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The effect of lead and zinc on selenium poisoning in mice

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THE EFFECT OF LEAD AND ZINC ON SELENIUM POISONING IN MICE

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Chapter 1

INTRODUCTION

Trace elements, because they are effective in catalytic amounts, must be held in delicate balance with each other as well as with the macronutrients of the diet. Disturbances of these balances result in deficiency and toxicity symptoms. Toxic doses of selenium, lead, and zinc, for example, are known to cause anemia (1, 2, 3, 4) and growth depression (5, 3) in experimental animals.

SELENIUM

Selenium is of particular interest as a toxicant because it is "among the few elements known to be absorbed by food and forage plants in sufficient amounts to create toxicity hazards to animals" (5). It is of even more concern because its soluble salts are toxic at such low levels as 0.5 mg% (3, 6), yet deficiency symptoms are also critical, and can be observed in areas practically "next door" to toxic areas.

Although the toxicity of selenium was first demonstrated in 1842 (7), it was not until 1933 (5) that selenium was associated with livestock poisoning as manifested by "blind staggers" and "alkali disease". Although both diseases are caused by excess selenium, the different forms in which the

element is ingested result in different symptoms. Rosenfeld and Beath (5) list these two as well as a third type of chronic selenium poisoning:

1. Blind staggers - caused by selenium extractable with water from native indicator plants and characterized by anorexia, emaciation, staggering, diarrhea, salivation, closure of eyes and death due to respiratory failure.

2. Alkali disease - caused by selenium from grasses or grains which is bound to proteins and is relatively insoluble in water. Symptoms include loss of hair, roughened coat, sloughing of hoofs, anemia, lameness, and liver atrophy.

The third type of poisoning is the selenium toxicity of chronic experimental selenosis which manifests itself with its own series of symptoms, mimicing neither blind staggers nor alkali disease, but resembling a combination of both. The most pronounced symptom of experimental selenium poisoning is a decrease in food consumption (5). The animals also may assume a hunched posutre and their fur is often coarse and ruffled (8). Gross pathological examination reveals ascites and edema (9, 10, 11), decreased liver function as evidenced by a hard nodular, and cirrhotic liver (1, 3, 12), and enlargement of the heart and spleen. Anemia is common (2, 3, 10, 13).

All degrees of poisoning are possible, from mild, chronic changes to acute reactions terminating in death from respiratory failure (3). Factors affecting animal response include the type of selenium compound administered, the species and sex of the laboratory animal used, the age of the animal, conditions of the test, caloric intake, and the criterion of toxicity employed (5). In addition, there seems to be an extreme animal variation in response to selenium poisoning (6, 13).

LEAD

Lead poisoning has been recently brought to national attention through popular magazine articles and newspaper publicity. Children in deteriorating housing have ingested enough lead from paint chips to cause severe anemia and mental retardation (14, 15). About 25 mg % in the diet is considered toxic. Absorption by painters and gasoline handlers makes special precautions necessary to prevent lead poisoning. It is speculated that part of Vincent Van Gogh's madness and eye problems were due to lead poisoning from his lead-oxide paints (16). In animals, brain lesions (14) and anemia (17) are the most commonly recorded signs of lead poisoning. The most sensitive indicator is said to be the presence of microscopic intra-nuclear inclusion bodies in renal tubular lining cells (15).

As a disorder of heme synthesis in erythropoeisis (17), lead poisoning causes basophilic stippling of red blood cells. It seems to alter ribosomal RNA in immature erythrocytes in such a way as to cause ribosomes to remain in maturing cells and precipitate when stained (18). These stippled red blood cells are thought to be altered reticulocytes. Accumulation of lead in the liver (3) may also lead to its dysfunction.

ZINC

Perhaps less familiar than lead poisoning is ingestion of toxic amounts of zinc. Fruit juices mixed or stored in galvanized containers can dissolve enough zinc to cause gastrointestinal upset in man (19, 20). In

experimental animals, 500 to 1,500 mg % zinc in the diet causes anorexia, growth depression, and hypochromic, microcytic anemia (20, 21, 22). These toxic effects are accompanied by high levels of zinc in the liver (23). Zinc competes with copper and iron (24) for uptake and utilization in enzyme systems, and the anemia of zinc toxicity apparently results first from an induced copper deficiency, and second, from an induced iron deficiency (3).

INTERACTIONS OF TRACE ELEMENTS

There are several possible effects of the combined administration of two potentially toxic substances. They might be:

1. The first has no effect on the toxicity of the second whose effect remains as before.
2. The first depresses or eliminates the toxic symptoms of the second.
3. The first cancels the toxic effects of the second and substitutes its own set of characteristic symptoms.
4. The first adds its own set of toxic symptoms to those of the second.
5. The first potentiates the action of the second and accentuates its toxic effect.
6. The first causes the second to be toxic at levels lower than the second would be alone.

Relatively little work has been done on the interactions of selenium, lead, and zinc. Moxon and Rhian (2) stated that lead had no effect on the toxicity of selenium, but gave no reference to a specific study. Leavander and Argrett (26) found lead to have no effect on selenium volatilization when injected into rats. No other mention of selenium-lead interaction could be found in the literature.

Previous studies on the effect of zinc on selenium toxicity indicated that there was a potentiation of selenium's toxic effect as evidenced by growth depression (25) and lowered hemoglobin levels (2). Substantiation of this conclusion was needed because the level of zinc used in these studies was so low that its effect was questionable. One study of selenium-zinc interaction used only 0.5 mg% zinc (2) and the other used 1.2 mg % (25) whereas Underwood reported (3) no toxic effects in rats even at a 250 mg % dose level. Amounts from 500 to 1,000 mg % are considered toxic.

The purpose of this project was to see what the effects of subtoxic doses of lead or zinc might be on selenium poisoning in mice.

Chapter 2

METHODS

Twenty-four weanling male albino Swiss-Webster mice[1] weighing from 16 to 20 g each were divided into 4 groups of 6 animals each. Each mouse received a total of 4 g of feed per day. The test elements were fed separately to prevent chemical interactions in the gastrointestinal tract. Group I was fed control diet night (7 P. M.) and morning (9 A. M.). Group II was fed control diet in the morning and selenium diet at night. Group III was fed lead diet in the morning and selenium diet at night, and Group IV was fed zinc diet in the morning and selenium diet at night.

Pilot studies with Swiss-Webster mice of mixed sex and weights from 18 to 26 g had been conducted previously to estimate toxic levels of selenium, lead, and zinc, and to determine appropriate nutritional feed rates. Following the results of those studies, weekly diets were prepared and specific 2-g portions were weighed into folded-paper packets. The control diet was a 50:50 mixture of ground rye[2] and Purina Lab Chow. Variations were as follows:

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[1] Simonsen Laboratories, Gilroy, California.

[2] Obtained from San Miguel, California through Mrs. Patricia Saam.

Selenium Diet: control diet plus 2.5 or 1.5* mg % Se as $Na_2SeO_4 \cdot 10\ H_2O$ (sodium selenate).

Lead Diet: control diet plus 15 mg % Pb as $Pb(CH_3COO)_2 \cdot 3\ H_2O$ (lead acetate).

Zinc Diet: control diet plus 1,000 mg % Zn as $5\ ZnO \cdot 2CO_2 \cdot 4\ H_2O$ (zinc carbonate).

*Due to the death of one animal and the poor appearance of several others at the midpoint of the experiment, it seemed advisable to reduce the selenium dose level from 2.5 to 1.5 mg % for the remainder of the three-week period in order to maintain a chronic rather than sub-acute toxicity.

Each group was given a small can into which the contents of the feed packets for all mice in the group were pooled. In order to minimize waste, another can was provided in which the mice could sleep and play. Water was constantly available from drip bottles.

Food intake was roughly estimated from the amount left in the can after each feeding, and any unused food was discarded. Water uptake was determined by weighing the bottles daily. Each animal was weighed daily, and growth charts were kept. Any unusual behavior or appearance of the mice was also noted and recorded.

The mice were housed in animal room facilities at California Polytechnic State University at temperatures ranging from 25° C in the day to 18° C at night. Artificial lighting was controlled on a 10:14 light-dark ratio.

Two mice from each group were sacrificed after each week so that a progressive picture of anemia induction and possible subsequent accommodation could be observed. The heaviest and lightest mouse of each group was

selected each time in an attempt to equalize weights between groups and minimize competition for food or social domination within the groups. Animals sacrificed after the first week were labeled "A" and "B". Animals "C" and "D" were sacrificed after the second week, and animals "E" and "F" after the third week. The heavier animal of each pair was designated "A", "C" or "E". At sacrifice, each mouse, having been fasted 15-20 hours, was etherized until respiration had almost ceased, was immediately weighed, and was decapitated. Blood for hematological determinations was collected in a Unopette capillary tube and three microhematocrit tubes as it issued from the transected neck. The spleen, kidney, and liver were then removed, weighed, and placed on wet paper toweling for comparison and observation of gross features and color.

Hematological data to assess possible anemias included a red blood cell count, hemoglobin determination, hematocrit, reticulocyte count, and examination of Wright's-stained blood films for anisocytosis, poikilocytosis, basophilic stippling, or other anomalies of the red blood cell.

A Unopette (#2705) and Improved Neubauer Hemocytometer were used to dilute and count red blood cells. The diluted blood not used for the red cell count was used to determine relative hemoglobin concentrations. The percent absorbance of the acid-lysed cells in the solution was determined at 640 nm with a Bausch and Lomb Spectronic 70 spectrophotometer.

Two microhematocrit tubes of blood were centrifuged to obtain the hematocrit. For reticulocyte stains, equal portions of New Methylene Blue

and blood were mixed in a capillary tube, allowed to stand 10 minutes, spread on a slide, and air dried (27). After the films were counter-stained with dilute Wright's stain, reticulocyte counts were made using a modification of the conventional counting method as follows: All the red blood cells (including reticulocytes) touching the scale or numbers of a Swift 111 ocular micrometer are counted. Then all the reticulocytes visible in the whole field of view are counted. The ratio of field reticulocytes to red blood cells touching the micrometer is expressed as a percentage and then divided by 15.[1] The resulting number corresponds to a conventional raw percentage of reticulocytes in red blood cells (28). A further correction of this percentage which takes into account abnormal blood cell counts and gives a more useful result is obtained by multiplying the reticulocyte count by the ratio of the experimental red cell count to a normal or average red cell count.

Blood film slides (2 per animal) were examined in random order and qualitative evaluations were made on three separate occasions, each time without knowing which animal's blood was being examined. The presence of anisocytosis or poikilocytosis was recorded on a scale using the following criteria:

0 = uniform cells

1 = slight abnormality (less than 25% of cells affected)

2 = moderate abnormality (25-50% of cells affected)

3 = marked abnormality (50-75% of cells affected)

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[1] The number 15 is obtained by doing simultaneous conventional counts and averaging the ratio of correspondence for each count of 200 red blood cells.

Anisocytotic abnormality was considered to be notable reduction in cell size of part of the red cell population. Poikilocytosis was defined as lack of roundness of the cells. Unround cells were considered abnormal. Other abnormalities were recorded as present or absent or described qualitatively.

Toxic effects on the spleen, kidney, and liver were assessed by relative organ weight determinations and observations of gross features and color.

Chapter 3

RESULTS

The following examination of results will treat each dependent variable (parameter of evaluation) in relation to time as well as to each independent variable (treatment) in the diet.

CONTROL ANIMALS

Growth and Feed Consumption

The control animals consumed all the P. M. feed offered them after the fourth day, but all the A. M. feed only after the sixth day (Table 1). The expectation that, being nocturnal animals, the mice's consumption of P. M. feed would be greater (56% of the total 4 g allotment) than consumption of A. M. feed (44% of total) was affirmed. Weight gain was greatest during the first week, averaging 2. 6 g per mouse (Figure 1), after which it seemed to level off at about 1. 4 g per mouse.

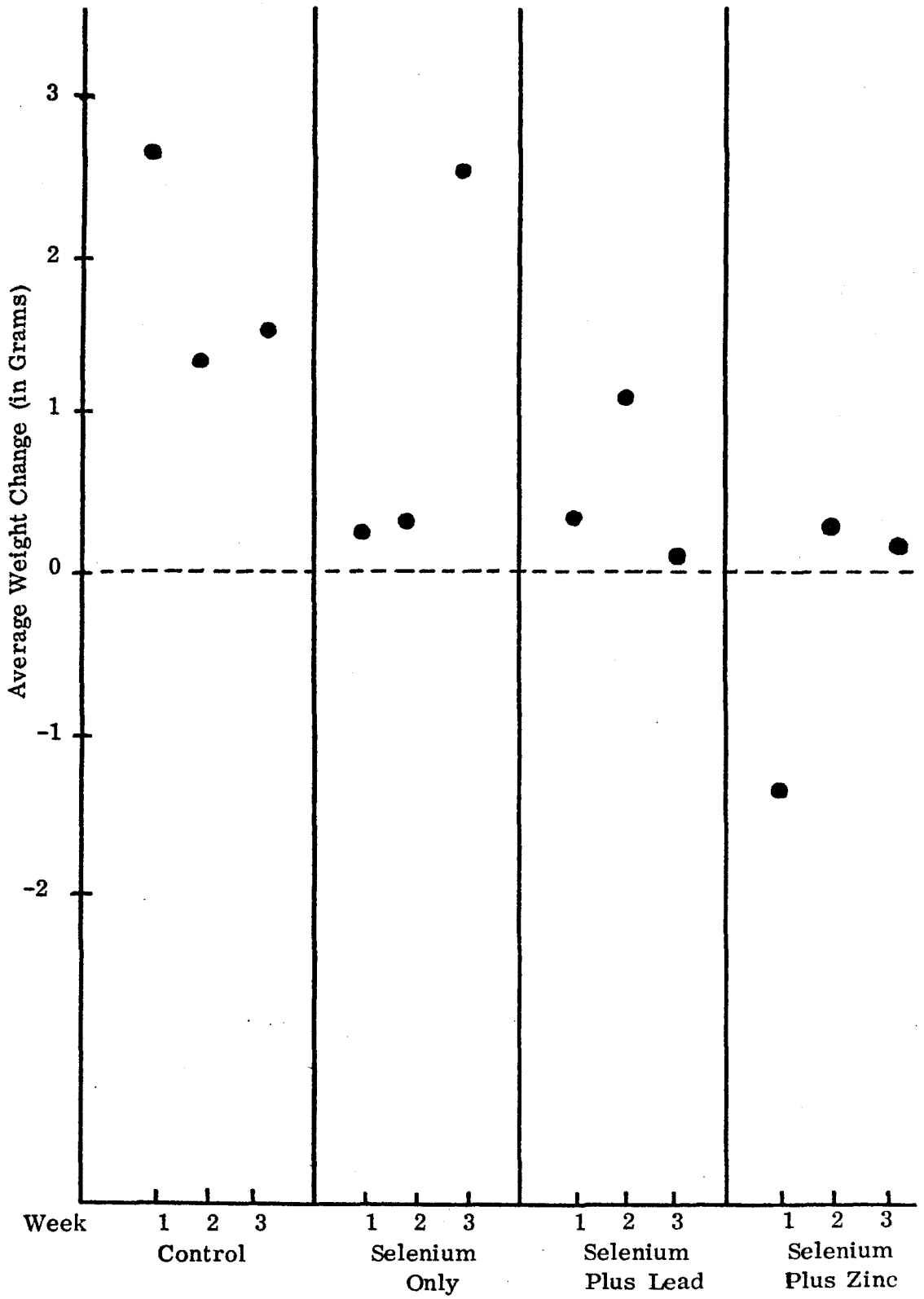
Hematology

Red blood cell counts averaged 6. 6 X 10^6 cells/mm^3 for the first week and then seemed to level off at approximately 8 X 10^6 cells/mm^3 during the second and third weeks (Figure 2). Hematocrits and hemoglobin concentrations seemed to follow suit (Figures 3 and 4). Reticulocyte counts were

Table 1

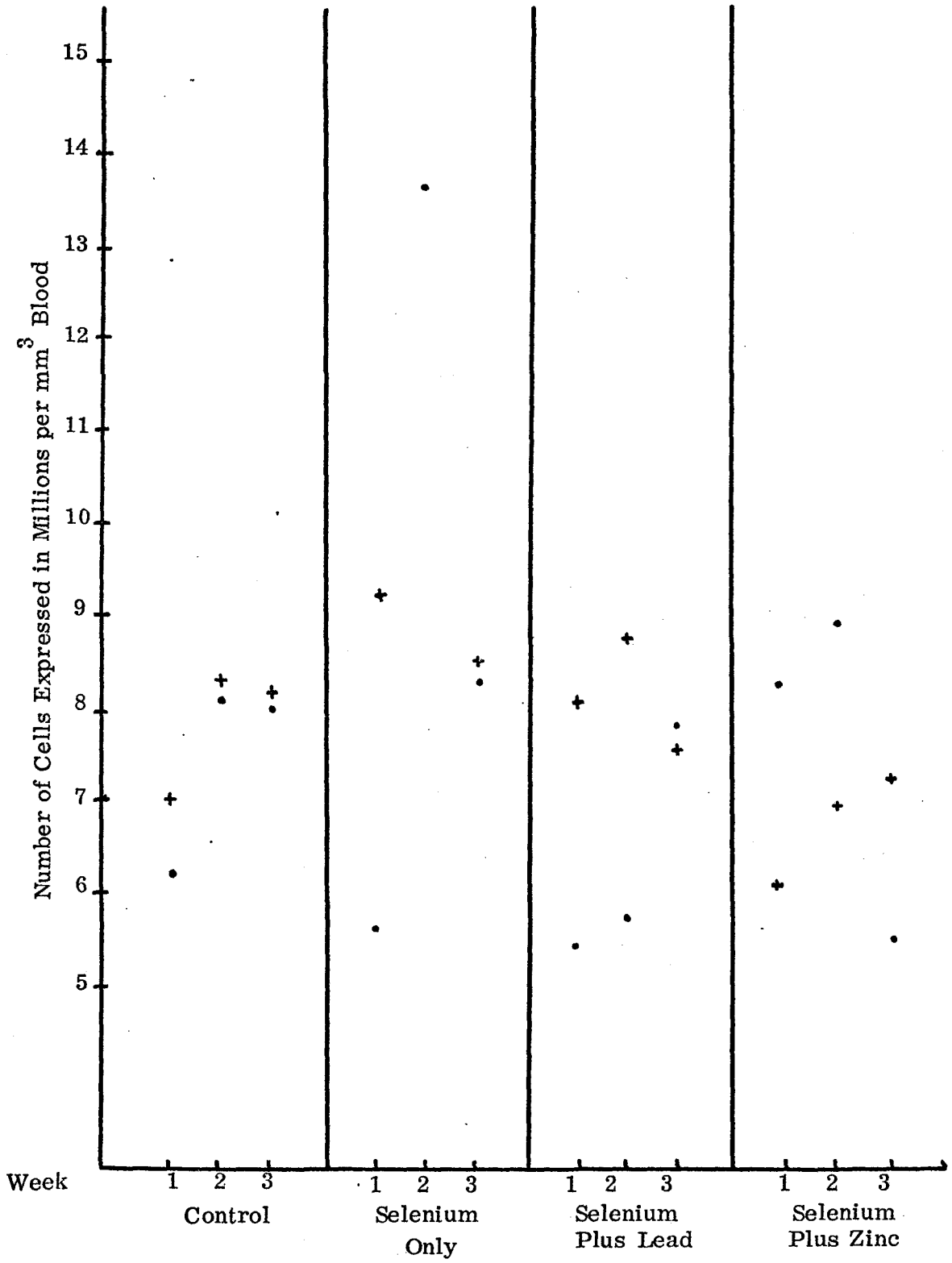
Average Weekly Feed Consumption of Mice Fed Selenium and Selenium-Lead and Selenium-Zinc Diets

	Control	Selenium only	Selenium-lead	Selenium-zinc
Week I				
A. M.	1.5 g	1.1 g	1.9 g	1.7 g
% of total consumed	44 %	55 %	66 %	68 %
P. M.	1.9 g	0.9 g	1.0 g	0.8 g
% of total consumed	56 %	45 %	34 %	32 %
Total consumed	3.4 g	2.0 g	2.9 g	2.5 g
% of possible	85 %	50 %	73 %	63 %
Week II				
A. M.	2.0 g	2.0 g	2.0 g	2.0 g
% of total consumed	50 %	53 %	57 %	54 %
P. M.	2.0 g	1.8 g	1.5 g	1.7 g
% of total consumed	50 %	47 %	43 %	46 %
Total consumed	4.0 g	3.8 g	3.5 g	3.7 g
% of possible	100 %	95 %	88 %	93 %
Week III				
A. M.	2.0 g	2.0 g	2.0 g	2.0 g
% of total consumed	50 %	50 %	50 %	50 %
P. M.	2.0 g	2.0 g	2.0 g	2.0 g
% of total consumed	50 %	50 %	50 %	50 %
Total consumed	4.0 g	4.0 g	4.0 g	4.0 g
% of possible	100 %	100 %	100 %	100 %



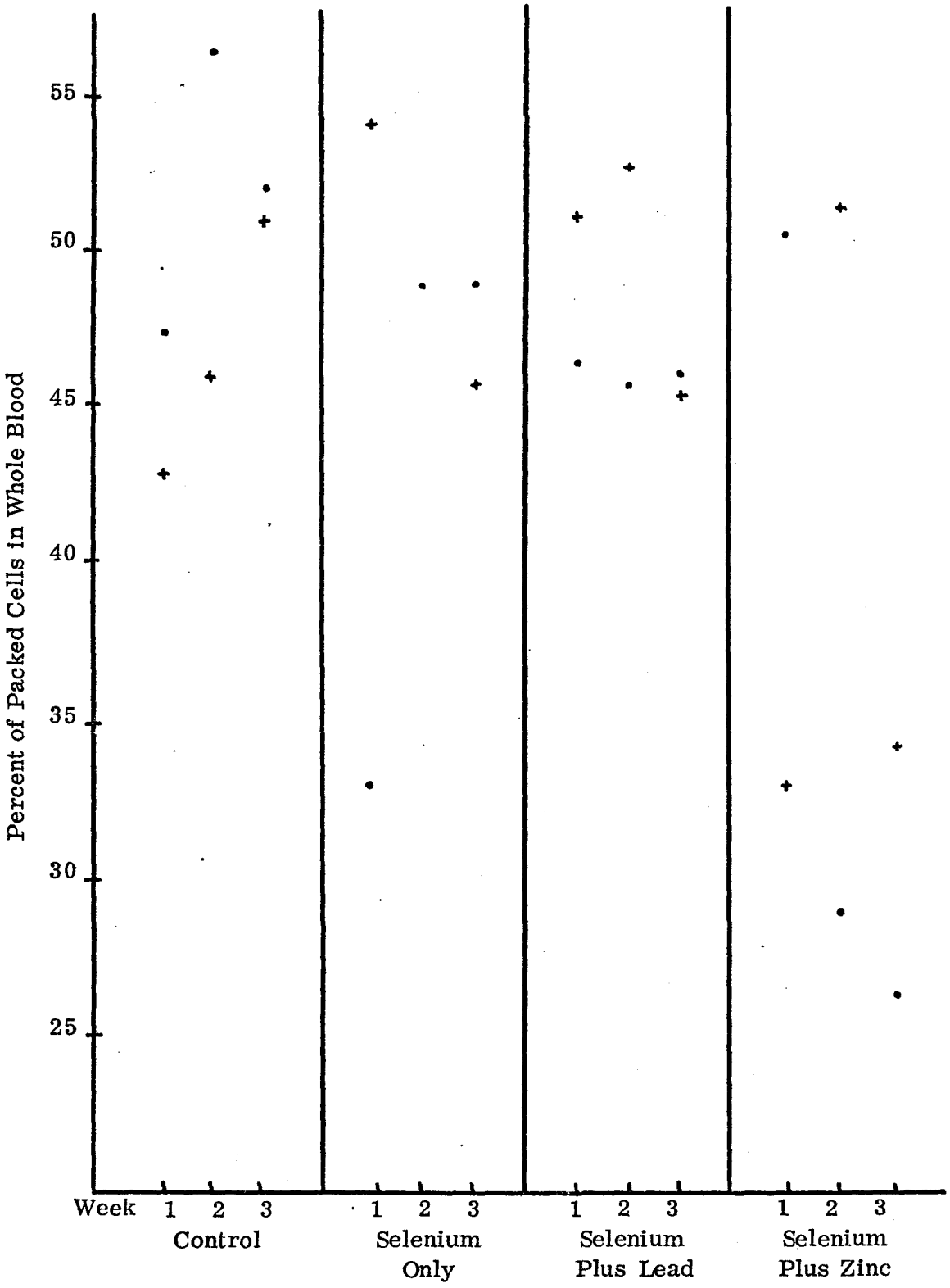
(Each plot point represents the two mice of each group sacrificed at the end of 1, 2, and 3 weeks)

Figure 1. Average Weight Gain of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets.



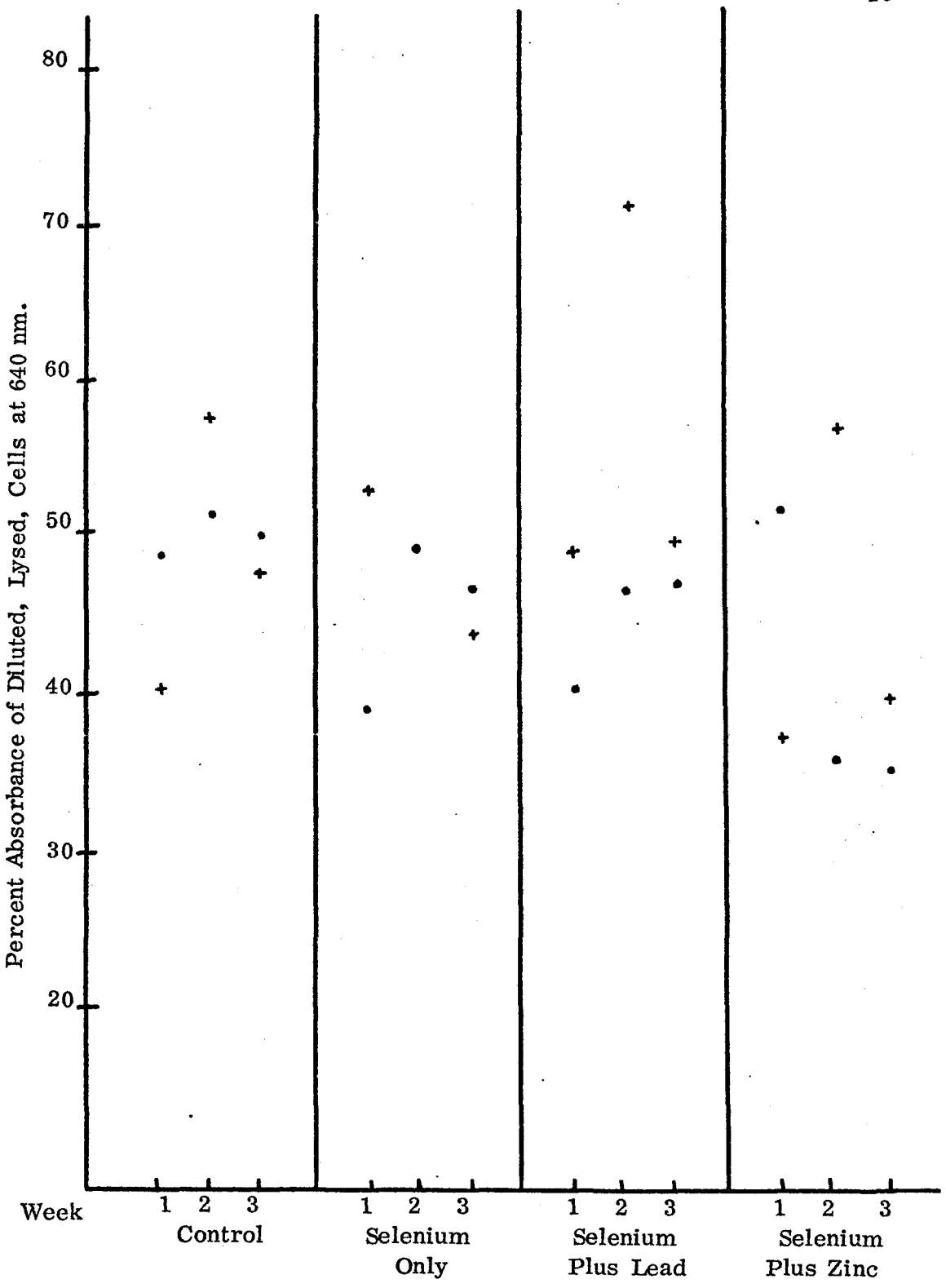
• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, C, or F or weeks 1, 2, and 3 respectively.

Figure 2. Red Blood Cell Count of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets.



• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, D, or F of weeks 1, 2, and 3 respectively.

Figure 3. Hematocrits of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets.



• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, D, or F of weeks 1, 2, and 3 respectively.

Figure 4. Hemoglobin Concentration of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets.

variable, averaging 2.4% (Figure 5). Animal 3E (the largest) had an elevated reticulocyte count (4.6%) which could not be accounted for.

Organs

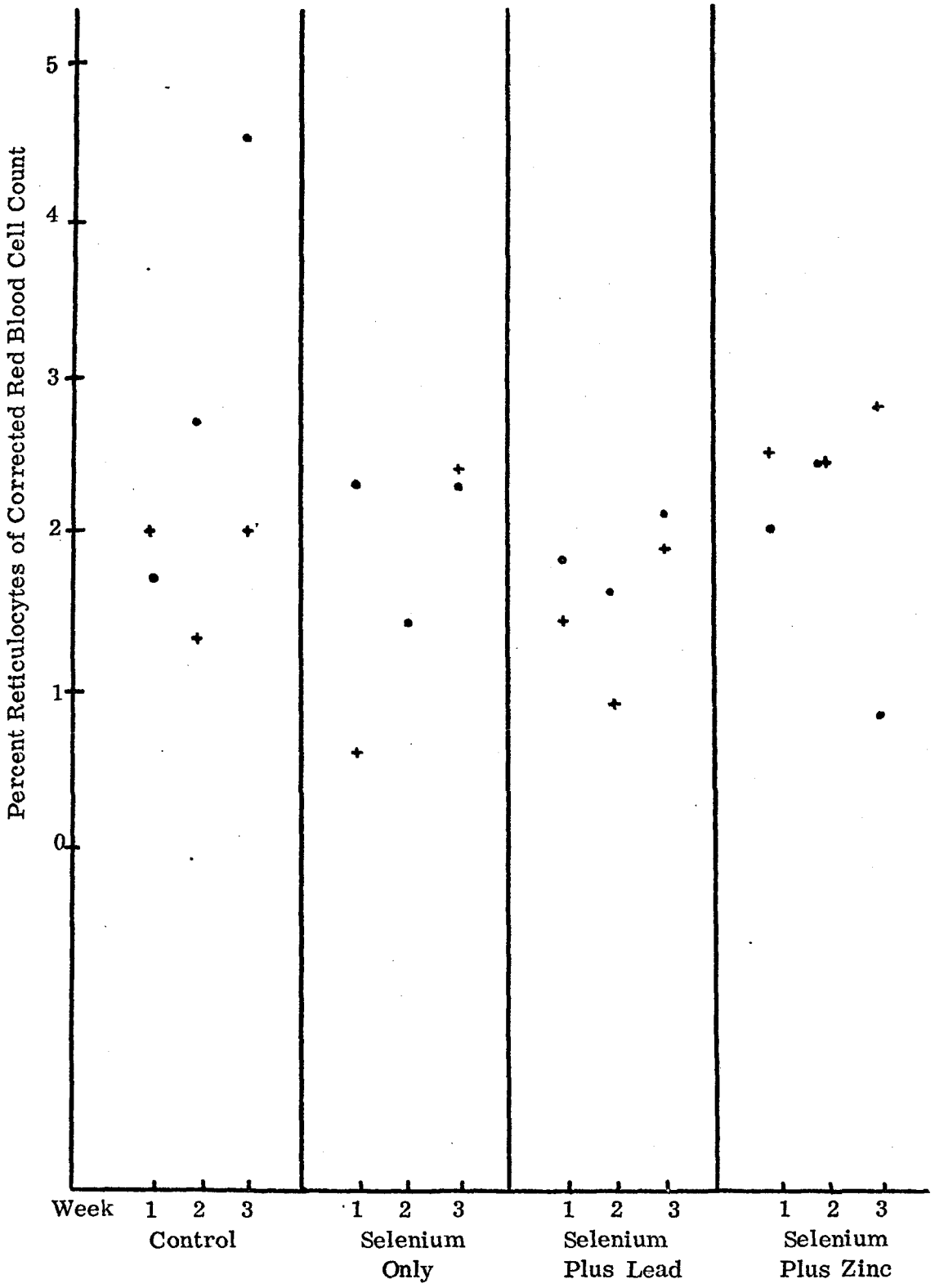
Animals in the control group showed a definite decrease in relative kidney weights with time, dropping from about 1.4 to 1.2 g % during the three week period (Figure 6). Liver weights also dropped from approximately 5.2 g % to 4.4 g % (Figure 7). The spleen however, increased in relative weight with time from about 0.45 g % to 0.51 g % (Figure 6).

Other parameters showed no appreciable time-based changes and simply served to establish approximate control levels. Some of these data are recorded in the Appendix. Complicating factors in the control animal picture included one evidently sick animal (Figure 8, animal 1B) with a low hematocrit (43%) and depressed growth rate, and another animal (1D) with a slightly darker liver, high red cell sedimentation rate, and rough-looking fur.

SELENIUM ONLY GROUP

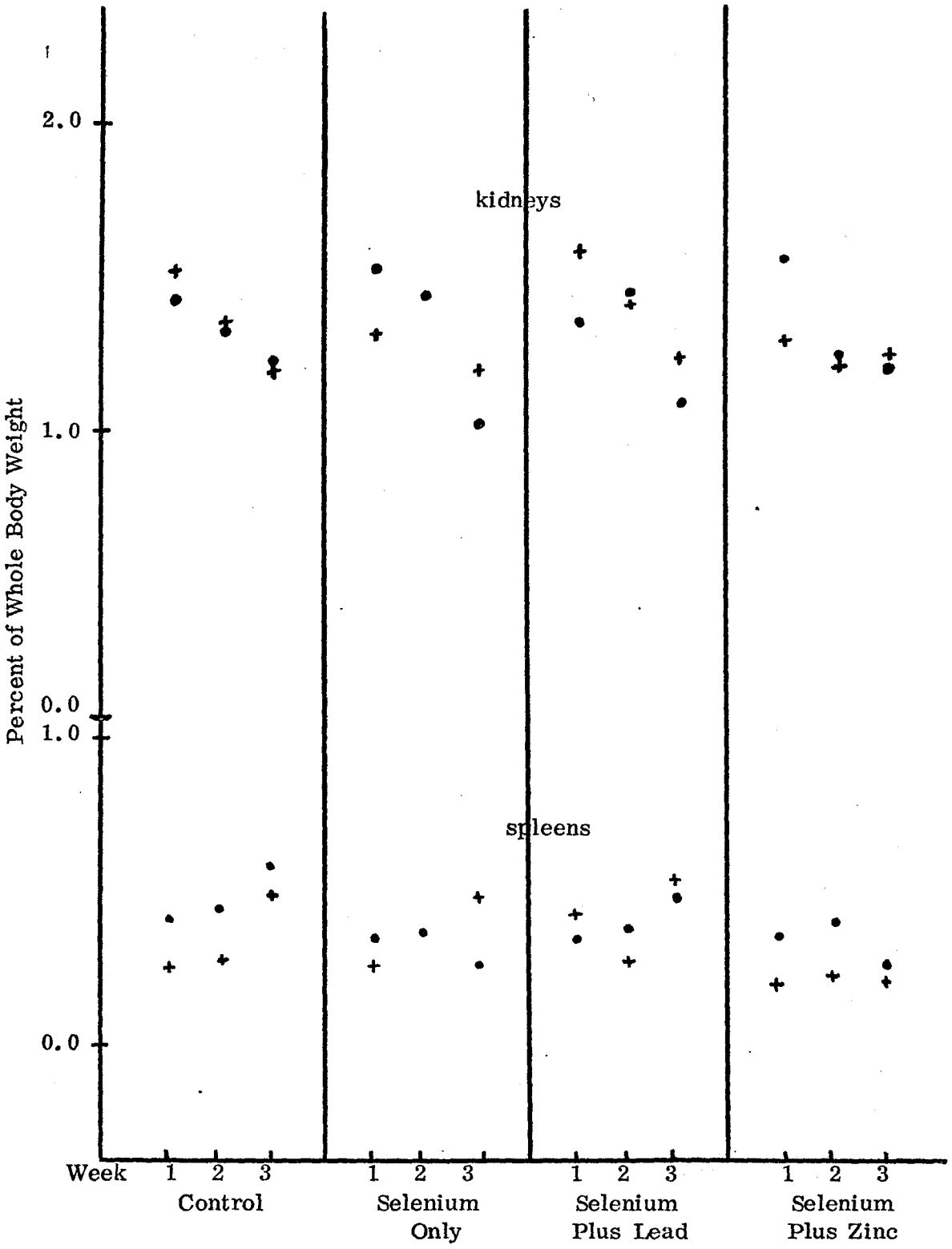
Growth and Feed Consumption

Some anorexia due to selenium in the diet was evident from the outset. Not only was consumption of selenium-containing feed low, but during the first week, the animals given selenium in the P.M. feed ate less control (A.M.) feed (1.1 g compared to 1.5 g for the control group). Although feed consumption was low during the first week, it increased during the second week to the control level (Table 1).



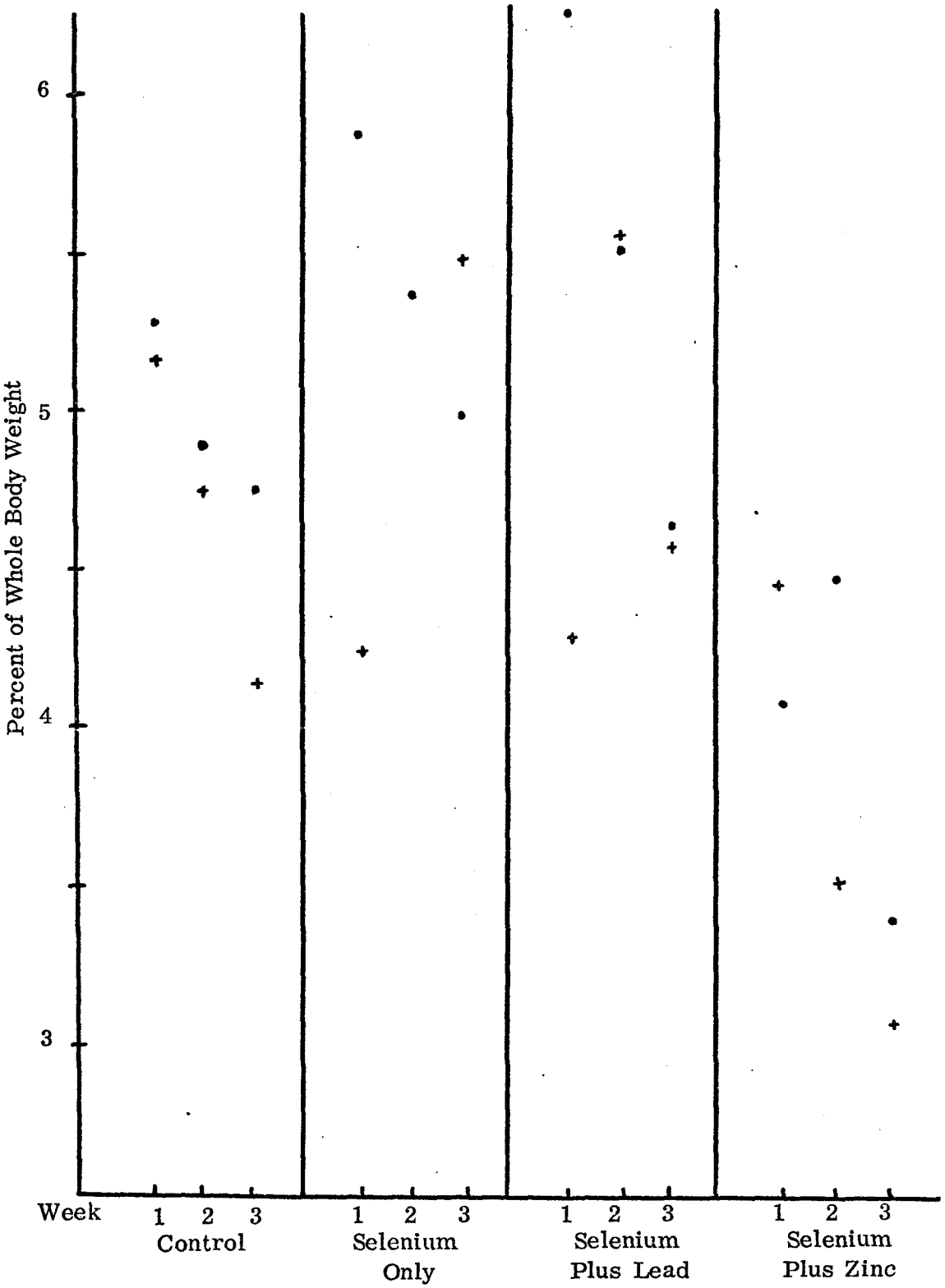
• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, D, or F of weeks 1, 2, and 3 respectively.

Figure 5. Reticulocyte Counts of Mice Fed Control, Selenium, Selenium-Lead and Selenium-Zinc Diets.



. = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ - animal B, D, or F of weeks 1, 2, and 3 respectively.

Figure 6. Relative Kidney and Spleen Weights of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets.



• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, D, or F or weeks 1, 2, and 3 respectively.

Figure 7. Relative Liver Weights of Mice Fed Control, Selenium, Selenium-Lead and Selenium-Zinc Diets.

Table 2

Qualitative Evaluation of Red Blood Cell Anomalies of Size (Anisocytosis) and Shape (Poikilocytosis) in Mice Fed Selenium, Selenium-Lead and Selenium-Zinc Diets (Values Range from 0=Cells 100% Uniform in Size or Shape to 4 More Than 75% Abnormal Cells)

	Control animals							
Animal designation	1A	1B	1C	1D	1E	1F	Median	Average
Anisocytosis	1.7	1.5	0.5	0.0	0.3	1.5	1.0	0.9
Poikilocytosis	0.0	0.5	0.5	0.0	0.2	0.75	0.4	0.3
	Selenium-Only Animals							
Animal designation	2A	2B	2C	2D	2E	2F	Median	Average
Anisocytosis	2.0	0.7	1.5	---	0.5	0.5	0.7	1.1
Poikilocytosis	1.0	0.3	1.5	---	0.8	0.3	0.8	0.78
	Selenium-Lead Animals							
Animal designation	3A	3B	3C	3D	3E	3F	Median	Average
Anisocytosis	2.7	1.5	0.5	2.8	1.5	1.8	1.7	1.8
Poikilocytosis	1.3	0.25	0.3	1.7	0.0	1.3	0.8	0.8
	Selenium-Zinc Animals							
Animal designation	4A	4B	4C	4D	4E	4F	Median	Average
Anisocytosis	1.5	1.3	2.5	1.0	3.0	1.2	1.4	1.8
Poikilocytosis	2.0	2.3	2.5	1.3	1.5	1.0	1.8	1.8

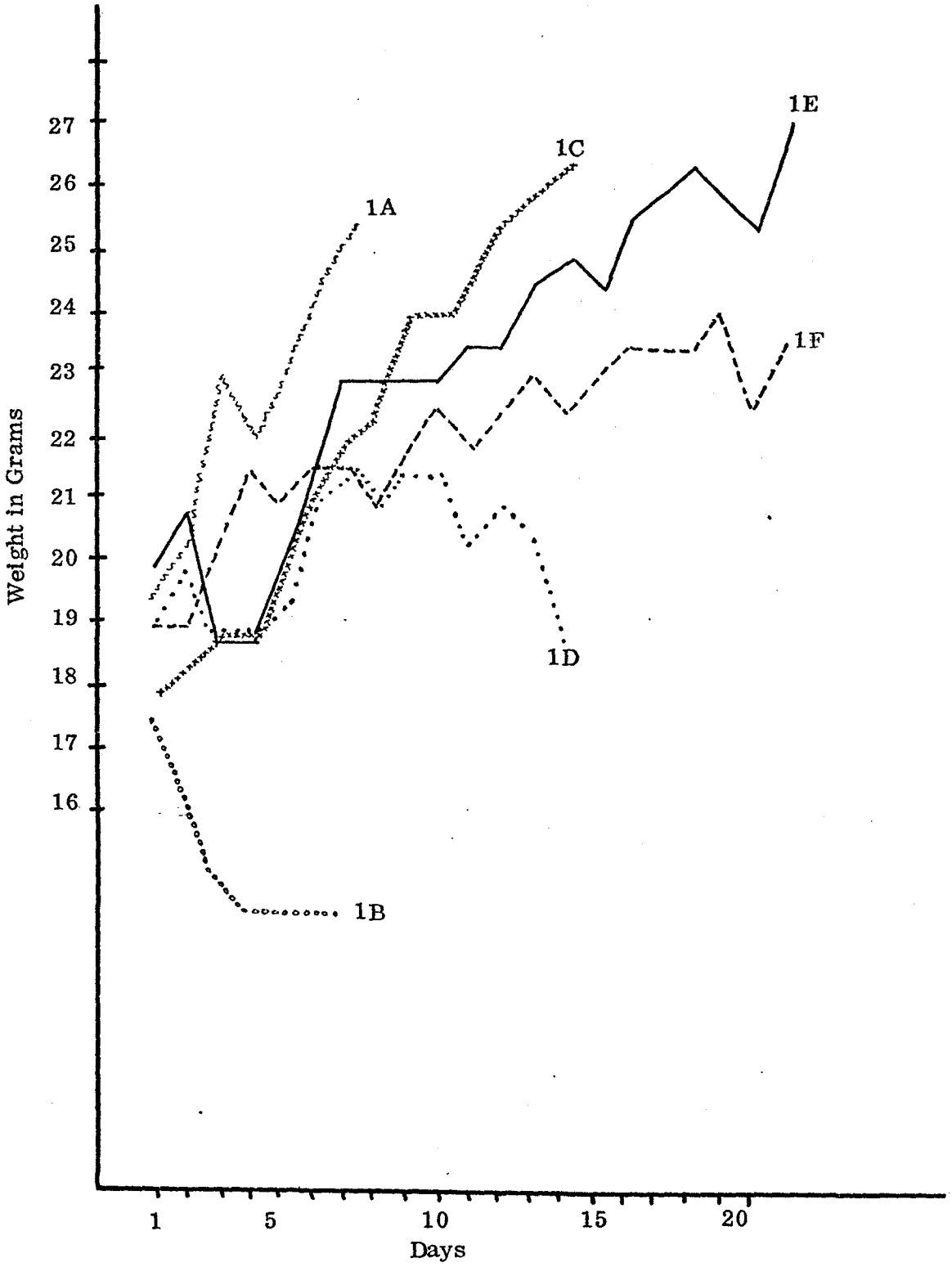


Figure 8. Daily Weights of Mice Fed Control Diet

One animal in this group died on the 8th day (Figure 9, animal 2D), and no blood or organ work could be done on it. To prevent further premature deaths, the selenium dosage was reduced from 2.5 to 1.5 mg %. Whether due to accustomization to feed or reduction of selenium levels, the toxic symptoms and signs of this group were less apparent after the second week when the animals had reached their nadir in almost all respects.

Hematology

In general, any anemia which might have been selenium-induced was very slight. Animal 2A was definitely anemic, however, as evidenced by its hematocrit of 33% (Figure 3), but since this animal was autopsied after only one week of treatment, it cannot be determined whether this effect was due to starvation or subacute selenium poisoning. The somewhat lowered mean corpuscular volume of animal 2C (36 u^3, Figure 10) is probably not accurate because of an error in the red blood cell count thought to be caused by overfilling of the Unopette capillary tube.

Anomalies in red cell shape as determined by random, blind, qualitative evaluation (Table 2) were twice as common in the selenium-only group as those in the control group as shown by average poikilocytosis values. Other blood indexes showed no noticeable variation from control levels.

Organs

Livers from animals in this group were slightly larger than controls, averaging 5.2 g % compared to 4.8 g % for the controls (Figure 7). They were

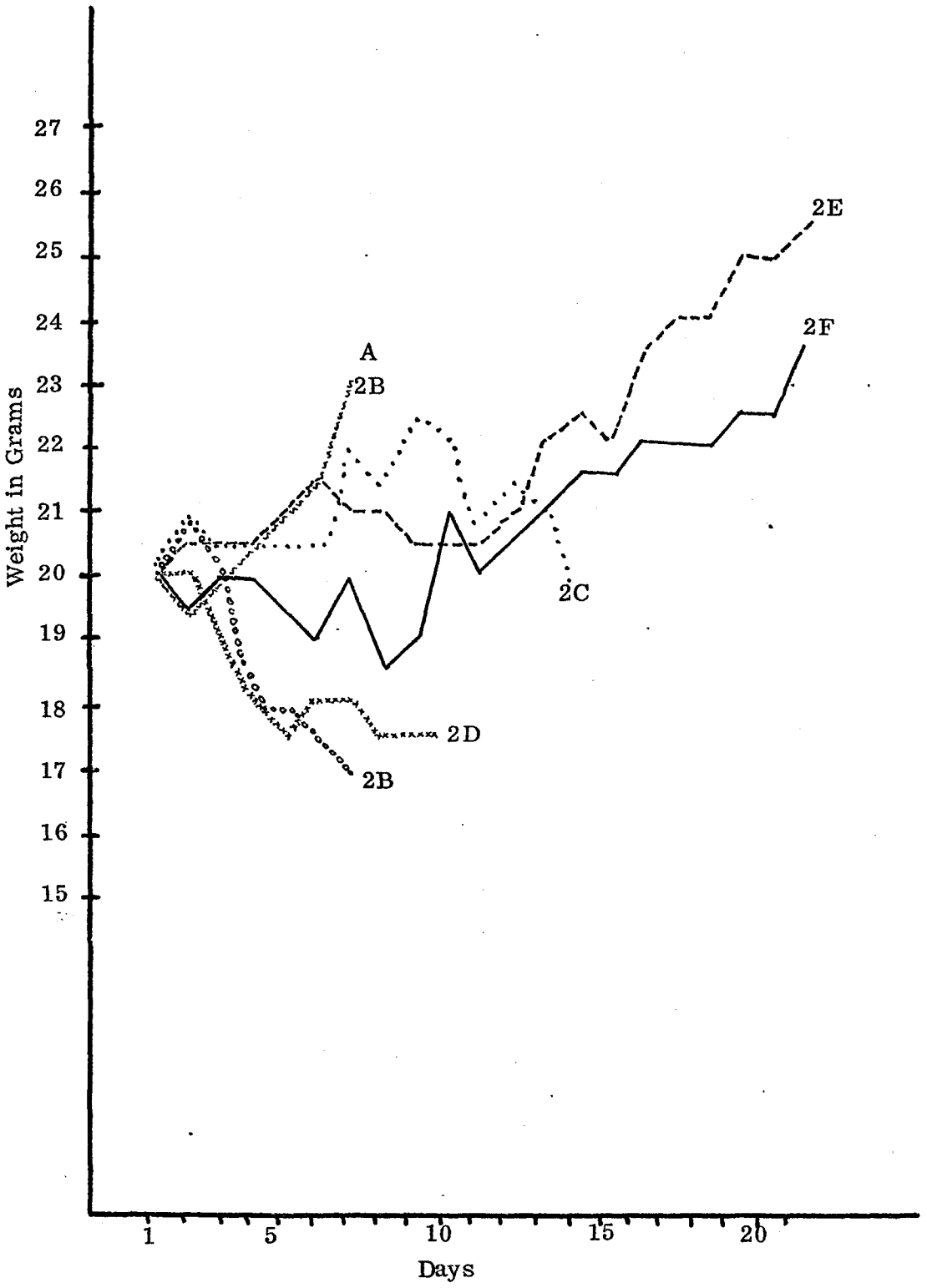
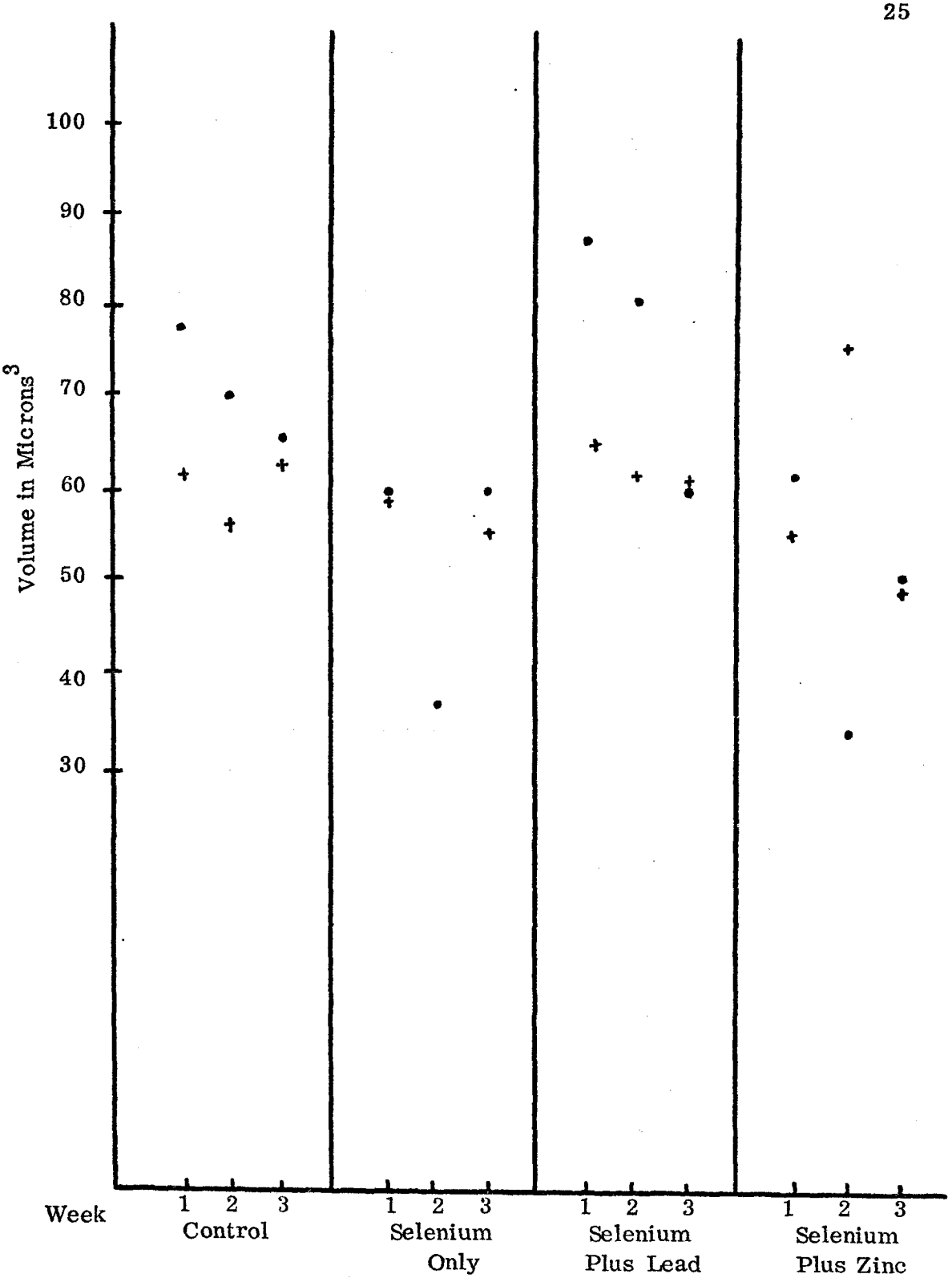


Figure 9. Daily Weights of Mice Fed Selenium Only Diet



• = animal A, C, or E of weeks 1, 2, and 3 respectively.
+ = animal B, D, or F of weeks 1, 2, and 3 respectively.

Figure 10. Mean Corpuscular Volumes of Mice Fed Control, Selenium, Selenium-Lead and Selenium-Zinc Diets.

lighter in color, and, until the third week, speckled or mottled in appearance. Apparently the livers were able to recover by the third week and showed no speckling although they were still slightly larger than controls (5.3 g % compared with 4.3 g %). Kidney weights (Figure 6) and color were normal except for a darkly colored one from animal 2E. Spleens appeared normal (Figure 6).

SELENIUM PLUS LEAD GROUP

Growth and Feed Consumption

During the first two weeks, the mice in the selenium plus lead group showed a feeding pattern similar to the selenium control group (Figure 11). Consumption of A. M. feed (containing lead), however, was not reduced from control levels of 2 g as it was in the selenium only group. The growth chart for the selenium plus lead group shows approximately the same trends as does the selenium only group (Figure 12).

Hematology

Red blood cell counts, hematocrits, and reticulocyte counts in the selenium plus lead group were not noticeably different from those of the control group, averaging 7.2×10^6 cells/mm^3, 48%, and 1.5% respectively (Figures 2, 3 and 5). Two animals (3C and 3D) showed evidence of two separate red blood cell populations--one large and hypochromic and one small and more darkly colored.

Animal 3D which had been losing weight (Figure 12), had a high red blood cell count of 8.7×10^6 cells/mm^3 compared with 7.6×10^6 cells/mm^3

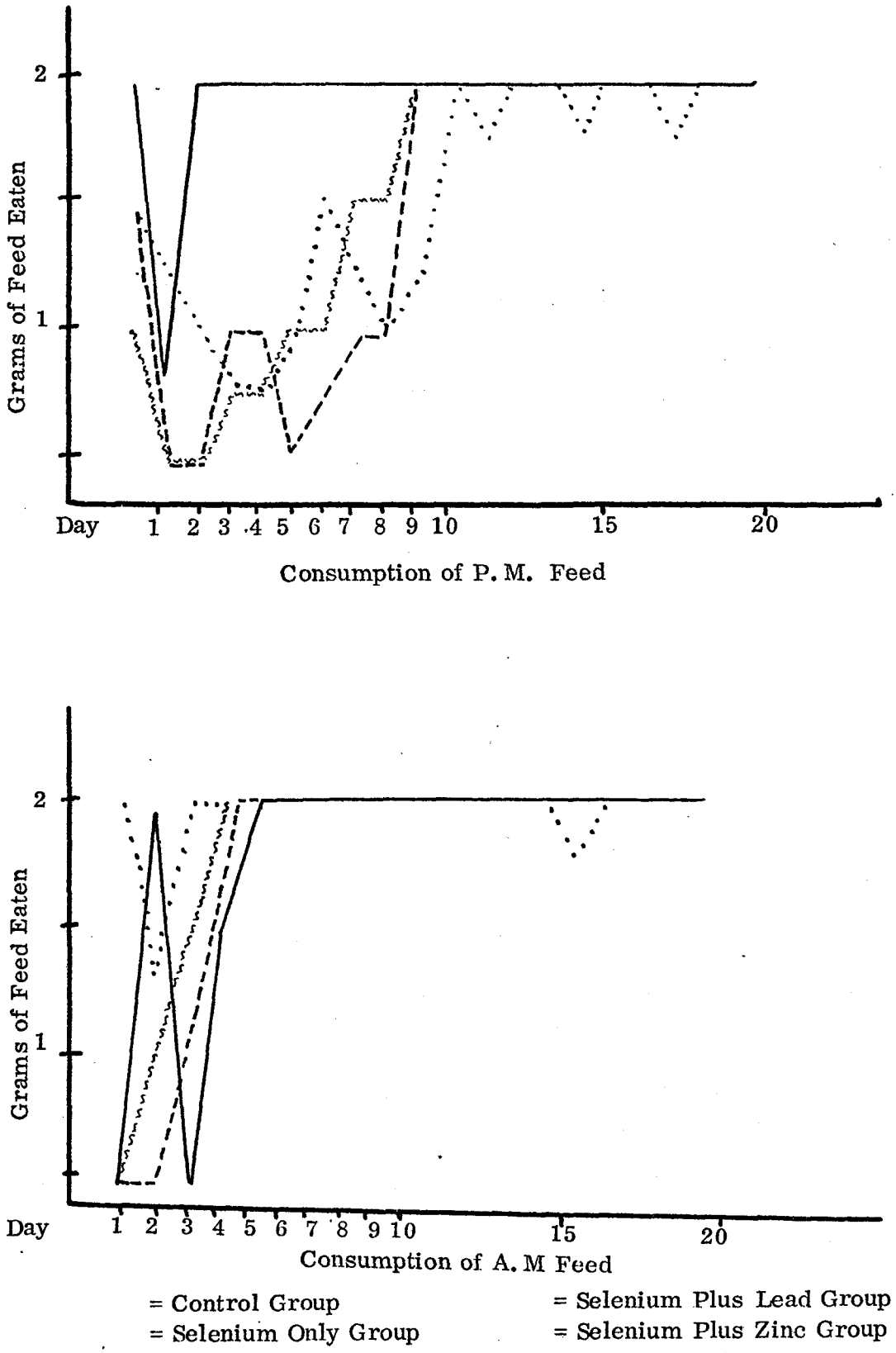


Figure 11. Estimated Average Feed Consumption per Mouse per Day of Mice Fed Control, Selenium, Selenium-Lead, and Selenium-Zinc Diets

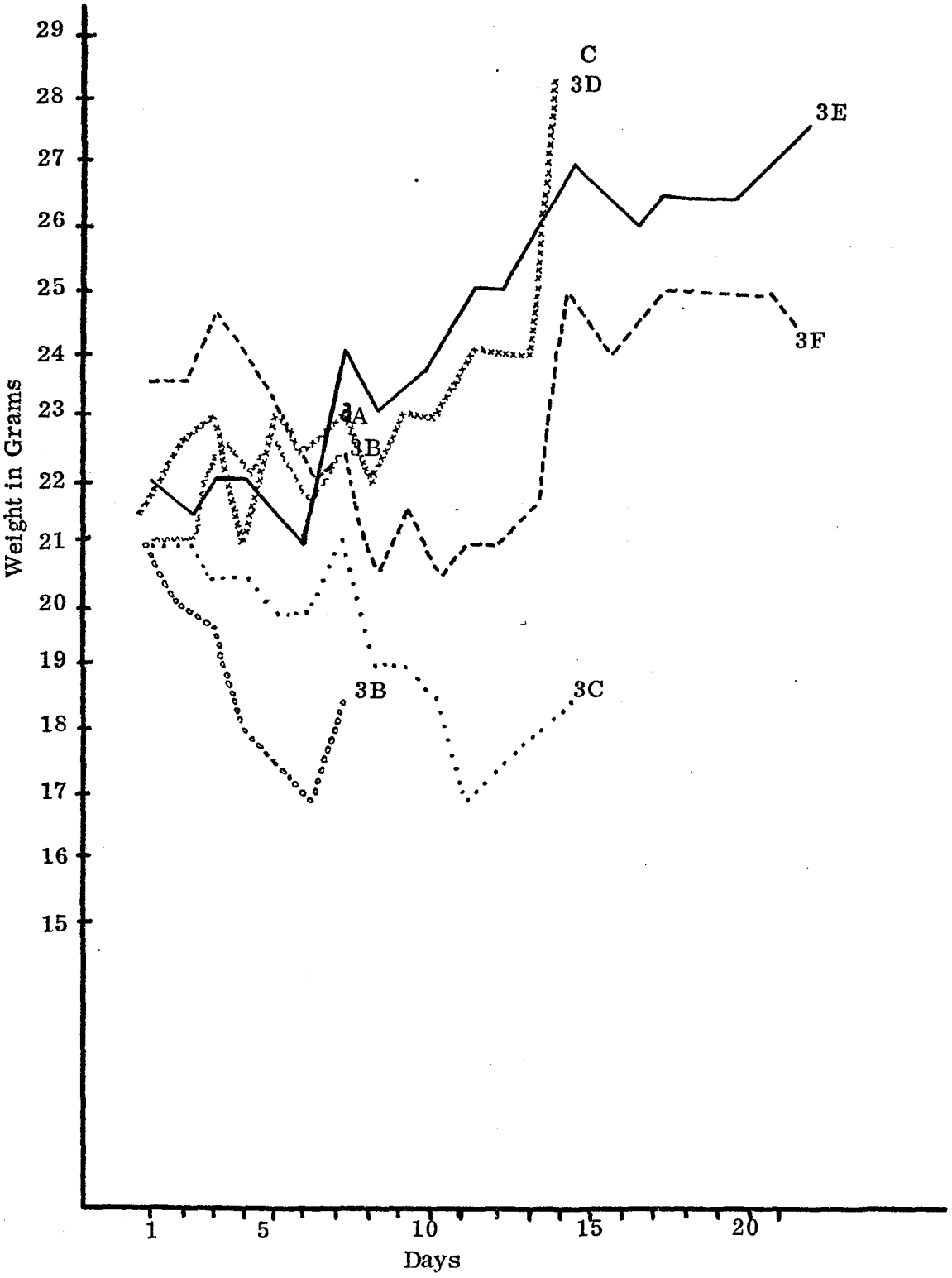


Figure 12. Daily Weights of Mice Fed Selenium Plus Lead Diet

in the control group (Figure 2). It also showed a high hemoglobin level as indicated by 70% absorbance at 640nm compared with 50% in the control group (Figure 4). The reticulocyte count, at 1.5% was also 0.9% lower than control level (Figure 5) and the spleen was only 0.19 g % of the body weight (Figure 6). Blood films from the last two animals (3E and 3F) both showed a high incidence of crenated cells. All animals in this group showed more anomalies of red cell shape and size than did the control or selenium-only groups (Table 2).

Organs

Livers in the selenium plus lead group varied in size averaging 5.1 g % as compared to 4.8 g % in the controls (Figure 7), several being light in color and mottled or speckled. There were no noticeable changes in kidney or spleen size, but several kidneys were very light in color and one spleen was a little darker than the rest.

SELENIUM PLUS ZINC GROUP

Growth and Feed Consumption

The selenium plus zinc group showed the most significant changes in the measured variables. Average selenium feed (P.M.) intake was very low during the first week (0.8 g as compared with 1.9 g in the control group), while zinc feed (A.M.) consumption was slightly increased from both control group and selenium only group levels (Figure 11). Total feed intake increased during the second and third weeks to the control level of 4 g per day (Table 1).

Growth charts, however, show a definite weight loss by most of the animals throughout all three weeks (Figure 13).

Hematology

Red blood cell counts were in the same range as those of the controls at 7.1 X 10^6 cells/mm^3 (Figure 2), but hematocrits as a whole were very low, averaging 37.4 % (Figure 3). For some unknown reason, however, the hematocrits of two animals (4A and 4D) remained at control levels. The mean corpuscular volume (Figure 10) showed microcytic anemia in some animals (4C, 4E and 4F). Reticulocyte levels were average except for animal 4E whose count was 0.8 % compared with an average of around 2.2 % for the rest of the group (Figure 5). Hemoglobin concentrations were low for all but two animals--the same two with high hematocrits (Figures 3 and 4).

The incidence of anisocytosis was twice as high as controls (1.8 compared to 0.9), and poikilocytosis was 6 times as high as the control group level (1.8 compared to 0.3) (see Table 2).

Organs

Liver size in the selenium plus zinc group was significantly decreased from 4.8 g % (control level) to 3.7 g %, and mottled, speckled, or light-colored livers were characteristic (Figure 7). Although kidney weights were not significantly different from control levels (Figure 6), a very light tan color was noticed in the kidneys from second and third-week animals. Spleen weights were not significantly different from control levels (Figure 6).

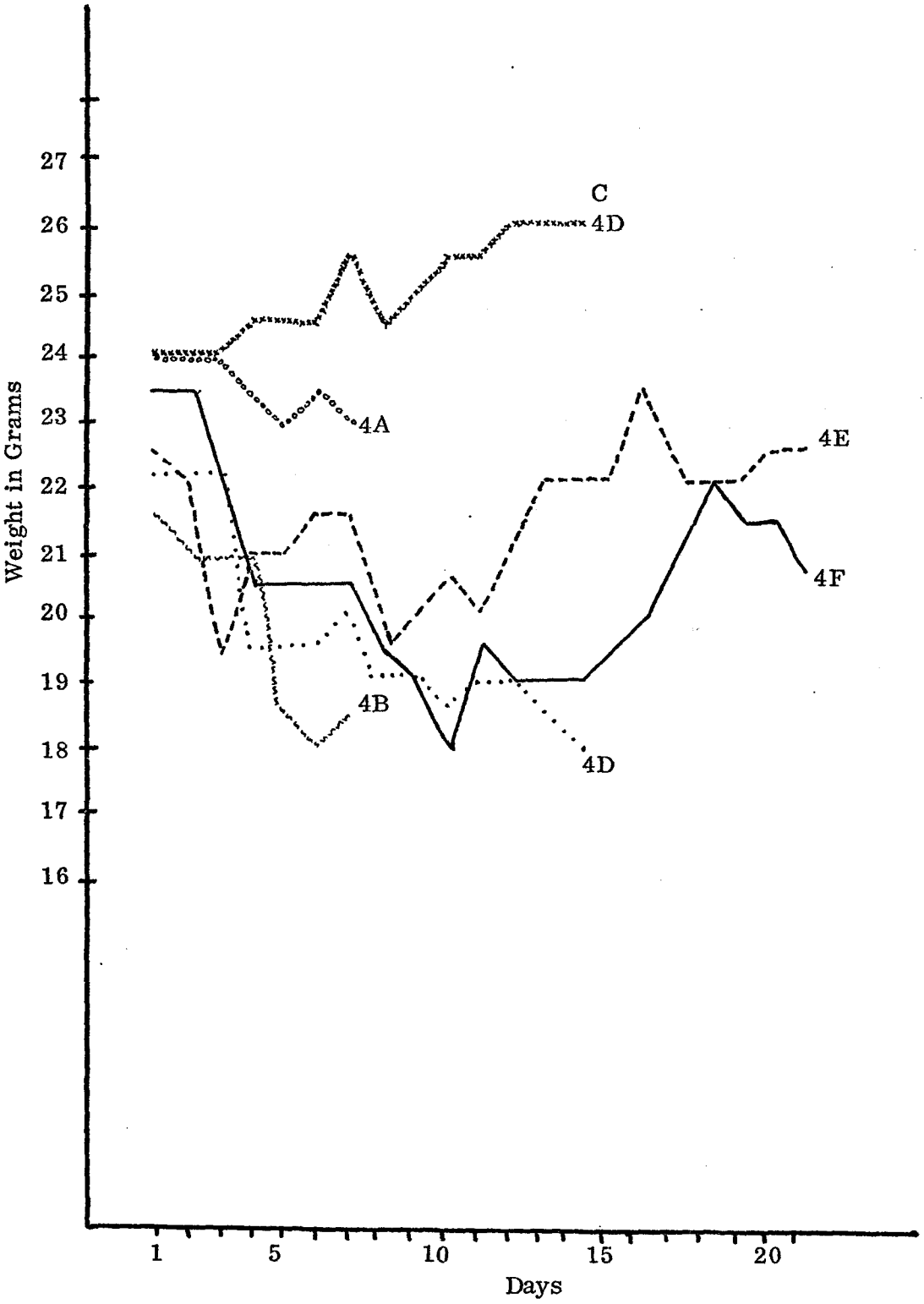


Figure 13. Daily Weights of Mice Fed Selenium Plus Zinc Diet

Chapter 4

DISCUSSION

SELENIUM-ONLY

In the selenium-only group, some of the symptoms of selenosis were obvious while others were absent or reduced. One of the most common symptoms of acute toxicity, anorexia (29), was evident in this group from the onset. As a result, growth was depressed, and many of the other parameters measured from the first week were probably affected. As time went on, however, feed consumption in this group as well as the others, increased to control levels making it more likely that growth depression and other results after this point (second week) were due to the selenium treatment rather than malnutrition.

Hematological data from the selenium-only group did not give evidence of anemia per se, but the qualitative assessment of red cell anomalies showed that there was a definite increase in the percentage of misshapen cells as compared with controls. From studies with ^{59}Fe, it has been found that the anemia which is often associated with selenosis does not seem to be due to a defect in erythropeoisis (as defined by red cell uptake and incorporation of iron), but rather a hemolytic factor (13). This hypothesis is supported in part by the observation that reticulocyte levels in this study remained constant

as did cell size (there was no increase in anisocytosis over the control level). The occurrence of gross changes in the liver (mottling and speckling) may be either an indication of or a cause of hemolysis. In the study by Halverson et al., liver damage and enlargement of the kidneys and spleen were considered to be related to a hemolytic factor as they were concomittant with signs of anemia (13).

The increase in poikilocytosis evident in this study may indicate a defect in erythropoeitic function other than that of hemoglobin synthesis and incorporation as described by Halverson et al. Red cell stroma defects traceable to faulty structural proteins may contribute to red blood cell fragility and therefore appear to be hemolytic factors. There are several theories as to how selenium might interfere with stroma protein synthesis.

One point of view focuses on the similarity of selenium to sulfur. Both selenium and sulfur are designated as part of Group VI-A of the periodic table of the elements, one being right above the other in the column headed by oxygen. It often follows that chemically similar elements can replace one another in their biological roles, and selenium has been found to replace sulfur in certain organic compounds (30). Selenium is known to occur as selenocystine and seleno-methionine in the tissues (30, 31), and apparently some enzymes handle selenium analogs just as readily as they do sulfur compounds (32). Part of selenium's toxic effect may be due to its interference with sulfur metabolism (32) and ultimately protein synthesis (31) which is so dependent on disulfide bonds. The weakness of these bonds could be responsible for the

occurrence of misshapen and fragile red blood cells. The fact that a high-protein diet provides some protection against selenium poisoning (33, 34) supports this amino acid analog approach. A corollary to this concept is the idea that selenium may replace sulfur in specific enzymes as well as structural proteins, thus producing a more catalytic effect on body systems by its excess or paucity.

Some symptoms of selenium poisoning were apparent in the animals of this group which were not tied to any of the quantitative variables which were assessed. Several animals had rough fur and appeared hunched and inactive. This effect of selenium may be due to its relationship to the oxidative processes of the cell.

Both injurious and positive effects result from the role of selenium as a regulator of oxidative reactions. By removing sulfhydryl groups essential to proper oxidation processes, selenium might produce toxic effects to whole enzume systems (3). On the other hand, some workers feel that "highly reactive free radicals and peroxides damage sensitive compounds and cell organielles", (31) and that selenium, as an antioxidant, protects against these damaging reactions.

A possible conclusion is that excess selenium interferes with protein synthesis (by whatever means) and this is the cause of the "hemolytic" anemia--defective stroma synthesis. Levels of blood proteins might be measured in future studies to see if liver dysfunction is related to a decrease in over-all effective synthesis of proteins. Amino acid analysis as well as careful electron microscopy might reveal subtle changes in stroma proteins.

SELENIUM-LEAD

Observations of the selenium-lead group showed that some of the symptoms of lead poisoning were evident. Lead inhibits the synthesis of hemoglobin at three points: It blocks the transformation of gamma-amino levulinic acid to prophobilingen, the conversion of coproporphyrinogen III to protoporphyrin III (17) and the synthesis of heme from protoporphyrin III. The red cells resulting from this defective synthesis are usually hypochromic and have a decreased life span due to excess fragility.

The blood from animals 3C and 3D had two populations of red cells, one of which was larger and hypochromic, perhaps indicating a blockage of hemoglobin synthesis in the upcoming erythrocyte population. The high incidence of red cell crenation among these animals also indicates the extent of their fragility. Anisocytosis was almost twice as common in the selenium-lead group as it was in the control or selenium-only groups. This too is an indication of defective erythropoesis. That we did not note any basophilic stippling of red cells (a common symptom of lead poisoning) was probably due to the low pH of the Wright's stain which we used (18).

The light color of the kidneys and the mottling and speckling of the livers may have been due to vascular damage or blockage. Since the selenium-lead group and the selenium-zinc groups both showed tan-colored kidneys, this effect may have been the result of selenium's toxic action.

The effects of lead (hypochromia, red cell fragility, and kidney damage) which are normally not evident at the subtoxic dose level used in this

study, were allowed expression, perhaps due to a malfunction of protein synthesis caused by selenium. Some of the symptoms of the mice in this group might have been due to selenium poisoning, but symptoms typical of lead poisoning were evident in addition.

SELENIUM-ZINC

The most significant results of this study were obtained from the selenium-zinc group. The mice in this group showed a markedly decreased food intake, especially at the outset. The depressed growth rates typical of zinc poisoning are partially due to its unpalatability and resultant anorexia in the animal (20). However, feed consumption was increased to the control level after the second week, and therefore symptoms, including weight loss, of the last two animals cannot be traced to malnutrition alone.

The low hematocrits of animals in this group indicate a zinc-induced anemia. There was an apparent lack of hemoglobin synthesis due to the competitive interference of zinc with copper and iron which are necessary to erythropoeisis. Cell shape anomalies were the most pronounced of any experimental group in this study, perhaps indicating dysfunction of the synthesis of both stroma and hemoglobin components of the red blood cell.

Since livers were again mottled and speckled in appearance, it is probable that this symptom was due to selenium poisoning. However, the marked atrophy of the livers apparent only in this group, must be traced to the effect of zinc. There is a propensity for zinc to accumulate in the liver (3), and the attachment of zinc ions to liver proteins (enzymes, globulins, etc.)

inactivate these cells (35).

SUMMARY

The effect of lead on selenium poisoning appears to be only slight, perhaps adding to the selenosis some anomalies of red cell shape and kidney color. However, subtoxic levels of zinc in combination with toxic levels of selenium markedly depress growth, lower the hematocrit, and decrease liver size in mice.

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APPENDIX

Table 3

Hematology of Mice Fed Selenium Plus Lead and Selenium Plus Zinc Diets

Animal	RBC Count (Mill.)	Hemato-crit	Mean corpuscu-lar vol.	Hemo-globin (%A)	Retic-ulocyte count
			Selenium Plus Lead Animals		
3B	8.1	51.0	64.0	48.8	1.4
3A	5.4	46.5	86.0	40.0	1.8
3D'	8.7	52.7	61.0	70.9	0.9
3C'	5.7	45.7	80.0	46.2	1.6
3F*	7.6	45.5	60.0	49.8	1.9
3E*	7.8	46.0	59.0	46.9	2.1
Median	7.7	46.3	62.5	47.9	1.7
Average	7.2	47.9	68.3	50.5	1.6
			Selenium Plus Zinc Animals		
4A	8.3	50.5	61.0	50.9	2.0
4B	6.1	33.0	54.0	37.2	2.5
4D*	6.9	51.3	74.3	57.0	2.4
4C'	8.8	29.0	33.0	35.9	2.4
4E*	5.4	26.3	49.0	33.1	0.8
4F	7.2	34.3	48.0	39.8	2.6
Median	7.1	33.7	51.5	38.5	2.4
Average	7.1	37.4	53.2	42.3	2.1

' = Two colonies of cells: a large, hypochromic population and small hyperchromic cells.

* = Many cells crenated.

Table 4

Hematology of Mice Fed Control and Selenium Only Diets

Animal	RBC count (Mill.)	Hemato-crit	Mean corpuscu-lar vol.	Hemo-globin (%A)	Retic-ulocyte count
			Control Animals		
1B	7.0	43.0	61.4	40.0	2.0
1A	6.2	47.5	77.0	48.2	1.7
1D+	8.3	46.0	55.4	57.6	1.3
1C	8.1	56.3	69.4	51.1	2.7
1F	8.2	51.0	62.0	47.5	2.0
1E	8.0	52.0	64.8	50.0	4.4
Median	8.0	49.3	63.4	49.1	2.0
Average	7.6	49.3	65.0	49.0	2.4
			Selenium Only Animals		
2B	9.2	54.0	58.0	52.9	0.6
2A	5.6	33.0	59.0	39.0	2.3
2C	13.6*	49.0	36.0*	49.6	1.4
2D	Animal died prematurely				
2E	8.3	49.0	59.0	47.3	2.3
2F	8.4	45.8	54.6	43.8	2.4
Median	8.4	49.0	58.0	47.3	2.3
Average	9.0	46.0	53.3	46.5	1.8

+ = High sedimentation rate

* = Unopette probably overfilled. These are very questionable figures.

Table 5

Statistical Consideration According to Time and Treatments of This Study

Source	d-f	SS	M-S	F-calculated	F-table
Hematocrit:					
SS-time	2	35.292	17.646	0.298	3.59
SS-tmts	3	515.512	171.837	2.905	3.20
Red Blood Count:					
SS-time	2	10.553	5.227	2.082	3.59
SS-tmts	3	14.077	4.692	1.851	3.20
Reticulocytes					
SS-time	2	0.8276	0.414	0.479	3.59
SS-tmts	3	1.778	0.593	0.686	3.20
Hemoglobin					
SS-time	2	313.343	156.672	2.713	3.59
SS-tmts	3	225.435	75.145	1.302	3.20
Liver					
SS-time	2	1.710	0.855	2.716	3.59
SS-tmts	3	7.501	2.500	7.942*	3.20
Kidney					
SS-time	2	0.342	0.171	16.054*	3.59
SS-tmts	3	0.017	0.006	0.540	3.20
Spleen					
SS-time	2	0.028	0.014	2.743	3.59
SS-tmts	3	0.060	0.020	3.845*	3.20

*Statistically significant at the 0.05% level.