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Scope and Method of Study: Purebred Beagle bitches were irradiated in the third trimester of pregnancy to the pelvic area, and after birth the pups were bled weekly up until twelve weeks of age and hemograms recorded. An effort was made to determine any temporary or permanent deviation in the hemograms among dosed groups and controls.

Conclusions and Summary: The $LD_{50/30}$ for the treated pups at these X-ray characteristics (100 KVp, 10 ma, 1/2 mm equivalent inherent filtration, 2 1/2 mm Al filtration, focal point to mid-tissue animal 53.5 cm.) was between 200 and 300 roentgens. Below this $LD_{50/30}$ dose, the hemograms showed a slight temporary white blood count decrease with abnormal cells, but no detectable change in the red cell indices. These changes were back to normal by the end of twelve weeks. Above the $LD_{50/30}$, the white blood cell count decreased, with more severe cellular abnormality, and the red cell indices indicated hypoplastic anemia. By the end of twelve weeks, even these dogs approached normal. This indicates a time-recovery factor for these pups irradiated *in utero* of these particular X-ray characteristics.

ADVISER'S APPROVAL L. Herbert Bruneau

HEMATOLOGICAL CHANGES IN BEAGLE PUPS X-IRRADIATED *IN UTERO* IN THE THIRD TRIMESTER OF GESTATION

By

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IN UTERO IN THE THIRD TRIMESTER OF GESTATION

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PREFACE

This research on irradiation of Beagle pups was done by me while working as a Medical Biologist for the Research Division of the United States Public Health Service in Montgomery, Alabama, in 1961-62. This research was under the direction of Dr. Paul D. Lamburt, M. S., D. V. M., now with the United States Public Health Service in Fort Collins, Colorado. The Research Division was under the direction of Mr. William A. Mills, now of the United States Public Health Service, Rockville, Maryland.

I am deeply indebted to many people for the completion of this report. I wish especially to thank Dr. Paul D. Lamburt, United States Public Health Service, who proposed and directed this study. My sincere appreciation goes to members of my committee, especially my major advisor, Dr. L. H. Bruneau, and to Dr. R. W. Jones.

My sincere appreciation goes to my father, E. D. Garrett, without whose encouragement, both moral and financial, this report would never have been written

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The fact that radiation can be harmful has long been recognized. Senn (1903) showed that X-rays could produce variations in the blood forming tissues.

> "That the subject still has its fascination and, indeed, its problems, is clearly demonstrated by the present series of papers. They also demonstrate how many facets there are to radiation hematology, and how many techniques may be used." Hulse, (1962).

The importance of the knowledge of the involuntary irradiation damage to the fetus in the case of dosage to the mother has increased with the use of medical irradiation treatment. The more striking results in experimental animals seem to be from heavy dosages in the early stages of pregnancy in the form of absorbed fetuses, malformations of the cranial region, or missing parts.

Much has been published on this subject. Langindorff, Kriegel, and Shibata (1962) reported the variation of radio-sensitivity with one day of gestation for abnormalities of the tail and limbs and for other visible gross abnormalities.

Russell (1954) also reported on these early abnormalities.

Less known is the damage done to the third trimester when the fetus is almost completed. The fetus gets a total body dose with unknown effects in the case of gastrointestinal X-ray investigations of the mother, and fetal X-rays to determine the fetal position and pelvic deminsions. In the face of the rising incidence of leukemia, investigation is being undertaken to determine, if possible, the correlation to these irradiation exposures to leukemia and other diseases. There is controversy of opinion as to low dosage harm to the fetus. Some recent studies done on children given total body *in utero* dose by pelvic irradiation to the mother both confirm and deny the harm. MacMahon (1962) reported an increase in damages, including leukemia, on a group followed to the age of six years. Kitchen (1961) reported no significant rise of damage over the control group in a similar experiment. These point out some of the problems which prompted this *in utero* project on dogs.

This report deals with the hematological studies done on Beagle pups irradiated *in utero* on the fifty-second day of gestation at doses varying from 0-400 roentgens of X-rays. These doses were chosen to include the control animals as well as those around the $LD_{50/30}$ and $LD_{100/30}$. Beagle dogs were chosen because of their docile nature and adaptability to laboratory research. Registered animals were used in an attempt to lessen the genetic variability supposedly found in mongrels and thereby help to stabilize the background.

It is the purpose of this report to discuss the methods used in this investigation and to compare the results over a twelve-week period of the 0, 100, 200, 300, and 400 roentgen doses. Very little investigation has been conducted in this area for comparison purposes; i.e., on the Beagle dog in the third trimester of gestation at these dose characteristics.

CHAPTER II

MATERIALS AND METHODS

Purebred Beagle bitches were irradiated partial body to the uterus on the fifty-second day of pregnancy with 100 KVP X-rays, and 10 ma. The X-rays produced by the unfiltered beam of the X-ray tube has only a 100 kilovolt potential, many of the beams being of lesser magnitude. In order to filter out these softer beams and to closer approach the 100 kilovolt potential, the X-ray machine had 1/2 mm equivalent inherent filtration; 2 1/2 mm Al filtration were also added. This total filtration brought the beam to a strength which was twice that which could be attenuated by 3.15 mm Al; the half-value layer. From the focal point to the mid-tissue of the animal was 53.5 cm, which delivered a dose rate of roughly about 19 roentgens per minute. This dose-rate and beam strength produced a lethal dose which killed fifty percent of our pups in thirty days ($LD_{50/30}$) of between 200 and 300 roentgens as shown in Table XXI in the Appendix.

The doses used were 0, 100, 200, 300, and 400r. The animals were restrained in a canvas sling without anesthesia or tranquilizers, the beam was coned, the lead shields were placed over the animals vertebrae, thereby assuring her of only partial body dose, and assuring radiation to the fetuses.

Periodic hemograms were taken of the pups, beginning one week after birth. The red and white blood cell determinations were done exclusively on the Coulter Counter, except in rare cases where the white cell count approached the leukopenic state. Sterile normal saline was used as the diluent, and a fresh aliquot was counted daily for background particles for the red blood count. For a white blood count background check, .1 cc of .1% Saponin was added to the saline.

The blood was collected from the juglar vein in a sterile, dry syringe, and the blood heparinized with the disodium salt of ethylene diaminetetraacetic acid, known commonly as EDTA.

The red blood counts were made on the Coulter Counter as described above. An aliquot of 20 lambda of the 1-50 dilution was added to 100 cc saline (1-50,000 dilution). The 1-50,000 dilution was placed on the stand and counted for red blood cells. A minimum of three readings on each sample was taken and averaged, the background count was subtracted, and the count corrected for coincidence counting by using the first three significant figures on the chart supplied by Coulter Electronics. Hemocytometer counts were made to check any abnormally low or high counts. Haymes solution was used as the diluent for the chamber counts. The results are shown in Table I and Table VIII in the Appendix.

The total white cell count was done on the Coulter Counter following the above procedure. A hemolytic agent of .1 ml of 1% Saponin was added. The sample was counted for white blood cells within twenty minutes. A minimum of three readings were taken, averaged, and the background count subtracted. The count was then corrected for coincidence counting using the chart as before. In case of an abnormally low or high count, it was checked on a hemocytometer using 5% Acetic Acid as a diluent and hemolytic agent. The results are shown in Tables I I and VII in the Appendix.

A dried blood film was stained with Wright's polychrome stain and examined for blood cell morphology.

The hemoglobin determination was made with the use of N/10 HCl as a hemolytic agent and a Hadenhauser hemoglobinometer.

The microhematocrit was done in a heparinized capellary tube spun three to five minutes in a microcentrifuge.

Red cell indices were figured by use of an adaptation of an outline by Hepler (1950). These use the hematocrit, hemoglobin and red blood count and the use of the canine "normal" of these values. The normal canine values for hemoglobin, hematocrit and red blood cell count were hard to establish. Schalm (1961) gives a list of fifteen different studies on "normal dogs", each with a different value for normal canine counts. Therefore, for the normal values, he averages these. These averages were used as normals for this study as shown in Table XXII in the appendix.

I. The hemoglobin content per erythrocyte was figured by use of the following:

A. <u>The Color Index (C.I.)</u>. This is the amount of hemoglobin in the average erythrocyte compared with the amount in a normal erythrocyte. These results are shown in the Appendix in Tables IX and X.

$$\frac{\dfrac{\text{experimental gms. Hemoglobin}}{\text{average gms. Hemoglobin}}}{\dfrac{\text{experimental Red Blood Count (in millions)}}{\text{average Red Blood Count (in millions)}}} = \text{C.I.}$$

B. The Mean Corpuscular Hemoglobin (M.C.H.). This is the weight in micromicrograms of the hemoglobin in the average erythrocyte. These results are shown in the Appendix in Tables XV and XVI.

$$\frac{\text{experimental Hemoglobin X 10}}{\text{experimental Red Blood Count (in millions)}} = \text{M.C.H.}$$

II. The volume of erythrocytes was figured by the following:

A. The hematocrit, which was obtained as previously described above. These results are shown in the Appendix in Tables III and VI.

B. The Volume Index (V.I.). This indicates the mean volume of the average erythrocyte compared with the mean volume of the normal erythrocyte. These results are shown in Appendix Tables XI and XII.

$$\frac{\dfrac{\text{experimental hematocrit}}{\text{average hematocrit}}}{\dfrac{\text{experimental Red Blood Count (in millions)}}{\text{average Red Blood Count}}} = \text{V.I.}$$

C. The Mean Corpuscular Volume (M.C.V.). This is the mean volume in cubic microns of the average erythrocyte. These results are shown in the Appendix in Tables XIII and XIV.

$$\frac{\text{experimental hematocrit X 10}}{\text{experimental Red Blood Count (in millions)}} = \text{M.C.V.}$$

III. The hemoglobin per unit volume was figured by the following:

A. The Saturation Index (S.I.). This indicates the concentration of hemoglobin per unit volume of erythrocytes relative to normal. These results are shown in the Appendix in Tables XIX and XX.

$$\frac{\dfrac{\text{experimental Hemoglobin (in gms)}}{\text{average Hemoglobin (in gms.)}}}{\dfrac{\text{experimental Hematocrit}}{\text{average hematocrit}}} = \text{S.I.}$$

B. <u>Mean Corpuscular Hemoglobin Concentration (M.C.H.C.)</u> in percent. This is the concentration of hemoglobin in the average erythrocyte; the ratio of the weight of hemoglobin to the volume in which it is contained. These results are shown in the Appendix in Tables XVI and XVII.

$$\frac{\text{experimental Hemoglobin (in gms.) X 100}}{\text{experimental Hematocrit}} = \text{M.C.H.C.}$$

CHAPTER III

DISCUSSION AND RESULTS

Ionizing radiation works by the formation of ion pairs. The electron from the radiation source hits the outer shell of an atom and knocks out an electron, forming a negative ion. This displaced electron goes into the shell of another atom forming a positive ion. This is known as an ion pair, and is illustrated by the ionization of water.

$$H_2O - e = H_2O^+$$

$$H_2O + e = H_2O^-$$

(Gorizontan, 1959)

Radiation damage is theorized to be either from a direct hit on the cell, or from the ionization of water, which makes up the greater part of the body. This ionized water makes normal metabolic processes impossible due to biochemical changes.

The most radio-sensitive cells are those in the most rapid mitotic cycles, which would be the undifferentiated cells mainly of the lymphoid tissue and bone marrow in the formed body, or the rapidly dividing embrological tissues of the fetus. The blood picture forms the first detectable change in the case of radiation damage.

In embryology, every tissue has a prime time in its development for being most radio-sensitive. If a dose is given at this time, the tissue is damaged or destroyed, depending on the dose. In this study the concern was on the radio-sensitivity of the blood after most fetal formation had occurred; in the third trimester of gestation. This was to relate the time of dosage to that given to women in late pregnancy.

The dosage to the pups was hard to define; the dosage to mid-tissue of the mother is the only guide. Other factors such as shielding of one pup by another could have occurred, but this was taken into consideration and the doses divided into two equal phases, one to each side of the mother.

Published results of blood studies of in utero radiated pups are hard to find. The results given here do not necessarily conform to the studies of pups radiated after birth. The first two weeks after radiation were not monitored because the count was not taken until the pups were one week of age, so the one week count was actually two weeks after radiation. Many of the pups had died, further evidencing the critical damage in that two-week period. Due to this fact, that there were no true replications, it was felt that statistical studies would be invalid.

Lapteva-Popava, 1959, made a study of hematalogical changes in irradiated dogs. He concluded that the radiation sickness was divided into four phases, not necessarily in sequence in all dogs:

1. Initial reaction - lability of hematogenesis;
2. Supression of hematopoietic function;
3. Intensified regeneration;
4. Leukemia and final determination of blood.

The fact that two dogs given the same dose under identical conditions had death rates months apart was explained to be due to an inherent biological resistence in the individual dog. This inherent biological resistence determined what phase of radiation sickness the dog was in at any given time.

"In the period of primary reactions, the quantative composition of the peripheral blood is extremely unstable." (Laptiva-Popova, 1959).

This is quite similar to the phases given in the X-ray Handbook of the United States Public Health Service, 1961, which gives these phases:

1. Latent period - almost any length dependent upon dose and inherent biological resistence;
2. Period of demonstratable effects on cells and tissues;
3. Recovery period (where applicable).

The results obtained from this study indicate biological variation in the pups and their hemocytological responses. Some of this variation may be due to stress caused by operative manipulation. In the treated animals, the degradation of red blood cells was demonstrated by the following, seen on the dried blood film stained with Wright's polychrome stain:

1. Phagocytosis of whole cell or parts of broken cells by mononuclear white blood cells;
2. Microcytes (blood dust) as by products of fragmentation process;
3. Red blood cell precusor cells in circulating blood (prorubricyte, rubricyte, metarubricyte);
4. Polychromatophilia;
5. Target cells (ratio of surface area to volume is increased over normal cells);
6. Anisocytosis (mixed sizes);
7. Reduced platelet number;
8. Increased platelet size.

The anomolies found in the white blood cells were as follows:

1. In the $LD_{50/30}$ survivors, a temporary decrease in number, followed by a recovery that approached that of the control animals. In the $LD_{50/30}$ death animals, a decrease in numbers that in some animals approached extinction; (Table XXI in Appendix).
2. A gradient in the nuclearcytoplasmic growth ratio;
3. Azurophilic granules;
4. Increased number of basophiles;
5. Binucleated cells;
6. Cells in mitosis found in circulating blood which indicates premature expulsion from bone marrow or lymph-forming system;
7. Karolysis of nucleus, and various other states of cellular degeneration;
8. Phagocytic mononuclear cells indicating dead cell debris in the blood stream;
9. Unidentified cells which indicate either a malfunction of the growth process of the cell, or partial degeneration which defies cellular identification.

The red cell indices which were run and shown in the Appendix Tables IX-XX, indicate a hypoplastic or aplastic anemia in the animals. The granulocytes and thrombocytes were also temporarily reduced.

There seems to be a recovery factor in the blood system of surviving pups irradiated in utero at these doses, which is evident at the end of twelve weeks, at which time this study was terminated.

In comparing the groups of 0, 100, 200, 300, and 400r, the most obvious thing was the lack of information on the 400r group. The dog counted before death had a white blood count of 150, which were all degenerated cells. The animal was hemoconcentrated, so the values for the red cell indices were of a false high. This dose is above the $LD_{50/30}$ and these results were expected.

The survivors of the 300r group showed a very slight difference in the white blood cell count, but the types of cells had a higher number of abnormal cells as described earlier in this report than the lower dose groups. This may mean that the low peak of white cell count was reached before the pup was born. The red cell indices indicated a low peak at three weeks count, as opposed to a later peak at lower doses.

The 100 and 200r groups (below the $LD_{50/30}$) were essentially the same. The white blood cell count of the 200r indicated a low at two-weeks count, which may be due to lack of animal numbers. The red cell indices indicate a low peak at four weeks, as opposed to an earlier low peak at higher doses. The control animal indicated a low peak of red cell indices over a twenty-four-week-count period.

It must be noted here that at the twelve-week count, the differences were not evident, which indicates a recovery factor by this time.

CHAPTER V

SUMMARY AND CONCLUSIONS

Purebred Beagle bitches were irradiated on the fifty-second day of gestation to the uterine area. Beginning at one week of age (two weeks after birth), periodic hemograms were run until the pups were twelve weeks of age. Red cell indices were calculated on these results.

The doses of X-ray were approximately 0, 100, 200, 300 and 400r, which include the $LD_{50/30}$ at 200r (Table XXI in Appendix). The pups which were monitored one to three days before death showed a hemoconcentration, low white blood count, and decreased platelets. The pups which survived showed a temporary decrease in the white blood count, which had been corrected by the end of twelve weeks. The red cell indices indicated a hypoplastic or aplastic anemia, with reduced granulocytes and thrombocytes, which was not evident by the end of the twelve-week period.

This indicates a recovery factor in the survivors of *in utero* radiated animals with X-rays at these doses and characteristics.

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APPENDIX

TABLE I

CONTROL BEAGLES RED BLOOD COUNT IN MILLIONS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
C-1 RBC	-	-	4.07	3.65	3.53	3.94	4.48	5.18	5.20	4.91	5.18	5.52
2	-	3.79	3.62	3.87	3.50	3.96	5.16	5.19	5.12	4.36	5.20	5.48
3	-	3.60	3.54	4.19	3.82	4.32	4.27	5.14	5.02	5.01	4.72	5.12
4	-	3.39	2.76	3.83	3.50	3.98	5.30	4.55	5.14	4.18	4.77	5.34
5	-	4.10	4.06	3.96	4.09	4.28	4.05	4.83	4.92	-	5.36	6.02
6	-	3.89	3.80	3.84	4.27	2.68	5.11	4.66	4.72	4.32	4.96	5.41
7	4.18	3.48	3.16	3.70	-	-	5.04	4.32	4.40	4.74	5.02	4.99
8	4.72	3.58	3.52	-	-	-	4.41	4.66	4.87	5.58	5.46	5.15
9	4.41	3.38	3.41	4.72	-	-	4.57	5.05	4.49	5.69	5.42	5.29
10	2.83	3.10	3.45	3.75	3.00	4.07	4.15	-	5.25	4.82	5.32	5.41
11	3.61	3.07	3.37	3.92	3.21	4.26	4.00	-	4.76	4.77	4.82	5.60
12	2.46	3.54	3.75	4.10	4.18	4.71	4.86	-	5.52	5.40	5.38	5.88
13	-	3.16	4.32	3.41	3.43	4.51	5.26	-	5.09	5.41	5.46	5.88

TABLE II

CONTROL BEAGLES WHITE BLOOD COUNT IN THOUSANDS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
C-1 WBC	9.2	-	13.3	14.3	13.9	14.2	18.6	15.0	11.0	16.6	34.1	12.6
2	13.9	12.9	10.1	14.8	15.5	15.6	17.9	18.1	9.6	15.3	19.8	13.3
3	31.4	12.3	14.4	21.4	18.7	21.1	25.2	18.3	9.2	12.4	22.5	13.1
4	9.8	10.6	10.2	14.1	14.0	16.4	19.0	15.3	10.4	12.4	14.3	13.7
5	9.0	10.1	9.2	13.4	12.5	18.0	17.9	13.9	7.5	-	12.5	9.8
6	10.4	10.2	9.0	15.6	14.1	12.2	21.6	13.0	12.6	15.7	16.2	13.2
7	5.2	-	14.8	12.1	-	-	17.5	13.8	9.3	11.8	16.0	12.9
8	9.9	-	11.9	14.4	-	-	14.8	17.6	12.2	16.8	12.5	14.0
9	14.8	-	14.1	16.5	-	-	14.1	18.6	12.7	12.3	13.3	14.2
10	10.8	8.6	12.1	8.9	11.9	8.3	-	-	10.6	10.3	11.2	12.2
11	13.6	11.1	12.2	12.4	14.4	18.1	-	-	12.5	13.2	13.8	18.3
12	9.1	10.6	10.4	9.9	14.3	9.8	-	-	10.4	13.6	12.4	13.9
13	-	7.4	12.9	12.1	11.6	13.6	-	-	13.8	13.3	12.1	13.6

TABLE III

CONTROL BEAGLES HEMATOCRIT IN PERCENT

Dog	Weeks of Age 1	2	3	4	5	6	7	8	9	10	11	12
C-1 HCT	40	-	37	29	30	33.8	36	39	37	40	40	37
2	45	35	33	33	29	28	30	33.5	33.5	33	35	38
3	43.5	32	34	33	33	33.5	30	41	35	-	35	35
4	38	30	30	28	29	31	31	35	35	-	33	39
5	43.5	32	35	28	30	26.5	33	33	35	-	33	38
6	38.5	35	35	33.5	33	32	40	34.5	35	32	35	36
7	42	31.5	30	32	-	-	40	36	34	39	43	38
8	43	31.5	33	35	-	-	39	36	35	40	41	40
9	41	31	31	36	-	-	37	36	36	42	43	38
10	34	35	31	25	28	33	31	-	36.5	37	36	39
11	38	35	31	29	29	31	25	-	37.5	35.5	37	36
12	30	31	30	30	30	31	32	-	36	35	36	36
13	-	32	34	28	28	31	34	-	36	33	35	35

TABLE IV

CONTROL BEAGLES HEMOGLOBIN IN GRAMS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
C-1 HGH	-	-	11	8.5	8.5	8.5	8.5	11	11	12	10	11
2	-	11	9	10	8.5	8.5	8.5	9.5	9.5	9.5	8.5	14
3	-	9	10	10.5	9.5	8.5	10	12	11	11	10	11
4	-	9	8.5	9.5	8.5	8.5	8.5	9.5	10.5	9	11	11
5	-	10	10.5	9	9	8.5	9	10	10.5	-	11	12
6	-	11	10	10	10	8.5	10.5	11	10	9	10	11
7	11.5	9	9	8.5	-	-	11	9.5	10	10	11	11
8	12.5	9.5	9.0	10.0	-	-	11	9.5	10	11	11	12
9	12.5	9.0	8.5	10.0	-	-	11	10.0	10	12	12	11
10	9.5	10	8	7	7.5	8.5	8.5	-	10.5	11	11	11
11	10	10	8.5	8.5	7.5	8.5	8	-	10.5	11	10.5	11
12	8.5	10	7.5	8	8	8	8	-	10.5	10	10.5	10
13	-	7.5	9.5	7.5	7.5	7.5	8.5	-	10.5	10	10.5	10

TABLE V

IRRADIATED BEAGLES HEMOGLOBIN IN GRAMS

Dose	Dog	Weeks of Age 1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	10	7.5	8.5	7	9	9	9.5	8.5	11	9.5	10	11
	17	9.5	8	7.5	6.5	7.5	9.5	7.5	7.5	10	9.5	9	11
	18	13	8.5	9.5	7.0	7.5	8.5	6.5	9	10	11	11	11
	19	12	7.5	7.5	6	6.5	6.5	7	8.5	8	10	10	11
	20	11	-	9.5	7	6.5	6.5	6.5	8.5	9	10	10	11
AVERAGE		11.1	7.8	8.5	6.7	7.4	8.0	6.1	8.4	9.6	10	10	11
200r	R-1	13	6	9	6	8	7.5	8	8.5	9	9.5	10	11
	2	11.5	8	8.5	9	9.5	8	9.5	10	9	9	11	11
AVERAGE		12.3	7	8.8	7.5	8.8	7.8	8.8	9.3	9	9.3	10.5	11
300 r	R-5	13	11	8	9	8.5	9.5	9	9	9.5	10	11	11
	7	13	11	9	9	8	12.5	10	9	9	11	12	11
	6	15	Died										
	8	11	10.5	Died									
	25	11.5	8.5	8	9	7.5	Died						
	28	10.5	9	8	Died								
AVERAGE		12.3	10	8.3	9	8.0	11	9.5	9	9.3	10.5	11.5	11
400 r	R-11	3 days 17.5 gms. died											

TABLE VI

IRRADIATED BEAGLES HEMATOCRIT IN PERCENT

		Weeks of Age											
Dose	Dog	1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	34	26	29	27.5	32	29	31	-	34	37	32	40
	17	32	25	26	22.5	27.5	26	27	-	33	34	35	46
	18	44	26	31	28.5	28	34	23	32	36	38	37	41
	19	40	23	23	20.5	21	24	23	29	30	33	36	43
	20	36	-	30	25	22.5	21.5	21	26	32	32	36	41
AVERAGE		37.2	25	27.8	24.8	26.2	26.9	25	29	33	34.8	35.2	42.2
200r	R-1	46	21	29	20	25	23.5	29	31	34	34	32	36
	2	41	26	28	31	31	29.5	34	35	33	35	33	39
AVERAGE		43.5	23.5	28.5	25.5	28	26.5	32.5	33	32.5	34.5	32.5	37.5
300r	R-5	44	37	27	30	30	32	34	32	34	36	35	37
	7	44	36	28	29	30.5	42	35	32	31	37	38	38
	6	50	died										
	8	34	36	died									
AVERAGE		43	36.3	27.5	29.5	30.3	37	34.5	32	32.5	36.5	36.5	37.5
400r	R-11	3 days 66% - died											

TABLE VII

IRRADIATED BEAGLES WHITE BLOOD COUNT IN THOUSANDS

		Weeks of Age											
Dose	Dog	1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16 WBC	10.6	10.7	10.6	17.3	15	13.8	13.7	13.4	13.1	10.5	12.9	12.9
	17	11.4	12.2	12.9	17.3	24.1	12.8	13.2	14.8	22.2	15.3	18.7	18.4
	18	16.6	13.9	11.2	16.8	28.7	15.7	13.8	16.4	16.7	14.7	15.5	14.7
	19	16.4	11.2	14.3	12.5	21.9	15.4	13.9	12.5	12.9	11.5	9.0	10.5
	20	16.0	-	12.6	16.2	25.6	18.2	14.9	18.4	16.7	17	14.8	13.4
AVERAGE		14.2	12	12.8	16	27.7	15.2	13.9	15.1	16.3	13.8	14.2	14.1
200r	R-1	10.3	5.4	7.9	6.9	13	10.5	18.5	14.0	15.3	14.5	18.0	10.7
	2	5.5	5	8.3	10.2	15.2	15	14.1	19.6	13.0	15.3	18.0	13.9
AVERAGE		7.9	5.2	8.1	8.6	14.1	12.8	16.3	16.8	14.2	14.9	18.0	15.8
300r	R-5	12.3	9.8	9.7	13.3	13	13.1	16.4	12.1	13	13.7	16.3	14.1
	7	13.0	13.2	15	11.4	14.9	15.7	19.3	13.7	12.6	16.7	16.6	11.4
	6	10.5	died										
	8	9.6	12.4	died									
AVERAGE		10.7	11.8	12.4	12.4	13.9	14.4	7.9	12.9	12.8	14.9	16.5	12.8
400r	R-11	3 days	.150 -	died									

TABLE VIII

IRRADIATED BEAGLES RED BLOOD COUNT IN MILLIONS

		Weeks of Age											
Dose	Dog	1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	3.52	2.79	3.10	3.22	4.06	4.02	4.16	4.08	4.49	5.48	3.68	5.58
	17	3.41	2.85	2.69	3.00	3.87	3.55	3.72	3.61	4.56	5.43	3.81	6.41
	18	4.93	2.99	3.53	3.01	3.89	4.08	3.15	4.15	4.98	5.43	4.13	6.09
	19	4.42	2.54	2.48	2.51	3.08	3.26	3.41	3.86	4.00	5.34	4.06	5.49
	20	3.67	-	3.28	3.22	3.22	3.29	3.16	4.16	4.45	5.30	3.93	5.87
AVERAGE		3.99	2.79	3.02	2.99	3.62	3.64	3.52	3.97	4.50	5.44	3.92	6.69
200r	R-1	5.13	2.44	3.50	2.46	3.84	3.52	4.70	5.02	3.72	4.16	-	5.38
	2	4.72	3.02	3.06	3.43	3.88	4.39	5.60	5.25	4.61	4.52	-	4.53
AVERAGE		4.92	2.73	3.23	2.94	3.85	3.90	5.10	5.15	4.20	4.30	-	5.10
300r	R-5	4.44	4.03	2.68	4.01	3.79	5.32	4.02	3.96	4.21	-	4.51	5.61
	7	4.32	4.08	3.14	3.48	3.98	5.85	5.06	4.79	4.68	-	4.40	5.43
	6	5.13	died										
	8	3.24	3.84	died									
AVERAGE		4.03	3.98	2.91	3.74	3.88	5.58	4.54	4.37	4.44	-	4.45	5.52

TABLE IX

COLOR INDEX TREATED

		Weeks of Age											
Dose	Dog	1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	1.30	1.23	1.25	.99	1.01	1.02	1.04	.95	1.12	.79	1.24	.90
	17	1.27	1.28	1.27	.99	.88	1.22	.92	.95	1.00	.80	1.08	.78
	18	1.20	1.30	1.23	1.06	.88	.95	.87	.99	.92	.92	1.21	.82
	19	1.24	1.35	1.38	1.09	.89	.84	.80	1.00	1.03	.85	1.12	.91
	20	1.37	-	1.32	.99	.85	.83	.87	.93	.92	.86	1.16	.86
AVERAGE		1.27	1.29	1.29	1.02	.90	.97	.90	.96	1.00	.85	1.16	.86
200r	R-1	1.15	1.12	1.17	1.11	.95	.97	.78	.77	1.10	1.04	-	.93
	2	1.11	1.21	1.27	1.20	1.12	.83	.77	.87	.89	.91	-	1.11
AVERAGE		1.13	1.66	1.22	1.16	1.03	.90	.78	.82	1.00	.98	-	1.02
300r	R-5	1.34	1.24	1.36	1.02	1.02	.81	1.02	1.04	1.03	1.11	1.12	.89
	7	1.37	1.23	1.31	1.20	.94	1.30	.81	.78	.89	-	-	1.11
	6	1.33	died										
	8	1.55	1.25	died									
AVERAGE		1.40	1.24	1.33	1.11	.98	1.06	.92	.91	.96	1.11	1.12	1.00

TABLE X

COLOR INDEX CONTROLS

Dog	Weeks of Age											
	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	1.23	1.06	1.10	.99	.87	.97	.96	1.11	.88	.91
2	-	1.26	1.13	1.18	1.11	.98	.75	.83	.85	.99	.75	1.17
3	-	1.14	1.29	1.14	1.13	.91	1.07	1.06	1.00	1.00	.97	.98
4	-	1.21	1.40	1.13	1.11	.97	.73	.95	.93	.98	1.05	.94
5	-	1.11	1.18	1.04	1.00	.91	1.01	.95	.97	-	.94	.91
6	-	.93	1.20	1.19	1.08	1.45	.94	1.08	.97	.95	.92	.93
7	1.25	1.18	1.30	1.05	-	-	1.00	1.00	1.04	.96	1.00	1.01
8	1.21	1.21	1.17	-	-	-	1.14	.93	.94	.90	.92	1.06
9	1.29	1.21	1.14	.97	-	-	1.10	.90	1.02	.96	1.01	.95
10	1.53	1.47	1.06	.85	1.14	.95	.93	-	.91	1.04	.94	.93
11	1.26	1.49	1.15	.99	1.07	.91	.91	--	1.01	1.05	.99	.88
12	1.58	1.29	.91	.90	.87	.78	.75	-	.87	.85	.89	.78
13	-	1.08	1.00	1.00	1.00	.76	.74	-	.95	.84	.88	.78
AVERAGE	1.35	1.21	1.17	1.04	1.06	.96	.92	.96	.95	.90	.93	.94

TABLE XI

VOLUME INDEX TREATED

Dose	Dog	Weeks of Age											
		1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	1.43	1.37	1.37	1.46	1.04	1.07	1.15	-	1.15	1.01	1.16	1.07
	17	1.43	1.31	1.49	1.10	1.04	1.09	1.07	-	1.07	.95	1.37	1.07
	18	1.31	1.28	1.31	1.40	1.07	1.25	1.07	1.18	1.07	1.04	1.34	1.01
	19	1.34	1.36	1.50	1.25	1.01	1.09	1.00	1.12	1.12	1.19	1.31	1.19
	20	1.47	-	1.34	1.16	1.04	.98	.98	1.19	1.07	.86	1.39	1.04
AVERAGE		1.40	1.33	1.40	1.27	1.04	1.08	1.05	1.16	1.09	1.01	1.31	1.08
200r	R-1	1.34	1.25	1.68	1.21	.97	1.00	.89	.92	1.37	1.24	-	.98
	2	1.30	1.28	1.37	1.37	1.19	1.28	.91	1.00	1.07	1.16	-	1.28
AVERAGE		1.32	1.26	1.52	1.29	1.09	1.14	.90	.96	1.21	1.20	-	1.13
300r	R-5	1.43	1.40	1.49	1.12	1.16	.89	1.25	1.19	1.13	-	1.16	.97
	7	1.53	1.31	1.34	1.25	1.13	1.07	1.04	1.00	.98	-	1.28	1.04
	6	1.46	died										
	8	1.58	1.37	died									
AVERAGE		1.40	1.36	1.41	1.18	1.14	.98	1.15	1.09	1.06	-	1.22	1.00

TABLE XII

VOLUME INDEX CONTROLS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	1.37	1.19	1.28	1.25	1.21	1.31	1.07	1.22	1.18	.97
2	-	1.37	1.44	1.27	1.24	1.04	.86	.95	1.01	1.13	.94	1.01
3	-	1.31	1.43	1.16	1.28	1.16	1.03	1.24	1.03	-	1.10	1.03
4	-	1.30	1.60	1.07	1.24	1.16	.86	1.15	1.01	-	1.04	1.07
5	-	1.16	1.30	1.04	1.10	.92	1.22	1.01	1.06	-	.94	.94
6	-	1.34	1.37	1.30	1.13	1.76	1.16	1.10	1.10	1.10	1.06	.98
7	1.52	1.31	1.43	1.28	-	-	1.19	1.24	1.15	1.22	1.28	1.13
8	1.37	1.29	1.37	-	-	-	1.29	1.15	1.07	1.07	1.13	1.16
9	1.39	1.36	1.34	1.13	-	-	1.21	1.07	1.19	1.10	1.19	1.07
10	2.05	1.61	1.34	1.37	1.39	1.24	1.12	-	1.04	1.15	1.01	1.07
11	1.56	1.67	1.37	1.10	1.40	.80	1.37	-	1.16	1.12	1.13	.94
12	1.79	1.31	1.19	1.09	1.06	.98	.98	-	.97	.95	.97	.91
13	-	1.19	1.36	1.01	.98	.98	1.04	-	.97	.91	.97	.88
AVERAGE	1.61	1.35	1.38	1.17	1.21	1.13	1.12	1.14	.97	1.10	1.07	1.01

TABLE XIII

MEAN CORPUSCULAR VOLUME CONTROL

Dose	Dog	Weeks of Age 1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	96	92	92	98	70	72	77	-	77	68	78	72
	17	96	88	100	74	70	73	72	-	72	64	92	72
	18	88	86	88	94	72	84	72	79	72	70	90	68
	19	90	91	101	84	68	73	67	75	75	62	88	80
	20	99	-	90	78	70	66	66	62	72	58	93	70
AVERAGE		92.4	89.2	94.3	85.6	70	73.5	70.8	72	73.5	64.5	88.2	72.5
200r	R-1	90	84	113	81	65	67	60	62	92	83	-	66
		87	86	92	92	80	86	61	67	72	78	-	86
AVERAGE		88.5	85	102.5	86.5	72.5	76.5	60.5	65.5	82	80.5	-	76
300r	R-5	96	94	100	75	78	60	84	80	76	-	78	65
	7	103	88	90	84	76	72	70	67	66	-	86	70
	6	98	died										
	8	106	92 died										
AVERAGE		100.8	91.3	95	79.5	77	66	77	73.5	71	-	82	67.5

TABLE XIV

MEAN CORPUSCULAR VOLUME CONTROL

Dog	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	92	80	86	84	81	76	72	82	79	65
2	-	92	97	85	83	70	58	64	68	76	63	68
3	-	88	96	78	86	78	69	83	69	-	74	69
4	-	87	108	72	83	78	58	77	68	-	70	72
5	-	78	87	70	74	62	82	68	71	-	63	63
6	-	90	92	87	76	-	78	74	74	74	71	66
7	102	88	96	86	-	-	80	83	77	82	86	76
8	92	87	92	-	-	-	87	77	72	72	76	78
9	93	91	90	76	-	-	81	72	80	74	80	72
10	138	108	90	92	93	83	75	-	70	77	68	72
11	105	112	92	74	94	74	92	-	78	75	76	63
12	120	88	80	73	71	66	66	-	65	64	65	61
13	-	80	91	68	66	66	70	-	65	61	65	59
AVERAGE	108	91	93	79	84	79	75	72	69	74	72	69

TABLE XV

MEAN CORPUSCULAR HEMOGLOBIN TREATED

Dose	Dog	Weeks of Age											
		1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	28.41	26.88	27.42	21.74	22.17	22.39	22.84	20.83	24.49	17.34	27.17	19.71
	17	27.86	28.07	27.88	21.67	19.38	26.76	20.16	20.78	21.93	17.49	23.62	17.16
	18	26.37	28.43	26.91	23.26	19.28	20.83	19.05	21.69	20.08	20.26	26.63	18.06
	19	27.15	29.53	30.24	23.90	19.48	18.40	17.60	22.02	22.50	18.73	24.63	20.04
	20	29.99	-	28.96	21.73	18.63	18.24	18.99	20.43	20.22	18.87	25.44	18.74
AVERAGE		27.96	28.23	28.28	22.46	19.79	21.32	19.73	21.15	21.84	18.54	25.50	18.74
200r	R-1	25.34	24.59	25.71	24.39	20.83	21.30	17.02	16.93	24.19	22.84	-	20.45
	2	24.36	26.49	27.78	26.24	24.48	18.22	16.96	19.05	19.52	19.91	-	24.28
AVERAGE		24.85	25.54	26.74	25.31	22.66	19.76	16.97	17.99	21.86	21.37	-	22.36
300r	R-5	29.28	27.29	29185	22.44	22.43	17.86	27.39	22.73	22.56	-	24.39	19.61
	7	30.09	26.96	28.66	26.24	20.62	28.47	17.86	17.14	19.52	24.34	-	24.28
	6	29.24	died										
	8	33.95	27.34	died									
AVERAGE		30.64	27.19	29.25	24.34	21.52	23.16	20.17	19.93	21.04	24.34	24.39	21.94

TABLE XVI

MEAN CORPUSCULAR HEMOGLOBIN CONTROL

Dog	Weeks of Age											
	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	27.03	23.29	24.08	21.60	18.97	21.23	21.15	24.44	19.30	19.93
2	-	27.71	24.86	25.84	24.29	21.46	16.47	18.30	18.56	21.79	16.35	25.55
3	-	25.00	28.25	25.06	24.87	19.68	23.42	23.35	21.91	21.95	21.19	21.48
4	-	26.55	30.79	24.80	24.28	21.36	16.04	20.88	20.43	21.53	23.06	20.60
5	-	24.39	25.86	22.73	22.00	19.86	22.22	20.70	21.34	-	20.52	19.93
6	-	20.28	26.32	26.04	23.42	31.72	20.55	23.60	21.19	20.83	20.16	20.33
7	27.51	25.86	28.48	22.97	-	-	21.82	21.99	22.73	21.10	21.91	22.04
8	26.48	26.54	25.57	-	-	-	24.94	20.39	20.53	19.71	20.15	23.30
9	28.34	26.63	24.93	21.19	-	-	24.07	19.80	22.27	21.09	22.14	20.79
10	33.57	32.26	23.19	18.67	25.00	20.88	20.48	-	20.00	22.82	20.68	20.33
11	27.70	32.57	25.22	21.68	23.36	19.95	20.00	-	22.06	23.06	21.78	19.37
12	34.55	28.25	20.00	19.51	19.14	16.99	16.46	-	19.02	18.52	19.52	17.01
13	-	23.73	21.99	21.99	21.86	16.62	16.16	-	20.63	18.48	19.23	17.01
AVERAGE	29.69	26.65	25.58	22.81	23.23	21.01	20.12	21.14	20.91	21.28	20.46	20.59

TABLE XVII

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION TREATED

Dose	Dog	Weeks of Age 1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	29.41	28.85	29.31	25.45	28.12	31.03	30.65	-	32.35	25.67	31.25	27.50
	17	29.69	32.00	28.85	28.88	27.27	36.54	36.23	-	30.30	27.95	25.71	23.91
	18	29.54	32.69	30.65	24.56	26.79	25.00	26.01	28.12	27.78	28.95	29.73	26.83
	19	30.00	32.61	32.61	29.27	28.57	25.00	30.43	29.31	26.67	30.30	27.78	25.58
	20	30.56	-	31.67	28.00	26.67	27.91	28.57	32.69	28.12	31.12	27.78	26.83
AVERAGE		29.84	31.54	30.02	27.23	27.48	28.09	30.39	29.50	29.04	28.79	28.45	26.13
200r	R-1	28.26	28.57	31.03	30.00	32.00	31.90	27.58	27.42	26.47	27.94	31.25	30.56
	2	28.05	30.77	30.36	29.03	30.64	27.12	27.94	28.57	27.27	25.71	33.33	28.21
AVERAGE		28.16	29.67	30.69	29.51	31.32	29.51	27.76	27.99	26.87	26.82	32.29	29.38
300r	R-5	29.55	29.73	29.63	30.00	28.33	29.69	26.47	28.12	27.94	27.78	31.43	29.73
	7	29.55	30.56	32.14	31.03	26.23	29.76	28.57	28.12	29.03	29.97	31.58	28.95
	6	30.00	died										
	8	32.35	29.17	died									
AVERAGE		30.36	29.82	30.88	30.51	27.28	29.72	27.52	28.12	28.48	28.87	31.50	29.34
400r	R-11	26.52	died										

TABLE XVIII

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION CONTROLS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	30	29.5	28.5	25	24	29	30	30.5	26	30
2	-	32	28	30.5	29.5	30.5	29	29	29	29	25	38
3	-	28.5	30	32	29	25.5	34	30	32	-	29	32
4	-	30	29	34	29.5	28	28	28	30	-	34	29
5	-	31.5	30	32.5	30	32	28	31	30.5	-	34	32
6	-	32	31	30	31	27	26	32	29	28.5	29	31
7	27	29	30	27	-	-	28	27	30	26	20	30
8	29	30	28	29	-	-	29	26.5	29	28	27	30
9	30.5	29.5	28	29	-	-	30	28	28	29	28	29.5
10	28	29	26	28	27	26	27.5	-	29	30	31	29
11	27	29	27.5	29.5	26	28	23	-	31	30	29.5	28.5
12	28.5	33	25	27	27	26	25	-	30	29	29.5	28
13	-	23.5	28.5	27	27	24.5	25	-	31	30.5	30.5	29
AVERAGE	28.3	29.7	28.5	29.5	28.5	27.3	27.4	29	30	29.1	29.3	30.3

TABLE XIX

SATURATION INDEX TREATED

Dose	Dog	Weeks of Age											
		1	2	3	4	5	6	7	8	9	10	11	12
100r	R-16	.91	.90	.91	.78	1.00	.88	.91	-	.97	.78	1.07	.84
	17	.88	.98	.85	.90	.93	.89	.86	-	.93	.84	.79	.73
	18	.92	1.01	.94	.76	.85	.63	.75	.84	.86	.89	.91	.82
	19	.92	.99	.92	.87	.75	.64	.80	.90	.92	.72	.86	.77
	20	.93	-	.99	.85	.88	.63	.88	.78	.86	1.00	.83	.82
AVERAGE		.91	.97	.92	.81	.88	.73	.86	.83	.91	.84	.89	.79
200r	R-1	.86	.90	.70	.92	.98	.97	.87	.84	.81	.84	-	.95
	2	.85	.94	.92	.87	.94	.66	.85	.87	.83	.78	-	.86
AVERAGE		.86	.92	.82	.89	.96	.81	.86	.85	.82	.81	-	.90
300r	R-5	.93	.89	.91	.91	.88	.91	.82	.87	.97	-	-	.92
	7	.90	.94	.97	.96	.83	1.21	.78	.78	.91	-	-	1.06
	6	.91	died										
	8	.97	.91	died									
AVERAGE		.93	.91	.94	.94	.86	1.07	.80	.83	.94	-	-	.99

TABLE XX

SATURATION INDEX CONTROLS

	Weeks of Age											
Dog	1	2	3	4	5	6	7	8	9	10	11	12
1	-	-	-	.89	.86	.79	.72	.74	.90	.91	.75	.94
2	-	.92	.90	.93	.89	.94	.87	.88	.84	.88	.79	1.15
3	-	.87	.79	.99	.89	.78	1.04	.86	.97	-	.88	.96
4	-	.93	.90	1.06	.89	.84	.85	.83	.92	-	1.01	.88
5	-	.96	.88	1.00	.91	.98	.83	.93	.92	-	1.00	.97
6	-	.69	.91	.91	.94	.82	.81	.98	.88	.86	.87	.94
7	.83	.91	.91	.82	-	-	.84	.81	.90	.79	.78	.89
8	.88	.87	.85	-	-	-	.88	.81	.88	.84	.81	.92
9	.93	.89	.85	.86	-	-	.91	.84	.85	.87	.85	.89
10	.75	.91	.79	.62	.82	.77	.83	-	.88	.90	.93	.87
11	.81	.89	.84	.90	.76	1.13	.67	-	.87	.94	.89	.94
12	.88	.98	.77	.82	.82	.79	.76	-	.90	.93	.92	.85
13	-	.91	.74	1.00	1.01	.77	.71	-	.97	.93	.91	.88
AVERAGE	.84	.89	.84	.89	.87	.85	.81	.85	.97	.81	.87	.93

TABLE XXI

SURVIVAL DATA OF _IN UTERO_ IRRADIATED PUPS

Dose (r)	No. of Pups	Number Alive 7 days	Number Alive 14 days	Number Alive 30 days	% Survivors 30 days
0	13	13	13	13	100
100	5	5	5	5	100
200	4	2	2	2	50
300	4	4	4	2	50
400	9	0	0	0	0

TABLE XXII

BLOOD VALUES OF THE DOG

Erythrocytic Series	Range	Ave.	Leukocytic Series	Range	Ave.
Erythrocytes	5.5-8.5	6.8	Leukocytes	6-18,000	11,000
Hemoglobin	12-18	14.9			
PCV	37-55	45.5			
MCV	60-77	69.8			
MCH	19.5-24.5	33			

RBC mean life span 108 (average) days; 107-122 (range) days

Schalm, 1961

VITA

GWENDOLYN GARRETT MICHAUD

Candidate for the Degree of

Master of Science

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