Physiological Iron Deficiency Anemia in Suckling Beagle Pups

Timothy F. Porter
Western Michigan University

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PHYSIOLOGICAL IRON DEFICIENCY
ANEMIA IN SUCKLING BEAGLE PUPS

by

Timothy F. Porter

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August, 1976
ACKNOWLEDGEMENTS

I would like to express gratitude and appreciation to Professors Gian C. Sud, Jean Lawrence, and Joseph Engemann and Richard L. Dorner, D.V.M. for their advice and constructive criticism in the research and writing of this thesis. This thesis would never have made it to the writing stage without the planning and technical assistance of Vaughn Augst and Susan Coady. Special thanks goes to the nursery crew which cared for the daily needs of the bitches and pups through the duration of the research. I would also like to thank the management personnel of Laboratory Research Enterprises, Inc. for their confidence in the value of this research.

Timothy F. Porter
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PHYSIOLOGICAL IRON DEFICIENCY ANEMIA IN SUCKLING BEAGLE PUPS.

Western Michigan University, M.A., 1976
Zoology

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INTRODUCTION

Many researchers have produced data showing a decrease in the gross hemogram of beagle pups during the first three weeks and another, often more severe decrease, at seven weeks post-partum (1, 2, 3, 42, 44). The apparent reason for these decreases is the change from fetal blood cells to adult blood cells (2, 56). Iron deficiency as a cause or contributing factor has not been thoroughly investigated probably due to the large mean corpuscular volume of the red blood cells.

The main purpose of this investigation was to ascertain whether the hemogram improved in comparison with a control section after various injections with iron dextran. At the time of the present investigation the number of pups that were weaned per litter was 5.2 pups. The number of pups at birth was between seven and eight live born. With sixty to eighty-five percent of the pre-weaning deaths occurring within the first ten days post-partum, a secondary intention of this investigation was to determine whether with the iron injection or injections a higher percentage of pups would survive after the first ten days post-partum.
LITERATURE REVIEW

Kernkamp in 1935 was one of the earlier researchers to investigate the effects of mineral deficiencies in suckling pigs (29). Since pigs were a desirable and marketable product, much work was done to increase the health and number of pigs that reached weaning age. It was found that if given access to most soils, the pigs would not develop iron deficiency because they would rut and swallow some of the soil containing the necessary minerals (29). Research in man has revealed that iron deficiency anemia develops only in times of rapid growth, pregnancy, or other stress (12, 24, 35). Rapid growth in man occurs immediately after birth and years later as the child reaches adolescence (12, 20, 27). Puberty in women brings about menstruation which is an additional stress that can in certain circumstances aid in bringing about an iron deficient condition (12). Pregnancy is a very common cause of iron deficiency in women (12, 24). Normally only small amounts of additional iron is needed in the diet to prevent total utilization of maternal stores (24). Stress is usually attributed to trauma due to blood loss often in cases of accidents or chronic gastro-intestinal bleeding (12). Research in iron deficiency in dogs has not been well documented (24, 42, 54). It was found that dogs, preferably beagles, were excellent animals for pharmaceutical research (2, 3, 41). This preference for the beagle dog caused a need and opened a market for a healthy well documented animal. Many beagle colonies were formed to fill the need
for pharmaceutical research (35). Most of the colonies were de-
veloped to establish a heterogeneous population of dogs, well accli-
mated to a cage and frequent handling (38, 41, 49). Economics dic-
tated a need for many healthy animals at weaning (1). Seventy-five
to eighty-five percent of all deaths that occur in pups before
weaning occur in the first ten days post-partum (1). After the
first ten days deaths that do occur are due to climatic conditions
and infections often leading to pneumonia in weaker susceptible pups.
The other significant cause of deaths after ten days is handling
accidents. By increasing the health of the weaker pups it is hoped
that the final fifteen to twenty-five percent death loss can be de-
creased.

Most research in iron deficiency has been on man with much of
the data in hematology (7, 30, 55). In comparison little iron de-
ficiency research has been reported on dogs (36, 42, 54). There is
even great variation in the hematological data produced on labora-
tory dogs (11, 38, 40, 41, 42, 44, 49). However, with known healthy
dogs the data is more uniform (2, 3, 18, 35, 38, 48, 49). Data from
these investigations has shown that the ranges for normal healthy
dogs are hemoglobin values of 15-18 grams/100 milliliters of whole
blood (gms.), hematocrit values of 45-58%, and red blood-cell counts
from 5.50-8.00 million per cubic centimeter of whole blood. In com-
putation of these values for red blood-cell indices it is evident
that the values are very similar to human red blood-cell indices.
When it is considered that dogs have biconcave red blood-cells that
are also similar in size to human red blood-cells, it is evident that
the morphological appearance of the red blood-cells should be similar (42). These similar properties allow for basic correlations between the two species.

At birth babies have a hemoglobin value of 19-24 gms. (47, 51, 57). This value is very high in comparison to the normal adult values of 13-18 gms. (56). In contrast the new born pups have hemoglobin values comparable to the normal adult values of 15-18 gms. (2, 3, 18, 38, 42, 44, 48). Babies do well the first two months post-partum because the iron requirement is available from the supply resulting from the catabolism of the high hemoglobin and by the supply stored by the fetus (12, 24, 46, 55, 57). Like babies the pups use the materials from the catabolized red blood-cells to produce new red blood cells. However, the lower initial levels of hemoglobin result in a lower supply of iron (2, 3, 42). Without additional iron a milk fed baby does not show signs of iron deficiency until five to six months post-partum (12, 46, 57). This was recounted by one author as the "physiological anemia of late infancy" (51). Without additional iron from other foods a pup begins to show signs of deficiency from three to six weeks post-partum. The assimilation of iron from the bitch's milk is minute and it is seldom that dog meal is available to aid in the digestive assimilation of additional iron (1, 3).

The classic picture of iron deficiency anemia consists of a drop in the hemoglobin and hematocrit of more than 30% below the normal adult values, a decrease in the red blood-cell count, a very low mean corpuscular volume, a low mean corpuscular hemoglobin concentra-
tion, a decreased mean corpuscular hemoglobin, a decreased total serum iron, an increased iron binding capacity, and the absence of hemosiderin in bone marrow smears (12, 20, 22, 24, 29, 30, 34, 42, 54, 55, 56, 57). The hematological data, excluding the last three parameters, can be obtained from newborn pups without overstretching them. With the use of only the hematological data a decrease in the red blood-cell count might be construed as an indication of copper deficiency (4, 42, 54). To measure this possibility a reticulocyte percentage and actual count are tabulated. Four to six days after the administration of iron an increase in the reticulocyte percentage of three to six times the normal value is expected if iron deficiency is evident (8, 9, 20, 21, 42, 55). In dogs and man the normal reticulocyte percentage is up to 2% of the red blood-cell count (11, 30, 35, 42).

Iron is unique in the body because once it is assimilated little is removed during normal physiological function (16, 24, 32, 51). A deficit in iron develops during times of rapid growth, pregnancy, or stress unless additional iron is added through the diet (5, 12). During these times of additional need, the intestine increases the assimilation of iron (24). It is known that in healthy individuals only 10-15% of the iron available in the diet is absorbed (12, 43, 51). In man and dogs this 10% is only 1-2 mg. of iron (12, 24, 36). In healthy adults this results in the storage of iron primarily in the liver and spleen (9, 12, 36). When healthy people are stressed, the intestinal assimilation of iron is not increased until the storage iron is depleted (12, 16, 39). In iron deficient people and
in others when the iron binding capacity is increased large increases in digestive iron assimilation to occur (12, 19, 43, 51). As the amount of iron in the diet or supplement is increased, the percentage assimilated decreases even though the absolute amount increases (12). In order to build up body iron through dietary assimilation a diet with supplemental iron must be administered for a lengthy period of one to six months (12, 51, 55). Supplemental iron is available in both elemental forms, ferrous and ferric. In the dietary supplement the ferrous form is preferred because in man it is more readily assimilated (12, 20, 36, 51). In dogs the acidity of the stomach allows both forms to be readily assimilated with a slight preference for the ferrous form (36, 42). Dietary administration of additional iron is inadequate for people and pups in stress situations when the iron is needed over a short period of time (23).

Iron can also be administered by intravenous and intra-muscular injections (6, 23). For intra-venous and intra-muscular injections either form of elemental iron is acceptable. Intra-venous injections are excellent for well controlled conditions and if other means for administration are unacceptable such as intestinal problems with supplemental dietary iron or painful intra-muscular injections or reactions to one of these means of iron administration (6, 12, 55). Intra-muscular injections of iron give large amounts of iron readily available for physiological utilization usually with little discomfort and rare staining (6, 9). Once iron enters the body regardless of the pathway the elemental state is determined by whether it is storage or circulating iron. Circulating iron or transfer iron is
in the ferrous state as an apoferritin molecule (ferritin) and the storage iron is in the ferric state (hemosiderin) (20, 28, 31, 36, 47). Though intra-muscular injected iron is considered to be storage iron similar to myoglobin iron, there is no evidence that the ferric form is preferable to the ferrous form. With consideration that iron, once in the physiology of the body is not excreted, it is apparent that intra-muscular injections have three preferable advantages to the other two means of iron administration when dealing with dogs. The advantages are that it is easily administered, it is difficult to give the animal an iron overload, and the iron is readily available as storage iron to be transferred to the liver or utilized as needed (6, 9, 10, 12, 46, 55).
METHODS-MATERIALS

Previous to this study pups from the colony used were bled to give a normal hemogram base-line for the pups through the first several weeks of life. Most of these pups had hematocrits under 30% with many less than 25% at seven weeks. Weaning at five to six weeks was considered to be the external pressure initiating the decrease in the hematocrits. It was decided to give iron injections to several litters of pups at 28 days of age. Those injected pups showed only a slight increase in the hemoglobin and hematocrit. Also, the pups receiving 200 mg. of elemental iron did not respond better than those that received only 50 mg. of elemental iron. From this data a more definitive investigation seemed to be indicated.

This investigation was designed to encompass 70 newborn beagle pups from 12 to 15 liters. Several of the bitches that whelped at the onset of the study either whelped 2-4 pups or were very poor in the care of them. To compensate for these contingencies 20 bitches were used with a total number of 94 pups. At two weeks post-partum the total population for the study was 17 bitches with 74 pups (Table I). The 74 pups were distributed at random through the seven sections. Where possible, pups from each litter were divided into each of the seven sections. At birth each pup was assigned an individual tattoo number which was tagged on a collar for two weeks before being tattooed on the ear. Each newborn pup used in this investigation weighed at least 0.20 kilograms (K.) at birth. All of the pups and
TABLE I

Summary of Pups

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<th>Section</th>
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<td>69</td>
<td>19</td>
<td>88</td>
<td>77 74 72 72 72 72 71 71 71 67</td>
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<tr>
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<td>70</td>
<td>75</td>
<td>19</td>
<td>94</td>
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</tr>
</tbody>
</table>

* Pups which died naturally
** Pups which died accidentally
bitches remained indoors in a controlled environment in conventional mesh cages 4 x 3 x 3 feet throughout the duration of the study (49). Water was available ad libitum to the bitches and pups with a commercial dog meal, Jim Dandy, checked daily. This dog meal exceeded the daily minimal NRC requirements (58). At three weeks all of the pups had a wet preparation of this dog meal available.
EXPERIMENTAL PROCEDURE

The seventy-four pups were divided into three groups and seven sections: the Control Group and Section consisting of ten pups; the Prophylactic Group consisting of thirty-four pups divided into sections A, B, and C; and the Therapeutic Group consisting of thirty pups divided into sections D, E, and F. The Control Group and Section did not receive any injection. The Prophylactic Group consisted of sections in which the pups received two iron injections before the depression in the hemogram at three weeks in order to prevent or alleviate the depression. The Therapeutic Group consisted of sections in which the pups received an injection at or after the depression at three weeks without two injections previous to the three week depression. Section A of the Prophylactic Group consisted of eleven pups that received iron injections on days three and ten post-partum. (Table II on page 12 depicts the relationship between the groups, sections, and their respective injections.) Section B had eleven pups that received injections on days eight and fifteen. Section C consisted of twelve pups that received injections on days eight, eighteen, and twenty-eight. Section D of the Therapeutic Group had eleven pups injected with iron on days fifteen and twenty-eight post-partum. Section E consisted of ten pups injected once on day twenty-one. Section F had nine pups that received only one injection on day twenty-eight. The iron solution for the injections was ferric hydroxide in a low molecular weight dextran known as No-Nemic, a pro-
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<td>1</td>
</tr>
<tr>
<td></td>
<td>F</td>
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duct of the Armour-Baldwin Company in Omaha, Nebraska. One hundred milligrams (mg.) of elemental iron was available per one cubic centimeter (cc.) of solution (9). The pups were injected with 1/2 cc. in the extensor muscles of the hind legs giving an availability of 50 mg of elemental iron per injection (12, 23, 24, 55).
Each pup and its respective bitch were bled weekly from either external jugular vein. The initial through the second weekly bleedings of all of the pups were accomplished by a microphlebotomy technique. This was done with the removal of approximately 0.5 cc by filling the needle and hub of a 19 gauge (ga.) needle from insertion into an external jugular vein. The total hemogram was derived from this sample (17, 53, 56). After two weeks post-partum, for all of the sections except the control, the blood-letting was accomplished with the removal of 2 cc of blood using the vacutainer system (17). The control section was divided into two equal parts for the bleedings on weeks three and four, one receiving the vacutainer technique and the other the micro technique. The control pups that had 2.0 cc removed had higher average hemogram values for weeks four, five, and six. After week four all of the control pups were bled by the vacutainer technique. The 2.0 cc vacutainer contained 0.05 cc of a 15% solution of ethylenediamine tetraacetic acid (EDTA) as the anti-coagulant. All of the samples were collected in the morning from non-fasted animals (2, 15, 40).

The hemogram, consisting of the red blood-cell count (RBC), the leukocyte or white blood-cell count (WBC), the hemoglobin, the hematocrit, the reticulocyte count, and the red blood-cell indices (indices), was determined from birth through ten weeks post-partum. The RBC and WBC were determined electronically using a Coulter Counter,
Model ZBI. The hemoglobin values were determined using the hemolyzed WBC dilution in the associated Coulter Counter Hemoglobinometer. The hemoglobinometer utilized the stable cyanmethemoglobin fraction in its determination of the hemoglobin value. The hemoglobinometer was non-functional during the third week of the investigation so the hemoglobin values were determined using the Hycel Cyanmethemoglobin Reagent in solution as the diluent and the Hycel Hemoglobin Standard to determine the hemoglobin graph. The diluted blood samples were then read on a Coleman Junior Spectrophotometer at the 540 wavelength. The hematocrit was determined with heparinized micro hematocrit tubes centrifuged at 18,000 G. for five minutes in a micro hematocrit centrifuge. The indices, the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC), were then calculated from these values (17, 22, 34, 56).

The reticulocyte counts were determined from slides made by a reticulocyte stain dilution. A drop of saline saturated with brilliant cresyl blue, one drop of 1% ammonium heparin from a 25 ga. needle, and a drop of fresh blood were placed in small glass tubes and mixed well to produce the reticulocyte stain dilution. Drops from these tubes were placed on clean microscopic slides where they were spread thinly and allowed to dry to be read later (17, 37, 56).
RESULTS

Control

The control pups received no injections in order to depict the hematological picture of the normal laboratory dog. The hemoglobin dropped sharply by the end of three weeks from an initial 15.2 gms. to 9.5 gms. In this same period of time the hematocrit dropped from 46.2% to 29.3% a decrease of 36.6%. The RBC did decline steadily but not sharply from 4.94 million (M.) to 3.57 M. The drop in these three parameters was less than many of the pups from the other sections giving a strong control average (Graphs I-VI). After three weeks the pups from the control section leveled off with a moderate RBC but did not show any significant increase of the hemoglobin or hematocrit until week seven. At week seven the control group had a strong increase in the hemoglobin of over one gram to 9.8 gms., a gain of over one percent in the hematocrit to 31.1%, and a slight increase in the RBC to 3.88 M. These values persisted through nine weeks with a slight increase at ten weeks. The indices did not show any significant decrease until week five when the MCH and MCHC were low (Table III). The reticulocyte counts of the Control Section did not show any significant increase during the ten weeks of the investigation (Table IV).

Section A

The hemoglobin, hematocrit, and RBC values for the pups of
TABLE III

Red Blood-Cell Indices

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<tr>
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<td>MCH 31.0 29.227.4 27.4 25.9 24.5 25.025.2 27.3 26.2 25.2</td>
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<tr>
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<td>MCHC 32.8 33.634.3 32.3 32.0 31.0 30.731.4 31.5 32.1 31.7</td>
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<tr>
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<td>MCV 94.8 87.083.7 96.6 83.4 78.2 80.077.7 82.8 83.7 84.9</td>
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<td>MCHC 33.5 34.135.7 32.7 30.8 30.0 31.932.5 33.0 33.5 32.5</td>
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MCV—Mean Corpuscular Volume
MCH—Mean Corpuscular Hemoglobin
MCHC—Mean Corpuscular Hemoglobin Concentration

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Section A declined parallel to but higher than all of the other sections until week three (Graph I, II and III). At three weeks the RBC became comparable to the other sections while the hemoglobin of 10.3 gms. and hematocrit of 31.9% remained higher resulting in a very high MCH (Table III). The RBC rebounded to a significantly higher value in week four from 3.36 M. to 3.90 M. and remained higher through week seven. During this same period the hemoglobin and hematocrit dropped slightly. When compared with the other sections, the hematological picture painted by these parameters showed these pups to be stronger. The decline often found after weaning became evident at week eight when these parameters declined to values less than the control. These two sections remained similar and statistically inseparable through week ten. The reticulocytes did not peak indicating that the pups did not show a hemopoietic response to the injections they received on days three and ten.

Section B

The hemogram of Section B declined with the results from the Control Section through the first three weeks. One variation was the peak in the reticulocyte count after this sections first iron injection, indicating a hemopoietic utilization of the iron. Also, in week three, the hemoglobin and hematocrit declined slower than did the control values for these parameters, while the RBC of both sections remained similar, resulting in a comparably high MCH for Section B (Table III). Between weeks three and seven the hemoglobin, hematocrit, and RBC values increased to 10.3 gms., 32.3%, and 4.05 M.
GRAPH I

HEMOGLOBIN MEANS
PROPHYLACTIC GROUP WITH CONTROL SECTION

- Section A
- Section B
- Section C
- Control Section

Time in Weeks

Initial 1 2 3 4 5 6 7 8 9 10

Grams of Hemoglobin

8.0 9.2 10.4 11.6 12.8 14.0 15.2 16.4
GRAPH II
HEMATOCRIT MEANS
PROPHYLACTIC GROUP WITH CONTROL SECTION

- Section A
- Section B
- Section C
- Control Section

Percentage of Red Cells in Blood

Time in Weeks

Initial 1 2 3 4 5 6 7 8 9 10
GRAPH III
RED BLOOD-CELL COUNT MEANS
PROPHYLACTIC GROUP WITH CONTROL SECTION

Section A
Section B
Section C
Control Section

Red Blood-Cells in Millions

Initial 1 2 3 4 5 6 7 8 9 10

Time in Weeks
respectively where they remained. These values were significantly higher than the control values for these parameters during this same period. In the eighth week the hemoglobin and hematocrit dropped to values indistinguishable from the control values, although the RBC remained definitely higher. In the ninth week these pups rebounded from the slight decline and continued to increase the values of these three parameters through the tenth week, concluding with the highest hemoglobin of 10.8 gms. and hematocrit of 33.0% and one of the highest RBCs of 4.07 M.

Section C

The hemoglobin, hematocrit, and RBC values of the pups in this section decreased rapidly through the third week sampling despite the second iron injection at eighteen days. At the four week bleeding, the same time as the third injection, these pups showed a large increase in the RBC from 2.93 to 3.62 M. with a correspondingly high hematocrit of 29.6%, and a steady hemoglobin of 10.0 gms., which resulted in a decreased and low MCH (Table III). Through weeks five and six Section C continued to increase steadily with a large increase in the seventh week from 3.87 to 4.29 M., 9.6 to 10.9 gms. and 30.7 to 33.8%. During weeks eight and nine, the hemogram declined to become equivalent to the control. These pups rebounded during the tenth week ending with a strong hemogram.

Section D

These pups paralleled the control group through week three ex-
cept for a higher hemoglobin value and the slight reticulocyte increase at three weeks (Graphs IV, V, VI and Table IV). This reticulocyte increase could indicate the four to six day time lag after the first iron injection at day fifteen (9, 42, 55). In week four the RBC and hematocrit increased to 3.60 M. and 30.1% as the hemoglobin continued to decrease to 9.1gms. This combination resulted in a low MCH and MCHC (Table III). The hemoglobin, hematocrit, and RBC all increased through week six which resulted in values significantly higher than the concurrent control values. During week seven the hematocrit decreased slightly to 31.0 gms., great increasing the MCHC. The RBC then decreased steadily for the next fourteen days before increasing slightly in the tenth week to the highest value of 4.30 M. The hematocrit remained steady through week nine before increasing through the tenth week to 32.8%. In the eighth week the hemoglobin decreased slightly from 10.4 gms. to 10.1 gms., before increasing in weeks nine and ten to 10.7 gms. The result of these increasing values in the tenth week was to show that a section from the Therapeutic Group was definitely stronger than the Control Section.

Section E

The hemogram of the pups in this section was low with values lower than the control pups through week three. Three values at three weeks were a hemoglobin of 9.5 gms., a hematocrit of 27.2%, and the RBC at 3.09 M. After the injection at day twenty-one, the hemogram increased significantly without an indicative reticulocyte peak
GRAPH IV

HEMOGLOBIN MEANS
THERAPEUTIC GROUP WITH CONTROL SECTION

Section D
Section E
Section F
Control Section

Grains of Hemooglobin

Time in Weeks

Initial 1 2 3 4 5 6 7 8 9 10
GRAPH VI

RED BLOOD-CELL COUNT MEANS
THERAPEUTIC GROUP WITH CONTROL SECTION

Red Blood-Cells in Millions

<table>
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<th>3</th>
<th>4</th>
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<td>✗</td>
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</table>
(Table IV). The hemoglobin at about 9.4 gms. and the hematocrit at about 30.8% remained slightly greater than the Control Section through week six, while the RBC at 3.71 M. was consistent with the control. In the seventh week the mean values of these three major parameters decreased to become lower than or comparable to the control. The means continued to decrease through the ninth week making this section the weakest. In the tenth week the results of the hemogram showed that this section was increasing comparable to the control pups.

Section F

The pups from this section had the weakest hemogram through the first four weeks. By week five, after their injection, the pups responded well enough to become comparable to the control pups without a corresponding reticulocyte increase. In the sixth and seventh week these pups continued to show an increase in the hemoglobin to 10.5 gms., the hematocrit to 32.5%, and the RBC to 4.15 M. with the results being significantly higher than the control. In week eight the hemograms of these pups began to decrease. The hemoglobin continued this decrease through week ten to 9.8 gms. while the hematocrit and RBC rebounded in the tenth week to 30.3% and 4.05 M. to show a slight increase. However, the result of the hemogram at ten weeks was the lowest hemoglobin, and hematocrit with a RBC comparable to the control at 4.03 M. These parameters combined to give the pups in this section the lowest MCH and MCHC (Table III).
Weight

The weights of the pups were recorded only through week seven. Due to logistical reasons further weight data was not gathered. Even though the mean weight gain per week and the total weight gained did not reflect any group pattern a general trend did emerge. The iron injected pups had a better weight gain through the seven weeks than did the non-injected control pups (Tables V and VI).

Grams of Hemoglobin Produced

The grams of hemoglobin produced were determined by multiplying the hemoglobin (gms.) times the estimated total volume as determined by the amount of weight gained. The amount of weight gained (mass produced) also gave a certain increase in the volume of blood. For every kilogram gained the blood volume increase was considered to be 80 ml. similar to the 82-83 ml. found by Schalm (3). For this investigation the original blood volume for all of the pups was considered, due to the pups' size and after consultation, to be 50 ml. giving a standard base-line.

The weekly sum and total gain in grams of hemoglobin produced are shown in Table VII. During the first week, all of the sections showed a loss in the grams of hemoglobin produced. Section A, which received an injection during this first week, had the smallest loss in the total grams of hemoglobin. Through the second week most of the sections showed a strong or steady increase except Section E which remained the same as the first week. By the end of three weeks all of
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### TABLE VI

Comparisons of Weight

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<th>Weight Gained (kilograms)</th>
<th>Percent Gained</th>
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the sections showed an increase from birth of the total grams of hemoglobin produced except Section A which remained with a net loss. Week four gave all of the sections a strong steady increase except Section F which remained stable indicating a leveling off of the rapid growth seen from the first through the third week. During the fifth week all of the sections except the control had a large increase. In the next two weeks all of the sections showed a steady increase with sections C, D, E, and F having the greatest. By the end of seven weeks all of the injected sections had a greater total mean of the grams of hemoglobin gained than did the Control Section.

Mortality

This investigation began with 75 newborn pups 0.20 K. or heavier in weight. In the first week seventeen of the pups had either died or were too weak to be maintained in the investigation. Those that were too weak at the first weekly bleeding died during the second week. Eight of these pups belonged to two very poor bitches. Six of these pups were not assigned to any section. Of the eleven that were assigned to sections three were in the Control Section, three were in Section A, three were in Section B, one was in Section C, and one was in Section F. That left 58 pups among seven sections giving sections Control A, and B less than ten (Table I). It was decided to add three more litters with nineteen pups starting one week later to attempt to bring the number of pups per section to at least ten. Four of these pups were randomly selected for the Control Section, six for Section A, four for Section B, two pups for Section C, and one each for
Sections D, E, and F. This brought the number of pups that were bled initially to 94. After the loss of seventeen pups in the first week the total number of pups was 77. With the loss of three more by ten days post-partum, the actual number of pups utilized for data in this investigation was the 74 pups bled at two weeks.

After the first ten days only seven pups died through the next nine weeks. Three of these pups suffered deaths by handling accidents. Of the original ninety-four pups, twenty-seven died in the ten weeks of the investigation. At the two week bleeding twenty pups or 74.0% of the pups that would die had expired. At the three week bleeding two more pups or 7.4% of the total that would expire had died. During the last seven weeks of the investigation only five pups died or 18.5% of the total that died. Of these five pups three or 11.1% died due to handling accidents; two in the Prophylactic Group and one in the Therapeutic Group. Only one or 3.7% of the expired pups died in the Prophylactic Group after the second week due to poor health. The Therapeutic Group had two deaths or 7.4% attributable to poor health. However, one of these deaths was before the pup had received an iron injection.

Bitches

The gross hemogram of the bitches was also determined while the pups remained with them. The results were reversed in comparison to the control pups. The bitches remained healthy eliminating a possible deleterious variable. This data was not considered further in this investigation.
Data Analysis

All of the data from this investigation was analyzed in a computer for means, standard deviations, and probability of variance using the mean values obtained from the control pups as the baseline. The probability of variance for the parameters hemoglobin, hematocrit, and RBC was significant for all sections from week three through week eight. The analysis of variance is not often understood by people with little background in statistics. Another way to view variance is to compare the values of the study subjects with the base-line or in this case the mean of the individual control values. If the mean values of the subjects vary from the control means, it is still possible that the subject values are only variations found naturally in the sampling and recording process. If the subject values are essentially the same as the control values, the number of mean values greater than the curve of the control mean values should be on the average equivalent to the number of mean values less than the control curve. A variance comparison was applied to this investigation for the eleven sampling periods using the hemoglobin, hematocrit, and RBC. In each section the number of means obtained that were greater than the curve established by the Control Section exceeded the number of mean values that were less than the control curve. The data from the pups that died during this investigation after the first ten days was deleted and the values recalculated. These new values showed no significant difference from the previous calculations of the original data.
DISCUSSION

The canine and human hemogram and red blood-cells are very similar (26, 35, 42). The indices are similar, the red blood-cell is bi-concave, and the two species are similar in growth from post-partum to five months in the canine and three years in man. Children given a complete diet of milk maintain a normal MCH and MCHC through the first four months (46, 47). Then if sufficient iron is unavailable through the diet, they begin to develop varying degrees of iron deficiency in the fifth and sixth months (12, 46, 57). This was recounted by several investigators as a physiological anemia (5, 47, 51). This additional iron is needed for the rapid growth of muscle, enzyme usage, and the increased blood volume for the increase in the body size (12, 20, 24, 30, 47). The canine pup is confronted with these same iron requirements but they occur more rapidly and for a shorter duration (3). A pregnant woman on a good diet normally gives birth to a healthy baby with iron stores and a very high hemoglobin in comparison with the normal adult hemoglobin (24, 46, 47, 51, 57). In comparison the gravid bitch on a good diet whelps 4-8 healthy pups with hemoglobin values similar to hemoglobin values found in a normal healthy adult (3). Information on iron storage in new born pups has not been readily available (2). From the information given about children and making a parallel comparison with the data shown in Graphs I and IV, there is an indication that at least some iron storage exists. From the graphs this is indicated by the hemoglobin production of the con-
trol pups in weeks eight through ten when compared to the growth of the other sections. Also, the high absolute numbers of reticulocytes (Table IV) at the initial and first two weekly bleedings indicate some iron stores (12). The indications from the literature have the growth of children and their needs for iron in the first two months post-partum being adequately served by destruction of the high hemoglobin at birth (12). Comparatively, the hemoglobin of the new born beagle pup is very similar to the 15-18 gm. hemoglobin found in the healthy adult (18, 42). The destruction of the red cells in the young pup can at best be very limited without stressing the pups supply of iron (2). The assimilation of iron from the bitches milk is minute and it is seldom that dog meal is available to aid in the digestive assimilation of additional iron (1, 3). There is evidence to question whether the pups could digest enough iron for their increased need if enough were made available (12, 28, 36). The degree of iron insufficiency is dependent upon the size of the pup, the number of litter mates, and the rate of growth during the first five weeks post-partum (3, 57). This also has an effect upon how the pups react to the stress of weaning. Mech, (33) in his study of the wolf, explains how much of the need for iron is alleviated in the wild canine. At three weeks the she wolf leaves the den to feed on a carcass and returns to the den where she regurgitates partly digested meat which is more easily digested by the pups (33). Also, the assimilation of iron from tissue sources has been shown in non iron deficient states to be greater than from other food sources and from mineral ingestion (13, 14, 52).
The classical picture of iron deficiency is a very low MCV with a low MCHC, and a decreased MCH. The cells are small with a decreased color index when examined in a differential smear (7, 22, 30, 42). As has been shown by the data, the decrease of the hematocrit in most of the pups has been just over 30% and the hemoglobin decrease has been only slightly more (24, 29). These parameters have combined to show a decreased MCHC in the pups comparable to anemic MCHC values found in children (22, 24, 30, 44). However, due to a corresponding decrease in the RBC, the MCV never approached the size indicative of the classic iron deficiency anemia (5, 20). The use of the differential smear to determine hypochromia and microcytosis has been shown in a study of slides by experts on known and varying degrees of iron deficiency to be subject to considerable disagreement (7). The degree of deficiency encountered in this investigation is slight making the interpretation of the slides very difficult and highly subjective (7, 47). Also, in humans the anemic condition must exist for one to several months before the classical picture of iron deficiency anemia becomes evident (12, 5). Hence, the interpretation of slide morphology was not included with the hemogram. With a normal or high MCV and a low MCHC the data from this investigation could be interpreted as being consistent with copper deficiency. In copper deficiency the MCV remains normocytic and the hemoglobin is reduced not by a lack of hemoglobin in the red blood cells, but by a decreased production of the red blood cells (4, 42, 54). The decrease in the RBC is not great as is shown in Graphs III and VI. The probability of copper deficiency is made much smaller by the increase in the per-
centage of reticulocytes after iron injections (2). The increase in
the reticulocytes after administration of iron is one of the primary
criteria for the establishment of iron deficiency (21, 22, 34, 55).

The data of this investigation indicates that the new born beagle
pups develop to varying degrees a physiological iron deficiency ane-
mia (5, 51). That this condition can be alleviated somewhat before
the condition fully develops is shown in the hemogram of Section A
during the first three weeks. Section A also shows that a dependency
upon administered iron during the first weeks post-partum can result
in an inadequate supply shortly after weaning. Apparently the pre-
sence of storage iron inhibits the normal assimilation of ingested
iron that occurs daily (20). After one week of stress, in Section A
during week eight, the normal assimilation of ingested iron probably
became normal. This is indicated by the significant rebound found in
the tenth week. A strong response of the control pups from week
eight through ten indicated an assimilation of iron sufficient for a
steady growth of hemoglobin.

Sections B, C, and D showed that supplemental iron in the sec-
ond week post-partum is very beneficial whether other injections were
previous or to follow. The single injection at three weeks was not
sufficient to give an indication as to whether or not it was bene-
ficial as shown by the data of Section E. The single injection at
four weeks was definitely beneficial to the pups in Section F as was
indicated by the increased hemogram. This section lost two pups
after the first ten days resulting in a survival of only 60% of the
pups at ten weeks. This indicated that though the injection at four
weeks was beneficial, it did not help to sustain a weaker pup that
might previously have needed the additional iron. However, there is
insufficient numbers for statistical evidence whether the injection
would or would not sustain a weaker pup.

The total grams of body hemoglobin gained through seven weeks
indicated that all of the sections which received iron injections had
a greater mean total body hemoglobin gain than did the strong Con-
trol Section. The total grams of hemoglobin produced is by itself an
insufficient criterion to establish beneficial erythropoiesis. When
this value is increased in conjunction with a substantial reticulo-
cyte increase and normal indices beneficial erythropoiesis is de-
finitely indicated.
SUMMARY

Seventy-four new born beagle pups were randomly distributed into seven sections. One section was used as a control while six sections received various iron injections. The hemogram determinations of a hemoglobin, hematocrit, red blood-cell count, red blood-cell indices, and a reticulocyte count were made from birth through ten weeks post-partum. The weight of the pups was determined through week seven.

The control section, which did not receive any injection, was used as a base-line for comparison with the iron injected sections. Four sections received various series of injections. One series injected section received two injections before two weeks of age, on days three and ten, and did not show any beneficial erythropoietic indications. The other three series injected sections that received one of the injections in the second week did show erythropoietic indications in the hemogram that the iron was beneficial. One of these sections received injections on days eight and fifteen, a second section received injections on days eight, eighteen, and twenty-eight, and the third series injected section received injections on days fifteen and twenty-eight. The other two sections received single injections, one on day twenty-one, and the other on day twenty-eight post-partum. Both of the single injected sections had a positive response but not significant when compared to the hemogram of the control section.

Using the hemoglobin value and an estimated total body blood
volume calculated from the weight of the pups, a total body hemoglobin value was determined through week seven. At the end of seven weeks all of the iron injected sections had a greater total body hemoglobin than the control section. The total body hemoglobin value without a corresponding increase in the reticulocyte count and a maintainence of normal indices is insufficient to establish beneficial erythropoiesis.

The secondary intention of this investigation was to ascertain whether the iron injections would be beneficial in preventing deaths after the first ten days post-partum. There was no evidence in the data obtained from the iron injected sections to indicate a decrease in the death rate after the first ten days.
BIBLIOGRAPHY


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