THE EFFECT OF TERRAMYCIN ON THE ERYTHROCYTES AND LEUCOCYTES OF THE BLOOD OF THE ALBINO RAT

Thesis for the Degree of M. S.
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This is to certify that the

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The effect of terramycin on the erythrocytes and leucocytes of the blood of the albino rat

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Major professor

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By

George E. Garske

AN ABSTRACT

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

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THESIS ABSTRACT

Due to the scarcity of information regarding the hematopoietic effects of terramycin an experiment was conducted to try to determine what effect, if any, terramycin had on the erythrocytes and leucocytes using the albino rat as an experimental animal.

Rats were divided into two groups, experimental and control, and placed on identical diets except that the ration of the experimental group contained 500 grams of terramycin per ton of feed.

Litters from the experimental group were used to check placental transmission and milk carryover of terramycin and to determine the average values for erythrocytes and leucocytes in immature animals.

Assay data revealed that terramycin was supplied to the young in utero via the placenta and in the milk to suckling young.

Results of counts made on 12 experimental and 7 control animals showed no marked differences in erythrocyte and leucocyte values for the two immature groups.

The experimental breeding colony of 5 animals gave results similar to the control breeding group of 4 animals in erythrocyte and leucocyte counts and in differential formula.

Four experimental and 2 control animals 73 days old showed no marked difference in differential formula.

All values found for erythrocytes, leucocytes and differential counts fell within the normal range of variation for these elements in the albino rat.

From the results obtained it can be concluded that at a level of 500 grams per ton of feed terramycin has no appreciable effect upon the erythrocytes and leucocytes of the albino rat.

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INTRODUCTION

Terramycin, a broad spectrum antibiotic, was discovered by Finlay and associates (1950) in the Biochemical Research Laboratories of Chas. Pfizer & Co., Inc. The result of a planned research program, terramycin is a product of a new actinomycete, Streptomyces rimosus, and is amphoteric forming both the crystalline hydrochloride and the sodium salt. Terramycin is effective against a wide variety of micro-organisms including many Gram-negative and Gram-positive bacteria, the rickettsia and certain of the large viruses.

Terramycin is closely related to aureomycin and tetracycline both in structure and in activity, tetracycline being the base for each of the other two. Aureomycin is modified by having a Cl atom at position 16 and terramycin by having a hydroxyl group at position 12.

P'an (1950) reported that some dogs receiving varying amounts of terramycin intramuscularly showed mild degrees of anemia but believed that the anemia was secondary to chronic ulceration at the site of injection rather than due to the toxic effect of terramycin.

The earliest reports that antibiotics might have an adverse effect upon hematopoiesis in man concerned chloramphenicol (Volini 1950, Gill 1950, Rich 1950).

As increasing numbers of reports were received Lewis and his associates (1952) of the United States Food and Drug Administration made a special nation-wide survey of blood dyscrasias in man in relation not only to chloramphenicol but also to other drugs and antibiotics.

The blood dyscrasias involved included aplastic anemia, leukopenia, thrombocytopenia purpura, and granulocytopenia. A total of 539 cases studied were grouped into four classes as follows:

A. Chloramphenicol alone involved

B. Chloramphenicol with other drugs

C. Chloramphenicol not involved

D. Unclassified

Some of the other compounds involved in classes "B" and "C" included other antibiotics, sulfa drugs, arsenicals, analgesics, anticonvulsants, barbiturates, and antipyretics. In class "C" cases involving broad spectrum antibiotics only, of which terramycin is one, totaled 15. Information as to the types of abnormalities concerned was not given.

Following this special survey Welch and his associates (1954) made a second survey which was much more comprehensive than the first survey and which placed greater stress on human blood dyscrasias associated with drugs other than chloramphenicol. The categories used in this survey were the same as those in the first.

Of a total of 1,448 cases reviewed in this survey 59 involved the use of terramycin. Eighteen of these cases fell into group "B" and on 4 occasions terramycin was the only drug involved besides chloramphenicol. The other 41 cases did not involve chloramphenicol. A further breakdown of these 41 cases by dyscrasias showed the following:

11 cases of aplastic anemia - 2 in which terramycin was the only drug used.

2 cases of anemia.

13 cases of thrombocytopenia purpura - 4 in which terramycin was the only drug used.

11 cases of granulocytopenia or agranulocytosis - 1 in which terramycin was the only drug used.

3 cases of leukopenia - all of which involved only terramycin.

1 case of non-thrombocytopenia purpura.

Mirone (1953) reported that a level of 150 mg. of terramycin per kilogram of feed had no adverse effects on red, white or differential counts in mice over a period of nine months.

Additional data (unpublished) furnished by Chas. Pfizer & Co., Inc. on bone marrow studies in chickens fed varying amounts of terramycin indicate that at a 200 gram

per ton level the blood forming tissues remain essentially normal with a slight polycythemia appearing after six weeks and then disappearing shortly afterwards. At a 2,000 gram per ton level degeneration of blood forming tissue occurred accompanied by deficient blood cell formation and production of abnormal cells.

The most recent evidence presented by Nelson and Radomski (1954) who worked with dogs showed that terramycin administered orally for a period of approximately three months had no hematopoietic effects.

Since terramycin is widely used as a therapeutic agent in man and other animals and as a growth supplement in animals it is important to know of any adverse effects which it might produce.

The purpose of the problem undertaken for this study was to try to determine what effect, if any, terramycin had in the production of these blood dyscrasias. It was decided to try to produce any or all of these effects using the rat as an experimental animal.

Although it was originally planned to administer terramycin suspended in mineral oil subcutaneously to newborn rats it was subsequently learned that it is difficult to give a sufficiently large dose and that a constant blood serum level is hard to maintain by this method. Therefore plans were altered and the research extended to include

placental transmission and milk carryover by giving the terramycin orally as a part of the food supply. A level of 500 grams per ton was chosen as this was greater than most commercial feed levels and would assure a constant exposure to the antibiotic.

MATERIALS AND METHODS

The rats used came from the Chemistry Department of Michigan State College and were highly inbred - no outside animals having been introduced for a period of 15 years - although no planned mating procedure was followed. The rats were divided into two groups - experimental and control - and were placed upon identical diets except that the diet of the experimental group contained 500 grams of terramycin per ton of feed. The terramycin was supplied in the form of Terramycin 10, a feed supplement containing 10 grams of terramycin per pound.

Composition of the diet is shown below:

Yellow Corn	57.0 %
Dried Skim Milk	20.0
Linseed Oil Meal	12.0
Soybean Oil	5.0
Crude Casein	3.7
Alfalfa Leaf Meal	1.5
Iodized Salt	.4
Calcium Carbonate	.4
	100.0 %

Blood was obtained by cutting the tail and bleeding was controlled by using silver nitrate applicators. Blood counts were made in a certified improved Neubauer hemacytometer using Hayem's solution as diluent for red cell counts and 3% acetic acid as diluent for white cell counts.

Differential counts were made on both groups of rats at the end of the experiment using blood drawn from the tail and Wright's stain for preparation.

Blood for terramycin assay purposes was drawn directly from the heart by heart puncture, allowed to hemolyze, centrifuged and then the serum was sent in for assay to the assay department of Chas. Pfizer & Co., Inc. In very young (newborn) rats blood was obtained by decapitating the animal and allowing the blood along with small amounts of body fluids to drain into a test tube. Several animals were necessary to obtain sufficient blood for assay and usually a whole litter was sacrificed.

Milk was sent to the assay department of Chas. Pfizer & Co., Inc. for terramycin assay to try to determine whether or not terramycin was transmitted from female to young via the milk and if so, the amount. This milk was obtained by sacrificing suckling young and removing the contents of their stomachs.

To gain information on placental transmission of terramycin newborn litters were decapitated and the blood and body fluids were drained off and sent in to be assayed.

The experimental group consisted of 1 male and 4 females plus several young born prior to the start of the experiment. All were placed on the diet containing terramycin. The young born to the breeding colony were utilized,

by litters, to check placental transmission of terramycin, milk carryover of terramycin and for red and white cell counts. Blood counts were started at the time of weaning and taken approximately every three days throughout the length of the experiment.

The procedure for the control group was the same except that no assay work was performed on them. This group consisted of 1 male and 3 females plus some younger animals that had been born before the start of the experiment.

Red and white cell counts and differentials were done on the two breeding colonies near the end of the experiment to check the effect of terramycin after prolonged exposure to the drug.

In addition to the breeding colonies a total of 25 animals was used in the experiment. Nineteen of these, 12 experimental and 7 control, were used to determine red and white cell averages in the immature animal. Differential counts were made on the remaining 6 animals, 4 experimental and 2 control, at age 73 days.

DATA

Erythrocytes

The erythrocyte picture in newborn and young rats shows a gradual increase from about 3 million per cubic mm. at birth to the normal adult level of 9 million per cubic mm. at approximately four months of age (Creskoff 1949). Jolly (1909) gives a series of values for erythrocytes at various ages as shown below in Table I.

Table I

AVERAGE VALUES
FOR ERYTHROCYTES AT VARIOUS AGES

Age (in days)	Number of Erythrocytes (in millions per cu. mm.)
21	4.4
30	5.5
42	5.6
60	6.7

Margot (MS. 1916) gives the values for erythrocytes at the various ages as shown in Table II.

Table II

AVERAGE VALUES
FOR ERYTHROCYTES AT VARIOUS AGES

Age (in days)	Number of Erythrocytes (in millions per cu. mm.)
28	6.5
46	9.7
60	8.8
70	8.8

The values as determined for the rats in this study are shown in Table III.

As will be observed from these values there is a tendency for rather marked fluctuations in number at all ages with a gradual rise in the number of cells with age.

In the adult rat the erythrocyte count averages 9.35 million per cubic mm. with very little difference between the sexes, the normal range being between 7 and 10 million per cubic mm. (Creskoff 1949). Rivas (MS. 1914) gives values in millions per cubic mm. of 8.7, 7.9, and 8.0 for the normal albino rat in three series of determinations. Jolly (1909) gives values in millions per cubic mm. of 8.9 at 365 days of age and 8.8 at 1,095 days of age.

Table III

AVERAGE VALUES
FOR ERYTHROCYTES AT VARIOUS AGES

EXPERIMENTAL			CONTROL		
Age	Number	Range*	Age	Number	Range*
24	5.2	5.0 - 5.4	24	4.4	3.5 - 5.1
25	5.2	4.9 - 5.5	27	5.0	4.5 - 5.2
26	4.4	3.0 - 5.0	33	5.6	5.4 - 5.9
29	5.1	4.6 - 5.7	36	6.0	5.5 - 6.6
36	6.9	6.0 - 8.4	39	5.8	5.3 - 6.4
37	5.6	5.5 - 6.0	42	5.7	4.8 - 6.2
40	5.9	5.5 - 6.2	45	6.0	5.7 - 6.5
43	6.4	4.7 - 7.4	53	7.6	7.5 - 7.8
46	6.7	6.3 - 7.3	56	7.2	6.8 - 7.5
47	5.7	5.0 - 6.4	59	6.8	6.5 - 7.1
49	5.9	4.5 - 7.0	62	6.9	6.3 - 7.6
52	6.4	5.5 - 7.4			
55	6.9	6.5 - 7.9			

* Age in days, number and range in millions per cubic mm.

Average counts of the experimental and control breeding colonies gave the results shown in Table IV.

Table IV

AVERAGE ERYTHROCYTE VALUES
IN BREEDING COLONIES

EXPERIMENTAL		CONTROL	
Number	Range*	Number	Range*
8.6	6.0 - 9.8	7.0	4.8 - 8.7

* Number and range given in millions per cubic mm.

The average of the control group was lowered somewhat by the fact that during the period counts were being made, one female gave birth to a litter of young. It was noted that the red cell count dropped about 2.5 million per cubic mm. but returned to normal in a few days.

It must also be noted that the breeders of the experimental group had higher individual red cell counts and therefore higher average counts at all times during the period counts were being made.

Leucocytes

Creskoff (1949) states that the newborn rat has a leucocyte average of 3,200 per cubic mm. and that this reaches the adult level in approximately the third week of life.

According to Jolly (1919) the number of leucocytes tends to increase with age as shown in Table V.

Table V

AVERAGE NUMBER OF LEUCOCYTES AT VARIOUS AGES

Age (in days)	Number of Leucocytes (per cubic mm.)
1	2,766
8	3,240
15	5,666
30	7,400
60	6,866
365	10,000

Margot (MS. 1916) gives the average values at various ages as shown in Table VI.

Table VI

AVERAGE NUMBER OF LEUCOCYTES
AT VARIOUS AGES

Age (in days)	Number of Leucocytes (per cubic mm.)
28	7,102
46	10,025
60	9,143
70	8,480

Values found in experimental animals are given in Table VII.

The average value of leucocytes in the adult rat is 9,000 per cubic mm. with a range of 6,000 to 18,000 per cubic mm. (Greskoff 1949).

Rivas (MS. 1914) gives values of 8,133, 8,467, and 11,407 per cubic mm. for normal albino rats in three series of determinations.

Table VII

AVERAGE NUMBER OF LEUCOCYTES
AT VARIOUS AGES

EXPERIMENTAL			CONTROL		
Age	Number	Range*	Age	Number	Range*
24	4,115	4,080 - 4,150	24	3,690	2,000 - 6,120
25	3,130	2,550 - 3,600	27	4,230	3,850 - 4,610
26	2,160	1,275 - 3,072	33	5,080	3,450 - 8,930
29	2,830	2,300 - 3,800	36	3,750	3,200 - 4,500
36	6,350	2,950 - 10,950	39	6,000	5,600 - 6,210
37	3,440	2,048 - 4,600	42	4,800	3,650 - 6,450
40	3,960	2,200 - 5,700	45	4,500	2,650 - 5,630
43	5,250	2,100 - 12,150	53	5,350	4,500 - 6,200
46	4,660	2,800 - 7,450	56	10,800	8,410 - 13,300
47	3,470	2,850 - 4,750	59	7,600	5,500 - 9,850
49	7,750	4,600 - 13,260	62	9,650	7,500 - 13,300
52	4,730	2,500 - 6,750			
55	3,550	1,750 - 6,900			

* Age is given in days, number and range per cubic mm.

The values found for the experimental and control breeding colonies in this study are shown in Table VIII.

Table VIII

AVERAGE LEUCOCYTE NUMBERS
IN BREEDING COLONIES

EXPERIMENTAL		CONTROL	
Number	Range*	Number	Range*
6,310	3,750 - 15,100	8,240	4,100 - 14,900

* Number and range per cubic mm.

Several factors contribute to errors in leucocyte number determinations. One of these is the fact that there is a decided leukopenia in the female rat at the time of estrus (Farris 1942). Since more females were used for these counts than males and since the estrous cycle in the rat is only 4 to 5 days in length, the possibility of this occurring is present at all times. Another cause of error is the fact that any emotional stress is reflected by an immediate leukocytosis (Farris 1938). The rat holders used to hold the animals while withdrawing blood consisted of tubes of varying diameters into which the rats were supposed to crawl. At times the animals were not as cooperative as

could be desired and had to be forced to enter the holder. Although no record was kept of such cases, they could be expected to cause an increase in the leucocyte count. This would also be true of the younger animals considered in the preceding section. However this could be expected to occur in both groups with the same frequency. A third factor is that during the birth process while the red count drops markedly, the white count rises sharply. The control breeding group, as was previously mentioned, contained a female who gave birth to a litter during the period counts were being made. The white count of this animal during this period rose from 6,000 per cubic mm. to 15,000 per cubic mm. and then dropped to about 7,000 per cubic mm. two days later.

Differential Counts

The distribution of the various elements composing the white cells of the blood of the normal albino rat as reported by Rivas (MS. 1914) is shown in Table IX.

Nowrey (MS. 1921) gives a breakdown by sexes as shown in Table X.

Table IX

DIFFERENTIAL FORMULA OF LEUCOCYTES*

Neutrophils	Lymphocytes	Eosinophils	Basophils
57.00	41.60	1.12	.28
52.50	46.60	.60	.30
55.90	43.00	.90	.20

* Percentages determined in three separate series of rats.

Table X

DIFFERENTIAL FORMULA
OF LEUCOCYTES BY SEXES

	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
M.	62.07	32.00	1.83	1.54	.42
F.	55.71	34.00	2.49	4.00	.45

Creskoff (1949) states that there is no important difference in differential formula between the sexes and gives average values as shown in Table XI.

Table XI

AVERAGE DIFFERENTIAL FORMULA

	Average	Range
Neutrophils	20	8 - 39
Lymphocytes	78	55 - 96
Monocytes	less than 1	0 - 3
Eosinophils	2	0 - 4
Basophils	less than 1	0 - 0

In rats conditioned for emotional studies the values given are shown in Table XII (Creskoff 1949).

Table XII

AVERAGE DIFFERENTIAL FORMULA
IN RATS CONDITIONED FOR EMOTIONAL STUDIES

	Average	Range
Neutrophils	35	24 - 47
Lymphocytes	60	47 - 73
Monocytes	2	0 - 3
Eosinophils	2	0 - 7
Basophils	1	0 - 1

The difference between these values can be explained by the fact that emotional stimulation causes an average increase of 12.08% in lymphocytes (Farris 1938).

Table XIII gives values found in the rats of the experimental and control breeding colonies. Also shown are values of 73 day old experimental animals and their controls.

Table XIII

AVERAGE DIFFERENTIAL COUNT VALUES
FOR EXPERIMENTAL AND CONTROL ANIMALS

Breeders:	EXPERIMENTAL		CONTROL	
	Average	Range	Average	Range
Neutrophils	30.5	15 - 56	34.7	26 - 40
Lymphocytes	67.5	40 - 83	61.3	56 - 67
Monocytes	1.5	0 - 3	3.3	0 - 5
Eosinophils	0	0	1.0	0 - 2
Basophils	.5	0 - 1	0	0
73 Days Old:	EXPERIMENTAL		CONTROL	
	Average	Range	Average	Range
Neutrophils	18.75	6 - 30	12.0	8 - 16
Lymphocytes	80.00	68 - 94	86.0	80 - 92
Monocytes	1.00	0 - 2	2.0	0 - 4
Eosinophils	.25	0 - 1	0	0
Basophils	0	0	0	0

Terramycin Assay

Terramycin assay determinations were done by the staff of the assay department of Chas. Pfizer & Co., Inc. at the Agricultural Research and Development Farm in Terre Haute, Indiana. The spot plate method of assay was used. On the whole, assay data was unsatisfactory due to the contradictory results obtained.

Placental Transmission

Schoenbach (1950) states that the fetal blood serum level of terramycin is approximately one-quarter that of the maternal level. Herrell (1950) finds that terramycin readily traverses the placental barrier.

The assay data in one case yielded results of .2 gamma per ml. in maternal blood and 1 gamma per ml. in the newborn animals. This is higher than is to be expected in the offspring.

In other cases both maternal and newborn sera showed levels of less than .1 gamma per ml. An explanation for these differences cannot be given.

Milk Carryover

The contents of the stomachs of several litters of suckling rats of various ages gave results upon assay ranging from greater than .1 gamma per ml. to less than .1 gamma per ml. of terramycin.

From these assay data it can be seen that terramycin was supplied to the experimental animals via the placenta and also through the milk but the amounts cannot be considered as reliable since few determinations were made.

DISCUSSION

The elements of the blood of the rat have wider normal ranges than those of human blood. This fact tends to make dyscrasias more common in the rat than in man. Since the total blood volume is small, repeated bleedings may produce anemia. Also repeated bleedings from the same area may produce inflammation and leucocytosis. Health and nutrition also markedly influence the blood picture (Creskoff 1949). All these facts tend to make the rat a difficult animal on which to do hematological studies.

Concerning terramycin assay data it can be stated that terramycin passes from female to offspring through the placenta and also that it is transmitted to the suckling young via the milk. This is important in that it shows that the experimental animals were exposed to the antibiotic from before birth until the end of the experiment.

The erythrocyte picture in young animals of both the experimental and control groups (Table III) shows similar tendencies to increase with age with much variation at any particular age.

This tendency is not as marked in the white cell picture of the young animals (Table VII). Although fluctuations occur from age to age there is a more constant rise in the control group.

The red cell averages (Table IV) in the experimental and control breeding colonies are both within the normal range for the albino rat. However the control group average is lower than would be expected.

Considering all the factors tending to cause error in leucocyte counts, the values for the two breeding colonies (Table VIII) are acceptable since they fall within the normal range for the rat. This appears to be even more so when the differential counts are considered.

The differential formulas both for the breeders and for 73 day old animals revealed a similar picture in the experimental and control animals (Table XIII).

SUMMARY

Data on the average erythrocyte and leucocyte values in immature rats showed similar results in the 12 experimental and 7 control animals.

The experimental and control breeding colonies gave average values for erythrocyte, leucocyte and differential counts that were similar.

Differential counts made on 73 day old animals (4 experimental and 2 control) gave results that were consistent for the two groups but that were slightly different from those of the experimental and control breeders.

All average values found for erythrocyte, leucocyte and differential counts were within the range of normal variation of these elements for the albino rat.

Terramycin assay data revealed that the antibiotic is supplied to the young in utero via placental transmission and to suckling animals through the milk.

From the results obtained in this study it can be said that at a level of 500 grams per ton of feed, terramycin has no appreciable effect upon the leucocytes and erythrocytes of the blood of the albino rat.

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