

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS

	Page
Effect of Diet on the Resistance of the Albino Rat to Bacterium abortus - - - RALPH HOAGLAND and JOHN M. BUCK	201
Transmission of Pullorum Disease (Bacillary White Diarrhea) in Incubators - - HUBERT BUNYEA and WALTER J. HALL	209
Inheritance of the Second Factor for Resistance to Bunt, <i>Tilletia tritici</i> , in Hussar Wheat F. N. BRIGGS	225
An Automatic Watering System with Recorder for Use in Growing Plants - - ROBERT A. STEINBERG	233
The Influence of the Potash Concentration in the Culture Medium on the Production of Carbohydrates in Plants - - - - - GEORGE JANSSEN and R. P. BARTHOLOMEW	243
A Study of the Mosaic Disease of Crucifers - - - - - E. E. CLAYTON	263
The Insensible Loss in Body Weight of Cattle - - - - - MAX KRISS	271
Quantitative Relations of the Dry Matter of the Food Consumed, the Heat Production, the Gaseous Outgo, and the Insensible Loss in Body Weight of Cattle - - - MAX KRISS	283



PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION
OF LAND-GRANT COLLEGES AND
UNIVERSITIES

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, CHAIRMAN
Chief, Bureau of Chemistry and Soils

C. L. SHEAR
*Senior Pathologist in Charge, Mycology and
Disease Survey*

F. L. CAMPBELL,
*Associate Entomologist, Bureau of Ento-
mology*

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
*Director of Research, Pennsylvania Agri-
cultural Experiment Station*

G. A. DEAN
*Head, Department of Entomology, Kansas
Agricultural Experiment Station*

F. B. MORRISON
*Head, Department of Animal Husbandry,
New York (Cornell) Agricultural Experi-
ment Station*

EDITORIAL SUPERVISION

M. C. MERRILL

Chief of Publications, United States Department of Agriculture

All correspondence regarding articles from State experiment stations should be addressed to S. W. Fletcher, Director of Research, Agricultural Experiment Station, State College, Pa.

Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price: Domestic, \$4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, \$5.00 a year (two volumes)
Single numbers, 25 cents

If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer, and they will be supplied practically at cost. Single copies of separates may be obtained free of charge from the Office of Information, United States Department of Agriculture, until the supply is exhausted.

Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 40

WASHINGTON, D. C., FEBRUARY 1, 1930

No. 3

EFFECT OF DIET ON THE RESISTANCE OF THE ALBINO RAT TO BACTERIUM ABORTUS¹

By RALPH HOAGLAND, *Senior Biochemist, Biochemic Division*, and JOHN M. BUCK, *Assistant Superintendent, Bethesda Experiment Station, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The albino rat appears to have been used but little in experiments with *Bacterium abortus*, perhaps on account of its comparatively high resistance to this organism.^{2 3} However, since the nutritive requirements of the rat have been studied extensively, it has seemed desirable to ascertain the extent to which the resistance of this animal to *Bact. abortus* is affected by certain dietary deficiencies.

EXPERIMENTAL DATA

The rats used in these experiments were raised in the bureau laboratories from healthy, vigorous, and prolific stock. Mature or nearly mature rats were employed. Unless otherwise stated, the rats were fed a diet fully adequate for excellent growth and reproduction. This diet was made up as follows:

Adequate diet for rats	Per cent
Yellow corn meal.....	25
Graham flour.....	25
Oatmeal.....	24
Dried beef.....	15
Dried bakers' yeast.....	4
Calcium carbonate.....	4
Cod-liver oil.....	2
Sodium chloride.....	1

A single strain of *Bact. abortus* which has been propagated in these laboratories for several years was used in all experiments. This strain is pathogenic for guinea pigs and exhibits typical cultural and agglutinating properties. In general, 4 or 5 day bouillon cultures were used for inoculation and feeding tests, but glycerin-agar cultures were fed in a few experiments.

Rats which had been exposed to *Bact. abortus* were usually killed at the end of from four to five weeks. Six dilutions of the blood serum were used for agglutination tests, viz, 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000. The internal organs were carefully examined and small quantities of the spleen were transferred to serum-agar slants and spread evenly over the surface. An effort was made to transfer approximately the same quantity of spleen to each tube. The cultures were incubated from six to eight days at 37.5° C., when the colonies of *Bact. abortus* were counted.

¹ Received for publication June 18, 1929; issued February, 1930.

² BURNET, E., and LAGOANÈRE, J. L. DE POUVOIR PATHOGÈNE DU M. MELITENSIS ET DU B. ABORTUS POUR LE RAT ET LA SOURIS. Arch. Inst. Pasteur Tunis 13: 182-191. 1924.

³ HAGAN, W. A. THE SUSCEPTIBILITY OF MICE AND RATS TO INFECTION WITH BACILLUS ABORTUS. Jour. Expt. Med. 36: 727-733. 1922.

FEEDING EXPERIMENTS WITH BACTERIUM ABORTUS

EXPERIMENT 1. ADEQUATE DIET

Eighteen rats which had received the previously described adequate diet from birth were fed pure cultures of *Bact. abortus* on several successive days. Four to five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, but agglutination reactions in dilutions ranging from 1 to 25 to 1 to 100 were yielded by the blood serum of eight rats.

EXPERIMENT 2. DIET LOW IN VITAMIN A

Six rats which had been fed a diet low in vitamin A from birth were fed a pure culture of *Bact. abortus* on several successive days. The diet was made up as follows:

Diet low in vitamin A	Per cent
Graham flour.....	36
Oatmeal.....	40
Dried beef.....	15
Dried bakers' yeast.....	4
Calcium carbonate.....	4
Sodium chloride.....	1

Three weeks after being fed the organism the rats were killed and examined. *Bact. abortus* was isolated from the spleens of two rats.

The serum from one of these reacted to the agglutination test in a dilution of 1 to 25, and that from another rat reacted in the same dilution.

EXPERIMENT 3. DIET VERY POOR IN VITAMIN A

Six rats which had been fed the above-described diet from birth were transferred to a ration very much poorer in vitamin A, as follows:

Diet very poor in vitamin A	Per cent
Casein.....	24
Dried brewers' yeast.....	5
Ash mixture.....	4
Hydrogenated cottonseed oil.....	10
Cassava starch.....	57

The casein was purified from vitamin A by heating in a current of air at from 115 to 130° C. The other constituents have been found to be practically free from this vitamin. This ration is probably poor in vitamin D also, but the rats used appear to have had a very small requirement for this vitamin, apparently because of considerable sunlight which entered the room through the partly opened windows.

After this group of rats had received the foregoing diet for 102 days they were fed a pure culture of *Bact. abortus* on five successive days. Five weeks later they were killed and examined. *Bact. abortus* was isolated from the spleen of only one rat, which also yielded an agglutination reaction in a dilution of 1 to 25. Agglutination reactions were obtained with the blood of two other rats in a dilution of 1 to 50. At the end of the test one rat was in very poor condition but the others were in fair condition. None had ophthalmia.

EXPERIMENT 4. ADEQUATE DIET

Four rats which had received an adequate diet from birth were fed approximately 10 c. c. of the contents of the stomach of an aborted calf infected with *Bact. abortus*. Five weeks later the rats were killed and examined. *Bact. abortus* was isolated from the spleen of one rat, but no agglutination reactions were obtained.

The results of these four experiments indicate that the albino rat is not readily infected with *Bact. abortus* when fed a pure culture of the organism, whether the rats have had an adequate diet or one deficient in vitamin A. Thus, of 34 rats which were used in these feeding tests only five were found to be infected with *Bact. abortus*. This method of exposing rats to *Bact. abortus*, therefore, was not regarded as satisfactory for testing the effects of deficient diets on the resistance of the rat to this organism.

INOCULATION EXPERIMENTS

EXPERIMENT 5. ADEQUATE DIET

Six rats which had been fed an adequate ration were inoculated intra-abdominally with 0.5 c. c. of a bouillon culture of *Bact. abortus* and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of all six rats, the number of colonies on the tubes of serum agar ranging from 1 to 13. All rats afforded a positive agglutination reaction, the titer being 1 to 1,000 or higher. This method of inoculation appeared to be too severe for testing the effects of deficient diets on the resistance of the rat to *Bact. abortus*.

EXPERIMENT 6. ADEQUATE DIET

Six rats which had been on an adequate diet were inoculated subcutaneously with 0.5 c. c. each of a bouillon culture of *Bact. abortus*, and 37 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of three rats, the number of colonies per tube ranging from one to five. The agglutination test was positive in all cases in dilutions of 1 to 100 or 1 to 200, the average being 1 to 150.

EXPERIMENT 7. ADEQUATE DIET

This experiment was conducted to determine the effects of inoculating rats subcutaneously with *Bact. abortus* as regards (1) the duration of the infection in the spleen, and (2) the persistence of agglutinins in the blood. Forty-six rats which had been fed an adequate ration were inoculated subcutaneously with *Bact. abortus* on the same date, and at intervals thereafter groups of three rats were killed and examined, with the results shown in Table 1.

The data in Table 1 indicate roughly the course of the infection in the spleens of rats inoculated subcutaneously with *Bact. abortus*. As judged by cultures taken from the spleens of rats killed at intervals during this experiment, the rats appeared to harbor large numbers of *Bact. abortus* in their spleens on the sixth day after inoculation, but the infection gradually subsided and had apparently disappeared by the forty-sixth day. The spleens of rats examined on the thirty-fifth and forty-second days, respectively, were only slightly infected.

TABLE 1.—Duration of *Bacterium abortus* infection in albino rats following subcutaneous inoculation

Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen ^a		Weight of spleen as percentage of body weight	Rat No.	Period following inoculation	Agglutination titer	Colonies in duplicate cultures from spleen ^a		Weight of spleen as percentage of body weight
			A	B					A	B	
			Number	Number					Number	Number	
1	6	1-100	400-500		0.530	24	42	1-1,000	1	0	.529
2	6	1-200	500-600		.627	25	46	1-200	0	0	.518
3	6	1-200	500-600		.725	26	46	1-50	0	0	.436
4	11	1-200	75-100		.475	27	46	1-200	0	0	.629
5	11	1-1,000	75-100		.635	28	56	1-500	0	0	.475
6	11	1-200	100-150		.465	29	56	1-1,000	0	0	.372
7	16	1-50	100-125	75-100	.420	30	56	1-100	0	0	.522
8	16	1-200	2	25	.461	31	65	(^b)	0	0	.265
9	16	1-200	75-100	75-100	.483	32	65	1-500	0	0	.324
10	21	1-200	50-75	50-75	.563	33	65	1-200	0	0	.324
11	21	1-100	15-25	15-25	.353	34	80	1-200	0	0	.281
12	21	1-200	100-150	75-100	.508	35	80	1-200	0	0	.298
13	24	1-200	21	15	.433	36	80	1-100	0	0	.275
14	24	1-200	15	6	.508	37	100	(^b)	0	0	.244
15	24	1-200	11	15	.296	38	100	1-200	0	0	.305
16	29	1 300	3	0	.502	39	100	1-200	0	0	.327
17	29	1 00	12	9	.486	40	113	1-100	0	0	.297
18	29	1-200	1	1	.569	41	113	(^b)	0	0	.252
19	35	1-1,000	0	0	.521	42	113	1-25	0	0	.214
20	35	1-200	0	0	.567	43	122	(^b)	0	0	.235
21	35	1-200	1	0	.421	44	122	1-50	0	0	.221
22	42	1-200	0	0	.665	45	122	1-100	0	0	.299
23	42	1-1,000	1	0	.582	46	122	(^b)	0	0	.217

^a In numerous instances the number of colonies of *Bact. abortus* on the surface of agar slants could not be counted accurately. In such cases the number was approximated.

^b Negative.

There was considerable variation in the agglutination titer of the blood of rats killed at different intervals. Toward the end of the experiment the titer became lower and the blood of some rats did not react.

The relation between the weight of the spleen and the live weight of the rat indicates a material enlargement of that organ from the sixth to the fifty-sixth days, inclusive, after inoculation with *Bact. abortus*, with a gradual reduction in the size of the spleen from that time until the end of the experiment.

The results of this experiment seemed to indicate that subcutaneous inoculation would be a satisfactory method for exposing rats to *Bact. abortus* in order to test their resistance to this organism as affected by diet. Since rats fed an adequate diet were regularly infected with *Bact. abortus* by this method, and since the infection ran a mild course, it seemed probable that adverse effects of deficient diets would become apparent when infection was accomplished by this means. Experiments with rats fed inadequate diets were therefore carried out.

EXPERIMENT 8. DIETS POOR IN VITAMIN A

Twelve rats which had been fed a diet poor in vitamin A⁴ since birth were inoculated subcutaneously with *Bact. abortus* and 39 days later they were killed and examined. *Bact. abortus* was isolated from the spleens of 3 of the 12, the average number of colonies

⁴ Ration described under experiment 2.

per tube being five. In all rats the agglutination test was positive in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

A control group of 12 rats which had been fed an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-nine days later the rats were killed and examined. *Bact. abortus* was isolated from the spleens of 6 of the 12, the average number of colonies per tube being five. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

Ten rats which had been fed a diet low in vitamin A⁵ from birth were placed on the following diet, which was very deficient in this vitamin.

Diet very poor in vitamin A	Per cent
Caseine (purified).....	24.3
Bakers' yeast.....	10.0
Ash mixture.....	4.0
Hydrogenated cottonseed oil.....	7.0
Wheat-germ oil.....	3.0
Cassava starch.....	51.7

The rats were fed this ration for 28 days, when they were inoculated with *Bact. abortus*. After 31 days more on this ration they were killed and examined. *Bact. abortus* was isolated from the spleens of 9 of the 10. The average number of colonies per tube was four. Serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 500, the average being 1 to 370. These rats were in fair condition at the end of the test and showed no signs of ophthalmia.

A control group of nine rats which had received an adequate diet from birth was inoculated at the same time as the preceding group. Thirty-two days later these rats were killed and examined. *Bact. abortus* was found in the spleens of 7 of the 9, the average number of colonies per tube being two. All rats afforded a positive agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

The results of these tests indicate no significant difference in the resistance to *Bact. abortus* of rats fed diets deficient in vitamin A as compared with others fed a diet containing an abundance of this vitamin.

EXPERIMENT 9. DIETS POOR IN THE ANTINEURITIC VITAMIN B

Seven rats which had been fed an adequate diet from birth were placed for 38 days on a ration containing only a small quantity of the antineuritic vitamin B. The rats were then inoculated subcutaneously with *Bact. abortus* and kept on this diet for 32 days longer, when they were killed and examined for the organism. The diet was made up as follows:

Diet low in vitamin B	Per cent
Dried beef.....	18.1
Ash mixture.....	4.0
Dried bakers' yeast.....	1.5
Cod-liver oil.....	2.0
Hydrogenated cottonseed oil.....	8.0
Cassava starch.....	66.4

⁵ Ration described under experiment 2.

With the exception of yeast, the constituents of this ration were practically free from vitamin B, the beef having been heated to destroy any of this vitamin normally present. The proportion of yeast in the ration furnished considerably less vitamin B than is required for mature rats, as is indicated by the fact that the rats lost an average of 29 per cent in weight on this diet. After inoculation with *Bact. abortus* the rats were fed this diet for an additional period of 32 days, or a total of 70 days. Most of the animals were in poor condition at the end of this period. *Bact. abortus* was isolated from the spleens of five of the seven rats, the average number of colonies per tube being four. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 364.

A second group of 10 rats which had previously received an adequate diet was fed for 11 days a ration containing practically no antineuritic vitamin B. This ration was made up as follows:

Diet very poor in vitamin B	Per cent
Dried beef (autoclaved)	19.1
Ash mixture	4.0
Cod-liver oil	2.0
Hydrogenated cottonseed oil	8.0
Cassava starch	66.9

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on this diet for 32 days, or a total of 43 days. Two rats died during the test and the others were in very poor condition at the end, having lost 25 per cent in weight. The rats were killed and examined with the result that *Bact. abortus* was isolated from the spleens of seven of the eight rats, the average number of colonies per tube being four. The cultures from the spleen of the other rat were contaminated. Seven of the rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 339.

A third group of rats which had received an adequate diet from birth was fed the same deficient ration as that fed the preceding group. After 11 days on this diet the rats were inoculated subcutaneously with *Bact. abortus* and were continued on the same diet for 28 days, or a total of 39 days, when they were killed and examined. At this time most of the rats were in very poor condition, having lost an average of 25 per cent in weight during the test. *Bact. abortus* was isolated from the spleens of seven of the eight rats used, the average number of colonies per tube being 30. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 331.

As a control, a fourth group of nine rats which had previously received an adequate diet was inoculated subcutaneously with *Bact. abortus* at the same time as Groups 1 and 2 and continued on the same diet for 32 days. The rats were then killed and examined, with the result that *Bact. abortus* was isolated from the spleens of seven of the nine rats, the average number of colonies per tube being two. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 200 to 1 to 1,000, the average being 1 to 567.

These tests indicate that a deficiency of the antineuritic vitamin B had no significant effect on the resistance of the rat to *Bact. abortus*. Cultures from the spleens of the third group of rats fed a ration de-

ficient in vitamin B showed considerably more colonies of *Bact. abortus* than cultures from two other groups of rats fed a similar diet, or than cultures from control rats fed an adequate diet. However, this difference may have been due to the fact that the third group of rats was killed on the twenty-eighth day after inoculation, whereas the three other groups were killed on the thirty-second day. In Table 1 it will be seen that the time between inoculation and examination of the rats has an important bearing on the number of colonies of *Bact. abortus* in the spleens. Thus, cultures from rats killed on the twenty-ninth day showed more colonies than cultures from rats killed on the thirty-second day.

EXPERIMENT 10. DIET POOR IN VITAMIN E

Eighteen female rats which had previously received an adequate diet were placed on a diet very deficient in vitamin E for a preliminary period of 105 days. This diet, which has proved to be very inadequate for reproduction, was made up as follows:

Diet poor in vitamin E	Per cent
Casein.....	24.3
Ash mixture.....	4.0
Dried bakers' yeast.....	10.0
Lard.....	8.0
Cod-liver oil.....	2.0
Cassava starch.....	51.7

The rats were then inoculated subcutaneously with *Bact. abortus* and continued on the same diet for 31 days longer, or a total period of 136 days. They were then killed and examined for the organism. *Bact. abortus* was isolated from the spleens of 17 of the 18 rats; cultures from the other rat were contaminated. The average number of colonies per tube was 12. The serum from all rats reacted to the agglutination test in dilutions ranging from 1 to 100 to 1 to 1,000, the average being 1 to 322.

As a control, eight rats which had previously received an adequate diet were inoculated subcutaneously with *Bact. abortus* at the same time as the preceding group. The rats were continued on this diet and 31 days later they were killed and examined, with the following results: *Bact. abortus* was isolated from the spleen of each rat in the control group, the average number of colonies per tube being nine. The serum from each rat reacted to the agglutination test, the dilutions ranging from 1 to 50 to 1 to 500, the average being 1 to 313.

The results of these tests show that the rats fed a diet very poor in vitamin E for 136 days were not materially less resistant to *Bact. abortus* than were control rats fed an adequate diet.

EXPERIMENT 11. DIET POOR IN CALCIUM AND PHOSPHORUS

Fourteen rats which had previously received an adequate diet were fed one very poor in calcium and phosphorus for 73 days. The diet was made up as follows:

Diet low in calcium and phosphorus	Per cent
Graham flour.....	94
Dried beef.....	5
Sodium chloride.....	1

This food mixture contained 0.37 per cent phosphorus but calcium was not determined. According to Sherman,⁶ wheat contains 0.45 per cent calcium, and meat 0.058 gm. calcium per 100 gm. of protein. Since the dried meat in this diet contained 82.7 per cent protein it probably contained approximately 0.048 per cent calcium, or practically the same proportion as was present in the Graham flour. According to McCollum and Simmonds,⁷ wheat is too poor in both calcium and phosphorus to meet the needs of the growing rat. The diet above mentioned contained 15 per cent of a mixture of beef and wheat proteins, which is ample for normal growth in rats. The diet was probably poor in vitamin A.

After the rats had been fed the calcium-phosphorus-deficient diet for 73 days they were inoculated subcutaneously with *Bact. abortus*. The rats were continued on the same diet, and 34 days later, or after a total period of 107 days on this diet, 11 of them were killed and examined for the organism. Five days later the remaining three were killed and examined. The results were as follows: *Bact. abortus* was isolated from the spleens of 7 of the 14, the average number of colonies per tube being eight. The serum from all rats afforded agglutination reactions in dilutions ranging from 1 to 50 to 1 to 1,000, the average being 1 to 525.

As a control, 12 rats, receiving an adequate diet were inoculated subcutaneously with *Bact. abortus* on the same date as the rats fed the calcium-phosphorus-deficient diet. Thirty-nine days later the control rats were killed and examined, with the following results: *Bact. abortus* was isolated from the spleens of 5 of the 12 rats, the average number of colonies per tube being five. All rats afforded agglutination reactions in dilutions ranging from 1 to 25 to 1 to 1,000, the average being 1 to 644.

The results of this experiment indicate that the rats fed a diet poor in calcium and phosphorus for 107 days were not materially less resistant to *Bact. abortus* than were rats fed an adequate diet.

A careful post-mortem examination was performed on all the rats used in these experiments. Definite lesions, such as are commonly found in guinea pigs inoculated with *Bact. abortus*, that is, nodular spleens and livers dotted with necrotic foci, were not observed. Enlargement of the spleen, while not pronounced, seemed to occur frequently in infected rats, as determined by a comparison between the weight of that organ and the live weight of the rat.

SUMMARY OF RESULTS

In this paper are reported the results of feeding experiments with albino rats to determine the effects of certain dietary deficiencies on the resistance of these animals to *Bact. abortus*. Rations deficient in vitamin A, the antineuritic vitamin B, vitamin E, and in calcium and phosphorus, respectively, were without significant effect on the resistance of the rat to this organism.

⁶ SHERMAN, H. C. CHEMISTRY OF FOOD AND NUTRITION. Ed. 3, rewritten and enl., p. 590, 593. New York. 1926.

⁷ MCCOLLUM, E. V., and SIMMONDS, N. THE NEWER KNOWLEDGE OF NUTRITION; THE USE OF FOODS FOR THE PRESERVATION OF VITALITY AND HEALTH. Ed. 3, entirely rewritten, p. 129. New York. 1925.