# A MICROCYTIC ANEMIA OF JUVENILE CHINOOK SALMON RESULTING FROM DIETS DEFICIENT IN VITAMIN E

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FISH COMMISSION OF OREGON
Portland, Oregon
Contribution No. 29
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# A MICROCYTIC ANEMIA OF JUVENILE CHINOOK SALMON RESULTING FROM DIETS DEFICIENT IN VITAMIN E

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## ABSTRACT

An anemia of juvenile chinook salmon *(Oncorhynchus tshawytscha)* is described and stages of development separated by recognizable syndromes. A vitamin E-deficient diet of low rancidity produced a severe microcytic anemia with strong immature red cell response and granulocytosis occurring 2 to 4 weeks before a drop in hemoglobin and hematocrit values. A vitamin E deficiency in association with diets of high rancidity produced a more severe anemia with impaired and delayed blood regeneration, microcytosis, abnormal red cell development, and a pronounced granulocyte response; complete recovery resulted when vitamin E was added to the diet.

## INTRODUCTION

Anemia is one of the more important physiological problems encountered in salmon and trout propagation. Instances of severe anemia accompanied by significant mortalities occurred in some groups of chinook salmon reared at Oregon Fish Commission's McKenzie, Marion Forks, and South Santiam hatcheries during 1958 and 1959. These were believed to be related to nutritional deficiencies and rancidity of both meat and "Oregon pellet" diets (Hublou *et al.*, 1959).

The elimination of anemia was a major problem to be solved in perfecting the Oregon pellet. Early detection and classification of anemias are important preliminary steps leading to their ultimate successful prevention and therapy. Hemoglobin, hematocrit, and red cell counts have been adapted for fish hematology by Hendricks (1952), Hesser (1960), Snieszko (1960), Larsen and Snieszko (1961*a*, 1961*b*), and others. While these measurements show that an anemia exists, morphologolical studies are necessary to make a diagnosis of the type of anemia. Ostroumova (1957) states: "the differential blood cell contents is shown to be a true index of haemopoietic activity and to be a highly sensitive sign of the degree of pathological alterations taking place in the body of the fish." To assess a fish's physiological condition, she suggests that hemoglobin levels and blood cell counts should be supplemented by an examination of cell morphology.

The value of studying blood morphology in nutritional deficiencies has been amply demonstrated for higher vertebrates by several workers. Evidence of reduced granulocyte formation in a folic acid deficiency and a depressed lymphocyte formation resulting from a deficiency of pantothenic acid or methionine was reported by Dinning (1962). A vitamin E deficiency in rabbits can be detected by leukocytosis and elevated peripheral neutrophils (Dinning, 1952). In monkeys, vitamin E deficiency

is indicated by leukocytosis, granulocytosis, normochromic-normocytic anemia, and abnormalities of both nuclear and cytoplasmic structures of red cell precursors (Dinning, 1955; Dinning and Day, 1957; Porter *et al.*, 1962). In rats, a deficiency of both vitamins B-6 and E caused elevated neutrophil levels in the peripheral blood. Normal levels of these cells were restored by supplementing the diet with either of the missing vitamins (Dinning *et al.*, 1954).

Law *et al.*, (1963) discuss nutritional aspects of an anemia of chinook salmon *(Oncorhynchus tshawytscha)* produced by a deficiency of vitamin E and by oxidative rancidity in the diet. The following report describes this anemia and blood changes during a recovery period, with particular emphasis on cell morphology, clinical measurements, and diagnoses.

## MATERIALS AND METHODS

Blood smears, hemoglobin, and hematocrit data used in this study were collected from fish used in the experiments conducted in 1959 and 1960 by Law *et al.*, (1963). In the 1959 study, spring chinook fingerlings were fed the Oregon test diet (Sinnhuber *et al.*, 1961) modified to provide various levels of rancidity. Each diet modification was with and without vitamin E supplementation. In 1960, chinook fingerlings were fed two test diets containing vitamin E, one diet of low and one of high rancidity. In addition, a highly rancid diet with no vitamin E supplement was used. This experiment was followed by a "recovery" period when vitamin E was added to the highly rancid diet. The level of vitamin E (D-alpha-tocopheryl acetate) supplementation was 50 mgs/100 gms dry diet. Predetermined amounts of rancid salmon oil were added to non-rancid salmon oil to obtain desired levels of rancidity. Oxidative rancidity was determined periodically by the 2-thiobarbituric acid method described by Yu and Sinnhuber (1957).

### Hematological Procedure

The diagnostic plan consisted of estimating hemoglobin and hematocrit values, making differential red and white cell counts, measuring the size of red cells, and recording cell abnormalities. Blood samples were obtained by severing the caudle peduncle and transferring the blood to hemoglobin pipettes, hematocrit capillaries, and microslides.

Hemoglobin levels were determined by the acid-hematin method without correction by centrifugation. Hematocrit values were obtained with heparinized microhematocrit tubes[1] sealed with a flame and read after centrifuging 4 minutes at about 11,500 revolutions per minute.

The blood smears were air dried and stained with unbuffered Wright's stain. Three separate enumerations were used to determine the differential blood cell counts: (1) a minimum of 1,000 mature and immature red cells,

[1] Clay and Adams, Inc., New York, N. Y.

leukocytes, and thrombocytes; (2) a minimum of 200 leukocytes and thrombocytes; and (3) at least 100 lymphocytes, macrophages, and granulocytes. Calculations were made on the basis of formulae given in Table 1. The differential counts provided estimates of the percentages of red cells, leukocytes, and thrombocytes present in a blood smear. The differential leukocyte count gave the relative abundance of lymphocytes, macrophages, and granulocytes. The granulocytes were further examined to discern any shift in maturation. Before these counts were made, blood smears stained with Wright's solution were compared with smears stained by the peroxidase method of Sato and Sekiya (Wintrobe, 1953) in order to reliably separate immature lymphocytes from immature granulocytes. Hemacytometer counts were not employed in these observations because of the small amount of blood available. Some of the information usually obtained by cell counts was deduced from hemoglobin, hematocrit and differential count values.

Morphological variation used to classify the observed anemia included changes in the shape and size of cells, percentage of immature forms, size and shape of the nucleus, chromatin structure, nuclear-cytoplasmic ratio, cell division and primitive immature red cells in the peripheral blood, basophilic staining of the cytoplasm, and cell fragility. The size of mature red cells was determined by measuring 25 cells from each fish and computing the average area by the following formula (Wintrobe, 1934):

Area in sq. microns=(Ave. max. radius) (Ave. min. radius) (3.1416)

Immature red cells were measured only when information pertaining to the progression of the anemia was needed.

The presence and degree of anemia, the state of balance between production and destruction of erythrocytes, and the classification of the anemia were interpreted from the above tests. Results of blood measures from replicate lots were combined and tested for significant differences by Student's t-test. Frequency distributions of erythrocyte size were compared and tested statistically by the Chi-square test.

The hematological nomenclature used is that of Wintrobe (1953) and Miale (1961) and, in most cases, is the same as that of other papers describing blood cells of fish (Katz, 1949; Jakowska, 1956; Schlicher, 1961; Watson *et al.*, 1956; Weinreb, 1958; and Yokoyama, 1947). A glossary of hematological terms is appended to this paper.

## RESULTS

The blood measurements obtained at the end of the 1959 and 1960 experiments are summarized in Tables 2 and 3. In both studies hemoglobin, hematocrit, and most of the cell morphological characteristics in the fish which were fed diets supplemented with vitamin E were considered satisfactory, although hemoglobin and hematocrit values were slightly lower in

TABLE 1. **FORMULAS USED IN BLOOD MORPHOLOGY STUDIES.**

1. Mean corpuscular hemoglobin concentration (MCHC) (per cent): $\frac{\text{Grams hemoglobin per 100 ml blood} \times 100}{\text{Hematocrit per cent}}$
2. Per cent immature red blood cells:① $\frac{\text{Number immature red blood cells} \times 100}{\text{Total number red blood cells}}$
3. Per cent red cells in peripheral blood:② $\frac{\text{Number red cells} \times 100}{\text{Total number blood cells}}$
4. Per cent leukocytes and thrombocytes in peripheral blood:② $\frac{\text{Number leukocytes} + \text{Number thrombocytes} \times 100}{\text{Total number blood cells}}$
5. Per cent leukocytes of the differential leukocyte and thrombocyte count: ③ $\frac{\text{Number leukocytes} \times 100}{\text{Number leukocytes} + \text{Number thrombocytes}}$
6. Per cent leukocytes in peripheral blood:② Formula 5 × Formula 4
7. Per cent thrombocytes of leukocytes and thrombocytes:③ $\frac{\text{Number thrombocytes} \times 100}{\text{Number leukocytes} + \text{Number thrombocytes}}$
8. Per cent thrombocytes in peripheral blood:② Formula 7 × Formula 4
9. Differential leukocyte counts (per cent):④
$$\text{Lymphocytes} = \frac{\text{Number lymphocytes} \times 100}{\text{Total number leukocytes}}$$
$$\text{Macrophages} = \frac{\text{Number monocytes} \times 100}{\text{Total number leukocytes}}$$
$$\text{Granulocytes} = \frac{\text{Number granulocytes} \times 100}{\text{Total number leukocytes}}$$
10. Differential per cent phagocytes: $\frac{\text{Number macrophages} + \text{Number granulocytes}}{\text{Total number leukocytes}}$
11. Per cent granulocytes in peripheral blood: Differential per cent granulocytes × Formula 6
12. Per cent macrophages in peripheral blood: Differential per cent macrophages × Formula 6
13. Per cent phagocytes in peripheral blood: Formula 6 × Formula 10

Sample of at least:
① 1,000 red cells
② 1,000 blood cells
③ 200 leukocytes and thrombocytes
④ 100 leukocytes

TABLE 2. **MEAN BLOOD DATA FROM JUVENILE CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E AT TERMINATION OF 1959 EXPERIMENT.**

| *Blood Measure* | With Vitamin E | | | | Without Vitamin E | | | |
|---|---|---|---|---|---|---|---|---|
| Diet TBA Number | 10① | 50 | 83 | 111 | 20 | 70 | 120 | 125 |
| **Routine Blood Tests and Indices** | | | | | | | | |
| Hemoglobin (gms/100 ml) | 13.1± 3.0② | 12.1± 1.7 | 11.1± 1.2 | 10.7± 1.7 | 6.6± 1.3 | ③ | ③ | ③ |
| Hematocrit (%) | 41.3± 3.4 | 37.7± 5.1 | 36.3± 4.4 | 35.5± 4.2 | 19.8± 7.7 | 11.0± 7.5 | 9.2± 5.1 | 10.5± 5.9 |
| MCHC (%)④ | 32.0± 2.8 | 32.2± 2.1 | 30.9± 2.0 | 30.9± 1.8 | 29.4± 3.0 | ..... | ..... | ..... |
| **Blood Smear Analyses** | | | | | | | | |
| Erythrocyte Size | | | | | | | | |
| Length (microns) | 15.2± 0.4② | 15.5± 0.8 | 15.5± 0.6 | 15.0± 0.5 | 13.1± 0.7 | 13.7± 0.4 | 13.2± 0.5 | 13.7± 0.5 |
| Width (microns) | 7.6± 0.2 | 7.5± 0.3 | 7.6± 0.3 | 7.7± 0.4 | 7.0± 0.2 | 7.0± 0.2 | 7.1± 0.4 | 7.2± 0.5 |
| Area (sq. microns) | 90.6± 4.5 | 90.8± 4.5 | 92.1± 5.1 | 90.6± 5.0 | 72.1± 4.8 | 74.9± 2.9 | 73.5± 4.1 | 77.5± 4.8 |
| Differential Red Cell Count (%) | | | | | | | | |
| Immature | 3.9± 1.4 | 4.9± 2.7 | 5.5± 3.2 | 4.8± 2.1 | 29.7±12.1 | 34.8±21.5 | 32.2±21.2 | 33.6±24.7 |
| Differential Leukocyte Count (%) | | | | | | | | |
| Lymphocytes | 94.3± 3.6 | 91.9± 7.4 | 91.4± 6.1 | 88.8± 8.4 | 89.6± 6.0 | 79.9±25.4 | 85.8±10.6 | 67.5±21.3 |
| Macrophages | 1.0± 0.9 | 2.5±⑤ | 2.7±2.2 | 2.9± 2.5 | 1.5± 1.2 | 1.6± 1.4 | 1.9± 1.7 | 5.2± 4.1 |
| Granulocytes | 4.7± 3.0 | 5.6± 5.3 | 5.9± 5.1 | 8.3± 6.1 | 8.9± 5.4 | 18.5±⑤ | 12.3±10.5 | 27.3±18.7 |
| Differential Count of Total Cells (%) | | | | | | | | |
| Red Cells | 99.2± 0.5 | 98.9± 0.5 | 99.1± 0.6 | 99.3± 0.6 | 96.6± 5.4 | 97.3± 2.1 | 96.1± 3.5 | 97.5± 1.4 |
| Leukocytes | 0.5± 0.3 | 0.7± 0.3 | 0.6± 0.4 | 0.5± 0.6 | 1.2± 1.0 | 1.6± 1.3 | 2.7± 2.4 | 2.0± 1.2 |
| Lymphocytes | 0.47 | 0.64 | 0.55 | 0.44 | 1.08 | 1.28 | 2.32 | 1.35 |
| Others | 0.03 | 0.06 | 0.05 | 0.06 | 0.12 | 0.32 | 0.38 | 0.65 |
| Thrombocytes | 0.3± 0.3 | 0.4± 0.3 | 0.3⑤ | 0.2± 0.1 | 2.2⑤ | 1.2± 1.1 | 1.1⑤ | 0.5± 0.5 |

① Rancidity level as measured by 2-thiobarbituric acid method (Yu and Sinnhuber, 1957).

② Mean and standard deviation from 24 fish per diet group; subsample for blood smears from 12, 12, 21, 21, 17, 6, 17, and 9 fish, respectively.

③ Very few samples due to inadequate volume of blood; those analyzed indicated severe anemia.

④ Mean corpuscular hemoglobin concentration.

⑤ SD larger than mean.

TABLE 3. **MEAN BLOOD DATA FROM JUVENILE CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E AT TERMINATION OF 1960 EXPERIMENT.**

| *Blood Measure* | With Vitamin E | | Without Vitamin E |
|---|---|---|---|
| Diet TBA Number | 8① | 87 | 81 |
| **Routine Blood Tests and Indices** | | | |
| Hemoglobin (gms/100 ml) | 12.8± 1.1② | 12.2± 1.5 | 6.2± 3.1 |
| Hematocrit (%) | 43.7± 4.1 | 40.3± 4.8 | 21.4± 3.3 |
| MCHC (%)③ | 29.3± 3.7 | 30.0± 2.8 | 29.1± 3.3 |
| **Blood Smear Analyses** | | | |
| Erythrocyte Size | | | |
| Length (microns) | 16.1± 0.6② | 15.8± 0.6 | 13.2± 0.7 |
| Width (microns) | 7.8± 0.3 | 7.8± 0.3 | 6.9± 0.4 |
| Area (sq. microns) | 98.2± 4.7 | 96.4± 6.5 | 71.2± 6.4 |
| Differential Red Cell Count (%) | | | |
| Immature | 2.6± 1.5 | 3.6± 1.3 | 17.6±12.5 |
| Differential Leukocyte Count (%) | | | |
| Lymphocytes | 98.8± 1.0 | 97.4± 1.8 | 91.5± 5.9 |
| Macrophages | 0.1± 0.2 | 0.4± 0.7 | 0.7± 0.9 |
| Granulocytes | 1.0± 0.8 | 2.2± 1.3 | 7.5± 5.4 |
| Differential Count of Total Cells (%) | | | |
| Red Cells | 97.8± 0.7 | 98.2± 0.8 | 94.0± 8.3 |
| Leukocytes | 1.8± 0.8 | 1.4± 0.9 | 4.2± 5.6 |
| Lymphocytes | 1.78 | 1.36 | 3.8 |
| Others | 0.02 | 0.04 | 0.36 |
| Thrombocytes | 0.3± 0.2 | 0.4± 0.3 | 1.7± 3.0④ |

① Rancidity level measured by 2-thiobarbituric acid method (Yu and Sinnhuber, 1957).
② Mean and standard deviation from 30 fish per diet group; subsample for blood smears from 10 fish per diet group.
③ Mean corpuscular hemoglobin concentration.
④ Standard deviation is high due to several extreme values.

the rancid diets containing vitamin E. An additional indication of normality was the absence of the primitive stages of immature red cells.

### Hematology of a Vitamin E Deficiency Anemia

Hematological observations reveal the devastating effect of vitamin E deficiency on the blood regardless of diet rancidity level. Hemoglobin and hematocrit values and lymphocytic differentials were below normal, while the percentage of immature red cells, leukocytes, thrombocytes, and granulocytic and monocytic differentials were above normal. The average mean

corpuscular hemoglobin concentration (MCHC) compared favorably with that of fish on supplemented diets (control), thus suggesting a normochromic anemia; whereas observation of red cells in smears suggested a hypochromic anemia. Mature red cells deviated from those of the control by having larger nuclei, some nuclear reticulation, and smaller size. In general, immature red cells were also smaller than those of the control fish and their size decreased progressively with advancing anemia. The presence of undifferentiated stem cells, the division of mature as well as immature red cells, and the abundance of cellular debris were characteristic of the most severe cases of this anemia.

The anemia exhibited by fish on low-rancid deficient diets was even more evident as the diet rancidity increased (Table 2). The volume of packed red cells decreased as diet rancidity increased to that level at which all red cell formation ceased. Erythrocytic depression occurred more frequently with the highly rancid diet. Toxic signs such as vacuolation and asynchronous development of leukocyte nuclei and cytoplasm were often observed. Stem cells, pronormoblasts and basophilic normoblasts were commonly found in the peripheral blood of fish on highly rancid rations, yet they were less common in the blood of fish fed non-rancid diets.

### Progression of Blood Dyscrasias

The progressive changes in hematocrit and morphological indices produced by vitamin E-deficient diets of low and high rancidity are contrasted in Table 4 with the blood characteristics of fish maintained on vitamin E supplemented diets (1959 study). A similar progression noted in the 1960 study is not presented here.

The first indication of abnormality was a granulocytosis which was detected in several fish after 4 weeks on the deficient diet. By the sixth week, mean values showed the increase (Table 4). The percentage of granulocytes remained high thereafter, although a granulocytosis failed to develop in a few test fish. Lymphocyte percentages decreased in differential leukocyte counts but increased slightly as percentages of total blood cells. The number of red cells per cubic millimeter decreased as the anemia became more severe (based on equal reductions of hemoglobin and hematocrit values), while lymphocytes did not change materially in number, and granulocytes doubled or tripled. Large lymphocytes became more numerous during later stages of anemia.

Hemoglobin and hematocrit levels declined simultaneously in fish fed all deficient diets and were generally correlated. Figure 1 shows the relationship of the two measures during experiments lasting 22 weeks in 1959 and for 20 weeks in 1960. Each graph is divided into 4 quadrants which emphasize that vitamin E-deficient fish had low hemoglobin and hematocrit values, while fish fed supplemented diets had high hemoglobin and hematocrit values. The overlapping values obtained during the 1960 study were principally due to samples taken before the 16th week of the experiment.

The percentage of immature red cells increased in a few fish after 6

TABLE 4. **MEAN BLOOD VALUES FROM CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E, 1959 EXPERIMENT.**

| Weeks—Lot | | Number Fish | Hematocrit % | Erythrocyte Area | % Immature RBC's① | % Leukocytes | % Thrombocytes | Differential Leukocyte Count Lymphocytes | Phagocytes② |
|---|---|---|---|---|---|---|---|---|---|
| | *Initial* | 10 | 36.3 | 100.1 | 6.4 | 3.1 | 0.3 | 95.2 | 4.8 (0.15%) |
| 2 | E—Low TBA③ | 4 | 29.8 | 98.5 | 12.0 | 0.6 | 0.4 | 96.6 | 3.4 (0.02%) |
| | No E—Low TBA | 4 | 27.3 | 106.8 | 8.0 | 1.4 | 0.6 | 97.4 | 2.6 (0.04%) |
| | No E—High TBA | 4 | 27.5 | 98.7 | 6.3 | 1.2 | 0.4 | 96.7 | 3.3 (0.04%) |
| 4 | E—Low TBA | 4 | 36.5 | 99.5 | 12.4 | 1.6 | 0.3 | 94.1 | 5.9 (0.09%) |
| | No E—Low TBA | 4 | 35.0 | 102.5 | 11.5 | 1.8 | 0.5 | 94.7 | 5.3 (0.10%) |
| | No E—High TBA | 4 | 34.3 | 103.0 | 9.5 | 2.3 | 1.2 | 94.3 | 5.7 (0.13%) |
| 6 | E—Low TBA | 4 | 35.3 | 96.4 | 12.0 | 1.0 | 0.4 | 93.0 | 7.0 (0.07%) |
| | No E—Low TBA | 4 | 33.7 | 99.1 | 6.1 | 1.8 | 0.6 | 94.3 | 5.7 (0.10%) |
| | No E—High TBA | 4 | 35.3 | 93.3 | 5.3 | 1.8 | 0.3 | 88.6 | 11.4 (0.21%) |
| 8 | E—Low TBA | 4 | 35.0 | 99.6 | 7.6 | 2.7 | 0.6 | 96.0 | 4.0 (0.11%) |
| | No E—Low TBA | 4 | 29.0 | 85.2 | 13.0 | 4.0 | 1.8 | 93.0 | 7.0 (0.28%) |
| | No E—High TBA | 4 | 19.5 | 87.2 | 12.9 | 4.6 | 2.1 | 93.7 | 6.3 (0.29%) |
| 12 | E—Low TBA | 4 | 40.0 | 94.5 | 7.0 | 2.5 | 0.6 | 97.0 | 3.0 (0.08%) |
| | No E—Low TBA | 4 | 24.3 | 76.5 | 33.7 | 5.4 | 1.8 | 97.4 | 2.6 (0.14%) |
| | No E—High TBA | 4 | 14.5 | 80.5 | 9.9 | 5.3 | 2.8 | 93.0 | 7.0 (0.37%) |
| 14 | E—Low TBA | 4 | 36.5 | 87.3 | 7.2 | 3.3 | 0.5 | 96.8 | 3.2 (0.11%) |
| | No E—Low TBA | 4 | 19.3 | 75.4 | 34.2 | 4.2 | 2.4 | 94.4 | 5.6 (0.24%) |
| | No E—High TBA | 4 | 11.0 | 72.0 | 31.2 | 10.6 | 3.8 | 88.4 | 11.6 (1.23%) |
| 16 | E—Low TBA | 4 | 37.0 | 93.7 | 4.5 | 1.9 | 1.3 | 97.8 | 2.2 (0.04%) |
| | No E—Low TBA | 4 | 25.0 | 80.2 | 31.6 | 3.7 | 1.5 | 98.1 | 1.9 (0.07%) |
| | No E—High TBA | 4 | 12.3 | 77.5 | 37.6 | 4.1 | 3.0 | 78.3 | 21.7 (0.89%) |
| 20 | E—Low TBA | 4 | 38.0 | 91.0 | 6.4 | 2.6 | 0.4 | 93.2 | 6.8 (0.18%) |
| | No E—Low TBA | 4 | 20.5 | 77.8 | 39.4 | 6.3 | 3.4 | 96.9 | 3.1 (0.20%) |
| | No E—High TBA | 4 | 13.0 | 79.2 | 44.0 | 5.3 | 0.9 | 92.5 | 7.5 (0.40%) |
| 22 | E—Low TBA | 11 | 40.3 | 90.6 | 3.9 | 0.5 | 0.3 | 94.3 | 5.7 (0.03%) |
| | No E—Low TBA | 17 | 19.8 | 72.1 | 29.7 | 1.2 | 2.2 | 89.6 | 10.4 (0.12%) |
| | No E—High TBA | 6 | 13.5 | 74.9 | 34.8 | 1.6 | 1.2 | 79.9 | 20.1 (0.32%) |

① All immature red cells included in the percentage: orthochromatophils, polychromatophils, basophilic normoblasts, and pronormoblasts.

② Macrophages and granulocytes considered collectively; absolute percentage is given in parentheses.

③ E refers to salmon fed vitamin E-supplemented diets; TBA refers to oxidative rancidity level.

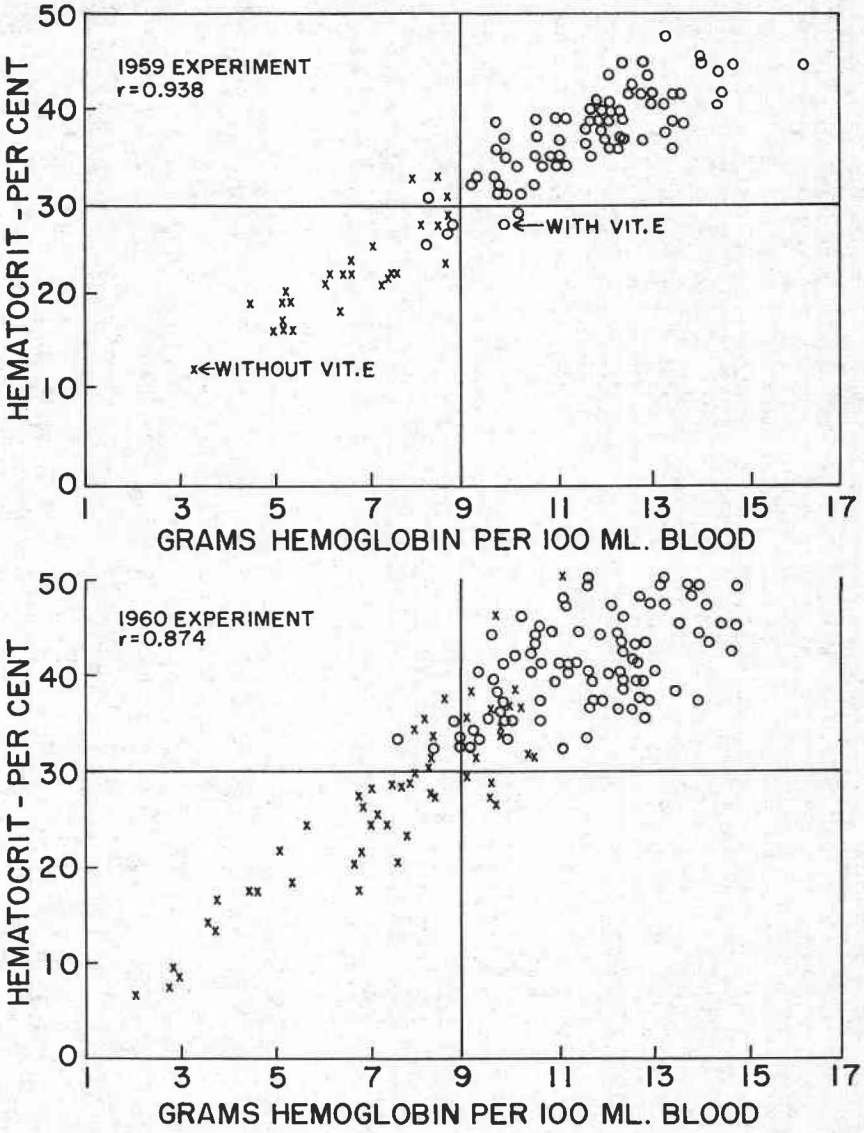


FIGURE 1. SCATTER DIAGRAMS OF HEMATOCRIT AND HEMOGLOBIN VALUES OF CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E, 1959 AND 1960 EXPERIMENTS.

weeks, and subsequent samples showed greater regeneration after 8 weeks on deficient diets of low rancidity. However, fish receiving more rancid, deficient diets did not exhibit a pronounced increase in immature red cells until the 12-16th week (Figure 2). Basophilic normoblasts and pronormoblasts were then observed upon regeneration.

The size of mature red cells decreased rapidly after the fish were on deficient diets for about 8 weeks, then decreased at a slower rate (Table 4). Anisocytosis (variable red cell size) was especially evident during early stages of anemia. Reduction of the size of erythrocytes was apparent even in cases of impaired blood production occurring with the highly rancid diets (at the 8th and 12th weeks, Table 4). Early in the anemia, the immature red cells were as large or larger than the mature cells; later, with strong blood regeneration, they were smaller. During the last phase of the anemia the number of immature red cells was reduced, with orthochromatophils being the last to disappear upon cessation of blood production.

The progression of the vitamin E deficiency anemia was observed in four stages:

1. Normocytic —Normal size of erythrocytes, little or no increase in immature red cells, relative increase in granulocytes, and normal or near normal hemoglobin and hematocrit values.

2. Microcytic regenerative —Microcytic red cells, high percentages of immature red cells, and granulocytes, rapid decline in hemoglobin and hematocrit values.

3. Abnormal regenerative —Abnormal red cell production, presence of basophilic normoblasts or pronormoblasts, moderate to high percentage of immature red cells, microcytic red cells, many granulocytes, low hemoglobin and hematocrit values.

4. Erythrocytic depression —Principally microcytic mature red cells remaining though reduced in numbers, very few immature red cells, many leukocytes and thrombocytes, continued high granulocyte differential counts, low hemoglobin and hematocrit values.

Representative samples of blood indices selected from the 1960 experiment are listed in Table 5.

### Recovery from Vitamin E Deficiency Anemia

Therapeutic feeding of vitamin E to anemic fish previously fed a highly rancid deficient diet resulted in all blood indices returning to normal within 20 days. The only exception was the percentage of immature red cells which had increased to 20% after 20 days of diet supplementation

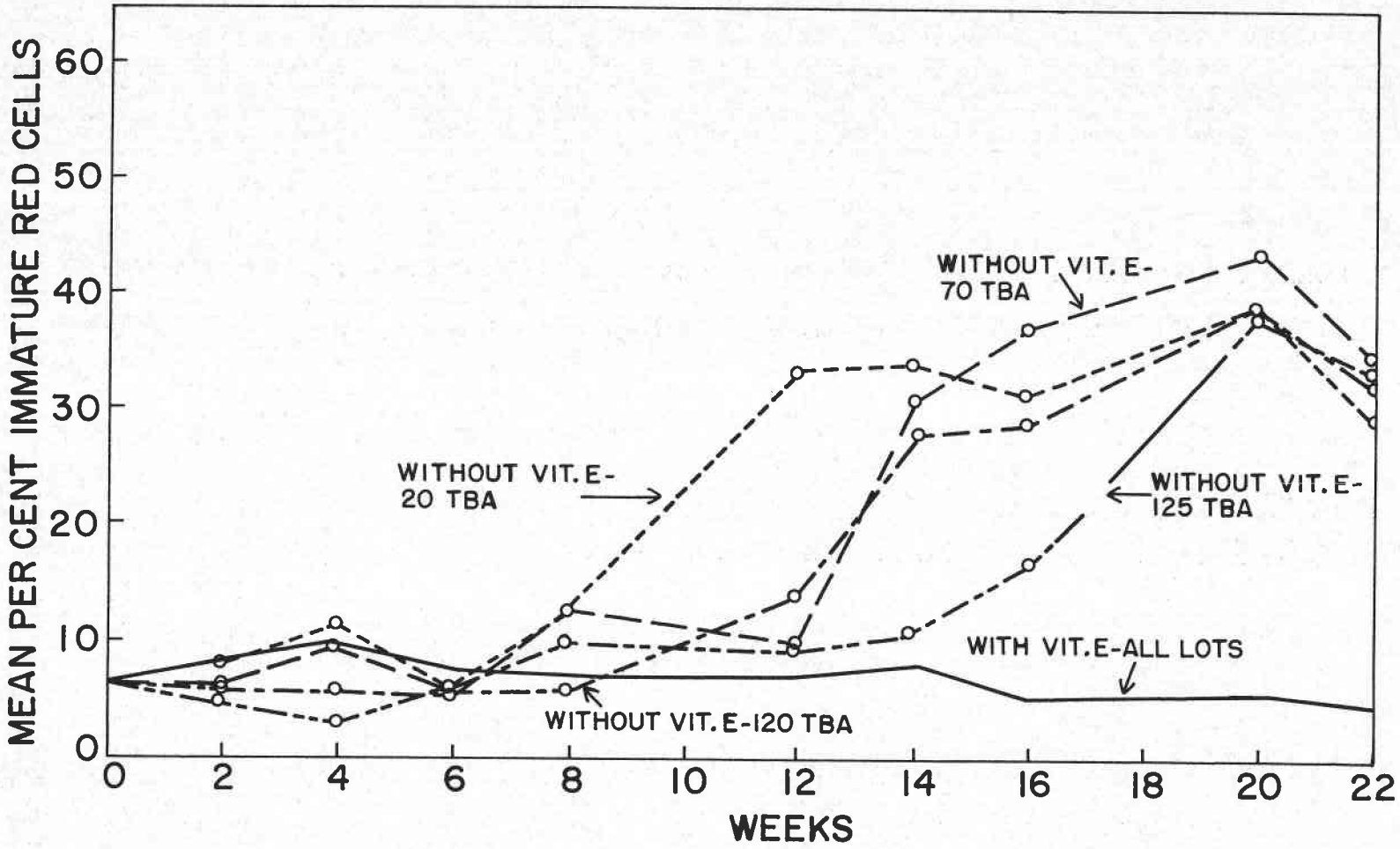


FIGURE 2. MEAN IMMATURE RED CELL PERCENTAGES OF DEFICIENT AND NONDEFICIENT CHINOOK SALMON BY WEEKS, 1959 EXPERIMENT.

TABLE 5. **BLOOD INDICES IN 4 STAGES OF ANEMIA EXHIBITED BY CHINOOK SALMON, 1960 EXPERIMENT.**

| *Stage* | *Number Fish* | *Hemoglobin (gms/100 ml)* | *Hematocrit (%)* | *% Immature RBC'S*① | *% Leuk's*② | *% Thromb's*③ | *Diff. % Phagocytes*④ | *Red Cell Area (sq. microns) Immature* | *Mature* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 9.9 | 34.0 | 11.6 | 2.5 | 0.4 | 7.3 | 83.1 | 82.8 |
| 2 | 5 | 6.0 | 22.6 | 28.9 | 3.5 | 0.9 | 8.4 | 69.1 | 70.7 |
| 3 | 1 | 3.8 | 19.0 | 44.8 | 0.6 | 1.6 | 35.7 | ..... | 62.3 |
| 4 | 4 | 2.2 | 5.8 | 6.2 | 11.2 | 9.1 | 7.5 | 57.5 | 62.0 |



① Red blood cells.
② Leukocytes, white blood cells.
③ Thrombocytes.
④ Per cent granulocytes and macrophages (monocytes) in the differential leukocyte count.

with vitamin E; these then returned to the normal value of 5% after a total of 48 days (Table 6). The initial high percentage of immature red cells indicated active blood formation resulting from vitamin E supplementation.

## DISCUSSION

The primary effects of a vitamin E deficiency as reflected in the blood of chinook salmon are (1) a decrease in hemoglobin, and (2) a greater release of immature red cells which indicate a disturbance of hemopoiesis. That hemopoiesis was disturbed is shown by (a) an increase of the severity of anemia with time; (b) microcytosis; (c) premature release of immature red cells; (d) mitotic division of peripheral red cells; (e) presence of basophilic normoblasts, pronormoblasts and stem cells; and (f) erythrocytic depression during some phase of the anemia.

TABLE 6. **MEAN BLOOD DATA OF CHINOOK SALMON RECOVERING FROM ANEMIA BY THE ADDITION OF VITAMIN E TO A RANCID OREGON TEST DIET, 1960 EXPERIMENT.**

| *Blood Measure* | *Initial* | *After 20 Days* | *After 48 Days* |
|---|---|---|---|
| **Routine Blood Tests and Indices** | | | |
| Hemoglobin (gms/100 ml) | 7.3± 2.5① | 10.1± 0.9 | 10.6± 0.8 |
| Hematocrit (%) | 28.8±10.0 | 38.3± 3.3 | 43.1± 3.4 |
| MCHC (%)② | 25.4± 2.5 | 26.6± 0.6 | 24.7± 3.0 |
| **Blood Smear Analyses** | | | |
| Erythrocyte Size | | | |
| Length (microns) | 14.2± 2.2① | 15.0± 0.5 | 15.1± 0.5 |
| Width (microns) | 7.3± 0.3 | 7.4± 0.4 | 7.8± 0.5 |
| Area (sq. microns) | 81.1± 7.0 | 87.3± 5.9 | 92.5± 5.8 |
| Differential Red Cell Count (%) | | | |
| Immature | 14.8± 9.0 | 20.6± 6.9 | 5.1± 1.4 |
| Differential Leukocyte Count (%) | | | |
| Lymphocytes | 91.0± 4.7 | 96.8± 1.8 | 96.4± 2.2 |
| Macrophages | 1.2± 1.5 | 0.7± 0.6 | 0.6± 0.4 |
| Granulocytes | 7.9± 4.0 | 2.5± 1.2 | 3.0± 2.1 |
| Differential Count of Total Cells (%) | | | |
| Red Cells | 96.7± 1.7 | 97.7± 0.7 | 97.4± 1.3 |
| Leukocytes | 2.3± 1.1 | 1.6± 0.5 | 2.1± 0.7 |
| Lymphocytes | 2.09 | 1.55 | 2.02 |
| Others | 0.21 | 0.05 | 0.08 |
| Thrombocytes | 1.0± 0.5 | 0.8± 0.5 | 0.5± 0.5 |

① Mean and standard deviation of 10 fish at beginning and after 48 days, and 20 fish after 20 days; subsample for blood smears of 8 fish at beginning, 6 after 20 days and 4 after 48 days.

② Mean corpuscular hemoglobin concentration.

A vitamin E deficiency appears to give a different blood picture in chinook salmon from that observed in nutritional deficiencies of other animals. Leukopenia was not demonstrated, as in rats under conditions of protein insufficiency (Kornberg, *et al.*, 1946; Guggenheim and Buechler, 1949). Likewise, a lymphopenia similar to that seen in rats and dogs suffering from pyridoxine deficiency (Hawkins and Evans, 1952) was not observed in salmon. Granulocytes increased in the blood of salmon fed vitamin E-deficient diets which contrasts to the granulocytopenias reported for mammals subjected to folic acid-deficient diets (Dinning, 1962). A vitamin E deficiency produced microcytic rather than macrocytic red cells as expected in folic acid deficiencies.

Vitamin E deficiency in some higher animals produces a normocytic anemia and granulocytosis which closely resemble the first stage of the anemia in chinook salmon reported in this paper. It is suggested that mammals fed diets antagonistic to vitamin E may develop blood dyscrasias similar in pattern to those of chinook salmon. Sinnhuber (personal communication)[2] found small red cells in rats fed diets containing a malonaldehyde derivative. Also, Oldfield (personal communication)[3] observed microcytosis in mink fed rancid diets although the condition was not consistent.

Differences in the size of erythrocytes were demonstrated in the chinook salmon experiments. Microcytosis produced by the vitamin E-deficient diet is illustrated in Figure 3 which shows that the deficient fish had shorter erythrocytes than the non-deficient ($P<.005$). Figures 4 and 5 indicate that the size of erythrocytes is closely correlated with hemoglobin and hematocrit values and that there is a fairly distinct separation in size of red cells of fish fed deficient and supplemented diets. Arbitrary lines are drawn to emphasize differences. Figure 6 indicates that small erythrocytes occurred with a vitamin E deficiency at hemoglobin levels ranging from 9 to 11 gms per 100 ml of blood, whereas at the same hemoglobin levels larger erythrocytes were produced by the supplemented diet ($P<.005$). Microcytosis in the presence of normal hemoglobins and hematocrits may be a sign of impending anemia.

The dyscrasias ascribed to this anemia of spring chinook can be divided into two groups: (1) those associated with the deficient diet of low rancidity, and (2) those pertaining to the deficient diet of high rancidity. It is probably simpler, however, to consider these results as describing one anemia attributable to a vitamin E deficiency complicated by various grades of rancidity. With diets of low rancidity, blood regeneration was active and consisted of normal stages of immature red cells in the peripheral blood. When the diet was very rancid, red cell production was impaired and retarded 6 to 8 weeks with an eventual release of many primitive forms. The various levels of oxidative rancidity, however, had little effect on the blood picture when vitamin E was added.

In normal chinook salmon, macrophages and a few granulocytes may be slightly vacuolated. These leukocytes of fingerlings receiving highly

[2] Russell O. Sinnhuber, Department of Food Science and Technology, Oregon State University.
[3] Dr. J. E. Oldfield, Department of Animal Science, Oregon State University.

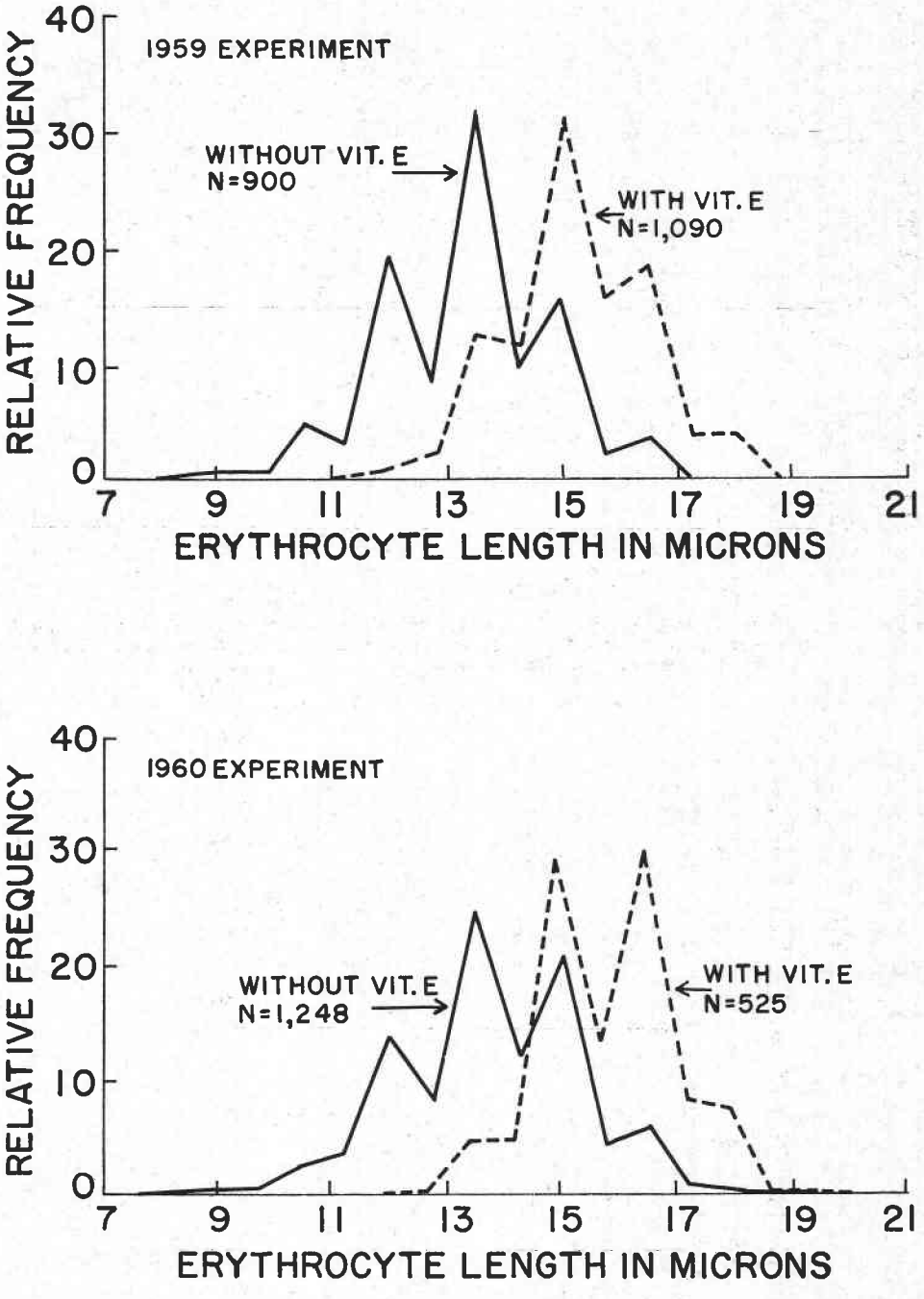


FIGURE 3. LENGTH-FREQUENCY DISTRIBUTIONS OF ERYTHROCYTES FROM CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E, 1959 AND 1960 EXPERIMENTS.

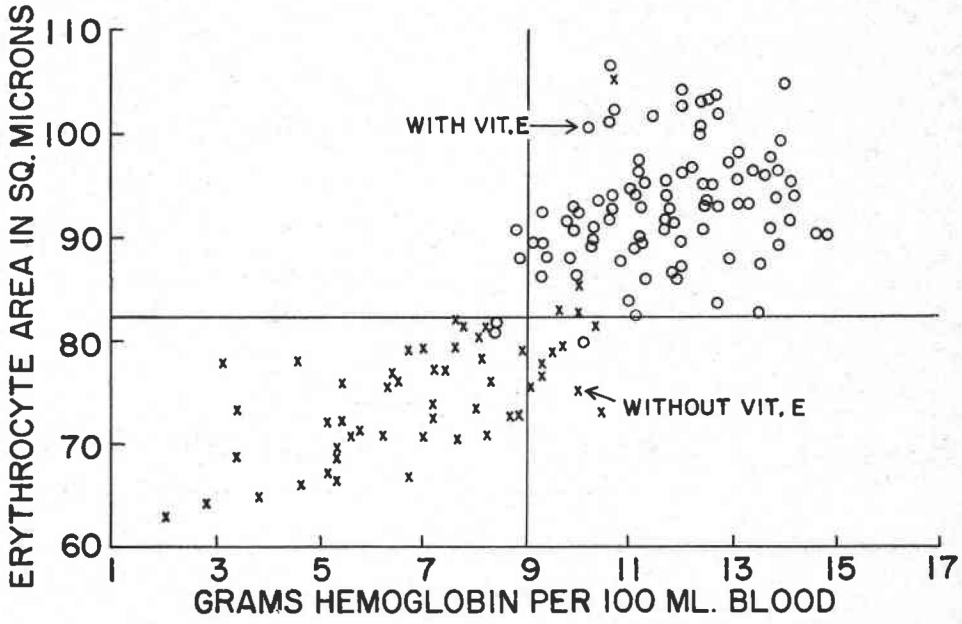


FIGURE 4. SCATTER DIAGRAM OF THE AVERAGE MATURE RED CELL AREA AND HEMOGLOBIN LEVEL OF CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E, 1959 AND 1960 EXPERIMENTS.

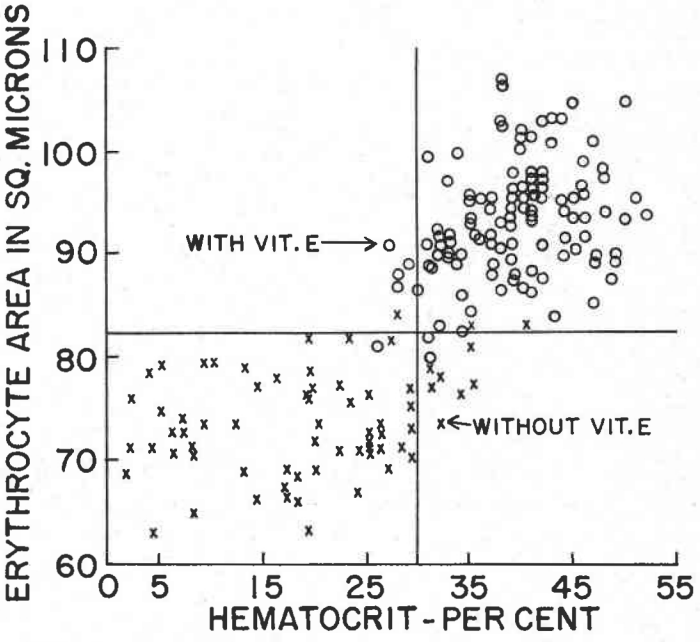


FIGURE 5. SCATTER DIAGRAM OF THE AVERAGE MATURE RED CELL AREA AND HEMATOCRIT PER CENT OF CHINOOK SALMON FED DIETS WITH AND WITHOUT VITAMIN E, 1959 AND 1960 EXPERIMENTS.

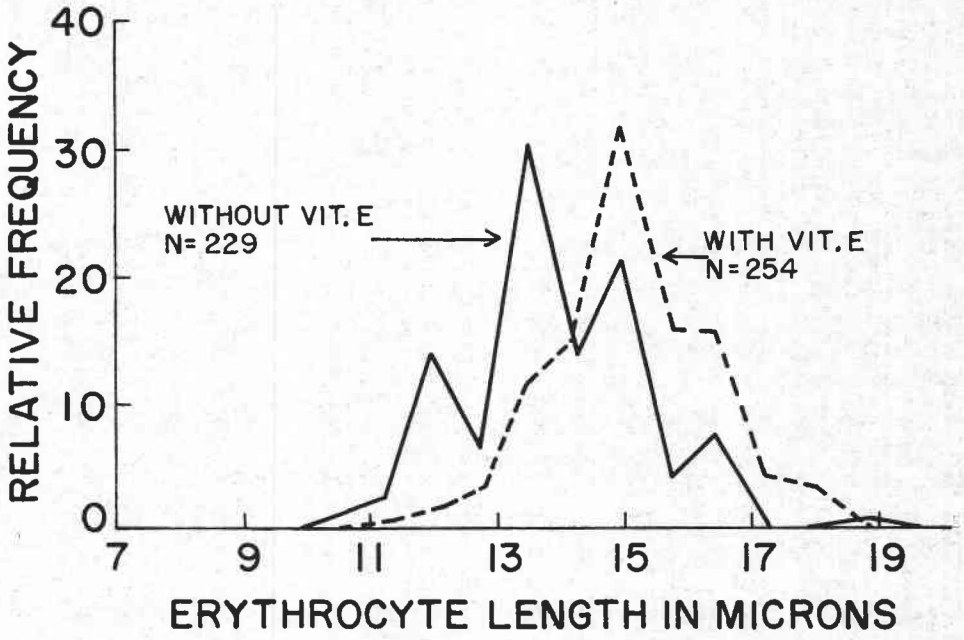


FIGURE 6. LENGTH-FREQUENCY DISTRIBUTIONS OF ERYTHROCYTES FROM CHINOOK SALMON WITH HEMOGLOBIN LEVELS OF 9-11 GMS/100 ML BLOOD, FED DIETS WITH AND WITHOUT VITAMIN E.

rancid diets consistently showed heavy vacuolation. Some of the heavily vacuolated granulocytes closely resembled toxic leukocytes described by Katz (1950) and Dombrowski (1953). The presence of heavy vacuolation is generally considered an indication of cell mortality.

The blood measures of fish receiving the deficient and supplemented diets revealed the same relative differences in 1959 and 1960, but the base percentage of granulocytes in differential leukocyte counts shifted upward during 1959 without regard to the effects of diet. This shift may have been due to *Myxobolus* sp. infection unknowingly contracted before the start of the experiment (Wyatt and Pratt, 1963), or due to small differences in handling between the two years. Recent studies suggest the validity of the latter explanation.

Ostroumova (1957) reported three stages of anemia in rainbow trout caused by multiple deficiencies of vitamins and minerals that are almost identical to two of the three anemia stages reported here.

## SUMMARY AND CONCLUSIONS

Differential counts, cell measurements and abnormalities were recorded in conjunction with hemoglobin and hematocrit values to describe an anemia of juvenile chinook salmon and separate it into progressive stages. The anemia was induced by feeding vitamin E-deficient rations and manipulating the rancidity level of each diet. Experiments were conducted during 1959 and 1960, the latter including an anemia recovery phase. The study showed that:

Diet rancidity had only a slight effect on the blood picture as long as vitamin E was added to the food. Highly rancid diets with vitamin E caused small reductions in hemoglobin and hematocrit values and mild granulocytosis.

Vitamin E-deficient diets of low rancidity produced a microcytic anemia characterized by a parallel decrease in hemoglobin and hematocrit values, strong blood regeneration, and granulocytosis. The regenerative response consisted of an increase in immature red cells of normal developmental stages (most cases), and increases in leukocytes and thrombocytes.

Vitamin E-deficient diets of high rancidity induced a severe microcytic anemia with impaired and delayed blood regeneration and granulocytosis. Blood of highly rancid groups differed from that of low rancid groups by a period of temporary depression and virtual elimination of the microcytic regenerative stage.

The anemia developed by a vitamin E deficiency can be divided into the following progressive stages: (1) normocytic with relative granulocytosis; (2) microcytic regenerative; (3) abnormal regenerative; and (4) red cell depression.

Blood dyscrasias of vitamin E-deficient chinook salmon indicated disturbance of blood formation. Although cell destruction was not quantitatively measured, abnormal cell fragility was inferred from the excessive cellular debris observed in peripheral blood.

Vitamin E added to a rancid diet for anemic chinook resulted in complete recovery of test animals (as measured by blood indices).

Techniques described in this study are believed to be satisfactory for diagnosing a vitamin E-deficiency anemia in spring chinook salmon. Counts with a hemacytometer are recommended to amplify the results of these tests.

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## LITERATURE CITED


Dinning, James S. 1952. Leucocytosis in vitamin E-deficient rabbits. Proc. Soc. Exptl. Biol. Med., 79: 231-232.

Dinning, J. S., J. M. Young, M. R. Simmons, and P. L. Day. 1954. Effects of a combined deficiency of vitamins E and B-6 on the blood picture of the rat. Proc. Soc. Exptl. Biol. Med., 85: 280-282.

Dinning, James S. 1955. The role of vitamin E in regulating the turnover rate of nucleic acids. J. Biol. Chem., 212: 735-739.

Dinning, J. S. and P. L. Day. 1957. Vitamin E deficiency in the monkey. I. Muscular dystrophy, hematologic changes, and the excretion of urinary nitrogenous constituents. J. Exptl. Med., 105: 395.

Dinning, James S. 1962. Nutritional requirements for blood cell formation in experimental animals. Physiol. Rev., 42 (1): 169-180.

Dombrowski, Heinz. 1953. Haematologisch-nosologische studien an bauchwassersuchtkranken Karpfen (*Cyprinus carpio* L.), Biologisches zentralblatt, 72 (7/8): 353-363.

Guggenheim, K., and Edith Buechler. 1949. The effect of quantitative protein deficiency on blood regeneration. I. White blood cells. Blood, 4: 958-963.

Hawkins, W. W. and Mary K. Evans. 1952. White blood cells and lymphoid tissue in vitamin $B_6$ insufficiency. Am. J. of Physiol., 170: 160-167.

Hendricks, Lawrence J. 1952. Erythrocyte counts and hemoglobin determinations for two species of suckers, genus *Catostomus* from Colorado. Copeia 1952 (4): 265-266.

Hesser, Ernest F. 1960. Methods of routine fish hematology. Prog. Fish-Cult., 22 (4): 164-171.

Hublou, Wallace F., Joe Wallis, Thomas B. McKee, Duncan K. Law, Russell O. Sinnhuber, and T. C. Yu. 1959. Development of the Oregon pellet diet. Oregon Fish Comm. Res. Briefs, 7(1): 28-56.

Jakowska, Sophie. 1956. Morphologie et nomenclature des cellules du sang des teleostiens. Rev. Hemat., 11: 519-439.

Katz, Max. 1949. The hematology of the silver salmon, *Oncorhynchus kisutch* (Walbaum), Ph. D. thesis, Univ. of Wash., 100 p.

Katz, Max. 1950. Some interesting cells in the blood of diseased silver salmon fingerlings. Copeia, (4): 295-298.

Kornberg, Arthur, Floyd S. Daft, and W. H. Sabrell. 1946. Granulocytopenia and anemia in rats fed diets of low casein content. Science, 103: 646-648.

Larsen, Howard N. and S. F. Snieszko. 1961*a*. Comparison of various methods of determination of hemoglobin in trout blood. Prog. Fish-Cult., 23 (1): 8-17.

Larsen, Howard N. and S. F. Snieszko. 1961*b*. Microhematocrit values of rainbow trout, brown trout, and brook trout. Prog. Fish-Cult., 23 (3): 114-119.

Law, Duncan K., John W. Westgate, Thomas B. McKee, and Russell O. Sinnhuber. 1963. Effects of vitamin E, selenium and certain antioxidants on the toxicity of autoxidized fats fed to chinook salmon. Oregon Fish Com., unpublished.

Miale, John B. 1961. Laboratory medicine-hematology, 2nd ed. Mosby Co., St. Louis, Mo., 918 p.

Ostroumova, I. N. 1957. Blood indices and haemopoiesis in the individual development of fish. The freshwater research inst., Leningrad, U.S.S.R., 43 (3): 63 p. In Russian, with English summary.

Porter, F. Stanley, Cay D. Fitch, and James S. Dinning. 1962. Vitamin E deficiency in the monkey. IV. Further studies of the anemia with emphasis on bone marrow morphology. Blood, 20 (4): 471-477.

Schlicher, Anna M. 1961. Endocrinological and hematological studies in *Fundulus heterclitus* (Linn.). Bulletin of the Bingham Oceanographic Collection, Peabody Mus. Nat. Hist., Yale Univ., 17 (3): 55 p.

Sinnhuber, Russell O., Duncan K. Law, T. C. Yu, Thomas B. McKee, Wallace F. Hublou, and James W. Wood. 1961. Sixth progress report on salmon diet experiments. Oregon Fish Comm. Res. Briefs, 8 (1): 53-61.

Snieszko, S. F. 1960. Microhematocrit as a tool in fishery research and management. U. S. Fish and Wildlife Service, Special Scientific Report; Fish. No. 341: 15 p.

Watson, Margaret E., Ray W. Guenther and Rodney D. Royce. 1956. Hematology of healthy and virus-diseased sockeye salmon, *Oncorhynchus nerka*. Zoologica, 41 (1): 27-38.

Weinreb, Eva L. 1958. Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri irideus*. I. Hematology: under normal and experimental conditions of inflammation. Zoologica, 43 (4): 145-153.

Wintrobe, Maxwell M. 1934. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Hematolog., 51: 32-49.

Wintrobe, Maxwell M. 1953. Clinical hematology. Lea and Febiger, Philadelphia, Pa., 1,185 p.

Wyatt, Ellis J., and Ivan Pratt. 1963. *Myxobolus insidiosus* sp. n., a myxosporidian from the musculature of *Oncorhynchus tschawytscha* (Walbaum). J. Parasit., 49 (6): 951-955.

Yokoyama, Hisado, Ogawa. 1947. Studies on the origin, development, and seasonal variations in the blood cells of perch, *Perca flavescens*. Ph. D. thesis, Univ. of Wisc., 149 p.

Yu, T. C. and Russell O. Sinnhuber. 1957. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technology, 11 (2): 104-108.

# APPENDIX

## GLOSSARY OF HEMATOLOGICAL TERMS

Acid-hematin—A brown compound formed when dilute hydrochloric acid is added to blood; a photometric or comparative method for estimating hemoglobin in the blood.

Anemia—Lack of blood; subnormal concentration of oxygen-carrying substance in a certain volume of blood; characterized by either a reduction in hemoglobin, red cell count, packed cell volume, or a combination of these phenomena. May be caused by a decrease in blood production, increase in blood destruction, or severe loss of blood.

Anemic—Of, pertaining to, or affected with anemia; bloodless.

Anisocytosis—A condition in which the red cells are not uniform in size.

Basophil—A leukocyte having large granules with an affinity for basic stains.

Basophilic—Generally refers to the coarse blue to purple cytoplasmic granulation of granulocytes. Also commonly used to describe other cells or portions of cells with strong affinity for basic dyes, blue to purple.

Basophilic normoblasts (prorubricyte or basophil red cell)—Stage of red cell between the pronormoblast and polychromatophil.

"-blast"—Suffix denoting the most undifferentiated of the cells of each series.

Blast cells—Inclusive term denoting the stages of development as a whole or when the series of blood cells has not been identified.

Blood cells—Cellular components of the blood which include red cells, leukocytes, and thrombocytes.

Blood regeneration—The replacement of lost or senile blood cells with new cells.

Chromatin—The more stainable portion of the cell nucleus which stains blue to lavender.

Circulating blood—Blood in vessels as well as storage organs and sites of blood production.

"-cyte"—Suffix denoting a cell, the type of which is designated by the root to which it is affixed, as erythrocyte, leukocyte, or thrombocyte.

Degenerative index—Shows the proportion of granulocytes with toxic cytoplasmic granules.

Degenerative shift to the left—Refers to a failure of neutrophils to mature as a result of depressed granulocyte formation or maturation; revealed by increased numbers of immature neutrophils in the peripheral blood (Schilling's classification).

Degenerative shift to the right—Depressed neutrophil production or release as revealed by observation of reduced numbers of principally segmented cells in blood smears.

Differential—Pertaining to percentages obtained from blood smears.

Differential leukocyte count (differential white cell count)—Counts of leukocytes from a stained blood smear which gives the percentages of lymphocytes, macrophages, neutrophils, basophils, eosinophils, and plasmacytes.

Differential shift—Changes in number of immature leukocytes or mature leukocytes. Also, changes in number of mature or immature red cells.

Disintegrated cell—Cell of any series in which the cytoplasmic outline has been disrupted or the nuclear chromatin is no longer surrounded by a membrane.

Dyscrasia—abnormality.

Erythroblast—Technically the most undifferentiated red cell, but also used to describe the two most primitive immature red cell stages.

Erythrocyte—Mature red blood cell; also used to include immature red cells. Used only in the former sense in this paper.

Erythrocytic—Pertaining to red blood cells.

Erythrocytic depression—Decrease in red cell numbers due to decreased production or release of red cells.

Erythropoiesis—The process of red cell production.

Erythropoietic depression—Lack or virtual termination of red cell production; may be temporary or permanent.

Fragility—Increased susceptibility of blood cells to rupture due to mechanical or chemical agents.

Granules—Generally considered the specific small particle or grain in the cytoplasm of granulocytes; but non-specific particles may be found in lymphocytes, macrophages, thrombocytes, and red cells.

Granulocyte—Inclusive term applied to leukocytes with neutrophilic, basophilic or eosinophilic granules in the cytoplasm, in addition to a segmented nucleus during the most differential stage. The plural form, granulocytes, include granuloblasts, progranulocytes, myelocytes, juveniles, band, and segmented cells.

Granulocytosis—An increase in the number of granulocytes in the blood (generally limited to a relative increase).

Granulocytopenia—A marked decrease in the number of granulocytes in the blood.

"hem-, hemato-, heme-"—Prefixes denoting blood.

Hematocrit—A centrifuge for separating the cellular elements of the blood from the plasma. Used in this paper synonymously with packed cell volume. Abbreviated Ht.

Hemocytometer—A chamber used to count blood cells.

Hemoglobin—The oxygen-carrying red pigment in red blood cells of most animals.

Hemoglobin level—Measured in grams of hemoglobin in 100 milliliters of blood. Hemoglobin estimation in fish blood using the acid-hematin method with centrifuging gives an index value rather than actual hemoglobin content due to interference of nuclei, immature red cells, leukocytes, and thrombocytes. Abbreviated Hb.

Hemopoiesis (hematopoiesis)—The formation or production of blood cells.

Hemopoietic response—Increased production of blood cells.

Heparin—A mucopolysaccharide acid salt occurring in various tissues, but most abundant in the liver. As heparin-sodium it is used as an anticoagulant.

Heparinized capillary tubes—Capillary tubes with a thin coating of heparin on the inner surface to prevent coagulation of blood during collection for obtaining packed cell volumes.

Hyperchromia—Condition in which the red cells contain more than the normal amount of hemoglobin.

Immature red cell—Red cell at some stage which has not developed its full potential of hemoglobin. Abbreviated Immat. RBC. Stages of immaturity from the most primitive to the mature are given below under the old and the new nomenclature for mammalian blood. (American Journal Clinical Pathology, Vol. 18, 1948).

| *Old Classification* | *New Classification* |
|---|---|
| pronormoblast | rubriblast |
| basophilic normoblast or megaloblast | prorubricyte |
| polychromatophil | rubricyte |
| orthochromatophil | metarubricyte |
| reticulocyte | reticulocyte |
| erythrocyte | erythrocyte |

Leukocyte—A white blood cell. This term includes all blood cells devoid of hemoglobin except thrombocytes, regardless of the stage of development. The major breakdowns are lymphocytes, macrophages, neutrophils, basophils, eosinophils, and plasmacytes.

Leukocytosis—Increase in leukocytes in the blood.

Leukopenia—Reduction in number of leukocytes in the blood.

Lymphocyte—A rounded mononuclear cell derived from lymphoid tissue. This cell has homogeneous blue cytoplasm, often a perinuclear clear zone, and deeply basophilic chromatin.

Lymphopenia—Reduction in number of lymphocytes in the blood.

Macrocytic anemia—An anemia characterized by large red cells.

Macrophage—A large mononuclear leukocyte with a large irregular nucleus and pale vacuolated cytoplasm. Considered synonymous with a circulating macrophage.

Mean Corpuscular Hemoglobin Concentration (MCHC)—An index indicating the per cent hemoglobin per unit volume of blood.

"meta-"—Prefix denoting the 4th stage in the granulocytic and erythrocytic series as recommended by the American Society of Clinical Pathologists and the American Medical Association.

Microcyte—Cell smaller than normal.

Microcytic—Pertaining to a condition in which red cells are smaller than normal.

Microcytosis—An increase in the number of small red cells.

Neutrophil—Leukocyte with small neutral staining granules in the cytoplasm. Mature forms have segmented nucleus. Neutrophils in salmon reveal granules only when overstained with Wright's or Geimsa solutions.

Neutrophilia—An increase in neutrophils of the blood; generally considered an absolute increase.

Normochromic—Pertaining to red blood cells with normal amount of hemoglobin.

Normocytic—Pertaining to red blood cells of normal size.

Nucleolus—A homogeneous pale blue body in the nucleus surrounded by a dense chromatin condensation.

Orthochromatophil—A cell of the erythrocytic series that follows the polychromatophil and precedes the reticulocyte of man or mature red cell in fish.

Packed cell volume (packed red cell volume, hematocrit reading, hematocrit value, hematocrit percentage)—Percentage of red cells after centrifuging blood at a speed and for a period of time required to produce maximum pack.

Peripheral blood—Blood being carried to or from the tissues in arteries, capillaries, and veins.

Phagocytes—Cells which destroy an organism or extraneous matter by a process of envelopment and absorption.

Phagocytosis—The destruction of an organism and extraneous matter by a process or envelopment and absorption.

Plasma—The fluid portion of the blood composed of serum and fibrinogin, obtained when an anticoagulant is used.

Poikilocyte—An abnormally shaped red cell.

Polychromatophil (rubricyte, polychromatophilic or polychromatic normoblast, polychromatophilic or polychromatic megaloblast)—A small round red cell with a large round nucleus and first indications of hemoglobin in the portion of the cytoplasm surrounding the nucleus.

"pro"—Prefix denoting stage of development following the most undifferentiated blood cell (blast).

Pronormoblast (rubriblast, hemacytoblast, erythroblast, stem cell)—Earliest developmental stage of red blood cell, indistinguishable from other stem cells except by association with known cells in the same blood smear.

Red blood cells (red blood corpuscles, red cells, erythrocytes)—Blood cells containing hemoglobin or which will contain hemoglobin at a later stage of development. Abbreviation RBC's.

Regenerative shift to the left—Rapid outpouring of neutrophils or other granulocytes with a relative increase in immature forms.

Reticulocyte—Cell between the orthochromatophil and the mature red cell of mammals distinguished by supravital staining, but not easily distinguished in the blood of salmon and trout. Semi-mature red cells in salmon were classified as orthochromatophils.

Series—As applied to blood, a group or succession of blood cells arranged in regular order, as follows:

Lymphocytic—lymphocytes and their developmental stages.

Granulocytic—granulocytes and their developmental stages.

Monocytic—monocytes and their developmental stages (machophages in fish).

Plasmacytic—plasmacytes and their developmental stages.

Erythrocytic—red blood cells.

Thrombocytic—thrombocytes and their developmental stages.

Serum—Fluid portion of the blood.

Specific granules—Neutrophilic, eosinophilic, or basophilic granules. This term does not include azurophil granules.

Stem cell—The earliest blood cell precursor.

Terminal—Ending; immediately before death, final.

Thrombocyte—Blood platelet in mammals; nucleated cells which disintegrate to initiate clotting of the blood of (most) fish, amphibians, reptiles, and birds.

Toxic leukocytes—White cells showing abnormal enlarged basophilic granulation and vacuolated cytoplasm.

Vacuole—A space or cavity formed in the cytoplasm of a cell.