GROWTH, DEVELOPMENT, AND THE FORMED ELEMENTS OF THE BLOOD OF TWENTY-TWO ALBINO RATS FED VARYING CONCENTRATIONS OF DRY GARLIC POWDER

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by

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THESIS

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INTRODUCTION

Early in the history of bacteriology, certain antagonistic relationships among various microorganisms were recognized. DeBary, ('78), attempting to grow two organisms on one substrate, found that eventually one would inhibit and completely destroy the other. This action became known as antibiosis, meaning "against life". The postulated chemical substance causing the reaction was called an antibiotic. Pasteur first made practical application of these phenomena by using microbial antagonism against the anthrax organism.

In the past decade, renewed emphasis has been placed upon the search for other antibiotic substances which may benefit man. Today, because of popular advertising, the word antibiotic is familiar to the general public. Literally, the word applies to any chemical substance of microbial origin that inhibits growth or metabolic activities of bacteria or other microorganisms. Recently, however, the term has been expanded to include any chemical substance produced by actinomycetes, bacteria, algae, or higher plants which will produce the same results. Many studies to find the higher plants exhibiting such properties have been made. Such widely used foods as onions

(Walker, Lindegren and Bachman, '25), cabbage (Pederson and Fisher, '44), rhubarb (Huddleson, Du Frain, Burrows and Giefel, '44), horseradish (Foter and Goliek, '38), and garlic (Walton, Herbold and Lindegren, '36) have been shown to possess antibiotic properties.

A search of medical literature reveals many instances of the use of garlic in attempting to cure disease. Isolated cases are recorded in medical journals where treatment of whooping cough (Petrocchi, and Gutman, '27), tuberculosis (Minchin, '29), dyspepsia (Roos, '25), flatulence and cholecystitis (J. A. M. A., '34), diabetes (Mahler and Pasterny '24), and arterial hyertension (Sunzeri, '26) with large quantities of garlic was made. Although little of this work has been subjected to controlled medical research, the possibility that the cures claimed may have been influenced by the presence of antibiotic substances in the garlic becomes apparent.

Huddleson and co-workers ('44) suggest possible prevention of infectious disease by ingestion of garlic or the garlic components having antibiotic properties. The adoption of such a suggestion would depend upon the action of large quantities of garlic on the living body. No studies have been recorded of a systematic attempt to determine the effect of large quantities of garlic on animals. This study was conducted to determine any

interference with growth and development which might result from feeding large quantities of garlic powder to albino rats.

Since Thorp and Harshfield ('43) found that in horses a lowered erythrocyte count and anemia resulted from ingestion of large quantitiies of onion, an antibiotic producing plant also of the lily family, studies of the formed elements of the blood were made to ascertain if garlic feeding would so affect albino rats.

Growth, food consumption, and autopsy records were kept to show any difference due to feeding dry garlic powder in the concentration of 2.5 and five percent of the basic diet. Analysis of the moisture content of garlic shows it to be 75 percent water. Therefore, the 2.5 percent dry garlic intake is equivalent to 10 perecnt when calculated on a moist basis. A five percent dry garlic intake would equal a 20 percent intake of green garlic.

LITERATURE

General Properties of Antibiotics

Microorganisms and products of their metabolism have been used for the control of disease in man, animals, and plants with varying degrees of success. As early as 1877, Pasteur was able to repress the production of anthrax by the simultaneous inoculation of the organism Bacillus anthracis. Following this early work considerable attention has been given to the study of bacteria possessing antibiotic properties. The voluminous literature on microbial antagonism which has accumulated since Pasteur's time is excellently reviewed by Waksman in his recent book ('45). The following properties of the various organisms displaying antibiosis, the methods of testing, and the practical applications are taken from various sections of Waksman's book. No attempt was made to gather the original material since the majority of it is recorded in French.

Of historical interest is the first antibiotic, pyocyanase, recognized by Emmerich and Low in 1899. Produced from Ps. aeruginosa, it was found to have a lytic effect upon the diphtheria, streptococci, meningococci, pneumococci and typhoid organisms.

Systematic search has been made to determine which

bacteria, acintomycetes, algae, fungi, and higher plants possess antibiotic properties. Several methods have been developed to show these antagonistic effects. Most methods are based upon the growth of a test organism in the presence of a living antagonist or the antibiotic substance produced by it in a liquid or on a solid nutrient media. Antibiotic action is established by failure of the test organism to grow. The nature of the substance must then be determined and quantitatively measured.

Three general methods for measuring the potency of an antibiotic are in use. One is the agar plate dilution method in which the unknown substance is diluted to various concentrations in liquid agar. The agar, after solidification, is streaked with several test bacteria. The highest dilution at which the test organism fails to grow is recorded as the potency of that antibiotic. In the serial dilution method, definite volumes of the media containing the test organism are placed in test tubes. Various dilutions of the active substance are added. The highest dilution giving complete inhibition of growth, judged by lack of turbidity in the medium, is considered the end point. A third method is the agar diffusion method in which the test organism is inoculated into agar medium, the active agent placed on the agar, and the rate of diffusion as judged by absence

of bacteria around the antibiotic recorded. The rate of diffusion is directly proportional to the concentration of the antibiotic substance.

Antibacterial substances have been classified chemically as lipoids, pigments, polypeptides, sulfur-containing compounds, quinone-like substances, and organic bases. Their mode of action has been studied by Waksman ('43). By far the greater percentage are bacteriostatic, arresting growth but not killing the test organism. Some are bactericidal, bacteriolytic, and even fungicidal. They are variable in their toxic properties. Some, such as pencillin, are relatively non-toxic while the toxicity of others prevents their use in therapeutics until their toxicity is attenuated. Certain antibiotics are highly selective in action, displaying their antibiotic properties on only one test organism; others are active on either gram positive or gram negative organisms.

Antibiotic Properties Of Allium Sativum (Garlic)

Of more immediate interest to this study are the reports of the antibiotic properties of garlic. Ten years ago interest in garlic as a curative agent was renewed when Walton, Herbold, and Lindegren , ('36) published their data of the bactericidal effects of crushed garlic. This was one of the first studies in which garlic was recognized as an antibiotic producing plant. Using a modification of the standard plate exposure method,they exposed crushed

garlic vapors to nutrient glycerine agar for varying periods of time. After treatment with the garlic several bacteria were streaked upon the plates. Complete arrest of Bacillus subtilis on the plates exposed to garlic vapors for four hours was observed. Further work with the active component showed it to be bactericidal in action. Its volatility was less marked at 10°C than at 22°. The heated garlic showed no bactericidal action. That which was water extracted and then heated showed no or only slight activity.

Huddleson et al. ('44), reporting the results of serial dilution tests on garlic, found inhibition of growth in 1:40 to 1:160 dilution of the fresh juice. They found that the volatile substance was given off over a long period of time. Preliminary chemical analysis indicated that the active substance was not an aldehyde or a carbohydrate.

Vollrath, Walton, and Lindegren ('37) tested the effects of substances present in garlic which might cause the antibiotic effects noted. Allyl polysulfides, compounds giving garlic its characteristic odor, proved of negligible bactericidal activity. Aldehydes in garlic such as acrolein (allyl aldehyde) were found to be powerfully bactericidal. Further study of acrolein showed it to be the only compound present in garlic with antibiotic activity comparable to that of garlic. The results of its ingestion by man are dizziness, nausea, and diarrhea. Similar symptoms were observed after eating several ounces of garlic. Proof of the presence of acrolein in garlic was attributed to a specific

color reaction.

Ingersoll, Vollrath, Scott and Lindegren ('38) tested the bactericidal properties of crotonaldehyde, a substance suggested as the antibiotic component of crushed garlic. They found inhibition of Escherichia coli in dilution of 1:10,000 when the plates were exposed to garlic, onion, or the chemically pure aldehyde. Water soaked garlic was shown to loose its bactericidal properties. The active principle gave a characteristic test for unsaturated aldehydes. When heated for distillation purposes, the garlic did not yield this test, probably because the proteins combined with the aldehydes before distilling off.

Huddleson and co-workers ('44), however, feel that crotonldehyde is not the antibiotic substance in garlic. The chemically pure crotonaldehyde which they tested had antibiotic activity in dilutions up to 1: 4,000. Their garlic extracts inhibited growth of the same organism in 1: 320,000 to 1: 640,000 dilutions. This wide difference in potency would indicate that some substance more active than crotonaldehyde is responsible for the antibiotic action of garlic. In more recent work, Jacobs ('44) has proposed the active principle to be allylpropyl disulfide plus a tri-sulfur compound.

At present, the published literature has shown these properties to be a part of the antibiotic activity of garlic. The active substance is:

1. Bactericidal in nature
2. Volatile, having its greatest activity between 10° and 22° C.
3. Active over a long period of time
4. Destroyed or attenuated by water extraction
5. Sulfur containing
6. Most active on fungi
7. Destroyed by heat

Work is now being conducted at Michigan State College to ascertain the active principles of garlic, to isolate the substances, and to show their effect upon organisms and higher life. Unpublished data from this study indicates that water extracts of the garlic bulb retain their antibiotic properties. Garlic, heated for distillation purposes under partial vacumm, also retains its bactericidal properties.

Use of Garlic in Treatment of Disease

Claims of therapeutic properties of the garlic bud have persisted from the time of folklore medicine. Periodically, the use of garlic in treatment of common physical disorders has been renewed.

Summary of the early literature in which mention is made of the use of garlic for medicinal purposes was made by del Valle Atiles ('20). He quotes the work of Minchin in which a volatile oil made from fresh garlic was used successfully to cure typhoid fever,

tuberculosis, and infantile diarrhea. The oil was said to be antiseptic in nature and capable of penetrating epithelial tissue. A similar oil preparation made from garlic was used successfully on 96 patients suffering from diarrhea and dyspepsia by Roos ('25).

An infusion of crushed garlic and milk was credited by del Valle Atiles ('20) as being an effective vermicide. Rico ('26) showed that alcohol extracts of garlic were effective in killing g. Ascaris, a species of worm including intestinal threadworms and similar parasites. An invitro study of the effects of a 10 percent extract of garlic upon these worms showed that the garlic caused a period of intense irritation in the parasite which was followed by paralysis and death in less than an hour.

Diabetic patients, fed 10 grams raw garlic daily by Mahler and Pasterny ('24) reacted with a decrease in glycosuria and an increase in hyperglycemia. Undesirable effects were noted in two cases when the patients became refractory to insulin after two days of garlic treatment.

The possibility that garlic extracts may be toxic is reported by Petrocchi and Gutman ('27) who fed two children, ill with whooping cough, large quantities of garlic. After 24 hours, one child displayed a greatly increased blood pressure, tachycardia, mydriasis, and myosis followed by death. Less severe toxic reactions were experienced by the second child.

On the other hand, garlic has often been

recommended for the treatment of arterial hypertension because of its reported dilating action on the blood vessels. Several attempts have been made to determine the effects of garlic upon the circulatory system. Sunzeri ('26), studying the pharmacology of garlic, injected aqueous and alcoholic extracts of garlic into dogs and observed an immediate drop in the blood pressure due to the stimulation of the vagus nerve. Later, the blood pressure was lowered by the dilating action of garlic upon the blood vessels. He suggested the use of garlic in treating hypertension.

Several studies have since been recorded in which this suggestion has been subjected to controlled medical research. Wakerlin and Gaines ('40) studied plants and hormones said to be of value in the treatment of hypertension. They administered two grams desiccated garlic orally to a dog made hypertensive by bilateral constriction of the renal arteries. No change in the blood picture was recorded.

A commercial preparation of garlic was fed to dogs in doses three times the recommended human allowance by Goldblatt, Kahn and Lewis ('42). No toxic effects were noted nor was there any change in the hypertensive level.

Such contradictory studies should be investigated further using sufficient animals to rule out individual variation in reaction before any claims for therapeutic values of garlic are made.

The Formed Elements of the Blood and the Hemoglobin Concentrations of Albino Rats As Recorded in the Literature

Thorp and Harshfield ('43) have shown that anemia results in horses due to large intakes of onion, an antibiotic producing plant of the lily family. Sebrell ('30) found that onions fed to dogs in quantities of 15 grams or over per kilogram of body weight caused severe anemia. Schlotthauer and Berryman ('43) fed dogs 90 grams fresh onion daily and observed no effect on the hemoglobin concentration. When the amount fed was increased to 150 grams per day, severe anemia followed by death occurred in two animals. Several dogs withstood 200 grams onion per day but their erythrocyte counts were reduced from 5.2 to 3.7 million cells per cmm. of blood. Garlic is also a member of the lily family producing an antibiotic substance. No studies of the formed elements and hemoglobin concentrations of the blood of animals fed garlic are reported. The possibility that garlic may affect the blood in a way similar to onion can not be ignored. This study of the formed elements and the hemoglobin concentrations of the blood of rats fed garlic powder was made to show any depressive action of garlic upon these components of the blood of the animals.

A. Erythrocyte counts. The formed elements of the blood of normal albino rats have been the subject of numerous investigations. Chisholm ('11) studied 50 rats weighing from

50 to 250 grams and reported an average erythrocyte count of 8.8 million cells per cmm. of blood. The red blood cell count rose from 6.3 to 7.6 million cells per cmm. for animals weighing from 25 to 50 grams to 8.4 to 9.0 for those whose weights were 200 to 250 grams.

Levy ('26) reports 10.5 million red cells as the average of the 18 animals studied. Vaughan and Gunn ('29), using standardized pipettes and counting chambers, found the average erythrocyte count of four month old rats to be 10.2 million cells per cmm. of blood while the counts of the six month old animals averaged 9.6. Kolmer and Boerner ('31) report an average of 9.3 million cells per cmm. of blood.

A comparison of the red blood cell counts of one group of animals fed an all-grain stock diet and a second group fed an adequate synthetic diet was made by Orten and Smith ('34). Using only male animals to rule out the fluctuation caused by pregnancy, they found no essential difference in the erythrocyte counts of the two groups of animals. At 200 grams, the animals had an average erythrocyte count of 7.0 and 7.9 million cells per cmm. of blood for those fed the stock and synthetic diet respectively.

Possible differences in the erythrocyte counts of different strains were investigated by Drabkin and Fitz-Hugh ('34). Wistar and Pennsylvania strains of rats were selected for the study. No significant strain difference

was observed. The average number of erythrocytes in the rats weighing 200 grams was 8.8 million cells per cmm. of blood.

Reich and Dunning ('43) have reported the most extensive study of the blood of eight species of rats. Rigidly controlled standard blood counting procedures were followed. The average red blood cell count for the 3,000 rats they examined was 8.7 million cells per cmm. of blood. They observed small but significant differences between the various strains with each exhibiting a characteristic peripheral blood picture. Unfortunately, the Wistar Strain was not among those studied.

B. Hemoglobin concentrations. Chisholm ('11) studied the hemoglobin concentration of the blood of 50 rats and reported an average of 88 percent. Levy ('26) obtained 94.3 percent as the average of 68 determinations while Goodall ('10) reports 110 percent. A comparison of these results is impossible because in no case are the number of grams of hemoglobin represented by 100 percent given. More recently, the hemoglobin concentrations of the blood have been reported as grams per 100 ml. of blood. Drabkin and Fitz-Hugh ('34) found a range of hemoglobin concentration in rats of 17.0 to 20.0 grams per 100 ml. of blood. The hemoglobin concentrations reported by Orten and Smith ('34)

for the animals fed the stock diet and synthetic diet ranged from 13.7 to 16.0 grams per 100 ml. blood. There were no differences in the dietary groups. Thewlis and Meyer ('42) found the hemoglobin concentration of the animals weighing 125 to 200 grams to be 14.1 grams per ml. of blood; while that of animals weighing 201 to 450 grams averaged 13.8. Reich and Dunning ('43) report a hemoglobin concentration of 14.2 as the average of the 3,000 animals studied.

C. **Leucocyte counts.** Total leucocyte counts are reported by Thewlis and Meyer ('42) as 18,819 cells per cmm. of blood; by Goodall ('10) as 10,600; by Stammer ('26) as 9,800; and by Kolmer and Boerner ('31) as 15,200 cells per cmm. of blood. Lotta and Hemderson ('37) present the average leucocyte count of the blood of rats as 18,000. Reich and Dunning ('43), averaging the values found in the literature, report a range of 8,000 to 15,000 cells per cmm. of blood. In their own extensive study, the average leucocyte count was 15,800 cells per cmm. of blood. The results of the studies cited from the literature are shown on Table 1.

The average differential white count recorded by Reich and Dunning ('43) was: neutrophils, 27 percent (range 15 to 40 percent); lymphocytes, 67 percent (range 50 to 80 percent); monocytes, five percent (range two to seven percent); eosinophils, two percent (range zero to four percent); and basophils, 0.7 percent (range zero to 1.5 percent).

Growth Studies

Few laboratories have reported the weights of albino rats over the entire growth period. Brody ('42) records the growth of suckling rats of the Wistar Strain in normal size litters of seven to nine young and in litters with the young reduced to two or four. The average weight at 21 days of age when the animals were weaned was 28 grams and 47.3 grams for animals of the large and the small litters respectively. The author points out that the difference is undoubtedly due to the more abundant milk supply available to the young of the smaller litters.

Orten and Smith ('34) found the weight of rats at 21 days of age to be 42 grams. At 30 days, the young rats fed an all grain diet averaged 66 grams. At 120 days, approximately the time of the first mating in this experiment, the animals averaged 390 grams. Animals in their laboratory fed a synthetic diet, were found to be slightly lighter, weighing only 343 grams at 120 days of age.

Drabkin and Fitz-Hugh ('34) report the weights of Wistar rats fed a diet of natural foodstuffs. The average weight of the animals at 30 days of age was 51 grams; at 120 days it was 258 grams.

Because of the great variation in animal growth due to differences in diet, climate, temperature, and strain variation, values reported in the literature are less valuable

TABLE 1

Erythrocyte and leucocyte counts and hemoglobin concentrations of albino rats as reported in literature

Investigator	Erythrocyte Count Million cells per cmm. blood	Leucocyte Count Cells per cmm. blood	Hemoglobin Concentration Grams per 100 ml. blood	Comments
Chisholm, '11	8.8		87.8%	27 male, 23 females 50 to 150 gram weight
Levy, '26	10.5	25,400		
Vaughan and Gunn, '29	10.2 9.6			Four month old rats Six month old rats
Kolmer and Boerner, '31	9.3	15,200		
Orten and Smith, '34	7.0 7.9		13.7 to 16.0	200 gram weight; fed all grain stock diet. 200 gram weight; fed synthetic diet.
Drabkin and Fitz-Hugh '34	8.8		17.0 to 20.0	200 gram animals
Reich and Dunning '44	8.7	15,800	14.2	Average of 3,000 animals
Goodall, '10	8.10	10,600	110 %	
Thewlis and Meyer, '42	7.63 8.55	23,075 18,819	14.1 13.8	125 to 200 gram weight 201 to 450 gram weight
Stammer, '26		9,800		
Lotta and Henderson, '37		18,000		

than the actual growth of the experimental animals compared with the growth of the stock colony animals from which they were derived. Such a comparison will be discussed in another portion of this paper.

EXPERIMENTAL PROCEDURE

Plan of Feeding Animals

Eleven male and eleven female rats of the Wistar Strain reared from stock colony animals in the Foods and Nutrition Department, Michigan State College, were used for the experiment. The animals, selected at 28 days of age, were fed a modification of the Evans diet (Evans and Burr, '28) shown in Table 2. Three different feeding regimes were planned. One was a control diet containing no garlic powder; the other two were diets containing 2.5 and five percent dry garlic powder, respectively.[1] Four male and four female rats were placed upon the diet at weaning. A like number were fed the diet containing the 2.5 percent concentration of garlic. Due to a lack of animals of the necessary age, only three male and three female animals were fed garlic at the five percent concentration. The grouping of the animals for the experiment is shown in Table 3.

The diet was mixed weekly with the exception of the garlic powder and refrigerated until used. The garlic was added just prior to feeding in the concentration of five and 2.5 percent, replacing the cornstarch from the control diet. This represents a concentration 20 and 10 percent fresh garlic. At the beginning of the experiment, the animals were

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1. The product was Dehyrated Garlic, manufactured by the Basic Vegetable Products Company, Vacaville, California. Acknowledgment is made to Dr. Lucas of the Horticulture Department, Michigan State College, for testing the antibiotic potency of the diet.

TABLE 2

Experimental diets

Ingredient	5 percent Garlic	2.5 percent Garlic	Control No Garlic
	Grams	Grams	Grams
Cornstarch	59.0	61.5	64.0
Casein	18.0	18.0	18.0
Dried-brewer's yeast	8.0	8.0	8.0
Osborne-Mendel Salts	4.0	4.0	4.0
Wesson Oil	5.0	5.0	5.0
Cod Liver Oil	1.0	1.0	1.0
Garlic Powder	5.0	2.5	0.0
	100.0	100.0	100.0

TABLE 3

Grouping of experimental animals according to litter number.*

Experimental Diet 1		Experimental Diet 2		Experimental Diet 3	
5% Garlic		2.5% Garlic		No Garlic	
Female	Male	Female	Male	Female	Male
1	1	1	1	1	1
2	2	2	2	2	2
3	3	3	3	3	3
		4	4	4	4

* All the animals designated by the same number were litter mates.

fed daily. Later, assay indicated that the antibiotic potency of the garlic diets did not change over a 48 hour period. Thereafter, animals were fed every other day. Further assay both of the complete diet and the individual dietary components showed no antibiotic potency in the basic ration. Therefore, any antibiotic action or variation of the experimental groups of animals from the control may be attributed to the effect of the garlic powder used in the diet.

Animal Grouping

Litter control was maintained by placing a male and female from each litter upon each of the three diets. Thus all the animals designated in Table 3 by the number one (1) were litter mates. The rats were caged separately on wire racks to enable the accurate collection of food consumption records. Weighed amounts of food were offered and the amount not consumed by the next feeding plus any spilled was subtracted from that fed to ascertain the food actually consumed.

Weight Records

Weekly weights were recorded before feeding to prevent any change in weight due to the recent ingestion of food.

Mating of Animals

The animals were first mated when three months of age following the brother-sister mating technique. A male was placed with its sister female of the same dietary group for ten days. The females were placed on clean wood shavings when the litters were expected. The number of young was reduced to six or seven, two days after birth, and weaned when the smallest animal weighed 40 grams. The animals were mated for the second time 10 weeks after the first mating. The young of the first litters were discarded. Two male and two female animals from each of the second litters are being kept upon the respective diets to determine any long time effects due to the feeding of dry garlic powder.

Formed Elements of the Blood.

Examination of the blood was begun when the animals had been on the diets four weeks. Thereafter, a study of the formed elements of the blood was made on each animal approximately every 30 days until a total of six studies had been completed. Blood from a tail clip was collected into paraffin cups containing a few crystals of dry heparin to prevent coagulation. Duplicate blood samples were taken in red and white Trenner pipettes

certified by the U. S. Bureau of Standards. The blood for the erythrocyte count was diluted 1: 200 with Hayem's solution. The pipettes were shaken for three minutes to insure even distribution of cells, a few drops expelled, and the two chambers of the Neubauer Improved Hemocytometer with U. S. Bureau of Standard certification, filled. Chambers which filled rapidly, without overflowing or air bubbles, were inspected for even distribution of cells. If the cells were unevenly distributed, the chamber was not counted. If the chamber was accepted for counting, the results were recorded regardless of differences in successive counts. The cells in 80 small squares were counted; the average of the four chambers was recorded as the erythrocyte count.

Blood for the leucocyte count was diluted 1:10 in a solution of acetic acid and methylene blue. The cells in the 400 small squares of each chamber was counted, totaled, and divided by two. The average of two such counts was recorded as the leucocyte count.

The method of Sheard and Sanford ('29) for the estimation of the hemoglobin concentration of the blood, as adapted for use in the photo-electric colorimeter, was followed. Two-tenths of a cubic milliliter of blood was blown into 20 ml. of a 0.1 percent solution of sodium carbonate and allowed to stand at least ten minutes to insure complete laking of the blood and color development. The solution was read in a Cenco Photolometer against distilled water set at a reading of 100, using a green filter with

maximum transmission at 525 μμ. Conversion from the reading to hemoglobin concentration was made by referring to the chart supplied by the manufacturer of this particular instrument. The hemoglobin concentration for each galvanometer reading had been determined previously by the oxygen capacity method.

Duplicate blood smears were made upon the glass slides, quick dried, and stained with Wright's stain. The percentage of lymphocytes, monocytes, neutrophils, eosinophils, and basophils in 300 consecutive cells was determined.

Autopsy

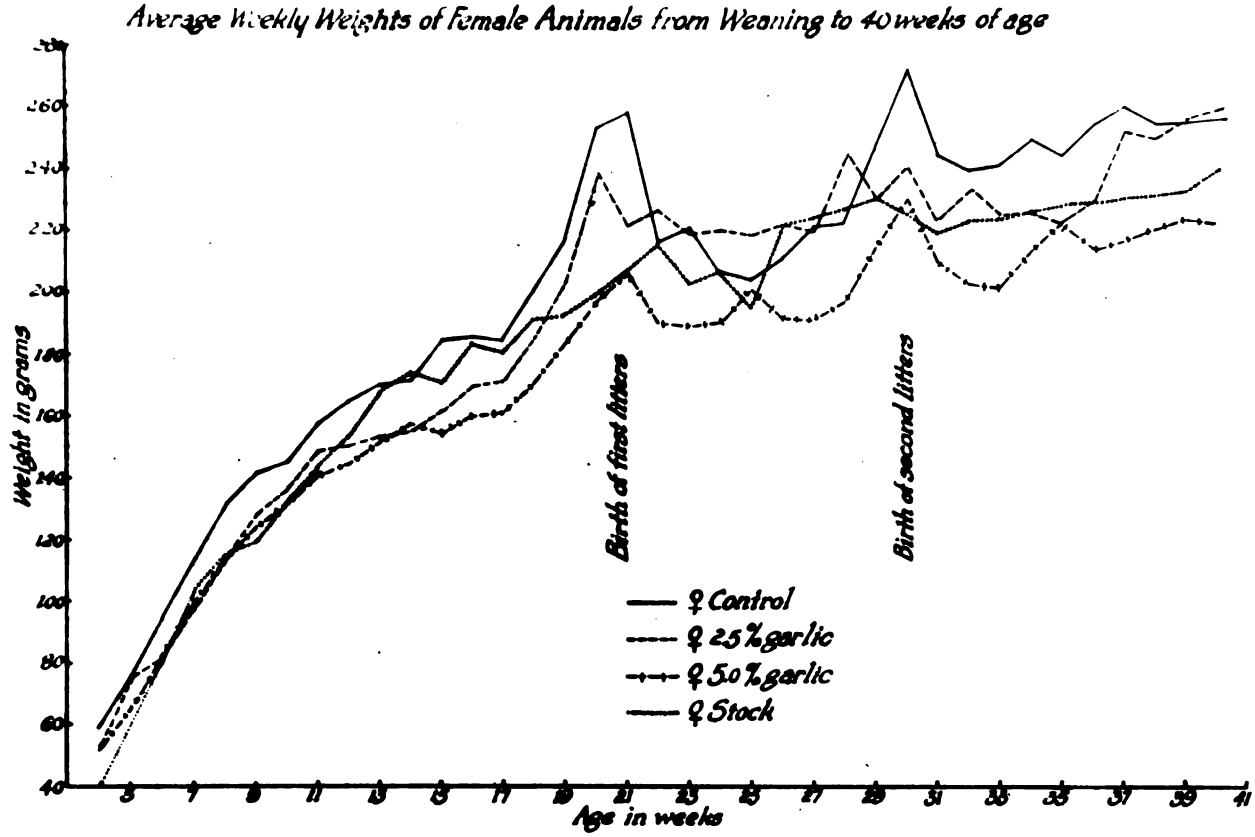
Gross autopsies were conducted upon the chloroformed animals at the end of the experimental period. Particular attention was paid to possible lung infection since previous work with the stock colony had shown a high incidence of lung lesions to be present. All abnormal tissues were saved for histological study, the results of which will be reported by another worker from this laboratory.

RESULTS AND DISCUSSION

This work is a part of a long time study of the effects of antibiotic substances when fed in large quantities to experimental animals. The experimental procedure is being repeated upon another series of rats by another investigator so that when the data are pooled the experiment will represent the action of garlic on five male and five female animals per diet group. No definite conclusions can be drawn from this study until the data from the second series can be examined. The results here reported were those shown by the first group of animals.

Growth and Development

The average weekly weights of the male and female animals maintained upon the three diets are shown plotted on Figure 1. The weights at weaning of the animals used in this study were comparable to those reported by Orten ('36) and Drabkin ('34). However, the experimental animals did not gain the weights following weaning which were reported by these authors as normal for their colonies. Since the failure may be due to dietary or species differences in the growth patterns of the two groups of animals, some other criteria for judging the normalcy of weight gains during the growth period should be adopted. A comparison of the weights of the


Average Weekly Weights of Female Animals from Weaning to 40 weeks of age
Weight in grams
Age in weeks
Birth of first litters
Birth of second litters
♀ Control
♀ 2.5% garlic
♀ 5.0% garlic
♀ Stock

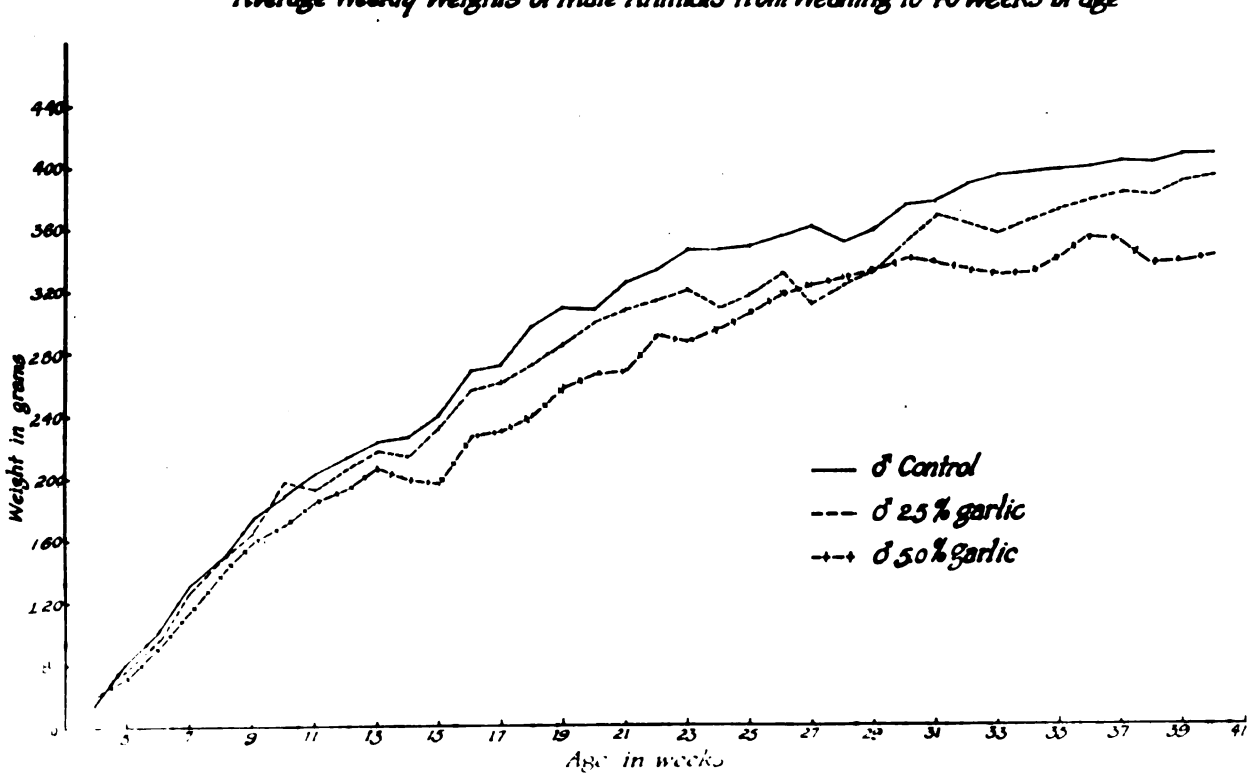

Average Weekly Weights of Male Animals from Weaning to 40 weeks of age
Weight in grams
Age in weeks
♂ Control
♂ 2.5% garlic
♂ 5.0% garlic

Figure 1

experimental animals with those of equal age from the stock colony from which they had been bred is a more satisfactory way to judge growth. Therefore, the average weights of the female stock colony animals of the same age are plotted with those of the experimental groups. Similar weight records were not available for the stock colony males. The average weekly weight of the stock colony and experimental female animals were comparable. The general growth pattern of the stock animals was maintained by those fed the simplified dietary. Therefore, the experimental diet is as adequate as the stock diet in promoting good growth. A study of the weight graphs alone would lead to the assumption that the simplified control diet sustained better growth than did the 2.5 or five percent garlic diets. The animals made greater gains in weight when fed garlic at the 2.5 percent concentration than when fed garlic at the five percent concentration. Some depression in growth might be attributed to the presence of garlic in the basic diet.

However, when the weight gains were calculated as a gain per gram of food intake (Table 4), it became apparent that there was no actual depression of growth due to the feeding of garlic. The lower weights of the animals fed the ration containing five percent garlic may be attributed to a smaller intake of food since the weight gain per gram of food was essentially the same as that made by the heavier control animals. The weight gain per gram of food consumed

was .058, .067, and .057 grams for the female rats fed five percent garlic, 2.5 percent garlic, and no garlic respectively. Male rats gained .095,.106 and .100 gram per gram of food on the same three diets. No explanation of the slightly higher gain per gram of food ingested, made by both the male and the female animals fed 2.5 percent garlic, is known at present. It is possible that garlic in some way influences the economy with which the animals utilize their food.

It is interesting to note that the male animals gained almost twice the weight per gram of food consumed as did the female animals. Autopsy records showed that this extra gain was not reflected in abnormal fat deposits.

All the females bore first litters well within the time interval for normal gestation. Only one female failed to produce a second litter. When she was remated to another male of the same dietary group but not the same litter, impregnation resulted. The male with whom she had first been mated later failed to impregnate another fertile female. Some loss of fertility must have occurred in the male between the two matings. No external signs of physical difficulty were observed nor were any gross abnormalities seen at autopsy. However, the study of the red blood cell system of this particular animal at the time he was mated to the experimental female, showed an extremely low erythrocyte count and hemoglobin concentration which could

TABLE 4

Average weight gains per gram food consumed

Diet	Average total weight gain	Average grams food consumed	Average weight gain per gram food consumed
5% Garlic			
Female	162	2,820	.058
Male	285	2,990	.095
2.5% Garlic			
Female	197	3,005	.067
Male	326	3,091	.106
Control			
Female	192	3,335	.057
Male	347	3,463	.100

not be attributed to poor technique. The lower than average erythrocyte and hemoglobin concentrations may have resulted in sterility.

Only four female rats nursed their first litters. From 59 to 98 percent of the young were lost. This lactation failure was traced to a manganese deficiency in the mineral salts used in the diet. Sufficient manganese had been supplied to the animals for the first two months after weaning. During the third, fourth, and fifth months a salt mixture deficient in manganese was inadvertently used. The manganese content of the salts was only one tenth that recommended by Griffith ('42) as necessary for normal lactation. Manganese deficiencies in rats are characterized by normal pregnancies but failure of the females to nurse their young followed by a high degree of cannibalism. These symptoms were those maifested by the experimental animals. The diet was immediately corrected by the addition of manganese sulfate. Shils and McCollum ('43), studying manganese deficiency in rats, reported that addition of manganese to the diets of deficient animals resulted in successful mating, lactation, and rearing of young. They concluded that manganese deficiencies are readily corrected by the addition of sufficient amounts of manganese in the diet. In the present study, the animals, supplied with manganese during a second gestation period, were able to wean 38 percent of the young of the control group. Seventy-

five percent of the young from females fed 2.5 percent garlic were weaned while only 47 percent of the offspring receiving five percent garlic survived. Four of the eleven females failed to nurse the young. It is interesting to note that the females fed the 2.5 percent garlic diet were able to nurse and wean a greater percentage of young than those fed the other two diets. One female of the control group bore twelve young but failed to rear them. This may account for the low percentage of the young of the control animals weaned.

Twenty of the animals were sacrificed after an experimental period of 36 weeks. The other two were kept for further study. Careful macroscopic autopsies were conducted upon the chloroformed animals to determine if any variation due to the feeding of garlic powder in 2.5 and five percent concentrations occurred. Because of the chronic lung infection in the stock colony animals, particular attention was paid to determine the extent of lung lesions present in the experimental animals. The lesions were characterized by dark spots both on the surface and throughout the lung lobes which sometimes were full of pus or serous fluid, sometimes apparently calcified. All degrees of infection were noted in the experimental animals from a very minimal amount involving only one lobe to an amount involving every lobe and apparently rendering most of the lung tissue incapable of action. Respiratory embarrassment

characterized by wheezing and difficulty in breathing had been observed in the stock colony animals. These animals were seen to have marked lung abnormalities when they were autopsied. Respiratory difficulty was noticed in only two experimental animals. One was a female fed the five percent concentration of garlic; the other a male maintained on the control diet. Their lungs were found upon examination to be infected to a moderate degree. Two animals of the six fed five percent concentration of garlic were infected to a moderate degree. One was not killed. This represents 40 percent moderate lung infection. Of the eight animals fed the 2.5 concentration of garlic, two were infected to a marked degree, one was not killed, and five were free of lung abnormalities. Only 29 percent of the animals fed 2.5 percent garlic were infected but the infection was more severe than that of the animals fed five percent garlic. Autopsy of the animals fed no garlic showed five animals with extreme lung abnormalities. This represents 63 percent extensive infection. Huddleson and others ('44) have suggested that ingestion of antibiotic substances may have practical use in preventing infectious disease. The feeding of the antibiotic substance in garlic did not prevent lung infection. Any protective effect of garlic would seem to be one of degree with fewer animals fed garlic having lesions and the lesions being less extensive.

The Formed Elements of the Blood

Wintrobe ('44) studied the hematological system of the rat and found that the formed elements of the blood reached adult values by the end of the second month. Since the erythrocyte and leucocyte counts and the hemoglobin concentrations reported in this study were first made when the animals were three months of age, adult values may be assumed. Melcoff ('44) has reported that three weeks are ample time for both hemoglobin and red blood cell regeneration after the moderate bleeding necessary to secure the blood for study. Blood was drawn monthly in this study.

The average monthly erythrocyte and leucocyte counts and the hemoglobin concentrations of the various groups of female animals are presented in Table 5, the male values are shown in Table 6.

Erythrocyte counts

The range of red blood cells of the female animals fed the higher concentrations of garlic was 6.18 to 8.98 million cells per cmm. of blood, those fed the lower amount of garlic ranged from 6.92 to 8.89, while the control animals ranged from 7.59 to 9.20 million cells per cmm. of blood. The total range of the male animals was much smaller than that of the female. As was to be expected, higher red blood cell counts were recorded for the male animals. The range of counts was 8.20 to 9.10, 8.54 to 9.44, and 8.53 to

TABLE 5

Average monthly erythrocyte counts, leucocyte counts, and hemoglobin concentrations of eleven female albino rats fed varying concentrations of dry garlic powder.

Diet	Month	Erythrocyte Counts	Leucocyte Counts	Hemoglobin Concentrations
		Million cells per cmm. blood	Cells per cmm. blood	Grams per 100 ml. blood.
5% Garlic	August	7.81	7,067	13.9
	September	7.92	7,550	12.8
	Oct.-Nov.*	6.18	7,975	11.8
	December	8.89	9,858	13.6
	January *	7.42	9,158	13.6
	February	8.54	9,658	15.5
2.5% Garlic	August	8.13	10,925	12.6
	September	8.50	7,413	16.0
	Oct.-Nov.*	6.92	10,369	12.1
	December	8.89	8,450	15.4
	January*	7.99	11,812	14.0
	February	7.82	10,505	15.2
Control No Garlic	August	8.80	7,775	16.3
	September	9.21	6,625	17.0
	Oct.-Nov.*	7.77	7,931	12.5
	December	9.28	9,456	15.8
	January*	7.59	9,981	14.1
	February	8.80	9,667	17.3

* Blood examined as near partuition as possible

TABLE 6

Average monthly erythrocyte counts, leucocyte counts and hemoglobin concentrations of eleven male albino rats fed varying concentrations of dry garlic powder.

Diet	Month	Erythrocyte Counts	Leucocyte Counts	Hemoglobin Concentrations
		Million cells per cmm. blood	Cells per cmm. blood	Grams per 100 ml. blood.
5% Garlic	August	8.20	8,142	14.3
	September	8.42	6,392	14.3
	Oct.-Nov.	8.76	7,700	14.5
	December	8.97	7,892	14.3
	January	9.10	7,850	15.2
	February	8.52	8,885	14.8
2.5% Garlic	August	8.66	6,150	15.5
	September	8.54	6,350	14.8
	Oct.-Nov.	8.90	6,798	15.7
	December	9.90	8,786	16.4
	January	9.31	8,188	16.1
	February	9.44	10,563	15.7
Control No Garlic	August	8.53	8,175	17.2
	September	8.88	7,013	15.2
	Oct.-Nov.	9.33	8,388	15.7
	December	9.13	10,094	15.3
	January	9.01	10,606	15.5
	February	8.82	10,423	15.6

9.13 million cells per cmm. of blood for the animals fed five percent, 2.5 percent, and no garlic respectively. The total range of counts previously reported in the literature was 6.30 to 10.5 million cells per cmm. of blood. This close agreement with the values recorded in the literature would seem to indicate that feeding garlic in the concentrations used in this study has little effect upon the total red blood cell count.

However, when the data were subjected to an analysis of variance in which the interaction of the diets and months was considered, a highly significant difference was found between the erythrocyte counts of the female rats fed five percent garlic and the control animals ("t" = 4.16 with 40 D. F.). A significant difference occurred between those fed 2.5 percent garlic and the control group ("t" = 2.9 with 46 D.F.). No difference in the red blood cell count was found between the animals on the five and 2.5 percent garlic diets ("t" = 1.42 with 40 D.F.). The lower red blood cell counts exhibited by the animals fed garlic show a small depression of the total circulating erythrocytes associated with the ingestion of garlic.

Male animals show no significant change in the erythrocyte count between the three diet groups. The "t" test between the groups ingesting five and 2.5 percent concentrations of garlic was 1.8 with 40 D.F., between animals on five percent garlic and control diets, was 1.36 with 40 D.F. and between the groups on 2.5 percent garlic

and the control diets, was 0.4 with 46 D. F.

Further analyses of the erythrocyte counts of the female and the male rats were made to determine between which months and which feeding regimes significant variation could be noted. Significant differences in the red cell counts were observed between the female animals fed the five percent garlic and the control animals for the first three months. The erythrocyte counts of the animals fed 2.5 percent dry garlic were significantly lower than those of the control animals for the first and third months. A difference was noted between the animals eating the five and 2.5 percent garlic diets during the third month. Failure to display a variation in later months may be due to animal adjustment to the diet fed. There was no apparent reason for the lack of variation in the second month for those fed 2.5 percent garlic and those fed control diets. The significant variation between the animals ingesting the two garlic diets, observed in the third month, may have been caused by a depressive action upon the red cell system of a larger total intake of garlic due to increased food consumption concurrent with pregnancy. The possiblity of interaction with the effects of the manganese deficiency should not be overlooked.

At no time was there a significant difference in the erythrocyte counts of the three groups of males. The "t" values for monthly comparisons for both the male and female animals are shown in Table 7.

TABLE 7

Differences in the monthly erythrocyte counts of the male and female animals as shown by the "t" test.

"t" Tests

Date	Between 5% Garlic and Control Diets		Between 5% and 2.5% Garlic Diets		Between 2.5% Garlic and Control Diets	
	Female	Male	Female	Male	Female	Male
August	4.95**	.91	1.40	1.14	3.46*	.22
September	3.16*	.56	1.46	1.41	1.70	.42
October-November	6.07**	.78	3.43*	.156	2.60*	.47
December	1.70	.30	0.0	1.01	1.70	.76
January	.67	.16	1.01	.33	.33	.50
February	.48	1.23	1.68	1.16	2.16	.53

* One possibility in 20 that results could be attributed to chance.

** One possibility in 100 that results could be attributed to chance.

$"t"_{.05}$ = 2.365 with D.F.

$"t"_{.01}$ = 3.499 with D.F.

B. Hemoglobin concentrations. The average hemoglobin concentrations are presented in Tables 5 and 6. The range of hemoglobin concentrations of the female rats was 11.8 to 15.5 grams per 100 ml. of blood for those fed five percent garlic, 12.1 to 15.2 for those fed 2.5 percent garlic, and 12.5 to 17.3 for the control animals. As with the red cell counts, the male animals showed less variation in total range. The ranges of hemoglobin concentrations of the male animals were 14.3 to 15.2, 14.8 to 16.4, and 15.2 to 17.2 for the five percent garlic, 2.5 percent garlic and the control diet groups respectively.

The lower range of the hemoglobin concentrations of the female animals is below that cited from the literature (13.7 to 20 grams per 100 ml. of blood). In most cases, values below 13 grams were found in studies made just after partuition when great drains had been made upon the blood supply of the animals. Discounting these pregnancy changes the hemoglobin concentrations of the female animals are within average ranges reported in the literature. Male hemoglobin concentrations were well within the range of average values reported in the literature.

A statistical analysis of the hemoglobin concentration of the blood of the female animals showed a significant difference in the concentrations between the animals fed the five percent garlic and no garlic ("t" = 3.59 with 40 D.F.) while no significant difference in

hemoglobin concentration occurred between the animals fed 2.5 percent garlic and no garlic ("t" = 1.72 with 46 D.F) or the animals fed the five and 2.5 percent garlic diets ("t" = 2.01 with D.F.).

Analysis of the monthly concentrations showed a significant variation between the hemoglobin concentrations of the animals fed five percent garlic and control animals for the first and fourth months. No other significant differences were noted. These values are recorded in Table 8. The lower concentration during the first month may be attributed to some difficulty in adjusting to the garlic in the diet but no reason is apparent for the change seen in the fourth month. The blood study of the fourth month was done approximately 30 days after the birth of the first litters which should allow ample time for regeneration of the blood. Since few females nursed their young, depletion of the hemoglobin forming materials due to lactation cannot be assumed.

Ohlson et al ('44) showed the red blood cell count to be the least changing and therefore probably the most accurate measure of the normalcy of the red blood cell system. Since no significant differences were noted in the erythrocyte counts of the male animals and since inspection of the hemoglobin concentrations showed homogenity of data, statistical analysis did not appear warranted.

TABLE 8

Differences in the monthly hemoglobin concentrations of the female animals as shown by the "t" test.

Date	"t" Tests		
	Between 5% Garlic and Control Diets	Between 5% and 2.5% Garlic Diet.	Between 2.5% Garlic and Control Diets
August	3.30*	1.60	1.60
September	2.10	1.64	.46
October-November	1.27	.54	.73
December	2.71*	2.22	.49
January	.40	.32	.18
February	1.60	.33	2.28

One possiblity in 20 that the results could be attributed to chance.

"t" $_{.05}$ = 2.365 with 8 D. F.

"t" $_{.01}$ = 3.499 with 8 D. F.

C. Leucocyte counts. The average total leucocyte counts of these animals are shown in Tables 5 and 6. The range for the female rats fed the five percent garlic diet was 7,067 to 9,858 cells per cmm. of blood; for those fed 2.5 percent garlic 7,413 to 11,812; and for the control animals 6,625 to 9,981. Male animals had leucocyte counts ranging from 6,392 to 8,885; 6,150 to 10,563; and 7,013 to 10,606 cells per cmm. of blood. The range in the literature cited was 8,000 to 19,000. The average leucocyte count of each dietary group studied is lower than those reported in the literature. The significance of, or the importance of, a lower than average white count is still unknown. It is possible that higher than average values resulting from counting techniques less strictly standardized than those used in this study are recorded in the studies cited. Because there was no trend shown in the white cell count either by the individual animal or in averages of the various groups, no statistical analysis of the data was made.

The differential white cell counts are recorded in Tables 9 and 10. Due to the wide individual variation, the results are presented for each animal instead of as the average of groups. The differential counts failed to show any trends due to the feeding of garlic. All the values were within the range found as average by Reich ('43).

TABLE 9

The differential white blood cell counts of eleven female albino rats

Month		August					September					October-November				
		Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
5% Garlic		Percent of 300 cells					Percent of 300 cells					Percent of 300 cells				
1		86.0	3.3	10.0	0.7	0.0	87.7	2.3	8.3	1.0	0.7	71.3	3.0	23.7	1.3	0.7
2		89.3	3.0	7.0	0.3	0.3	89.3	3.0	7.0	0.7	0.0	90.3	0.7	8.7	0.0	0.3
3		77.3	2.3	17.7	2.7	0.0	86.0	2.0	10.0	1.7	0.3	66.0	1.7	31.7	0.7	0.0
2.5% Garlic																
1		89.3	2.3	7.3	1.0	0.0	85.7	9.3	3.7	0.7	0.7	55.3	6.0	37.7	1.0	0.0
2	*	72.0	3.0	23.0	2.0	0.0	66.0	3.0	27.3	3.0	1.0	59.0	1.3	35.7	0.3	0.3
3		92.7	1.0	5.7	0.7	0.0	85.3	2.7	8.3	3.3	0.3	67.3	1.0	30.0	1.3	0.3
4		77.7	3.7	16.7	1.7	0.3	83.0	2.0	13.3	1.0	0.7	61.3	2.3	33.7	2.7	0.0
Control																
1		82.0	3.7	11.7	2.3	0.3	84.0	2.3	10.0	3.0	0.7	66.7	2.7	30.3	0.3	0.0
2	*	96.0	3.0	1.0	0.0	0.0	92.0	1.7	5.0	0.7	0.7	81.3	1.3	15.7	1.0	0.7
3		79.3	3.3	11.7	5.0	0.7	76.0	4.3	15.7	3.0	1.0	# 53.5	4.3	40.3	1.8	.25
4		83.0	2.7	12.3	2.0	0.0	84.0	1.7	11.0	3.3	0.0	57.7	3.7	36.3	2.3	0.0

* 100 cells counted
\# 400 cells counted

TABLE 9-- cont'd

The differential white blood cell counts of eleven female albino rats

Month	December					January					February				
Animal	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
1% Garlic	Percent of 300 cells					Percent of 300 cells					Percent of 300 cells				
1	88.3	3.0	6.7	2.0	0.0	79.0	3.7	17.0	0.3	0.0	80.0	2.0	17.5	0.7	0.0
2	84.7	2.0	11.3	2.0	0.0	75.0	3.0	21.3	0.7	0.0	86.3	2.0	10.0	1.7	0.0
3	83.3	2.7	11.3	2.7	0.0	74.0	3.0	24.7	1.7	0.0	59.0	4.0	35.3	1.7	0.0
.5% Garlic															
1	83.3	1.3	13.7	1.7	0.0	85.3	4.3	9.7	0.7	0.0	86.3	2.0	11.0	0.7	0.0
2	65.7	6.3	26.0	2.0	0.0	89.0	3.3	7.7	0.0	0.0	71.7	2.7	25.7	0.0	0.0
3	88.7	3.0	6.0	2.3	0.0	85.3	1.0	13.0	0.7	0.0	61.7	3.0	34.3	1.0	0.0
4	74.3	2.3	22.7	0.7	0.0	79.7	1.0	18.3	0.7	0.3	83.7	0.7	12.3	0.0	0.0
Control															
1	66.3	4.3	25.7	3.3	0.3	67.0	3.7	29.0	0.3	0.0	88.0	1.7	9.0	1.3	0.0
2	82.7	4.0	13.0	0.3	0.0	76.7	5.3	17.3	0.7	0.0	Animal Died				
3	66.0	2.7	30.0	0.7	0.7	70.7	4.3	24.3	0.7	0.0	82.3	0.7	15.0	2.0	0.0
4	78.3	3.0	13.3	5.3	0.0	72.0	5.7	22.3	0.0	0.0	74.3	2.0	21.7	2.0	0.0

TABLE 10

The differential white blood cell counts of eleven male albino rats

Month		August					September					October-November				
Animal		Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
5% Garlic		Percent of 300 cells					Percent of 300 cells					Percent of 300 cells				
1		83.0	3.7	12.3	0.7	0.3	82.7	2.3	13.3	1.0	0.3	83.7	3.3	11.0	1.3	0.7
2		82.7	2.7	13.3	0.7	0.7	79.0	3.0	15.7	2.0	0.3	78.0	2.0	14.5	5.0	0.5
3		86.7	6.3	5.7	1.0	0.3	86.7	2.7	8.7	2.0	0.0	81.7	1.7	14.0	2.7	0.0
2.5% Garlic																
1		77.3	4.0	17.7	0.3	0.7	73.0	4.0	20.3	2.7	0.3	75.3	4.0	17.3	3.3	0.0
2		88.0	3.3	8.3	0.0	0.3	78.5	2.3	16.7	2.7	0.0	89.0	0.7	9.7	0.7	0.0
3	*	84.5	3.5	11.0	1.0	0.0	69.0	1.3	27.0	2.7	0.0	# 86.0	2.0	11.0	1.0	0.0
4		77.7	3.7	16.7	1.7	0.3	78.3	2.3	16.0	2.7	0.7	80.3	2.7	15.3	1.3	0.3
Control																
1		83.3	4.3	11.0	0.7	0.7	84.3	3.0	11.3	1.0	0.3	78.7	4.7	13.0	3.7	0.0
2		76.3	2.0	21.0	0.7	0.0	86.0	2.3	8.7	2.3	0.7	84.8	2.3	10.3	2.7	0.0
3		78.7	4.0	16.7	0.7	0.0	71.7	2.7	22.3	3.0	0.3	61.3	4.3	28.0	6.3	0.0
4		84.0	4.0	10.3	1.7	0.0	76.0	2.0	19.0	2.3	0.7	72.3	2.7	21.3	2.0	0.7

* 200 cells counted
\# 100 cells counted

TABLE 10 -cont'd

The differential white blood cell counts of eleven male albino rats

Month	December					January					February				
Animal	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
5% Garlic	Percent of 300 cells					Percent of 300 cells					Percent of 300 cells.				
1	89.0	2.0	8.7	0.0	0.3	79.0	3.3	17.3	0.3	0.0	82.0	3.3	14.7	0.0	0.0
2	78.7	1.7	17.7	2.0	0.0	88.7	1.3	9.7	0.3	0.0	87.3	0.7	11.7	0.3	0.0
3	72.3	1.7	25.7	0.3	0.0	78.7	2.0	19.0	0.3	0.0	77.7	2.7	18.0	1.7	0.0
2.5% Garlic															
1	69.0	4.7	25.0	1.3	0.0	82.0	2.3	14.7	1.0	0.0	58.0	2.7	34.3	3.3	0.3
2	88.3	2.0	9.3	0.3	0.0	78.0	2.7	18.0	1.3	0.0	85.0	1.7	11.7	1.7	0.0
3	62.7	5.7	31.0	0.7	0.0	79.7	3.0	16.3	1.0	0.0	66.7	1.3	27.7	4.3	0.0
4	83.3	2.0	14.3	0.3	0.0	89.0	1.3	9.0	0.3	0.3	77.0	3.0	18.0	2.0	0.0
Control															
1	86.7	2.7	10.3	0.3	0.0	77.7	3.0	17.3	2.0	0.0	54.7	4.0	40.3	1.0	0.0
2	87.3	3.7	8.7	0.0	0.3	88.3	2.0	9.0	0.7	0.0	84.3	2.0	13.0	0.7	0.0
3	78.3	4.3	16.0	1.3	0.0	77.0	2.7	19.0	1.3	0.0	74.3	2.3	20.0	3.3	0.0
4	84.0	3.3	11.3	1.3	0.3	77.0	3.7	16.3	2.7	0.3	70.7	2.7	25.3	1.3	0.0

While studying the differential blood slides an unidentified protozoa-like organism which stained blue with Wright's Stain was observed on some of the slides. Attempts to identify the organism have failed. No mention has been found in any available literature of such an abnormality in rat blood. Attempts were made to correlate its presence with the total white blood cell count but no correlation could be found ($r = .038$) Studies to isolate, grow, and identify the organism are now in progress. The results of this work will be reported by another worker from this laboratory. There is no group difference in the prevalance of the organism on the slides and presumably in the blood stream of the animals. The antibiotic effect of garlic did not prevent infestation with the organism. However, antibiotic substances have been shown to be highly selective in action. Garlic is more active against fungi than other forms of microorganisms. Failure to prevent this infection must not be considered as invalidating the antibiotic claims made for garlic.

SUMMARY

The growth, development, and the formed elements of the blood of albino rats fed varying concentrations of dry garlic powder have been studied and general trends recorded.

Growth of the female animals on the experimental diets was shown to be comparable to that of the stock colony females. Weight gains per gram food consumed were greater for the male and female animals fed 2.5 percent garlic.

Normal gestation periods were observed. The failure to wean the first litters was traced to a manganese deficiency in the diet. Second litters, given the recommended manganese allowances, were more successfully reared.

Autopsy records showed the only abnormality to be a chronic lung infection. It is possible that the antibiotic effect of garlic was responsible for the lesser prevalence and less severe cases of lung infection noted in the animals fed garlic.

The total red blood cell count and hemoglobin concentrations reported are within the average range found in the literature. No permanent change in the red blood cell system was produced by the addition of garlic in the diet. Significant lowering of the red blood cell count for the first

three months observed in the female animals may have been caused by the action of garlic upon the animals. The lowered hemoglobin concentration of the female animals fed five percent garlic may also have been due to some depressive effect of garlic upon red blood cell formation.

The white blood cell system was apparently unaffected by the inclusion of garlic in the experimental diets.

The presence of an unidentified protozoa-like substance on the stained differential slides was noted. Further study is being conducted to determine the characteristics of this organism.

The experimental procedure has been duplicated upon an additional group of animals. Results of both studies will be pooled when the second study is completed. Until this is done and the trends recorded in this paper are either sustained or changed, no definite results of the feeding of dry garlic powder to albino rats can be claimed.

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