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The Influence Of Methotrexate On The Cardiovascular System Of The Albino Rat

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THE INFLUENCE OF METHOTREXATE ON THE CARDIOVASCULAR SYSTEM OF THE ALBINO RAT

A Thesis

By

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DEDICATION

The writer wishes to dedicate this thesis to his wife, Mrs. Shirley M. Brown, whose inspiration and support have enabled him to go thus far in pursuit of a successful career.

H. O. B.

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CHAPTER I

INTRODUCTION

The medical historian, looking backward to our own times from some point still in the future, may well single out the years 1948 and 1958 as important markers in the fight against human cancer.

Lederle Laboratories, a Division of American Cyanamid Company (8), developed methotrexate in 1948 and the second date stands out as the year when Creech and his associates first described their successful application of regional perfusion - a technique of blocking off circulation around a tumor while an anti-cancer drug is introduced into the blood vessels of the isolated region (2).

Although of limited use, regional perfusion or infusion with methotrexate and leucovorin (citrovorum factor) therapy has been called the most significant advancement in cancer chemotherapy in the past fifteen years. The previous statement assumes a greater force when it is noted that the production of a cancer drug is one thing while its utilization presents a problem in itself (2). Thus it happened that the full value of methotrexate was not realized for nearly a decade while the best methods of using it were being explored. The perfusion technique permitted a higher

concentration of the drug to be delivered to the tumor area while minimizing undesirable systemic side effects.

The relationship between the mode of action of drugs used to treat cancer has caused many investigators to wonder what this dreadful disease is. Is it a chronic virus infection as some scientists believe? Or is it caused by any one of a number of other factors, or combination of factors: a defective immunity, a mutated gene, a distorted enzyme pattern, a hormonal imbalance, an environmental poison, a breakdown of organs and systems which enable us to withstand stress, or a perverted allergic-like immunity which destroys normal body cells and tissues? Whatever it is, the change from non-cancer to cancer takes place in a world all but hidden from man - a world so minute that it cannot even be viewed by an electron microscope.

Against such a background of death, hope and imperfect knowledge, the word "challenge" seems wholly inadequate to describe the task confronting the scientist. Some of the lines of attack are as follows: (a) the action of chemical molecules which are deliberately designed to block a specific chemical reaction within cancer cells; (b) chemicals that prevent cell division; (c) substances that imitate the effects of X-ray and (d) substances which compete with vitamins and hormones in cell metabolism or destroy bacteria and other primitive cells.

Four categories of agents have been useful in treating cancer (2): the antimetabolites, including the folic acid

antagonists such as methotrexate; the alkylating agents, such as triethylene melamine, ethylenimines, nitrogen mustards, etc.; the antibiotics, chiefly the actinomycins, and the adrenal steroids, such as cortisone and hydrocortisone.

According to Watson (18), methotrexate has been known for more than ten years to be effective against some cancers such as acute leukemia and choriocarcinoma, a rare cancer which forms in the uterus. However, two problems are involved with its use. First, some researchers point out, a slow infusion technique is needed so that this drug and related antimetabolites, which are slower in their action than other anticancer drugs, can be administered over a long period of time. Second, an antidote is needed to combat the general toxicity that results with long-term local administration of methotrexate which "looks" into the general circulation. Citrovorum, which is closely related to the vitamin folic acid, is known to prevent the toxic effects of methotrexate. Methotrexate is believed to act by interfering with the supply of folic acid needed by rapidly growing cancer cells (18).

Watson also stated (18), that this new method, which provides both the slow infusion and the antidote, can be tried with different combinations of other cancers.

Garofalo and Miller (4), in their investigation, observed that methotrexate may cause severe depressions of all blood cellular elements. However, methotrexate is a drug that is

designed for the treatment of acute leukemia. Clinical evidence shows that it is more effective in children than in adults. In some cases, it has caused clinical improvement and increased survival time in acute leukemia for periods varying from weeks to two years (4).

The hematological picture, as reflected in blood and bone marrow films after methotrexate therapy, may become almost indistinguishable from normal for varying periods of time. The greatest effect has been observed in acute leukemia characterized by "blast" forms in bone marrow or blood (17).

Transplantation of bone marrow, necessary in leukemia and in other conditions affecting the soft tissue that forms blood cells, may be more successful when accompanied by early, brief injections of the drug methotrexate than when these drug dosages are prolonged or delayed (17).

Previous reports on the use of methotrexate have indicated possible help in overcoming the immunity problem in skin and kidney transplants, as well as in bone marrow injections, but problems of timing and quantity of the drug remain to be solved.

According to Terry (16), there are about 160 anti-cancer drugs that are being tested on humans, and twenty of these have been approved for use on patients. One of the exciting drugs which is being utilized is methotrexate, an anti-folic-acid drug which as early as 1944 had been found to give

temporary help to leukemia victims, thought it never cured any of them.

Some few years ago, Hertz (8), the chief of an endocrinology branch institute tried methotrexate on victims of choriocarcinoma, a rare but widely growing cancer of the uterus. The drug was given to sixty-three women with the cancer. Amazingly, thirty of the women are now in apparent "perfect" health." The remaining seven are still getting treatment, twenty-five were helped but eventually died, and only one never showed any improvement.

An experimental study of the differences between resistant and sensitive leukemias has been greatly facilitated by the development of sublines in mice that are resistant to folic acid antagonists (8). An A-methopterin-resistant leukemia developed by Law and Boyle found that this characteristic remained unchanged by repeated transfer in mice which were free of the antagonist (7). Repeated treatment of mice with A-methopterin carrying a transplantble lymphoid leukemia had become resistant to this compound which resulted in the selection of a subline which appeared to be A-methopterin-dependent (7). That is the dependent leukemic cells required the presence of A-methopterin for most rapid growth and this effect seemed to be inhibited by the Citrovorum Factor (CF). It was of particular interest to determine to what extent the nature of the resistance observed in bacterial cells was applicable in these resistant leukemias.

Although encouraging progress has been made in the treatment of the leukemias, the failure of the present chemotherapeutic agents to do no more than extend life, rather than cure these diseases, may be attributed to the inability to attain tissue concentrations adequate to prevent completely the reproduction of neoplastic cells. Partly because of this failure an opportunity is afforded for resistant cells to survive and to give rise to a drug-resistant strain. It appears that in large populations of leukemic cells, just as in bacterial populations, a mutation can occur at random which may confer upon that cell the capacity to survive in the presence of a drug (7). After such an event, the resistant cell becomes the progenitor of a population which is no longer affected by a drug that induced a remission when first used. Although the defense mechanisms of the body facilitate the elimination of invading organisms which have become drug-resistant (e.g., to antibiotics), the occurence of a resistant leukemic cell is of greater consequence, since defenses against aberrant cells produced by the body apparently are relatively inefficient.

McGeer (11) states that antifolic acid compounds, such as aminopterin and amethopterin are useful in the treatment of certain neoplastic diseases, particularly acute leukemia. In another treatment with methotrexate, mice were inoculated with leukemia L1210, but the anti-leukemic effect was blocked

by the metabolite. Another investigator (9) has reported on the treatment of malignant melanoma and other malignant tumors of an extremity by regional perfusion with the alkylating agents, while Mandel (10) reported on the treatment of epithelial tumors by antimetabolite-methotrexate.

With these ideas in mind one can easily see the importance of an investigation of this nature. Therefore, the purpose of this thesis is to determine the influence of methotrexate on the cardiovascular and respiratory systems of the albino rat.

CHAPTER II

METHODS AND MATERIALS

Six albino rats of the A. and M. University strain were utilized in this investigation. These animals were fed Purina Rat Chow and allowed to eat and drink water ad libitum. A close observation on the weight of each animal was recorded in order to determine the side effects that may accompany injections of methotrexate. Each animal served as a control and following the intramuscular injection of the antimetabolite the same male or female served as the experimental animal.

Each animal was placed in a rat warmer so that the heat would increase the circulation in the rat's tail. A small piece of the tip of each rat's tail was amputated in order to get enough blood to make a white blood cell count according to the procedures of Wright (6).

The average normal number of leukocytes in an albino rat is usually stated to be from 8.8×10^3 to 19×10^3 per cu. mm., however normal counts may be slightly lower or higher fluctuating with age, influenced by digestion, and other little understood factors. There is a greater normal fluctuation in the number of leukocytes as compared with that of the erythrocytes.

Another procedure utilized in this investigation was to differentiate among the types of leucocytes in the circulating blood of the albino rat. The blood for this analysis was obtained in the same manner as the one described above for determining the number of leucocytes and the procedure was executed according to the methods of Kimber (6).

Under normal conditions the neutrophils ranged from 24 to 47%, lymphocytes 47 to 73%, monocytes 0 to 3%, eosinophils 0 to 7%, and basophils from 0 to 1% in every one hundred cells counted.

The next procedure in this blood picture was to determine the volume of packed RBC's (hematocrit) in the albino rat. The hematocrit was performed according to the Adam's Micro-Hematocrit Procedures (3). Under normal conditions the albino rat's hematocrit is approximately 50 cc. of erythrocytes in every 100 cc. of blood.

The three procedures utilized to determine the average normal number of leucocytes per cu. mm., the volume of packed RBC's and the percentage of the different types of leucocytes in the control animals were also executed in the experimental animals following injections of methotrexate.

Several other parameters, such as the heart rate, blood pressure and respiratory rate, were executed in this investigation to determine more conclusively the effects of methotrexate on the cardiovascular system.

The indirect blood pressure, which only measures the systolic pressure was determined on the control and the experimental animals.

The technique for taking indirect blood pressure measurements from a rat's tail depend upon the effectiveness of the temperature control unit in maintaining the rat's tail at a constant temperature. The temperature setting is critical at 1/2 ^{o}F. and a variation in the base plate temperature will either stimulate ciruculation or permit occlusion. A base plate temperature of approximately 96^{o}F. was required, and for best results, the rat holder was prewarmed and adjusted to this temperature before the animal was placed in the container (15). Even then, close control temperature should be maintained because overheating can cause discomfort to the contained animal. Once an effective dial setting was found, it was used repeatedly with slight variations required to maintain proper circulation in the rat's tail.

Each animal was placed in the Lucite container and allowed to become adjusted in order to prevent an elevated blood pressure which may occur due to excitement.

The occluding Blood Pressure Cuff was placed on the rat's tail as near the body of the animal as possible and then connected to the Electrosphgmograph. The Pneumatic Pulse Pick-Up consists of an extremely sensitive transducer with a pneumatic coupling which was attached to the rat's tail.

The pneumatic element was taped lengthwise over the animal's tail, distal to the cuff, with the tape stuck to the base board rather than to the tail itself. When this element was in place on the tail, the connecting tubing was coupled to the transducer which was then connected to the Electrosphygmograph microphone input. The automatic cuff pump was adjusted to measure the Blood Pressure every half minute (15).

To obtain blood pressure readings from the rat's tail, the sound amplitude of the Electrosphygmograph was turned up until pulsations from the tail were observed. If no pulsations were present, the operation of the pneumatic transducer was checked by tapping the tail element very lightly. If the unit was operating properly and no pulsations were observed, the animal was warmed until the pulsations from the circulation in the tail were very distinctive and had reached an amplitude of 3 to 4 centimeters at dull sound amplitude settings of the Electrosphygmograph. The amplitude was decreased appropriately to produce approximately 5-millimeters to 1-centimeter deflections at 0 cuff pressures.

When circulation was established in the tail, the sound amplitude was turned to zero and the Electrosphygmograph was calibrated either with the hand bulb or with the cuff pump. When the calibration had been established, the sound amplitude was increased to produce pulsations approximately 1-centimeter in amplitude but the cuff pressure remained at zero. The Automatic cuff pressure pump increased the

pressure above the blood pressure of the animal and was then allowed to decrease slowly. The pressure at which pulsations first appeared on the descending pressure curve was a measure of systolic blood pressure of the animal. Diastolic pressures were not observed with this particular technique.

To obtain the respiratory rate, the Impedance Pneumograph, a transducer for recording from a single pair of electrodes, which measures the respiration of experimental animals or humans (15) connects directly to any of the channel amplifiers on the Physiograph with a single standard transducer cable.

The Impedance Pneumograph requires two electrodes to be connected on either side of the chest of the subject. This instrument impresses a small alternating current signal across the body of the subject and detects the small variations in this current occasioned with respiration.

The Impedance Pneumograph has distinct value as a transducer. Recordings are readily made from animals where mechanicaltype pneumographs are impractical. However, only a single pair of electrodes is required for recording respiration from large and small subjects alike (15).

For the normal monitoring of respiration, a condensor-coupled output with a long-time constant was provided. Utilizing the condensor-coupled output, even with long-term changes in electrode impedance, or with breath-holding, the pen will always return to its base-line in a manner similar to that of the cardiac or hi-gain preamplifiers (15). The

Impedance Pneumograph measures changes in impedance across the input terminals.

The animal was shaved and a ml. of Procaine HCL was injected subcutaneously to render the animal insensitive to pain. The electrodes were then connected to the animal insuring good electrical contact. A third ground electrode was used to reduce 60-cycle interference on the pneumograph.

On the Impedance Pneumograph, the Amplitude control was turned slowly (clockwise) until the respiration patterns reached a suitable size, allowing sufficient deflection clearance for deep breaths. A few seconds of stabilization of the internal coupling condensor was required.

Preliminary studies indicate that optimum placement of the electrodes is between the 5th and 6th rib interspace on man, dog, and rat (15). Electrode placement is usually not critical, although in certain forms of breathing some cross-sectional areas of the body will decrease in size with inhalation and be recorded as downward deflections of the recording pen. Light breathing patterns on a normal adult will produce approximately a 1% change in 500 ohms (15).

In order to measure the heart rate the Cariotach was utilized. The Cardiac Preamplifier is designed to have a maximum sensitivity of at least 1 millivolt per centimeter of pen movement (15). This sensitivity is sufficient for nearly all the physiological potentials encountered in cardiac physiology.

The main Amplifier was raised to the Record-Ready switch to record. A few seconds were required for the circuit to stabilize itself.

Each of the preceding analyses was recorded for the control and experimental animals and the mean readings were determined.

CHAPTER III

EXPERIMENTAL RESULTS

Thus far only a description of the basic experiments in this investigation has been given. A statistical analysis of the results has been reserved for the tables that follow indicating a variance in leukocytes, lymphocytes, neutrophils, hematocrit, heart rate, blood pressure, and respiratory rate preceding and following injections of 1.25 mg. of methotrexate in each of the controlled and experimental groups of albino rats. This analysis included a series of five tests on the above groups. The findings reflected in each table are also graphically illustrated to further validate the differentials brought on by injections of methotrexate into the specimens. Accompanying each table and graph is a key to interpretation of the data and their validation.

The normal leukocyte count of the control group as shown by Table IA and IB and Figure 1 ranged from 9.9×10^3 per cu. mm. to 1.2×10^4 per cu. mm. with a mean of 1.1×10^4 per cu. mm. and the experimental count ranged from 5.6×10^3 per cu. to 7.5×10^3 per cu. mm. with a mean of 6.2×10^3 per cu. mm. This showed a significant decrease of 45% below the control count. Under normal conditions as shown by Table IIA and B and Figure 2 the neutrophils ranged from 29.8% per cu. mm. to 39.2% per cu.mm.

with a mean of 32.8% per cu. mm. while the experimental results showed a range of 13% per cu. mm. to 16.4% per cu. mm. with a mean of 14.3% per cu. mm. The results showed a significant decrease of 56% per cu. mm. lower than what was observed in the control group. The lymphocyte count ranged in the controlled group from 61% per cu. mm. to 68.8% per cu. mm. with a mean of 65.6% per cu. mm. and the experimental count ranged from 32% per cu. mm. to 48.2% per cu. mm. with a mean of 40.6% per cu. mm. showing a decrease of 23% per cu. mm. (See Table IIIA and B and Figure 3). The hematocrit in the controlled group as shown (Table IV and Figure 4) ranged from 46% per cu. mm. to 51% per cu. mm. with a mean of 49.3% per cu. mm. and the experimental results showed 45% per cu. mm. to 50% per cu. mm. with a mean of 47.3% per cu. mm., an insignificant decrease of 4% per cu. mm. The controlled heart rate shows a normal range of 265 to 280 beats per minute with a mean of 272 beats and the experimental results ranged from 275 to 295 beats per minute with a mean of 287.5 beats. This was an increase of 5% over the control group showed which is not significant. (See Table V and Figure 5). The blood pressure ranged from 53 to 89 mm. Hg. with a mean of 76.6 mm. Hg. The experimental results showed a range from 86 to 123 mm. Hg. with a mean of 99.3 mm. Hg. This showed a significant increase of 30% over the control group. (See Table VI and Figure 6). The controlled respiratory rate ranged from 80 to 120 times per min. with a

mean of 131.8. This was a significant increase of 32% over the respiratory rate in the control group. (See Table VII and Figure 7).

Similar results have been shown in previous experiments utilizing methotrexate but using different experimental animals.

LEUKOCYTE COUNT

Control

1A	1B	2A	2B	3A	3B
12,600	12,400	8,550	9,950	10,850	11,700
12,500	12,000	10,050	11,250	11,700	10,650
12,500	11,850	10,200	11,350	11,400	11,350
12,250	12,250	10,400	10,400	11,100	11,150
12,300	12,250	10,355	10,400	11,700	11,600
62,150	60,750	49,555	53,350	56,750	56,450
$\bar{X}$=12,430	$\bar{X}$=12,150	$\bar{X}$=9,911	$\bar{X}$=10,670	$\bar{X}$=11,350	$\bar{X}$=11,290
SD=0.575	SD=110.30	SD=134.975	SD=171.248	SD=111.38	SD=208.598
SE=0.26	SE=49.33	SE=60.36	SE=77.08	SE=49.81	SE=93.29

Table IA: This table shows the leukocyte count prior to the injection of methotrexate based on 5 tests; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

LEUKOCYTE COUNT

Experimental

1A	1B	2A	2B	3A	3B
5,600	5,900	8,300	6,400	5,850	6,300
5,450	5,950	7,500	6,350	5,450	6,100
5,850	5,500	7,100	6,200	5,750	6,200
5,450	5,700	7,450	6,550	5,600	6,050
5,600	6,400	7,150	6,550	5,650	6,050
27,950	29,450	37,500	32,050	28,300	30,700
$\bar{X}$=5,590	$\bar{X}$=5,890	$\bar{X}$=7,500	$\bar{X}$=6,410	$\bar{X}$=5,660	$\bar{X}$=6,140
SD=208.075	SD=168.075	SD=22,558	SD=73.74	SD=75.84	SD=52.919
SE=96.00	SE=75.16	SE=10.88	SE=32.98	SE=33.96	SE=23.71

Table IB: An analysis of the leukocyte count after the injection of methotrexate based on 5 tests; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

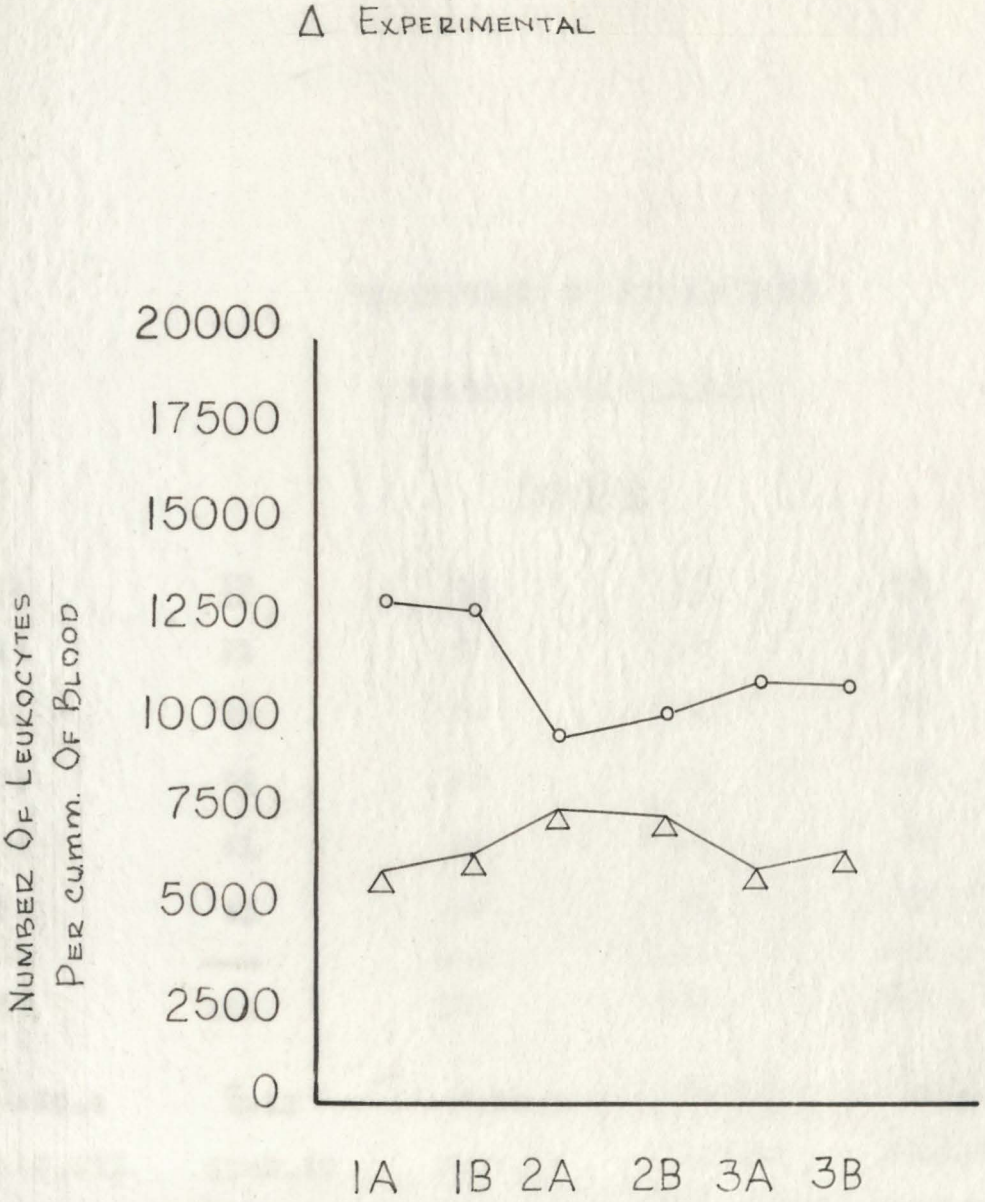

Figure 1: The mean leukocyte counts prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

PERCENTAGE OF NEUTROPHILS

(Differential Count)

Control

1A	1B	2A	2B	3A	3B
32	31	29	40	29	38
31	33	34	42	32	31
31	32	31	38	28	32
30	31	30	36	30	34
29	33	33	40	30	34
153	160	157	196	149	169
$\bar{X}$=30.6	$\bar{X}$=32	$\bar{X}$=31.4	$\bar{X}$=39.2	$\bar{X}$=29.8	$\bar{X}$=33.8
SD=0.575	SD=0.50	SD=4.14	SD=0.82	SD=0.742	SD=1.34
SE=0.26	SE=0.22	SE=1.85	SE=0.32	SE=0.33	SE=0.59

Table IIA: This table shows the differential count of neutrophils prior to the injection of methotrexate based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

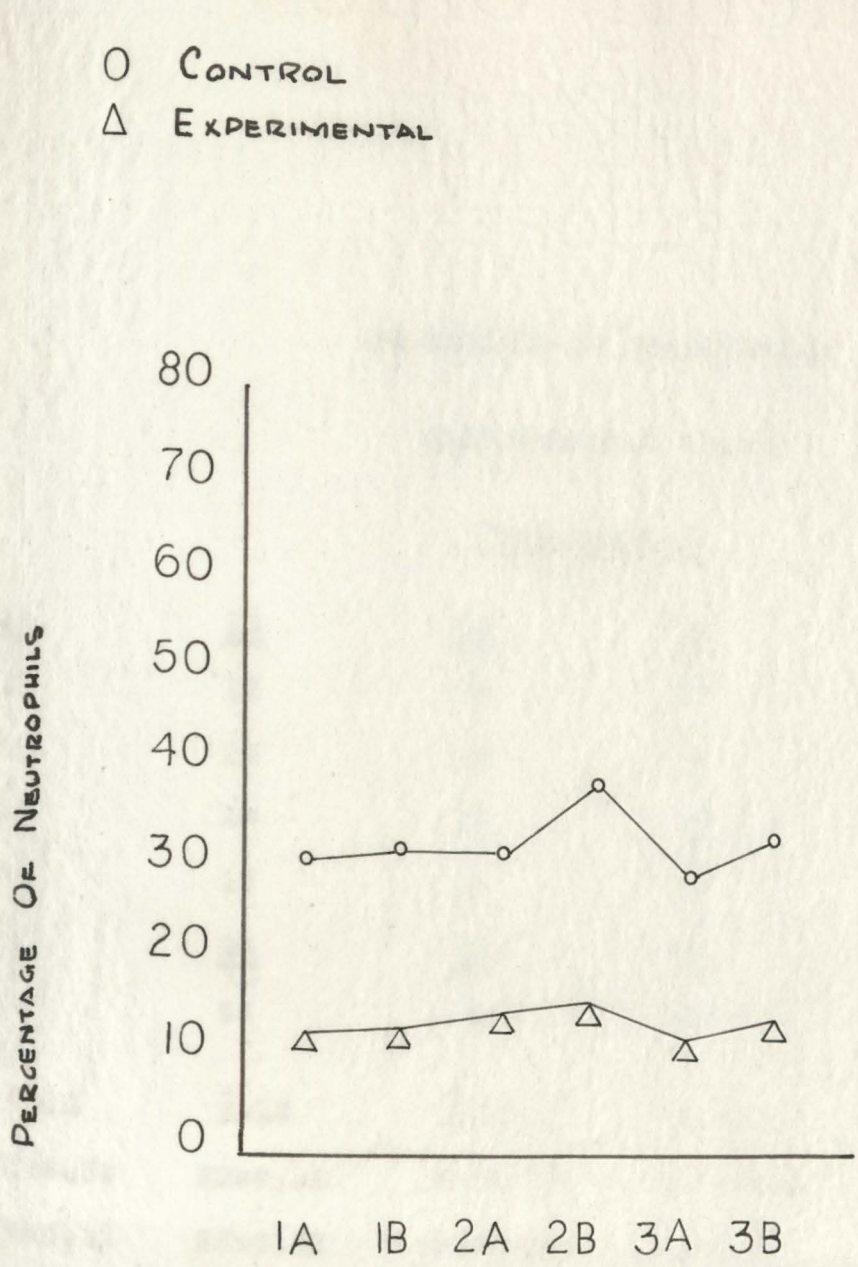

Figure 2: The mean neutrophil counts prior to and following the injections of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

PERCENTAGE OF NEUTROPHILS

(Differential Count)

Experimental

1A	1B	2A	2B	3A	3B
14	12	14	17	12	15
12	12	16	16	14	16
13	14	17	18	12	14
14	13	16	16	14	14
12	14	15	15	12	15
65	65	78	82	64	74
$\bar{X}$=13	$\bar{X}$=13	$\bar{X}$=15.6	$\bar{X}$=16.4	$\bar{X}$=12.8	$\bar{X}$=14.8
SD=0.50	SD=0.50	SD=0.575	SD=0.575	SD=1.88	SD=0.418
SE=0.22	SE=0.22	SE=0.26	SE=0.26	SE=0.84	SE=0.18

Table IIB: This table shows the differential count of neutrophils after the injection of methotrexate based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

PERCENTAGE OF LYMPHOCYTES

(Differential Count)

Control

1A	1B	2A	2B	3A	3B
63	65	69	60	69	61
69	66	60	59	66	68
69	67	67	62	72	67
67	64	69	64	69	65
67	68	65	60	68	64
335	330	330	305	344	325
$\bar{X}$=67	$\bar{X}$=66	$\bar{X}$=66	$\bar{X}$=61	$\bar{X}$=68.8	$\bar{X}$=65
SD=1.23	SD=0.79	SD=1.871	SD=1.0	SD=1.08	SD=1.118
SE=.55	SE=0.35	SE=0.84	SE=0.50	SE=0.48	SE=0.50

Table IIIA: This table shows the lymphocyte count prior to the injection of methotrexate based on 5 tests; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

PERCENTAGE OF LYMPHOCYTES

(Differential Count)

Experimental

1A	1B	2A	2B	3A	3B
38	40	42	50	34	45
36	38	45	46	32	43
37	42	44	49	32	47
35	40	40	47	30	45
36	37	41	49	32	46
182	197	212	241	160	226
$\bar{X}$=36.4	$\bar{X}$=39.4	$\bar{X}$=42.4	$\bar{X}$=48.2	$\bar{X}$=32	$\bar{X}$=45.2
SD=2.28	SD=3.89	SD=1.035	SD=0.82	SD=0.707	SD=0.742
SE=1.02	SE=1.74	SE=0.46	SE=0.31	SE=0.31	SE=0.33

Table IIIB: This table shows the lymphocyte count after the injection of methotrexate based on 5 tests; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation, SE=standard error.)

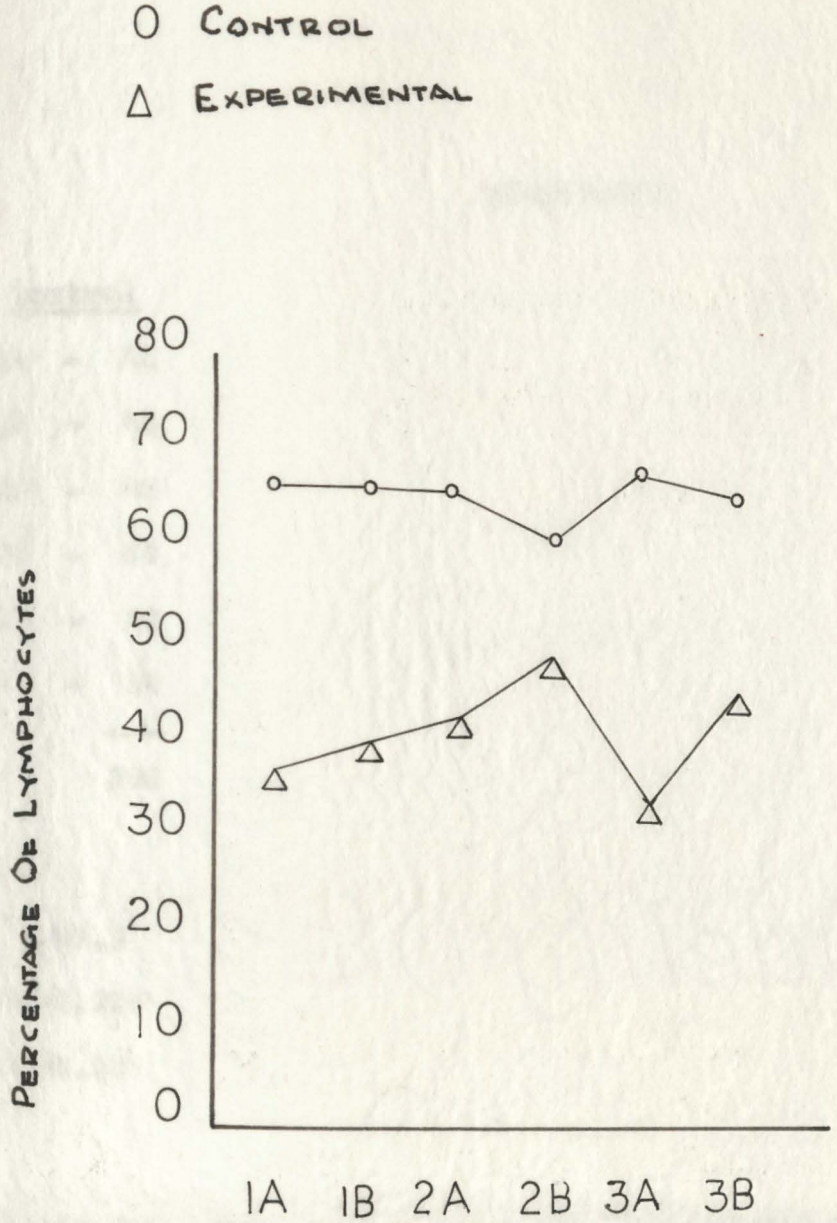

Figure 3: The mean lymphocyte counts prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

HEMATOCRIT

Control		Experimental	
1A	51	1A	46
1B	46	1B	45
2A	49	2A	49
2B	50	2B	50
3A	50	3A	46
3B	50	3B	48
	296		284
$\bar{X}$	49.3	$\bar{X}$	47.3
SD	0.800	SD	1.166
SE	0.33	SE	0.68

Table IV: This table shows the mean volume of packed red blood cells prior to and following the injection of methotrexate; each count based on the average of 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

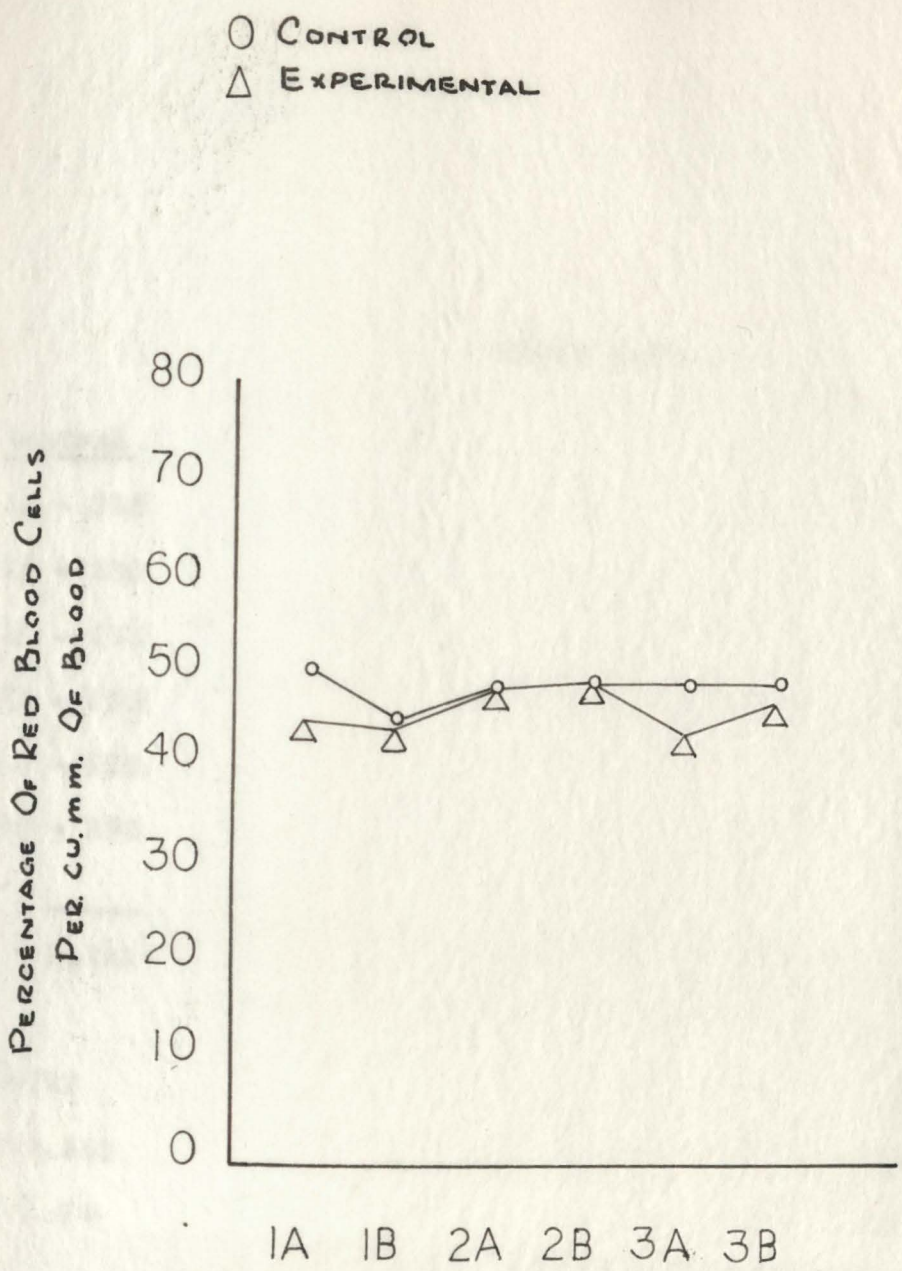

Figure 4: The mean blood volume counts prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

HEART RATE

Control	Experimental
1A - 265	1A - 275
1B - 280	1B - 290
2A - 278	2A - 290
2B - 270	2B - 285
3A - 270	3A - 295
3B - 270	3B - 290
1,633	1,725
$\bar{X}$=272	$\bar{X}$=287.5
SD=2.536	SD=3.085
SE=1.04	SE=1.28

Table V: This table shows the mean heart rate prior to and following the injection of methotrexate; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

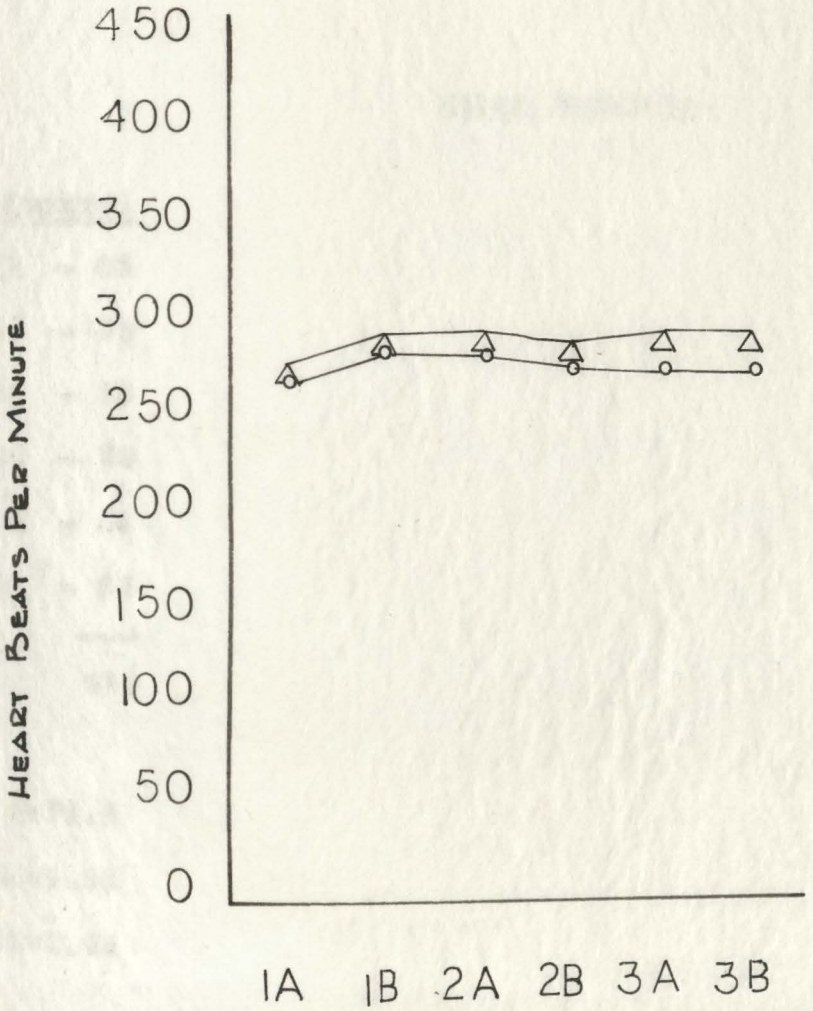

Figure 5: The mean heart rate prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

BLOOD PRESSURE

Control		Experimental	
1A	85	1A	87
1B	89	1B	123
2A	53	2A	101
2B	88	2B	95
3A	80	3A	104
3B	65	3B	86
	460		596
$\bar{X}$	76.6	$\bar{X}$	99.33
SD	6.50	SD	6.064
SE	2.05	SE	2.48

Table VI: This table shows the mean systolic blood pressure prior to and following the injection of methotrexate; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

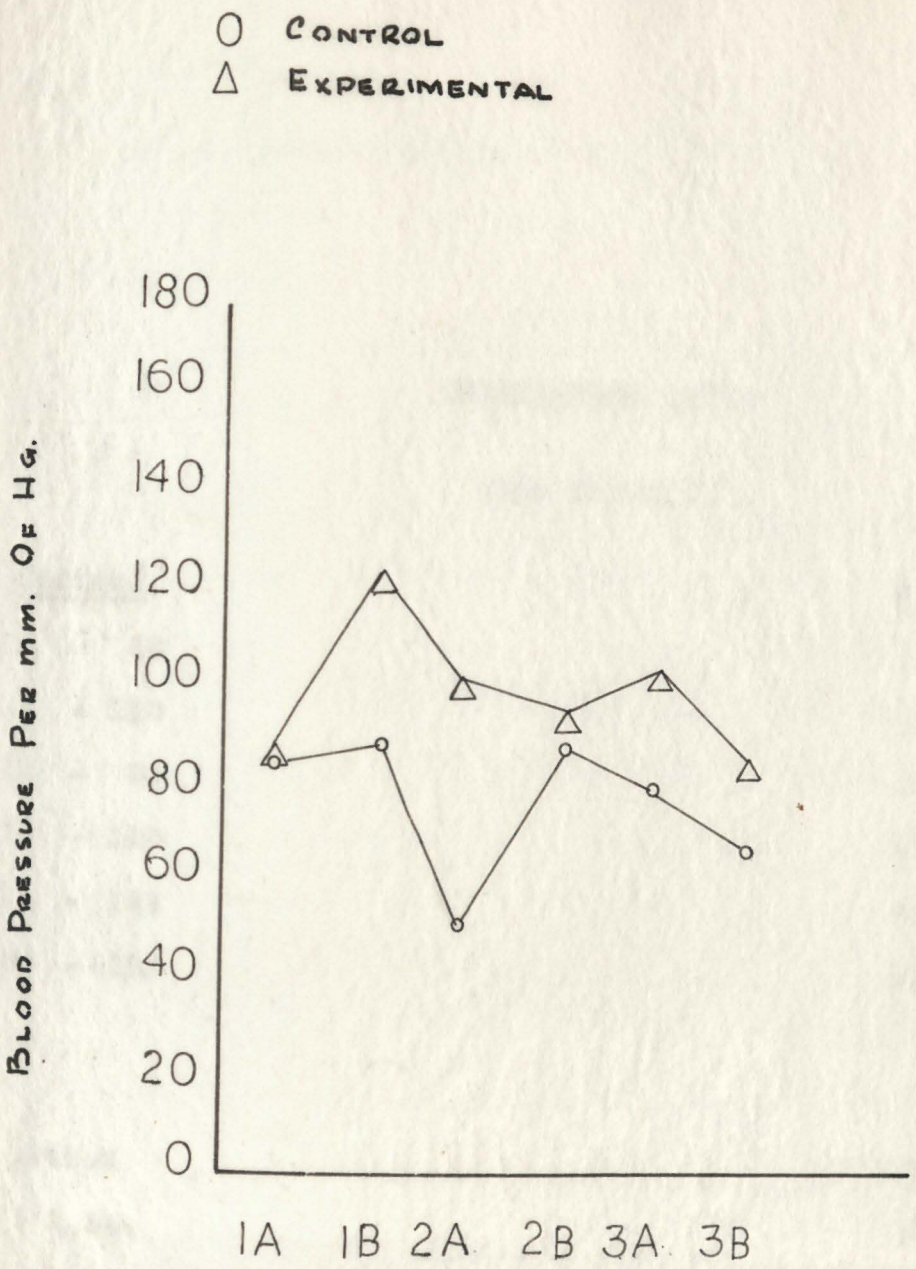

Figure 6: The mean systolic blood pressure prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

RESPIRATION RATE

(Per Minute)

Control		Experimental	
1A	80	1A	130
1B	100	1B	125
2A	80	2A	125
2B	110	2B	135
3A	108	3A	135
3B	120	3B	140
$\bar{X}$	99.6	$\bar{X}$	131.75
SD	7.384	SD	2.717
SE	3.02	SE	1.11

Table VII: This table shows the mean respiratory rate prior to and following the injection of methotrexate; (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals; $\bar{X}$=mean, SD=standard deviation and SE=standard error.)

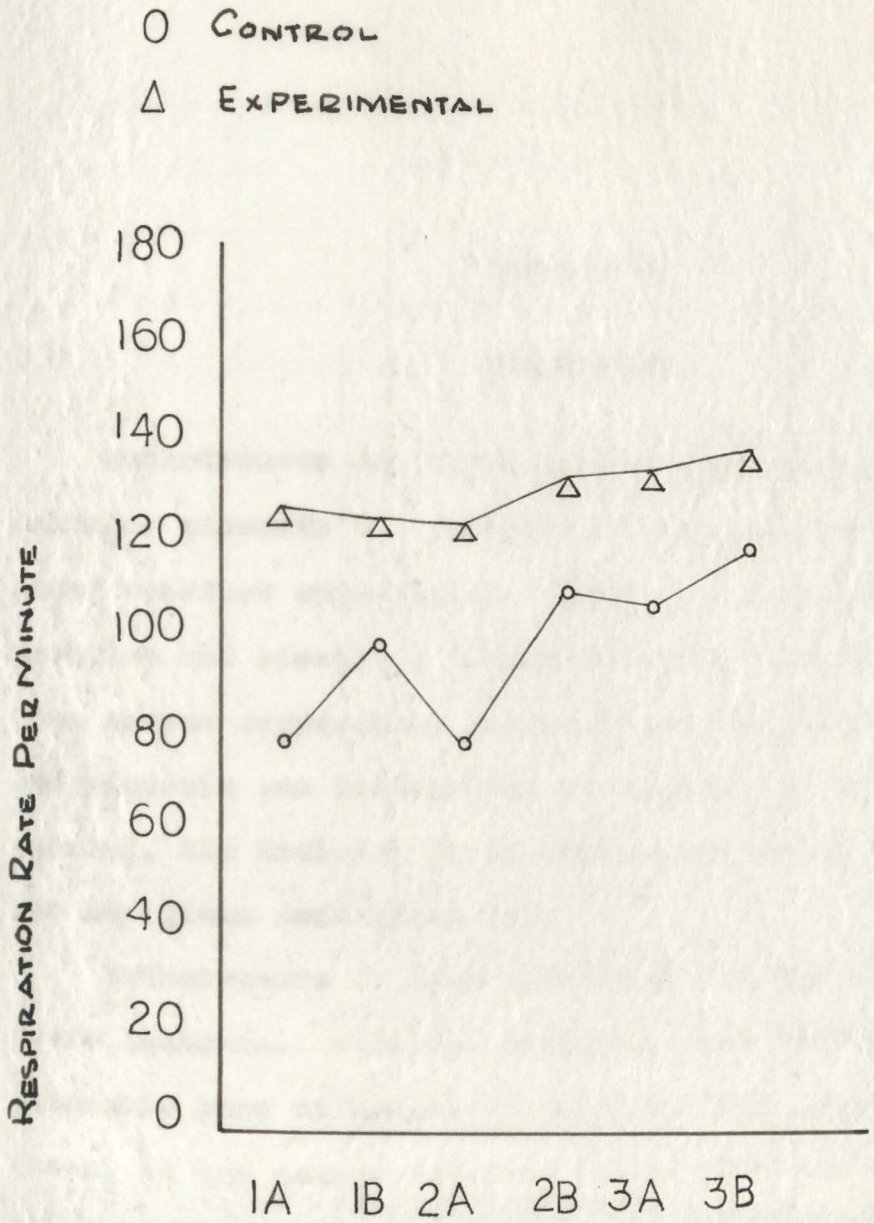

Figure 7: The mean respiratory rate prior to and following the injection of methotrexate; each curve based on 5 tests. (1A, 1B, 2A, 2B, 3A, and 3B represent the identification of the animals.)

CHAPTER IV

DISCUSSION

Methotrexate may cause severe depression of all blood cellular elements and patients undergoing therapy should be under constant supervision. Signs of gastrointestinal ulceration and bleeding, including bleeding from the mouth; bone marrow depression, primarily of the white cell series, and alopecia are indications of toxicity of this drug. In general, the toxicity is in direct proportion to the dose for any given individual (4).

Methotrexate is used clinically for the treatment of acute leukemia. Clinical evidence shows that it is considerably more effective in children than adults. In some cases, it has caused clinical improvement and increased survival time in acute leukemia for periods varying from weeks to two years. The hematological picture, as reflected in blood and bone marrow films after methotrexate therapy, may become almost indistinguishable from normal for varying periods of time. Greatest effect has been observed in acute leukemia characterized by "blast" forms in bone marrow or blood (4).

The current study has demonstrated that methotrexate can cause a decrease in leukocytes, lymphocytes, neutrophils and erythrocytes.

Despite the many pharmacologic studies of methotrexate during the last 12 years, many basic properties have not been clearly delineated. Absorption from the gastrointestional tract has been said to be complete (12) and incomplete (12). Estimates of the extent, rate, and route of excretion of methotrexate have varied widely (12). Finally, the toxic and therapeutic differences between this compound and closely related anti-folics, have not been adequately explained (12). The differences among the results of various studies on absorption, blood levels, and excretion of methotrexate appear to have resulted chiefly through differences in techniques of measurement of the drug and the chemical purity of the drug employed.

It has been previously pointed out that for combination therapy, as for single drug treatment, there is an optimal treatment level, and that the maximum effectiveness of the combination may be modified by factors such as treatment schedule and the ratio of doses employed (8).

In the present experiments both the enhanced toxicity specificity and the wider effective dose range resulting from treatment are dependent on the methotrexate dosage ratio. Maximum antileukemic effectiveness was generally attained with combination of 1.25 mg. of methotrexate. Therapeutically,

a specific dosage level which, when used alone, was less toxic for normal rats than for leukemic rats. It is of interest that such combinations appeared to produce little additive toxicity in either normal or leukemic rats (8).

Potentiation of the activity of methotrexate against experimental leukemic mice has been observed with a number of compounds including halogenated pyrimidines and their ribonuleosides, stilbomidine, and triparanol (14). The clinical usefulness of methotrexate has been reported to be limited by the development of cumulative neurological toxicity during therapy (11). The current data suggest that methotrexate may be more useful when employed at relatively low dosage levels in combination with other agents.

The current data further emphasize the necessity for employing a wide range of doses and dosages ratios in the quantitative evaluation of the relative effectiveness of single drug and combination therapy.

One of the most significant advances in cancer chemotherapy was regional perfusion, a technique in which an anti-cancer drug is introduced into the blood vessels while the circulation of the drug to the remainder of the body is prevented (9). In the Lederle Laboratory Report on Cancer, the perfusion technique, employing methotrexate, had been of value in the treatment of patients whose tumors were incurable by other means (8).

The testing procedure with the experimental drug, methotrexate, was administered to six albino rats. The drug, moreover,

was quite toxic, and it exerted its anti-metabolite influence on the normal blood cells.

It has been reported (19) that some antimetabolites will create a deficiency of the citrovorum factor which will indirectly inhibit the growth of certain white blood cells. This is possibly one of the reasons for the reduced white blood cell count in animals treated with methotrexate, since it is one of the folic acid antagonists. The findings observed in ths study also depicted a reduction of lymphocytes in the blood smear of the treated animals. Burchenal (1) reported a total of 30% or less of "blast" cells and lymphocytes in the marrow of leukemia patients during remissions following treatments with methotrexate. The administration of most antimetabolites is associated with a "megaloblastic" marrow, leukopenia and thrombobytopenia (2).

There are great numbers of factors that may account for the increase in blood pressure observed in animals following methotrexate injections. An increased heat frequency, which was observed in the investigation has been reported as a cause of mild hypertension (19). The reduction in lumen of the blood vessels is another probable cause of the increased pressure. Reduction in lumen size, which causes increased resistance of arterioles due to spasms of contraction, occurs functionally in emotional states, in exercise and as a result of nerve strain (19). The methotrexate may have interfered with the reflex centers which normally counteracts the

action of the constrictor fibers, thereby, producing an increase in blood pressure. Still another explanation is that an increased volume of circulating blood due to sympathetic stimulation will cause the spleen to contract thereby producing a rise in blood pressure. The reduced hematocrits observed in this study clearly show the reduced viscosity of the blood of the experimental animal, consequently, it is strongly postulated that the increase in blood pressure is due to a neural origin rather than plethora.

Folic acid is one of the vitamins which has long been known to play an important role in the maturation of erythorocytes (13). Methotrexate is one of the antifolic agents. The interference with normal action of folic acid could account for hematocrit reduction in the methotrexate treated animal.

The respiratory analysis was another parameter observed in this investigation. There was a corresponding increase in the respiratory rate which may or may not be due to a decrease in the quantity of oxygen and oxidases. Blood transports oxygen in two of its components, plasma and hemoglobin, of the erythrocytes. The amount of oxygen which by nature of its partial pressure, can be forced into a solution of water or plasma is little more than 0.3 volumes per cent (19). The amount of oxygen that can be dissolved in plasma would not be sufficient for an animal, like the albino rat. With a reduction in erythrocytes, in the

treated animal, it is possible that an insufficient amount of oxygen was available which caused an oxygen debt. To compensate for this lack of oxygen, the respiratory rate was increased. It can be postulated that methotrexate has an indirect effect on the respiratory center. Since the drug interferes with the oxygen content and the oxidase system of the body, one can assume that it mobilizes the carbon dioxide in the body to stimulate the aortic and carotid sinus reflexes thereby producing a hypernea. It is also physiologically probable that the increased carbon dioxide stimulates the chemoreceptors in the body and produces a reflex stimulation of the respiratory center.

It is apparent that additional studies must be made in order to fully understand all of the effects produced by this drug, consequently, the investigator is not at liberty to make any definite commitments as to why there was such a widespread variation in the various metabolic responses utilizing methotrexate.

CHAPTER V

SUMMARY AND CONCLUSION

Intramuscular injections of methotrexate were administered in 1.25 mg./kilogram of body weight dosages. The effects of the drug administered were as follows:

1. Leukocyte count in the control animals ranged from 9.9×10^3 per cu. mm. to 1.2×10^4 per cu. mm. with a mean of 1.1×10^4 per cu. mm. The count in the experimental animals ranged from 5.6×10^3 per cu. mm. to 7.5×10^3 per cu. mm. with a mean of 6.2×10^3 per cu. mm. A significant decrease of 45% below the control count was shown.

2. Neutrophil count in the control animals ranged from 29.8% per cu. mm. to 39.2% per cu. mm. with a mean of 32.8% per cu. mm. The count in the experimental animals showed a range of 13% per cu. mm. to 16.4% per cu. mm. A significant decrease of 56% per cu. mm. was shown.

3. Lymphocyte count in the control animals ranged from 61% per cu. mm. to 68.8% per cu. mm. with a mean of 65.5% per cu. mm. The count in the experimental animals ranged from 32% per cu. mm. to 48.2% per cu. mm. with a mean of 40.6% per cu. mm. A decrease of 23% per cu. mm. was shown.

4. Hematocrit count in the control animals ranged from 46% per cu. mm. to 51% per cu. mm. with a mean of 49.3% per

cu. mm. The count in the experimental animals ranged from 45% per cu. mm. to 50% per cu. mm. with a mean of 47.3% per cu. mm., showing an insignificant decrease of 4% per cu.mm.

5. The heart rate count in the control animals showed an average normal range of 265 to 280 beats per minute with a mean of 272 beats and the count in the experimental animals ranged from 275 to 295 beats per minute with a mean of 287.5 beats. An increase of 5% was shown which was not significant.

6. The blood pressure count in the control in the animals ranged from 53 to 89 mm. Hg. with a mean of 76.6 mm. Hg. The count in the experimental animals showed a range from 86 to 123 mm. Hg. with a mean of 99.33 mm. Hg. A significant increase was shown to be 30%.

7. The respiratory rate count in the control animals ranged from 80 to 120 times per minute with a mean of 99.6 and the count in the experimental animals ranged from 125 to 140 times per minute, with a mean of 131.81. A significant increase of 32% was shown.

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