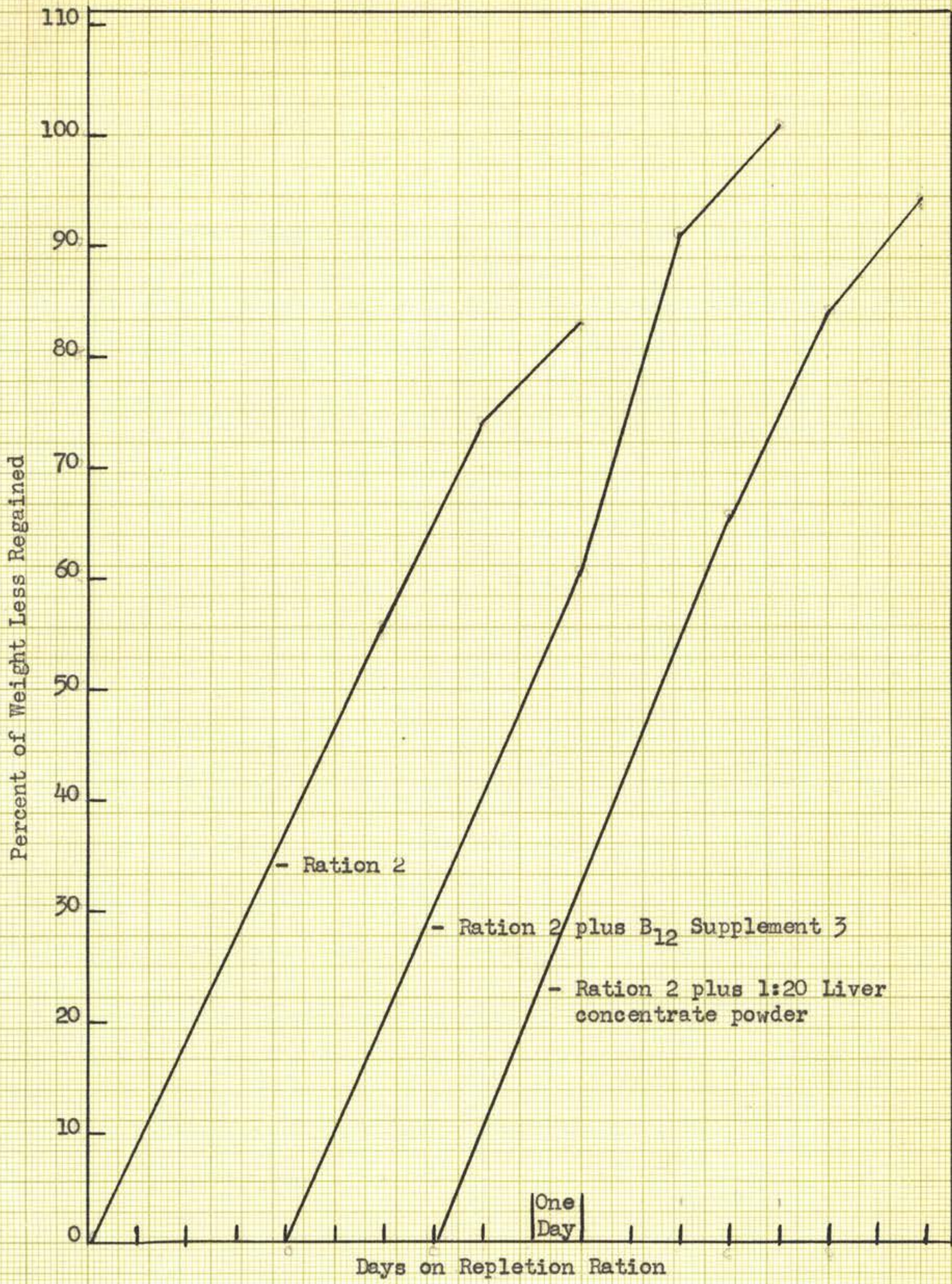

Figure 1. Growth Rates of Depleted Rats in Experiment 1.

TABLE III

A SUMMARY OF THE GROWTH RESULTS OF EXPERIMENT 2

Supplement to ration 2	Number of rats	Average initial weight	Average depleted weight	Percent of loss regained at 3 days	5 days	7 days
		grams	grams			
None	5	204	154	50	72	92
Vitamin B_{12} (25 mcgms per kilo ration)	4	207	140	48	70	87
1% APF Supplement 5 (Lederle)	5	216	153	53	79	98
1% APF Supplement 5 (Lederle) and vitamin B_{12} (25 mcgms per kilo ration)	5	209	147	52	73	102
0.5% B_{12} Supplement 3 (Merck)	5	199	145	59	81	106
0.5% B_{12} Supplement 3 (Merck) and vitamin B_{12} (25 mcgms per kilo ration)	4	212	155	47	72	81

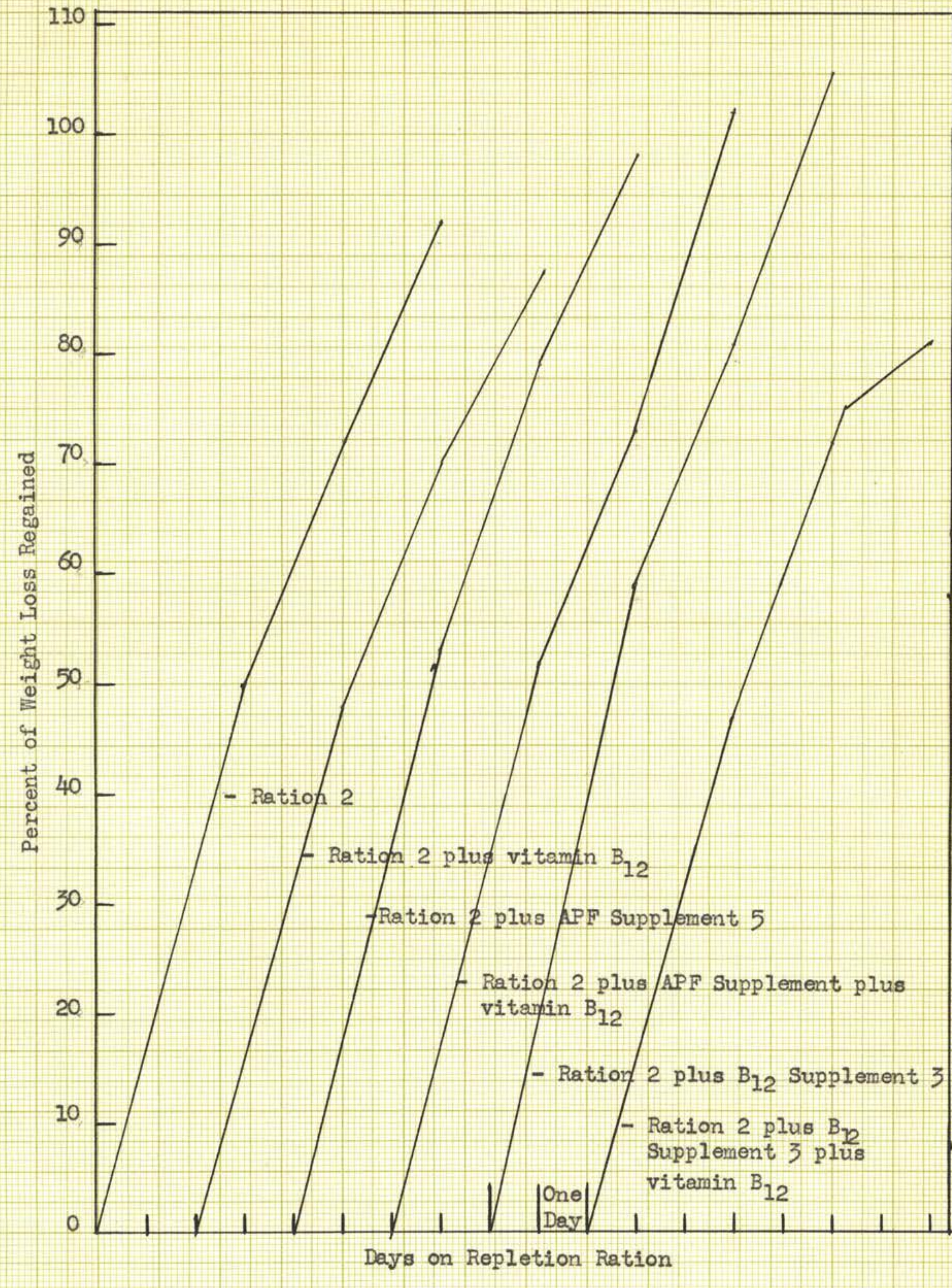

Figure 2. Growth Rates of Depleted Rats in Experiment 2.

The results of the blood analyses are given in Table IV. The plasma proteins of all groups increased about 4.0 gram percent during the repletion period but appreciable differences in the level of plasma proteins among the groups was not observed. The hemoglobin values increased slightly during the repletion period for all groups but differences among the various groups were not found. Essentially the same trend was observed relative to blood urea values.

Since no differences were apparent from the foregoing data regarding the rate at which the mature depleted female rats regained lost weight, it would appear that the supplementation of ration 2 with either vitamin B_{12} or sources of this factor did not materially affect the rate at which protein tissue was regenerated by the depleted rats. The ration fed during the repletion phase of this study contained adequate amounts of methionine, choline, and folic acid and this fact may have been responsible for failure of the rats in this study to respond to vitamin B_{12}. Schaefer et al (1950) have recently pointed out that an interrelationship exists between the rat's requirement of these nutrients and vitamin B_{12}. It is possible that ration 2 contained methionine, choline and folic acid in such amounts that the requirement of vitamin B_{12} was too small to be detected by the experimental procedure employed. It is also possible that vitamin B_{12} is not required for the regeneration of tissue under such conditions. The recent work of Chow and Barrows (1950) has shown that vitamin B_{12} did not improve the efficiency of utilization of soybean proteins by vitamin B_{12}-deficient rats when it was incorporated into rations of different caloric values. These workers concluded that vitamin B_{12} did not enhance the biological value of soybean protein but that it

TABLE IV

A SUMMARY OF THE BLOOD ANALYSES OF THE DEPLETED AND REPLETED RATS IN EXPERIMENT 2

Supplement to ration 2	Plasma proteins Initial*	Plasma proteins Final**	Hemoglobin Initial*	Hemoglobin Final**	Urea Final**(1)
	gram	percent	gram	percent	mgm percent
None	6.6	10.3	12.4	13.6	27.1
Vitamin B_{12} (25 mcgms per kilo ration)	6.1	10.4	12.6	13.8	23.5
1% APF Supplement 5 (Lederle)	6.2	10.0	12.2	13.8	32.8
1% APF Supplement 5 (Lederle) and vitamin B_{12} (25 mcgms per kilo ration)	6.5	10.0	12.3	13.0	29.3
0.5% B_{12} Supplement 3 (Merck)	6.8	10.1	12.2	13.7	28.3
0.5% B_{12} Supplement 3 (Merck) and vitamin B_{12} (25 mcgms per kilo ration)	6.7	10.3	12.4	13.9	26.3

* Initial values determined on blood samples taken at the end of the depletion period.

** Final values determined on blood samples taken at the end of seven days on the repletion rations.

(1) Initial values for urea were determined on blood samples from one-half of the rats and averaged 21.1 milligram percent.

may play an important role in carbohydrate or fat metabolism. It should be mentioned, however, that the rate at which the depleted rats regained lost weight in the study reported herein was not affected by supplementation with vitamin B_{12} whereas Chow and Barrows did observe more rapid gains with such supplementation.

Additional evidence that vitamin B_{12} may not be involved in the utilization and synthesis of protein has been presented by the work of Vars and his associates (1950). Using a technique which involved the 70 percent hepatectomy of rats and a subsequent study of the liver protein regeneration, these workers found no differences between the vitamin B_{12}-deficient rats and those fed the vitamin in respect to nitrogen balance, food intake, weight restoration, and liver protein regeneration. These results would tend to substantiate the results of the depletion-repletion trials presented here. It would appear that vitamin B_{12} is not involved in the utilization of protein by the rat.

SUMMARY

The effect of vitamin B_{12} and possibly other factors of the 'animal protein factor' complex on the rate at which mature female depleted rats regained lost weight has been studied. The depletion technique involved the feeding of a low protein ration to mature female rats until they had lost about 30 percent of their body weight. The supplementation of an all-plant ration with either crystalline vitamin B_{12}, antibiotic fermentation by-products, or combinations of these materials effected no significant differences in the rate at which the depleted rats regained lost weight. There were no marked differences in the blood hemoglobin, urea, and plasma protein values of any of the

groups at the end of the repletion period. It would appear that the apparent function of this vitamin is not concerned with the utilization or synthesis of protein by the rat.

BIBLIOGRAPHY

Archibald, R. M. 1945. Colorimetric determination of urea. Jour. Biol. Chem. 157:507.

Benditt, E. P., R. W. Wissler, R. L. Woolridge, D. A. Rowley, and C. H. Steffee. 1949. Loss of body protein and antibody production by rats on low protein diets. Proc. Soc. Expt'l Biol. Med. 70:240.

Charkey, L. W., H. S. Wilgus, A. R. Patton, and F. X. Gassner. 1950. Vitamin B_{12} and amino acid metabolism. Proc. Soc. Expt'l Biol. Med. 73:21.

Chow, B. F., and L. Barrows. 1950. Role of B_{12} on nitrogen retention of rats fed soybean protein diets at different caloric levels. Fed. Proc. 9:354.

Cunha, T. J., J. E. Burnside, H. M. Edwards, G. B. Meadows, R. H. Benson, A. M. Pearson and R. S. Glasscock. 1950. Effect of the animal protein factor on lowering the protein needs of the pig. Arch. Biochem. 25:455.

Hartman, A. M., L. P. Dryden, and C. A. Cary. 1949. The role and sources of vitamin B_{12}. Jour. Amer. Diet. Assn. 25:929.

Hawk, P. B., B. L. Oser, and W. H. Summerson. 1947. Specific gravity of blood by the copper sulfate method. Practical Physiological Chemistry, 12th Ed. pg. 435, The Blakiston Company.

Luecke, R. W., W. N. McMillen, F. Thorp, Jr., and J. R. Boniece. The effect of vitamin B_{12} concentrate on the growth of weanling pigs fed corn-soybean diets. Science 110:139.

McGinnis, J., P. T. Hsu, and W. D. Graham. 1948. Studies on an unidentified factor required by chicks for growth and protein utilization. Poul. Sci. 27:674.

Rubin, M. and H. R. Bird. 1947. A chick growth factor in cow manure. V. Relation to quantity and quality of soybean oil meal in the diet. Jour. Nutr. 34:233.

Schaefer, A. E., W. D. Salmon, D. R. Strength, and D. H. Copeland. 1950. Interrelationship of folacin, vitamin B_{12}, and choline. Effect of the hemorrhagic kidney syndrome in the rat and on growth of the chick. Jour. Nutr. 40:95.

Snedecor, G. W. 1946. Statistical methods applied to experiments in agriculture and biology. Ames: The Iowa State College Press.

Vars, H. M., G. M. Karn, and C. C. Fergerson. 1950. Vitamin B_{12} and liver protein regeneration. Fed. Proc. 9:373.

Wissler, R. W., R. L. Woolridge, C. H. Steffee, Jr., and P. R. Cannon. 1946. The relationship of the protein-reserves to antibody-production. II. The influence of protein repletion upon the production of antibody in hypoproteinemic adult white rats. Jour. Immunol. 52:267.

Zucker, L. M., T. F. Zucker, V. Babcock, and P. Hollister. 1948. Zoopherin: A nutritional factor for rats associated with animal protein sources. Arch. Biochem. 16:115.

PART III

GROWTH STUDIES WITH RATS FED ALL-PLANT RATIONS

Section A. Growth Studies with Female Weanling Rats

It has been established by numerous investigators that rations composed primarily of corn and plant proteins supplemented with minerals and all known vitamins except vitamin B_{12} would not support maximum growth of chicks. When rations of this type were supplemented with materials of animal origin, considerably better growth could be obtained. Excellent reviews of the work that led to the identification of vitamin B_{12} and studies of other new nutrients have been prepared by Woods (1948, 1949). Schweigert (1949) has reviewed the many factors that have been proposed as members of the "animal protein factor" complex and their possible relation to vitamin B_{12}. From the many studies that have been conducted it would seem that vitamin B_{12} is necessary for optimum growth of chicks fed rations composed only of feeds of plant origin.

The studies of Jaffe and Elvehjem (1948) would indicate that the rat requires essentially the same nutrients as the chick for maximum growth. The basal ration fed by these workers contained 45 percent soybean meal and fractions prepared from liver promoted greater growth than did the basal ration alone. Rubin and Bird (1949) found that chicks fed rations containing 70 percent soybean meal had a greater requirement for the "cow manure factor" than those fed lower levels of soybean meal. These workers (1949) later found that the "cow manure factor" was identical with vitamin B_{12}. Hartman and his associates (1949) found that purified rations containing high levels of exhaustively extracted casein and yeast resulted in poor reproductive performance of rats and in poor growth of their young. This deleterious effect could be corrected by the addition of supplements that contained vitamin B_{12}.

EXPERIMENTAL PROCEDURE

In the following studies the weanling rats used as the experimental animals were of the Sprague-Dawley strain and were obtained from the local stock colony. In most instances the weanlings were from females that had been fed a corn-soybean meal ration. The weanling rats were allotted on the basis of weaning weight, litter, and the maternal ration. During the growth experiments the rats were housed in individual cages. The rations were fed _ad libitum_. Supplements when added were at the expense of the entire ration. The composition of the rations are given in Table I.

Several supplements were used during the course of these studies. The B_{12} Supplement 1 was prepared by Merck and Company and this company reported the vitamin B_{12} activity as four times greater than that of condensed fish solubles. The liver powder was the 1:20 liver concentrate powder manufactured by Wilson and Company. The B_{12} Supplement 3 was a product prepared by Merck and Company which, according to the manufacturer's assay, contained an activity equivalent to 12.5 milligrams of vitamin B_{12} per pound. The crystalline vitamin B_{12} was supplied by Merck and Company as a mixture with common salt and one gram of the mixture contained one milligram of vitamin B_{12}.

The rumen contents tested in these studies were obtained from the rumen of two steers that had been full fed corn, sorghum silage, and cottonseed meal. The composite sample from the two steers was

TABLE I

RATIONS FED IN GROWTH STUDIES WITH WEANLING FEMALE RATS

Ingredient	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5	Ration 6
	percent	percent	percent	percent	percent	percent
Soybean meal	25	-	25	-	35	45
Vitamin-test casein	-	-	-	15	-	-
Meat and bone scraps	-	23	-	-	-	-
Ground yellow corn	68	70	73	83	63	63
Alfalfa leaf meal	5	5	-	-	-	-
Mineral mixture*	2	2	2	2	2	2
Vitamin mixture**	+	+	+	+	+	+
dl-methionine	0.2	0.2	0.2	0.2	0.2	0.2

* Mineral mixture was that of Hegsted et al (1941)

** The water soluble vitamin mixture supplied per kilogram of ration the following amounts of the various vitamins: Thiamin HCl 4 mg., riboflavin 6 mg., pyridoxine HCl 3 mg., calcium pantothenate 20 mg., niacin 20 mg., choline chloride 1 gm., folic acid 1 mg., inositol 20 mg., and p-aminobenzoic acid 20 mg. Two drops of a mixture of alpha tocopherol and cod liver oil were administered weekly.

frozen soon after it was obtained and remained frozen until it was used. Just prior to use the sample was allowed to thaw at room temperature and immediately mixed with the rest of the ration.

The growth studies were of five weeks duration during which individual weekly weights were taken.

RESULTS AND DISCUSSION

A summary of the results of the growth studies is presented in Table II. Weanling rats fed ration 1 and ration 2 supplemented with rumen contents grew slightly faster than those fed the unsupplemented ration, but the difference was not statistically significant. Retardation of chick growth has been reported by Nichol and his associates (1949) when assaying dried rumen contents for the presence of vitamin B_{12}. However, these workers found that one of the samples tested contained some vitamin B_{12} activity and they concluded that rumen contents were variable sources of this vitamin.

To further refine the experimental ration, alfalfa leaf meal was omitted from ration 3. A rather extensive study has failed to show that supplementary sources of vitamin B_{12} or other factors present in 1:20 liver concentrate powder increased growth of female weanling rats fed ration 3. Crystalline vitamin B_{12} when added to ration 3 produced slightly greater gains than did the unsupplemented ration 3. This difference was not statistically significant. The basal ration may have been adequate for growth or the young may have had adequate stores of vitamin B_{12} when placed on experiment. However, those fed ration 3 were produced by female rats that had been maintained for as long as three successive generations on the all-plant ration. It is possible that the synthesis of the factor by microorganisms in the intestine

TABLE II

SUMMARY OF THE RESULTS OF GROWTH STUDIES WITH FEMALE WEANLING RATS FED ALL-PLANT RATIONS

Experiment	Ration fed	Supplement to ration	Number of rats	5 weeks gain
				grams
1	1	None	7	87
	1	4 percent rumen contents	7	95
2	2	None	6	91
	2	4 percent rumen contents	6	96
3	3	None	15	103
	3	0.5% B_{12} Supplement 3	15	101
	3	2.0% 1:20 liver powder	14	104
4	3	None	10	101
	3	0.5% B_{12} Supplement 3	10	101
	3	Vitamin B_{12} (25 mcgms./kilo)	10	111
	3	0.5% B_{12} Supplement 3 and vitamin B_{12} (25 mcgms./kilo)	10	103
5	4	None	7	100
	4	2.0% Liver powder	8	104
	4	0.5% B_{12} Supplement 1	8	104
	4	4.0% Rumen contents	7	93
6	4	None	10	109
	4	2.0% 1:20 Liver powder	10	103
	4	0.5% B_{12} Supplement 1	10	104
7	3	None	10	101
	3	0.5% B_{12} Supplement 3	10	88
	5	None	10	68
	5	0.5% B_{12} Supplement 3	10	92
	6	None	10	69
	6	0.5% B_{12} Supplement 3	10	97

was sufficient to meet the requirements of the growing rat. Hartman and his associates (1949) have postulated that such synthesis does take place especially when high levels of riboflavin are fed. The amount of riboflavin fed in these studies was adequate but not excessive. Coprophagy might also have been a factor in these trials despite the wire-bottomed cages used to house the rats. Schaefer and his associates (1950) have proposed that an interrelationship exists between the requirement of the rat for choline, folic acid, methionine, and vitamin B_{12}. Ration 3 contained choline, folic acid, and methionine in amounts considered adequate by most workers. These nutrients may have reduced the requirement of the growing rat for vitamin B_{12} to the extent that this assay procedure was not sufficiently sensitive to show a need for the vitamin. One fact that may partially explain the apparent lack of a need for vitamin B_{12} in these studies is the fact that all of these growth trials were conducted with female rats and their requirement for maximum growth may be considerably less than that of the faster growing males.

Ration 4, containing vitamin-test casein as the source of supplementary protein, was fed during other trials. Supplementation of ration 4 with sources of vitamin B_{12} did not increase the rate of growth. The growth rates of the rats fed both ration 4 and the vitamin B_{12} supplemented ration were identical with those of the rats fed ration 3. Hartman _et al_ (1949) have suggested that casein, and even vitamin-test casein, contains significant amounts of vitamin B_{12}. This may explain why the rats grew at no faster rate when sources of this vitamin were added to ration 4. In view of the fact that the rats grew no faster when fed ration 4 than when fed ration 3, it would appear that ration 3 likewise contained sufficient vitamin B_{12} to support growth or that some other mechanism provided the vitamin in adequate amounts. The obvious conclusion from all the growth studies presented herein, is that vitamin B_{12} supplementation to the rations employed was not beneficial.

Since it appeared that the vitamin B_{12} requirement might be related to the level of protein in the ration, Experiment 7 was conducted. In this experiment soybean meal was fed at three different levels. Ration 3, ration 5, and ration 6 contained 25, 35 and 45 percent soybean meal respectively. A summary of this growth study is given in Table II. It will be noted that the growth of the rats fed the unsupplemented rations decreased as the level of soybean meal in the ration increased. This negative correlation between growth rate of chicks and level of soybean meal fed was observed by Rubin and Bird (1948). Although the addition of the B_{12} Supplement 3 to rations 5 and 6 stimulated growth, the gains were no greater than those obtained with the rats fed the unsupplemented ration 3. Analysis of variance (Snedecor, 1946) failed to show significant differences between the growth rates of the rats fed ration 3 and those of the rats fed the supplemented rations 5 and 6. As was noted in the previous experiments, no beneficial growth stimulus was obtained by supplementing ration 3.

Since most investigators are using higher levels of soybean meal in studies similar to these, it may be that the differences between these studies and those of other workers are due to the differences in the level of protein fed in the rations. It would seem that vitamin B_{12} is functioning in some manner in the utilization of the higher levels of protein. It is evident from the work of Chow and Barrows

(1950) and Vars et al (1950) that vitamin B_{12} is not functioning directly in the utilization of protein. It is possible that rations containing high levels of soybean meal have amino acids in improper ratios for maximum growth. Almquist (1949) has demonstrated that the ratio of some of the essential amino acids to each other is important for optimum growth performance of chicks. It may be that an imbalance of amino acids increases the requirement for vitamin B_{12}. Since the methionine requirement is related to the presence of vitamin B_{12} in the ration, it may be that the requirement for vitamin B_{12} is increased when the level of methionine in the ration is increased. It would seem that reasonably adequate evidence is available that vitamin B_{12} is functioning in transmethylation reactions (Schaefer et al, 1950) and methionine is one source in the body of 'labile methyl groups' (Keller et al, 1949). It might be suggested that the increased requirement for vitamin B_{12} with the higher levels of protein is due to the increased amount of methionine in the ration and the proper utilization of this methionine above a certain dietary level is contingent on the presence of increased amounts of vitamin B_{12}. If the requirement for vitamin B_{12} increases with increasing levels of protein or of any one of the essential amino acids, it is possible that the level of either of these factors in ration 3 is such that the requirement for the vitamin is minimal.

SUMMARY

Growth studies with female weanling rats fed various rations supplemented with sources of vitamin B_{12} indicated that the addition of this vitamin to these rations was of no value in improving the growth rate. Rumen contents, when fed at the 4 percent level with a corn-soybean

meal ration, did not improve the growth rate. Increasing the amount of soybean meal in the ration resulted in decreased gains of the rats fed these rations. Supplementation of the rations containing the higher levels (35 percent and 45 percent) of soybean meal with a source of vitamin B_{12} improved the gains of the rats fed these rations over the corresponding unsupplemented rations. The gains resulting from such supplementation did not exceed those of the rats fed the unsupplemented 25 percent soybean meal ration. It would appear that vitamin B_{12} was not required by the rats fed the ration containing 25 percent soybean meal or that it was synthesized by the microflora of the gastrointestinal tract in sufficient quantities to promote near maximum growth.

BIBLIOGRAPHY

Almquist, H. J. 1949. Amino acid balance at super-normal levels. Proc. Soc. Expt'l. Biol. Med. 72:179.

Chow, B. F., and L. Barrows. 1950. Role of B_{12} on nitrogen retention of rats fed soybean protein diets at different caloric levels. Fed. Proc. 9:354

Hartman, A. M., L. P. Dryden, and C. A. Cary. 1949. The role and sources of vitamin B_{12}. Jour. Amer. Diet. Assn. 25:929.

✓Hartman, A. M., L. P. Dryden, and C. A. Cary. 1949a. Bacterial synthesis in the rat of an unidentified growth-promoting factor. Fed. Proc. 8:205.

Jaffe, W. G. and C. A. Elvehjem. 1947. Fractionation of a growth-stimulating factor in liver. Jour. Biol. Chem. 169:287.

Lillie, R. J., C. A. Denton, and H. R. Bird. 1948. Relation of vitamin B_{12} to the growth factor present in cow manure. Jour. Biol. Chem. 176:1477.

Nichol, C. A., A. R. Robblee, W. W. Cravens, and C. A. Elvehjem. 1948. Distribution of an unidentified chick growth factor. Poul. Sci. 27:438.

Rubin, M. and H. R. Bird. 1948. A chick growth factor in cow manure. V. Relation to quantity and quality of soybean oil meal in the diet. Jour. Nutr. 34:233.

Schaefer, A. E., W. D. Salmon, D. R. Strength, and D. H. Copeland. 1950. Interrelationship of folacin, vitamin B_{12}, and choline. Effect of hemorrhagic kidney syndrome in the rat and on growth of the chick. Jour. Nutr. 40:95.

Schaefer, A. E., W. D. Salmon, and D. R. Strength. 1950. Role of vitamin B_{12} and methyl donors in lipotropism and transmethylation in the rat and the chick. Fed. Proc. 9:369.

Snedecor, G. W., 1946. Statistical methods applied to experiments in agriculture and biology. Ames: The Iowa State College Press.

Schweigert, B. S. 1949. The animal protein factor. Nutr. Rev. 7:225.

Vars, H. M., G. M. Karn, and J. C. Fergerson. 1950. Vitamin B_{12} and liver protein regeneration. Fed. Proc. 9:379.

Woods, R. 1948. The animal protein factor. Borden Review of Nutrition Research 9 (8).

Woods, R. 1949. The story of vitamin B_{12}. Borden Review of Nutrition Research 10 (1).

Section B. The Effect of Castration of Male and Female Rats on Their Response to Vitamin B_{12}

Certain observations on the growth of rats fed purified rations suggested there was a difference between the response of male and female rats to vitamin B_{12}. Unpublished results of MacVicar and Bolene(1950) indicated that the addition of vitamin B_{12} to a purified ration effected a greater growth in male than in female rats. The addition of liver to this purified ration promoted a growth response with both sexes. These differences were observed when rats were fed a ration containing soybean protein with or without the addition of small amounts of protomone. Carrick (1948) observed a difference between the response of male and female chicks when fed a ration containing condensed fish solubles. Male chicks grew at a faster rate when the basal ration was supplemented with fish solubles while female chicks did not show any improvement in rate of gain when fed these rations. Ershoff (1949) reported that female rats fed massive doses of dessicated thyroid failed to show any improvement in rate of growth when vitamin B_{12} was added to the ration. The addition of liver concentrate to the ration overcame the growth depressing effects of the thyroid substance. His later work showed that the growth depressing effect of thyroid substance could be partially overcome by the inclusion of certain vegetable fats in the ration (1949a) and completely overcome by the administration of chorionic gonadotropin (1950). Recently Ershoff (1950a) has found that male rats responded to vitamin B_{12} supplementation in the same manner as did the females. Under the conditions of the experiments reported by Ershoff, vitamin B_{12} did not completely overcome the growth depressing effects of dessicated thyroid while liver concentrates completely counteracted this condition.

Register et al (1949) have suggested an improved assay procedure for the determination of the growth factor present in liver extracts which makes use of the growth depressing effects of protomone administration and the ability of vitamin B_{12} to overcome this growth depression. Bethiel and Lardy (1949) found that the growth depressing effects of protomone could be overcome by the administration of either vitamin B_{12} or liver concentrate. These workers postulated that vitamin B_{12} is the factor necessary for proper growth of rats fed protomone.

A rat growth assay for vitamin B_{12} was proposed by Frost et al (1949) which not only contained protomone as a metabolic stimulant but also sulfaguanidine as an inhibitory agent of bacterial synthesis in the intestine. These workers used rats of both sexes in these studies and found no significant difference between the response of the males and the females. They concluded that precautions other than equal pairing as to sex did not appear to be necessary or desirable.

These conflicting results would seem to warrant further investigations on sex hormonal effects and vitamin B_{12} administration. With this in mind the studies to be reported here were initiated. In these studies rats of both sexes, castrated and uncastrated, were fed a purified ration deficient in vitamin B_{12} for ten days and then fed rations that contained small amounts of protomone in addition to the various supplements.

EXPERIMENTAL PROCEDURE

Sprague-Dawley weanling rats were used in this study. Thirty of the male rats were anaesthetized and the testicles removed. Eighteen of the female rats were anaesthetized and both ovaries removed. Thirty uncastrated rats of each sex were kept as controls. All the rats were fed ration 1, shown in Table III, without protomone for ten days. At the end of this ten day period the rats were allotted into groups according to sex, weight, and gain during the standardization period. The rations fed during the test period are given in Table III. The corn starch was a commercial product. The soybean sodium proteinate was the product of Archor Daniel Midlands Company. The liver powder was the 1:20 liver concentrate powder of Wilson and Company. The crystalline vitamin B_{12} was supplied by Merck and Company as a mixture with common salt and one gram contained one milligram of vitamin B_{12}. The rats were housed in individual cages and weighed weekly.

RESULTS AND DISCUSSION

The results of this study are summarized in Table IV. Uncastrated male rats fed ration 2 (basal ration plus vitamin B_{12}) made greater gains than those fed ration 1 (basal ration). This difference was statistically significant. Statistical analysis showed that the uncastrated male rats fed ration 3 (basal ration plus 1:20 liver concentrate powder) made significantly greater gains than those fed either ration 1 or ration 2. Castrate male rats fed ration 2 or 3 made greater gains than those fed ration 1 and the differences were significant. The castrate males fed ration 3 gained slightly more than those fed the vitamin B_{12} supplemented ration. This difference was

TABLE III

RATIONS FED IN GROWTH STUDY WITH MALE, FEMALE, AND CASTRATE RATS

Ingredient	Ration 1 (basal)	Ration 2	Ration 3
	percent	percent	percent
Corn starch	68.80	68.80	68.80
Sodium proteinate	21.75	21.75	19.75
Corn oil	5.00	5.00	5.00
Mineral mixture*	4.00	4.00	4.00
dl-methionine	0.20	0.20	0.20
Vitamin mixture**	+	+	+
Protomone	0.25	0.25	0.25
Liver concentrate powder (Wilson)	-	-	2.00
Vitamin B_{12}	-	0.25mcgms.	-

* The mineral mixture was that of Hegsted et al (1941).

** The vitamin mixture was fed to supply the following amounts of the water soluble vitamins per kilogram of ration: Thiamin HCl 6 mg, riboflavin 12 mg., niacin 40 mg., calcium pantothenate 60 mg., pyridoxine HCl 6 mg., para-amino benzoic acid 100 mg., folic acid 5.0 mg., inositol 1 gm., choline chloride 2 gm. During the standardization period only one-half of the above amounts of the various vitamins were fed. In addition to the water soluble vitamins the rats received twice weekly 2 drops of a mixture of cod liver oil and alpha tocopherol.

TABLE IV

THE RESPONSE OF MALE, FEMALE, CASTRATE MALE, AND CASTRATE FEMALE RATS TO RATIONS DEFICIENT IN VITAMIN B_{12}

Ration	Two weeks gain			
	Uncastrated males	Castrated males	Uncastrated females	Castrated females
	grams	grams	grams	grams
Ration 1 (basal ration)	46.7 (10)[1]	47.0 (10)	43.8 (10)	42.0 (6)
Ration 2 (ration 1 plus 25 mcgms vitamin B_{12} per kilo of ration)	57.7 (10)**	60.0 (10)**	63.0 (10)**	64.4 (6)**
Ration 3 (ration 1 plus 2% 1:20 liver concentrate powder)	69.2 (10)**#	64.2 (10)**	62.2 (10)**	71.3 (6)**

1 Numbers in parentheses are the number of rats fed that ration.

** Indicates that the gains were highly significant statistically when compared to those of the rats fed the basal ration.

The difference between the gains of the uncastrated males fed ration 3 and those of the uncastrated males fed ration 2 was statistically significant.

not statistically significant although it was in the same direction as that of the uncastrated males fed corresponding rations.

Uncastrated female rats fed the basal ration supplemented with either vitamin B_{12} (ration 2) or liver concentrate powder (ration 3) made greater gains than those fed the basal ration (ration 1). The differences were statistically significant. The liver concentrate powder supplemented ration did not support greater gains than the ration supplemented with vitamin B_{12} as was the case with the males. There was no difference in the response of the castrate females and uncastrated females to vitamin B_{12} supplementation. The castrate females, however, fed the basal ration supplemented with liver concentrate powder gained at a faster rate than did the uncastrated females fed this ration. This growth response of the castrate females was greater than that of either the uncastrated females or that of the castrate females fed the basal ration supplemented with vitamin B_{12}. The castrate females and the uncastrated males fed the basal ration supplemented with liver concentrate powder gained at essentially the same rate. In both cases those fed the liver concentrate powder supplemented ration gained faster than those fed the ration containing vitamin B_{12}.

It would appear from this data that the male sex hormone might act synergistically with some factor present in liver concentrate powder that is not identical with vitamin B_{12}. The presence of the female sex hormone, on the other hand, seems to act antagonistically with this unknown factor since castrate females fed the liver concentrate powder ration gained faster than uncastrated females fed the same ration. The castrate males fed the liver concentrate powder ration gained slightly less than the uncastrated ones. This may have been due to the removal of the influence of the male hormone. There does not appear to be a marked difference in the response of the castrate male rats and the uncastrated females to any of the rations fed.

It would appear that the liver concentrate powder used in this study may have contained a factor(s) other than vitamin B_{12} which was instrumental in overcoming the growth depressing effects of protomone administration. This has been suggested by Ershoff (1949) as a result of experiments with hyperthyroid rats. The growth response of the uncastrated males was comparable to that obtained by Ershoff (1950a), but the growth response of the uncastrated females was not identical with the results reported by this worker (1949). The results of the study reported in this paper are not wholly in agreement with those of Frost (1949) since this worker proposed that no precautions were necessary regarding the use of rats of opposite sexes in studies of this kind other than equal pairing as to sex.

SUMMARY

A growth experiment was conducted on both uncastrated and castrated male and female rats. Vitamin B_{12} and liver concentrate powder stimulated growth of male and female (castrated and uncastrated) weanling rats fed a purified ration containing protomone. Vitamin B_{12} was only partially effective in overcoming the growth depressing effects of protomone administration in male rats and in castrate female rats. The addition of vitamin B_{12} to the ration fed to castrate males and to uncastrated females seemed to be as effective in promoting growth as was the addition of the liver concentrate powder. Castration of male rats appears to inhibit to some extent the growth

response of rats fed the liver concentrate powder ration. The synergistic effect of the male hormone and the antagonistic effect of the female hormone with a factor(s) present in liver concentrate powder and not identical with vitamin B_{12} is suggested.

BIBLIOGRAPHY

Bethiel, J. J., and H. A. Lardy. 1949. Comparative effectiveness of vitamin B_{12} whole liver substance, and extracts high in APA activity, as growth promoting materials in hyperthyroid animals. Jour. Nutr. 37:495.

Carrick, C. W. 1948. Known growth factors in distillers solubles. Proc. 3rd Conference on Feeds of the Beverage Distillers. pg. 10

Ershoff, B. H. 1949. An antithyrotoxic factor for the rat not identical with vitamin B_{12}. Proc. Soc. Expt'l. Biol. Med. 71:209.

Ershoff, B. H. 1949a. Protective effects of soybean meal for the immature hyperthyroid rat. Jour. Nutr. 39:259.

Ershoff, B. H. 1950. Effects of liver residue and chorionic gonadotropin on ovarian development in the hyperthyroid rat. Proc. Soc. Expt'l. Biol. Med. 73:282.

Ershoff, B. H. 1950a. Effects of vitamin B_{12} and liver residue on growth of hyperthyroid male rats. Proc. Soc. Expt'l. Biol. Med. 73:459.

Frost, D. V., H. W. Fricke, and H. C. Spruth. 1949. Rat growth assay for vitamin B_{12}. Proc. Soc. Expt'l. Biol. Med. 72:102.

MacVicar, R. W. and C. E. Bolene. 1950. Unpublished data.

Register, U. D., W. R. Ruegamer, and C. A. Elvehjem. 1949. An improved assay for a growth factor in liver extracts. Jour. Biol. Chem. 177:129.

PART IV

STUDIES WITH PIGS FROM SOWS FED ALL-PLANT RATIONS

INTRODUCTION

In recent years the attention of many investigators has been directed to the factors causing excessive losses of baby pigs. Many investigators have suggested that these losses are due to improper nutrition and especially related to the ration of the pregnant and lactating sow. Some evidence is also available to indicate that infectious microorganisms are involved, but efforts to isolate infectious agents have not been too fruitful.

Madsen and his associates (1944) reported a disturbance in baby pigs that resulted in high mortality and suggested that it might be of dietary origin. The affected pigs developed symptoms soon after birth characterized by diarrhea. Autopsy revealed degenerative changes in the kidney which usually showed rather extensive accumulation of uric acid precipitate. Changes in the blood of the affected pigs were characterized by greatly elevated urea-nitrogen and uric acid levels. Hypoglycemia was also present but not a consistent symptom of the disturbance. Inanition was suggested as a possible cause since witholding food from apparently healthy pigs produced a condition similar to that observed in the affected pigs.

A naturally occurring disease in pigs characterized mainly by a hypoglycemia was reported by Morrill in Illinois (1946). This worker was able to produce a condition in baby pigs similar to the naturally occurring one by fasting the baby pigs. Although hypoglycemia was reported as the characteristic symptom, the urea and uric acid levels of the blood of the clinical cases of the disease and the fasted pigs were greatly elevated. A condition of similar nature has been observed by Green _et al_ (1949). These workers fed one-week-old pigs reconstituted fat-free skimmilk diets supplemented with minerals and vitamin A. These diets appeared to be toxic to the pigs. The toxic effects were manifested by high mortality with both gross and microscopic evidences of degenerative changes in the kidney, liver, and adrenal glands. The characteristic change in the blood of the affected pigs was a greatly elevated urea nitrogen level. The addition of dextrin accentuated the apparent toxic effects of the reconstituted fat-free skimmilk diets. The addition of a combination of dehydrated cereal grass and yeast seemed to alleviate the apparent toxic effects of these diets.

Schultze (1950) reported that excessive mortality occurred in the young from rats fed purified rations containing isolated soybean protein. The injection of vitamin B_{12} into the newborn rats prevented the occurrence of this syndrome which this worker designated as "acute uremia of the newborn". The condition in the affected rats was characterized by greatly elevated blood urea values. Schultze suggested that the condition was of metabolic origin rather than a pathologic condition established _in utero_. The studies of this worker indicated that vitamin B_{12}-deficient rations _per se_ do not necessarily cause the acute uremia. Zucker _et al_ (1948) found that rats fed purified diets containing plant proteins produced young that died soon after weaning. The young rats from these females had greatly elevated blood urea values. McGinnis (1949) observed that chicks fed an all-plant ration had high non-protein nitrogen values unless the rations were supplemented with a source of the animal protein factor.

Doyle and Hutchings (1946) isolated an infectious agent causing clinical and pathological features of gastroenteritis which resulted in heavy death losses in baby pigs. It was found to affect older swine, but less severely than baby pigs. The disturbance developed promptly following pen exposure, and also following per os administration of the triturated gastrointestinal tract and contents of the affected pigs. Exposed pigs showed symptoms of the disease within 40 hours after exposure and usually died within six days. Changes in blood constituents were not reported in this work.

EXPERIMENTAL PROCEDURE

Blood samples were obtained from pigs one, two and three weeks of age produced by sows that were fed an all-plant ration. Oxalate was used as an anticoagulant. Protein-free filtrates were obtained by the method of Folin and Wu (Hawk, 1947). The protein-free filtrate was used for the determination of urea by the method of Archibald (1945), sugar by the method of Folin and Wu (Hawk, 1947a), and non-protein nitrogen by nesslerization. Hemoglobin was determined by a modified acid hematin method. In some cases hematocrit determinations were made using the Wintrobe hematocrit tube and centrifuging at 3000 rpm.

Blood samples were obtained from seven typical pigs in a herd affected with a spontaneously occurring disturbance whose symptoms were similar to those observed in pigs with artifically induced gastroenteritis. The pigs were about four weeks old when the first symptoms of the disease were noted. The blood samples were obtained five days later.

A preparation was obtained from Dr. L. P. Doyle of Purdue University that was very active in producing a gastroenteritis in young pigs. In studying the blood picture of pigs infected with this disease two pregnant sows were obtained. After farrowing, the pigs from both litters were allowed to run together, the sows being kept separated. In order to get information on the transmissible nature of the infectious agent, one litter was exposed at five days of age by per os administration of the preparation of Doyle and the other litter allowed to run with them. Blood samples were taken from all pigs prior to the exposure of one litter and on the third, seventh, and eighth day after exposure. Urea, non-protein nitrogen, and sugar determinations were made on the protein-free filtrates of these samples. Hematocrit determinations were made on the oxalated blood. The pigs were observed frequently and rectal temperatures were taken.

In order to study the effect of this infectious agent on older pigs, two more litters were handled as described above except that the pigs of one litter was exposed by per os administration of the preparation at three weeks of age. Blood samples were obtained from all pigs just prior to the exposure of one litter and on the third, fifth, and seventh days after exposure. The pigs were observed frequently during the course of the disease.

RESULTS AND DISCUSSION

The results of the blood analyses of the pigs from sows fed an all-plant ration are given in Table I. The levels of the various constituents are within the range of normal values reported for pigs of this age. The uremia noted by Schultze (1950) and Zucker et al (1948) in rats fed all-plant rations was not observed in these pigs. The

TABLE I

LEVELS OF CERTAIN NITROGEN CONSTITUENTS AND SUGAR IN THE BLOOD OF PIGS FROM SOWS FED AN ALL-PLANT RATION

Age of pigs	Urea nitrogen	Non-protein nitrogen	Sugar	Hemoglobin	Hematocrit
weeks	mg%	mg%	mg%	gm%	
One (4)*	6.9	34.9	109	10.7	34
Two (4)	15.5	52.3	128	7.1	24
Three (3)	8.9	43.9	108	5.8	20

* Number in parentheses indicates the number of values from which the average value was calculated.

TABLE II

LEVELS OF CERTAIN NITROGEN CONSTITUENTS AND SUGAR IN THE BLOOD OF PIGS AFFECTED WITH A NATURALLY OCCURRING DISTURBANCE

Age of the pigs at exposure	Days after exposure	Urea nitrogen	Non-protein nitrogen	Sugar
days		mg%	mg%	mg%
28 (7)*	5	49.7	70.2	99

* Number in parentheses indicates the number of values from which the average was calculated.

pigs from which the blood samples were obtained were vigorous and healthy at the time they were bled.

The average values of the various nitrogen constituents and sugar in the blood of the pigs affected with a naturally occurring disturbance are presented in Table II. The urea nitrogen and the non-protein nitrogen values are considerably higher than those of the three-week-old pigs from the sows fed the all-plant ration. There was a tendency for the blood sugar values to be lower than those of the three-week-old pigs. Two of the most severely affected pigs had blood sugar values of 52 and 75 milligram percent respectively. The sows in this herd of affected pigs were being fed a good commercial ration, and it would not seem likely that this ration was deficient in factors necessary for proper lactation. At the time the blood samples were obtained from these affected pigs, 15 pigs out of 80 had died. Many others were severely affected.

The averages of certain blood constituents of the pigs artificially exposed to the infective agent of Doyle are given in Table III. There were nine pigs in each litter at the beginning of the study. One litter was exposed at five days of age by oral administration of the Doyle preparation and the other litter was allowed to run with them. Since both litters showed symptoms of the disease in 48 hours, the figures in Table III are the averages of all the pigs of both litters. Rectal temperatures failed to show the presence of fever at any time during the course of the disturbance. It will be noted that the disturbance was characterized by an immediate rise in blood urea nitrogen and non-protein nitrogen. There was no marked change in the blood sugar values although there was a tendency for the values to

TABLE III

LEVELS OF CERTAIN NITROGEN CONSTITUENTS AND SUGAR IN THE BLOOD OF PIGS ARTIFICALLY EXPOSED TO AN INFECTIOUS GASTROENTERITIS

Age of the pigs at exposure	Days after exposure	Urea nitrogen	Non-protein nitrogen	Sugar	Hematocrit
		mg%	mg%	mg%	
5 days	0 (18)*	6.7	46.4	137	28
	3 (18)	37.2	74.3	113	24
	7 (13)	41.0	116.3	116	23
	8 (7)	23.5	67.4	106	21
21 days	0 (10)	12.8	33.4	127	
	3 (10)	37.8	64.1	135	
	5 (9)	31.6	46.7	114	
	7 (7)	23.1	47.2	111	

* Number in parentheses indicates the number of values from which the average was calculated.

decrease as the time after exposure increased. The greatest mortality occurred on the fifth, sixth, and seventh days after exposure. During this period of time 11 of the 18 pigs died. All the pigs alive at the end of eight days after exposure were sacrificed and autopsied. Of the seven alive at this time, two were very weak and it is doubtful if they would have survived another day. For all practical purposes, thirteen of the pigs did not survive this infection. In all cases where the pigs were bled within 24 hours before death, extremely high urea nitrogen and non-protein nitrogen blood values were observed. In some cases the blood sugar values were below the normal range, but there was no consistency in this respect. There did not appear to be any hemoconcentration resulting from the severe diarrhea as evidenced by a study of the hematocrit values given in Table III. Autopsy of the severely affected pigs revealed an extensive accumulation of precipitate in the pelvis of the kidney. The changes in the kidney and in the blood of these pigs are sililar to those observed by Madsen et al (1944) and Green et al (1949). It is possible that the changes resulted from inanition as the pigs were so severely affected that they did not nurse as often as normal pigs and vomited frequently.

In the study of the effect of this infectious agent on three-week-old pigs essentially the same results were noted as had been observed with the younger pigs. The older pigs seemed to be more resistant to the infectious agent, however, and a greater number of those exposed recovered. The highest blood levels of urea nitrogen and non-protein nitrogen were found about three days after exposure. Only three of the older pigs died during the period of observation and the survivors appeared as though they had recovered from the effects of the disease.

Essentially the same pattern was evident in the occurrence of the symptoms of this disturbance in the pigs of the two ages studied. Diarrhea and vomiting were evident within 48 hours after exposure to the infectious agent. The blood urea nitrogen and non-protein nitrogen levels increased rapidly after exposure, reaching peak values between the third and seventh days after exposure. In the pigs bled just prior to death the blood urea nitrogen and non-protein nitrogen values reached the extremely high values of 151 to 177 milligrams percent for urea nitrogen and 224 to 308 milligrams percent for non-protein nitrogen.

SUMMARY

The levels of urea nitrogen, non-protein nitrogen, and sugar in the blood of pigs from sows fed all-plant rations have been determined. These values did not differ from those given as normal for pigs of the ages studied. The pathological changes and the blood changes of pigs artifically exposed to an infectious gastroenteritis agent have been studied. Kidney degeneration was found in cases showing symptoms of the disease. Blood urea nitrogen and non-protein nitrogen values rose rapidly after exposure. Blood sugar was not affected in the same magnitude. Mortality of the pigs exposed to the infectious agent at five days of age was very high but the exposure of pigs three weeks of age did not result in as high mortality. Analyses of the blood of pigs affected with a spontaneously occurring disturbance showed essentially the same changes as those noted for the artifically infected pigs of the same age.

BIBLIOGRAPHY

Archibald, R. M. 1945. Colorimetric determination of urea. Jour. Biol. Chem. 157:507.

Doyle, L. P. and L. M. Hutchings. 1947. A transmissible gastroenteritis in pigs. Jour. Am. Vet. Med. Assn. 108:257.

Green, W. W., H. C. H. Kernkamp, M. P. Roepke, and L. M. Winters. 1949. A toxemic-uremic syndrome in baby pigs fed on dried skimmilk. Amer. Jour. Vet. Res. 10:256.

Hawk, P. B., B. L. Oser, and W. H. Summerson. 1947. Preparation of protein-free blood filtrates. Practical Physiological Chemistry. 12th Ed. pg. 493. The Blakiston Company.

Hawk, P. B., B. L. Oser, and W. H. Summerson. 1947a. Determination of glucose. Practical Physiological Chemistry. 12th Ed. pg. 520. The Blakiston Company.

Madsen, L. L., I. P. Earle, L. C. Heemstra, and C. O. Miller. 1944. Acute uremia associated with "uric acid infarcts" in the kidneys of baby pigs. Am. Jour. Vet. Res. 5:262.

McGinnis, J., P. T. Hsu, and W. D. Graham. 1948. Studies on an unidentified factor required by chicks for growth and protein utilization. Poul. Sci. 27:674.

Morrill, C. C. 1946. A physiopathological study of the newborn pig with special reference to hypoglycemia. Ph.D. Thesis, University of Illinois.

Schultze, M. O. 1950. Nutritional value of plant materials. II Prevention of acute uremia of the newborn rat by vitamin B_{12}. Proc. Soc. Expt'l. Biol. Med. 72:613.

Zucker, L. M., T. F. Zucker, V. Babcock, and P. Hollister. 1948. Zoopherin: A nutritional factor for rats associated with animal protein sources. Arch. Biochem. 16:115.

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