A HAEMATOLOGICAL STUDY OF THE GENUS NOTROPIS

A Thesis

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Master of Science

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by

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ABSTRACT

The purpose of this study was (1) to establish the general blood picture of the genus Notropis; (2) to show the response of the blood cells to various experimental conditions of temperature and diet; (3) to locate the haematopoietic tissues under normal and experimental conditions, and (4) to arrive at a possible scheme of blood cell formation.

The study of the general blood picture was made more difficult by the small volume of blood available from the minnows. The differential blood counts, the red cell counts and the haemoglobin were tabulated with most of the emphasis placed on experimenting with the blood coagulation. The results of the blood clotting experiments showed that the clotting mechanism was similar to that of higher mammals.

The minnows were subjected, in the experimental studies, to changes of diet and temperature. The experimental results, both tabular and graphic, showed the direct response of the blood cells to the experimental conditions. With an increase in temperature, there was a direct increase in the red cell count and the haemoglobin within twenty-four hours. Temperature increase combined with a liver diet also

resulted in a rise in the erythrocyte count and the haemoglobin within twenty-four hours. This increase to the combined stimuli, was slightly greater than the response to temperature increase alone. In the third experiment, with vitamin B_{12} feeding, there was an increase in erythrocyte count and haemoglobin within twenty-four hours.

In all three experiments the red blood cells and the haemoglobin responded to the experimental conditions within twenty-four hours by increasing. When the fish have adapted to their stimuli, the erythrocyte counts and the haemoglobin readings stop climbing and assumed a fairly constant reading. This constant reading was referred to as the stabilized production rate. A stabilized production rate of blood cells was reached within 18 to 21 days in Experiment I, within 14 days in Experiment II, and in 12 to 14 days in Experiment III. The stabilized production rates varied according to the affects of the experimental stimuli used.

The haematopoietic organs under normal and experimental conditions were found to be similar to the embryonic sites of developments in higher mammals. In the genus Notropis the kidney and the spleen were the largest producers of the blood cells, the liver and the intestine produced on a much smaller scale. Under experimental conditions of increased temperature, or increased temperature and diet supplement, or a

vitamin B_{12} diet, the kidney, spleen, liver and intestine, were found to manufacture blood cells at a much higher rate than under normal conditions.

An intensive examination of haematopoietic tissues revealed large and small lymphoid hemoblasts. From the large lymphoid hemoblasts, granulocytes developed and from the small hemoblasts the remaining blood cells were formed. The mode of blood cell formation of the genus Notropis approached the neo-unitarian hypothesis.

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CHAPTER I

THE PROBLEM

Much research has been done and is still being conducted on higher mammals and in human haematology. In the lower vertebrates, particularly in the realm of fishes, the amount of detailed haematological research is limited. The majority of the research completed on the fishes was concentrated in the fields of biochemistry and physiology.

STATEMENT OF THE PROBLEM

The purpose of this research was (1) to establish the general blood picture of the genus Notropis, (2) to show the response of the blood cells to various experimental conditions of temperature and diet, (3) to locate the haematopoietic tissues under normal and experimental conditions, and (4) to arrive at the possible scheme of blood cell formation.

CHAPTER II

REVIEW OF THE HISTORY

Catton[2] studied the blood cells of certain Teleosts (trout, roach and perch) and found they consisted of nucleated erythrocytes, reticulocytes, nucleated thrombocytes, coarse and fine granulocytes and lymphocytes of varying sizes. Basophils and monocytes were generally lacking. A few representative white blood counts and erythrocyte counts of various fishes have been listed by Downey[5]. Determination of the erythrocyte counts and the haemoglobin contents were seriously complicated by a very rapid clotting time. The general blood picture of the genus Notropis has not been tabulated in the literature.

Saito[21] experimented with the elements of blood coagulation and blood coagulation time. He found that the blood of fish required about one minute for perfect coagulation. Further studies of Saito[21] disclosed that the blood of the fish contained fewer platelets than that of humans. Generally speaking, he found that the mechanism for coagulation of fish blood more primitive than that of mammals.

The red blood cell counts made by Smith[24] on the liver-fed trout were found to be higher than wild trout counts. Unfortunately this experiment was not conducted

under controlled conditions. Therefore, it was not conclusive that the liver diet was the factor responsible for the rise in erythrocytes.

Vitamin B_{12} therapy has been used successfully in treatment of pernicious anemia. The effects on the haematopoietic system were comparable with those seen in liver therapy. With relatively large amounts of vitamin B_{12}, the marrow picture may return to normal in 48-72 hours according to Had and Campbell[10]. Review of the literature did not reveal any experiments using vitamin B_{12} as a fish food supplement.

The advances in our knowledge of blood cell formation in the lower vertebrates, has been largely due to Downey[5], Yoffey[27], Duthie[6], Catton[2], Jordan[13], Speidel[13], Reznikoff[19], and Drzewina[7]. On the basis of histological examination of the kidneys of certain fishes, several theories as to the nature of the stem cells and the process of blood cell development have been advanced. These theories are more thoroughly discussed in Chapter VII.

Downey[5] reported that the mesonephros of the kidney was generally the predominent haematopoietic organ in the teleost. The spleen had only an accessory role. However, there was great variation with regard to the relative haematopoietic activity of spleen and kidney. Thus in tautog, scup and fundulus the spleen was relatively active; in the

goldfish, buffalo-fish and flounder it was relatively inactive; in trout and carp, spleen and kidneys were about equally active.

The spleen of teleosts was generally described by Yoffey[27] as containing lymphoid tissue, pulp and ellipsoid cells. He concluded that the blood flowed through the lymphoid tissues and received from it the developing cells.

Jordan and Speidel[13] reported eosinophils and lymphocytes enveloping the splenic arteriole. Jordan and Speidel[13] and Yoffey[27] found no basophils present in the spleen. All three authors found that the senile and disintegrating erythrocytes and eosinophils were phagocytized by macrophages chiefly of reticular origin.

Downey[5] studied the intertubular renal tissues and found they resembled the lymphomyeloid tissue of the spleen as described by Jordan and Speidel.[13] Lymphocytes, in various stages of amitosis in the haematopoietic kidney tissue were reported by Downey.[5]

Jordan and Speidel[13] described the eosinophils as having a dual origin in most teleost fishes; from the lymphocytes and from the intestinal connective tissues in the mucosa and submucosa.

In reviewing the literature of the fishes generally and teleosts in particular, it was seen that considerable research has been completed on the blood formation with

the view of enlarging upon the mammalian blood formation theories. Little actual mention was made in the literature of the genus Notropis from the haematological point of view.

CHAPTER III

A. SPECIMENS

Family:	Cyprinidae
Genus:	Notropis
Species:	blennius
Common name:	the Minnow or more often called the River Shiner

The minnows, Figure I, were obtained from Lake Winnipeg during the early part of October. The transfer of these minnows from their lake habitat to the main aquarium presented a few problems. The transport was made in large metal containers filled with lake water and a small amount of ice. The vessels were kept open during the drive to the University. A small tire pump provided the extra aeration necessary before the minnows were placed in the aquarium.

To acclimatize the minnows to life in the aquarium several precautionary measures were taken. The temperature of the water in the aquarium was $\pm$ 1° centigrade of the lake water. Since these fish in their natural habitat were accustomed to fast running water and an ample supply of oxygen, the aquarium was arranged accordingly. Running water and an air hose were installed to provide the oxygen and the turbulence necessary in the water. A medicated

Figure I...The Minnows...

aquarium "High-Ball" was used to neutralize any acids and impurities that might collect at the bottom of the tank. The number of minnows kept in stock was in the neighborhood of three hundred and fifty. The fish used for experimental work were transferred in small numbers to a test aquarium. The main aquarium was used largely as the control tank, Figure II. After a fourteen day period of acclimatization to their new habitat and diet, the minnows were used for the experimental work.

B. FEEDING

Goldfish food, tropical fish food and pellets were fed to the minnows. These foods were made up of crustacean material, protein, fat, crude fiber, ash, dried flies, epsom salts, meat meal, zweiback, dried skim milk and shrimp. This diet provided good overall nutrition, the minnows thrived and increased in weight. Figure III.

C. MORTALITY RATE

During the initial transfer of the minnows from Lake Winnipeg to the University aquarium no losses were incurred. In the course of the acclimatization period the mortality rate increased to one or two minnows a day for approximately three weeks. Following these three weeks the mortality rate dropped to an occasional death usually due to injury.

Figure 11... The control aquarium containing the minnows used for the research ...

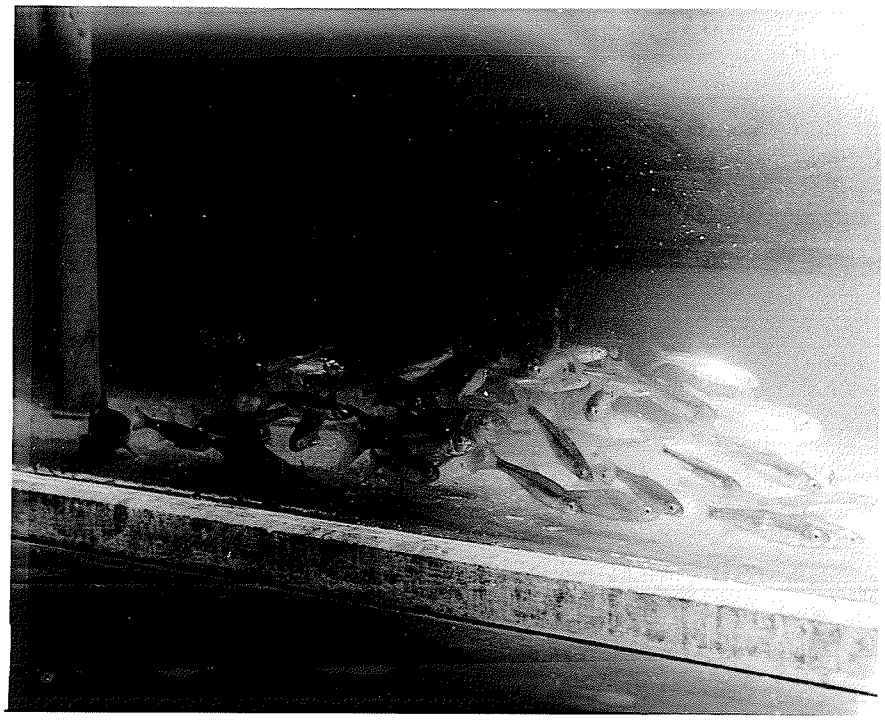


Figure III...Various views of the genus Notropis...

D. ACCLIMATIZATION

The response of these fish to vibratory sounds or sudden movements were the main difficulties encountered in their acclimatization. A wooden shield was placed before the glass side of the aquarium exposed to the room. The air hose pressure was increased to absorb most of the extraneous noise. An aluminum screen was placed over the top of the aquarium. These three precautionary measures eliminated losses due to fright or jumping out of the tank. The fish seldom schooled together in the corners but swam over the complete length of the aquarium.

E. BEHAVIOUR

The lake and the aquarium water were moderately warm at 15° centigrade. The running tap water and air hose provided the triple benefits of a slight water current, the necessary aeration and the masking out of most of the extraneous noise. The minnows swam about the aquarium in a free uninhibited fashion. They fed both on the surface and on the bottom of the tank and over a few months general increases in weight and length were observed.

As the late fall and winter set in, the temperature of the tap water gradually dropped to approximately 9° centigrade and remained there. At this lower temperature the

fish were more sluggish in their movements, although their activity increased considerably at feeding or under additional air or water pressure.

The behaviour of the experimental fish varied in accordance to the stimulus used. In the experiment with temperature change the activity of the fish increased directly with the temperature rise. The liver-fed fish increased in their activity but this activity seemed to be due mainly to the mounting temperature. The third experimental group exhibited activity similar to the liver-fed and temperature controlled fish, but upon supplementing their diet with large doses of vitamin B_{12} their activity increased markedly. They were more active swimmers, more food conscious and much more difficult to net.

The various rates of activity in the behaviour of the minnows did not alter their general healthy appearance. Under all the experimental stimuli they appeared to be quite well nourished.

CHAPTER IV

METHODS AND TECHNIQUES

The study of the blood of Notropis was hampered by two main factors. The first was the small volume of blood obtainable. To quote the physiologist, Doctor Wilhelm Wunder[26] from his book titled "Physiology of the Freshwater Fishes of Central Europe."

"fish blood is only 1/50 of the body weight."

The second factor was the rapid rate of blood coagulation. The coagulation plus the small volume did not allow much in the way of handling time. Therefore, the experimental work and equipment had to be adjusted to overcome these drawbacks.

Three methods were experimented with in obtaining the maximum amount of blood from the minnows. The first was an attempt at drawing the blood directly from the heart. It did not prove to be successful since a needle and syringe could not be used effectively. Decapitation was attempted but this provided too large a surface volume of blood exposed and as a result coagulation set in before much of the blood could be utilized. Neither of these methods provided the necessary amount of blood. The third method adopted provided a smaller surface volume of blood, a more continuous flow and an easier procedure of obtaining it.

The minnow was held in cheese cloth, the tail region was swabbed with alcohol and a sharp scalpel was used to sever the tail anterior to the anal fin, Figure IV. The severed iliac artery and other small blood vessels provided a slow steady stream of blood. If it was necessary to decrease the coagulation time, the severed area of the fish was lightly brushed with heparin. This method usually provided enough blood from a single minnow to complete the erythrocyte count, haemoglobin reading and several differential smears.

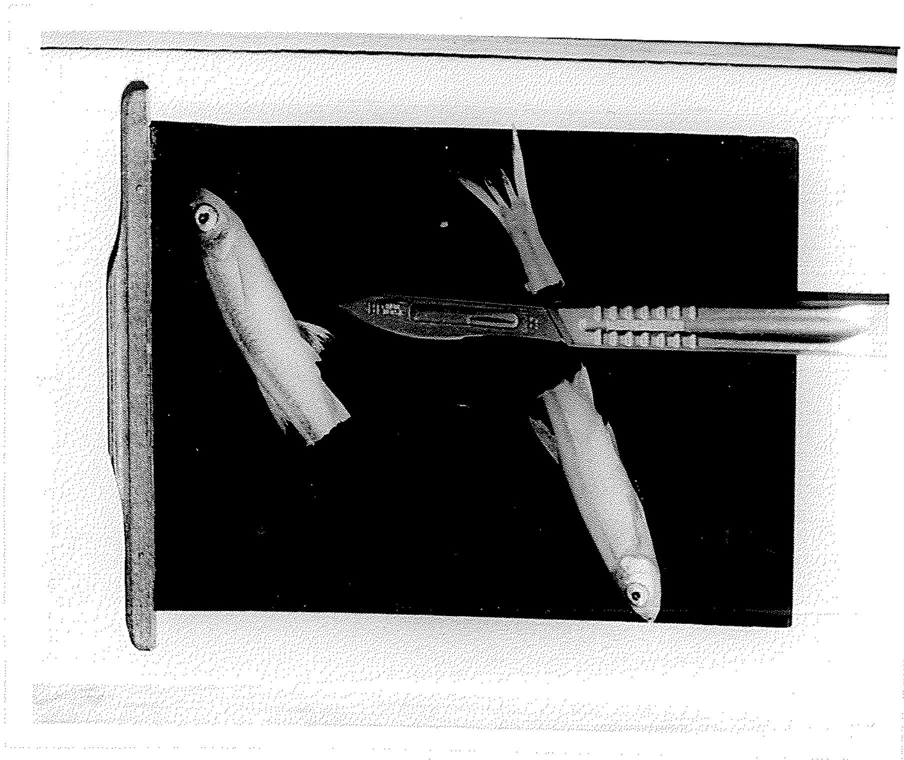
TECHNIQUES

1. Cytological Smears

Cytological smears were made of the spleen, gonads, kidneys, liver and mesentery. These smears were made directly from the minnows within half an hour of the severing of their tails. These smears were wet fixed in a solution of equal volumes of 95% alcohol and ether. The staining procedure used for these cytological smears was a modification of the Papanicolaou Cytology staining method.[18] The slides were transferred directly from the alcohol-ether solution to the following:

1. dipped in 80% alcohol
2. dipped in 70% alcohol
3. dipped in 50% alcohol
4. dipped in distilled H_2O

Figure IV...The method adopted for obtaining the blood, by amputating the tail with a scalpel...



5. stained in Harris' hematoxylin from five to six minutes
6. rinsed in distilled H_2O
7. dipped into 0.25% aqueous solution of hydrochloric acid
8. rinsed in tap water
9. dipped in distilled H_2O
10. dipped in 50% alcohol
11. dipped in 70% alcohol
12. dipped in 80% alcohol
13. dipped in 95% alcohol
*14. stained in OG-6 for two minutes
15. dipped in 95% alcohol
16. dipped in 95% alcohol
*17. stained in EA-65 for two minutes
18. dipped in 95% alcohol
19. dipped in 95% alcohol
20. dipped in absolute alcohol
21. dipped in a mixture of xylol-95% alcohol
22. dipped in xylol
23. dipped in xylol
24. mounted in permount

*OG-6 and EA-65. Cytology stains contained in Papanicolaous' "Atlas of Exfoliative Cytology."[18]

2. Histological Sections

The specimens used were fixed in 10% formalin. Two methods of tissue embedding were used, the rapid paraffin method and the cedarwood oil procedure. The latter method is contained in Guyers' "Animal Micrology."[9]

The rapid paraffin method was devised by Mallory and Wright.[23]

1. The tissues stored in 10% formalin were used directly.
2. two to three changes of acetone- 1-2 hours
3. two changes of benzene-30 minutes each change
4. three changes of paraffin wax in the oven - 30 minutes each change.
5. Tissues embedded in pure paraffin.

The sections were cut with a Bausch and Lomb rotary microtome, floated on warm water and fixed to the albumin-coated slides by warming the slides gently. The staining procedure was as follows:

1. the sections were immersed in xylol for 10-15 minutes to dissolve the paraffin
2. the sections were dipped in a solution of equal volumes of xylol-95% alcohol
3. dipped in 95% alcohol
4. dipped in 70% alcohol
5. dipped in 50% alcohol

6. dipped in 35% alcohol
7. stained in Harris' hematoxylin for 20 to 30 minutes
8. rinsed in tap water
9. rinsed in acid alcohol
10. dipped in 35% alcohol
11. dipped in 50% alcohol
12. dipped in 70% alcohol
13. dipped in 95% alcohol
14. stained in eosin in alcohol for 1-2 minutes
15. rinsed in equal volumes of xylol-95% alcohol
16. dipped in xylol
17. dipped in xylol
18. mounted in permount

The sections were now ready for microscopic examination.

3. Technique Used for Differential Blood Smears

1. a clean slide was touched lightly to the severed end of the fish.
2. the drop of blood was thinly spread on the slide and air dried.
3. ten drops of Wright's blood stain were added to the smear and agitated for two minutes.
4. twenty drops of distilled water were added to the Wright's stain and this mixture agitated for four minutes.

5. the slide was rinsed in running tap water and air dried.

The blood smears were ready for microscopic examination.

4. Technique Used for Red Blood Cell Counts

The red blood cell counts were determined by using the hemocytometer with the Neubauer counting chamber. This technique was taken from the text "Animal Micrology" by Guyer[9] pp. 116-118.

5. Techniques Used for the Determination of the Blood Clotting Time

Two techniques were used to determine the coagulating time of the blood of Notropis.

1. a small glass hematocrit tube
2. a paraffin coated glass slide

The two techniques were used a countless number of times before an accurate timing could be obtained. The first method was not as favored since the glass tube allowed more error.

It was difficult to file small nicks in the length of the hematocrit tube. The amount of blood obtainable and its rapid rate of congealing did not permit sufficient time for the accurate breakage of the hematocrit tube to determine the first fibrin formation. The hematocrit method was used mainly as a check on the second method. Since no large

surface of blood was exposed in the tube it was used as a check against the second method where a large surface of blood was exposed to the air. The differences were negligible. $\pm$ 2 seconds variation was found due to the handling of the tube and its breakage, whereas on the paraffin coated slide the blood was drawn out with a glass rod and the first fibrin threads were immediately observed.

Figure V shows some of the equipment used in the study and Figure VI shows the experimental aquarium.

Figure V...Some of the equipment used for determining the blood pictures of the genus Notropis...

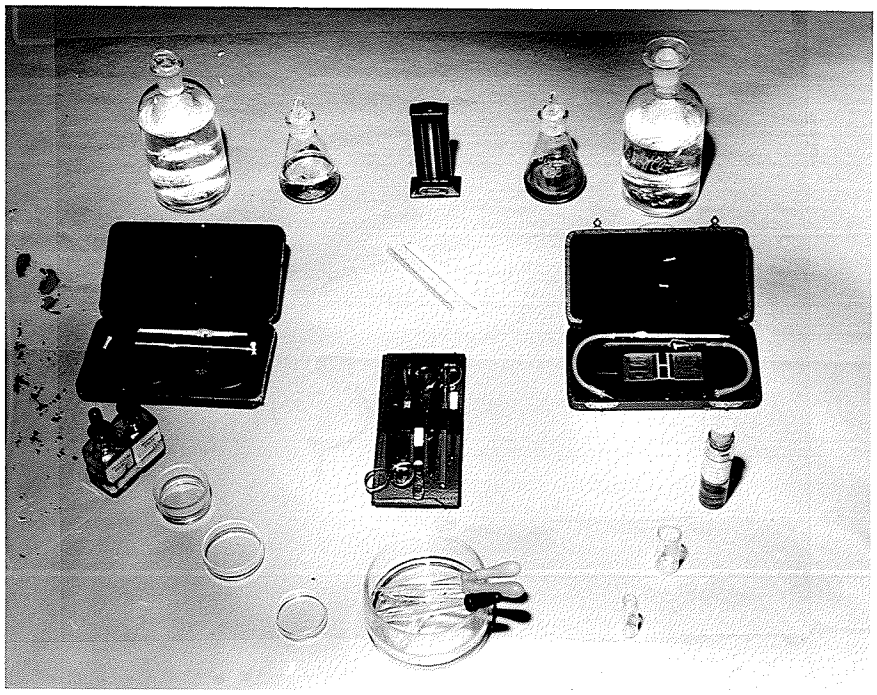
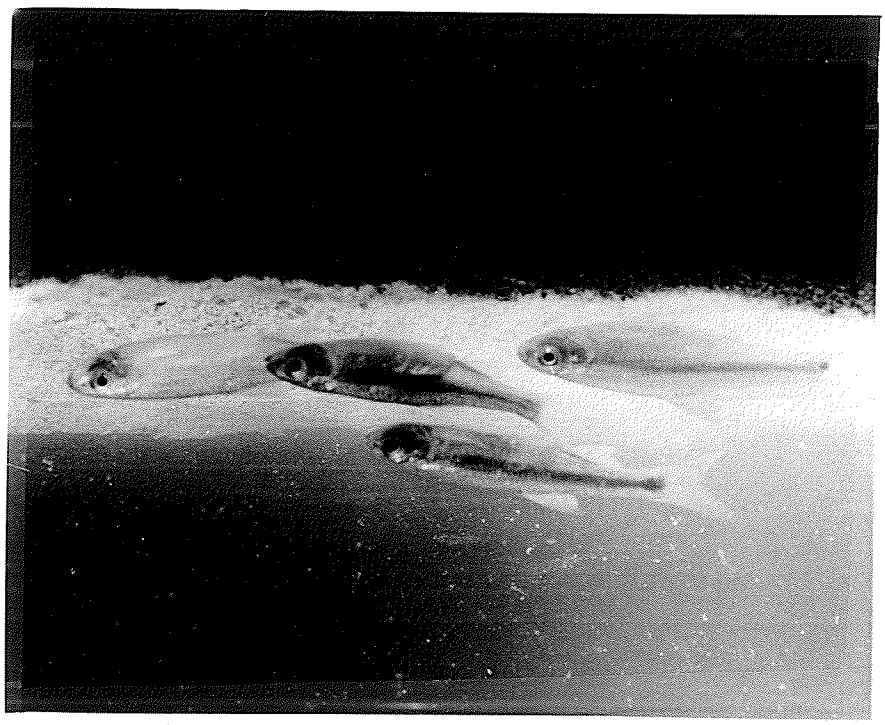


Figure VI... Minnows contained in the experimental aquarium ...



CHAPTER V

THE GENERAL BLOOD PICTURE OF NOTROPIS

A. DIFFERENTIAL BLOOD COUNTS OF THE MINNOWS

The first series of blood smears were made after the minnows had been installed in the controlled aquarium for three weeks. All the previous counts were used only for experimentation with stains. The minnows were fed the food referred to in Chapter II, Feeding. The temperature was kept fairly constant at 15° centigrade. The running water and air hose were used to keep the temperature and the oxygen content fairly constant. The blood smears were taken over a period of ten days at approximately the same time, between 10:00 a.m. to 11:00 a.m., to discount any changes due to activity. The weight of the minnows used was approximately 3.5 to 4 grams. The differential blood counts from the ten specimens used were within the following range:

Neutrophils	-	4-6%
Eosinophils	-	5-6%
Basophils	-	0%
Lymphocytes	-	64-67%
Monocytes	-	24-26%

In these smears, besides the white blood cells and the erythrocytes, there were found platelets, immature erythrocytes, smudges of erythrocyte nuclear material

disintegrating cells, cellular debris and artifacts. Figures VII, VIII, IX, X, XI, XII.

B. EXPERIMENTATION WITH THE BLOOD CLOTTING MECHANISM

A series of experiments conducted to determine if the blood clotting mechanism was somewhat similar to that of the higher mammals.

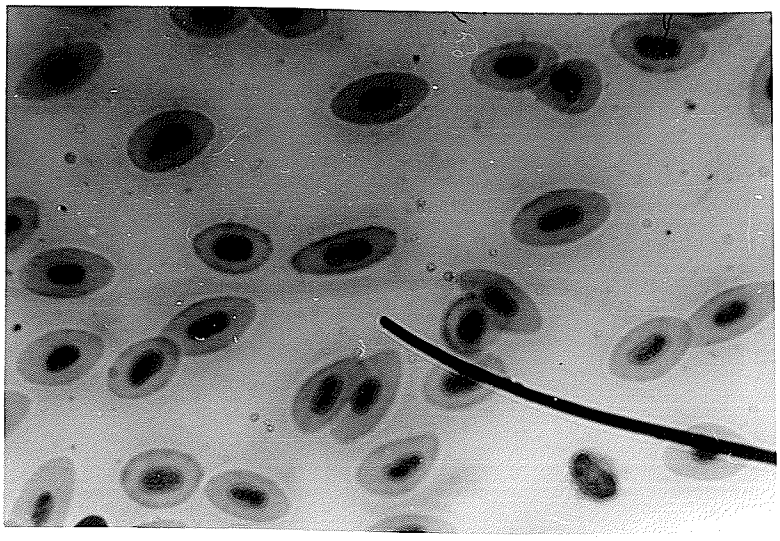
(1) A large drop of blood, obtained from the severed blood vessels of the minnow, was placed in a watch glass containing a drop of sodium citrate solution. This mixture did not coagulate. However, when a drop of calcium chloride solution was added, coagulation was initiated immediately and a complete clot was formed in a few seconds. During the coagulation, microscopic examination revealed the flow of erythrocytes, fibrin thread formation and the rapid formation of a congealing matrix.

(2) A second experiment was conducted by adding a drop of blood to a drop of heparin, no coagulation could be observed, even after one hour. When a drop of thrombin was added to this mixture, immediate coagulation resulted. Microscopic examination showed the same process at work as observed in the first experiment.

Experiment (1) and (2) were drawn up in point form as follows:

Figure VII...Erythrocytes of the genus Notropis...
... X 1200.

Figure VIII...Erythrocytes and a large lymphocyte...
...X 1200.



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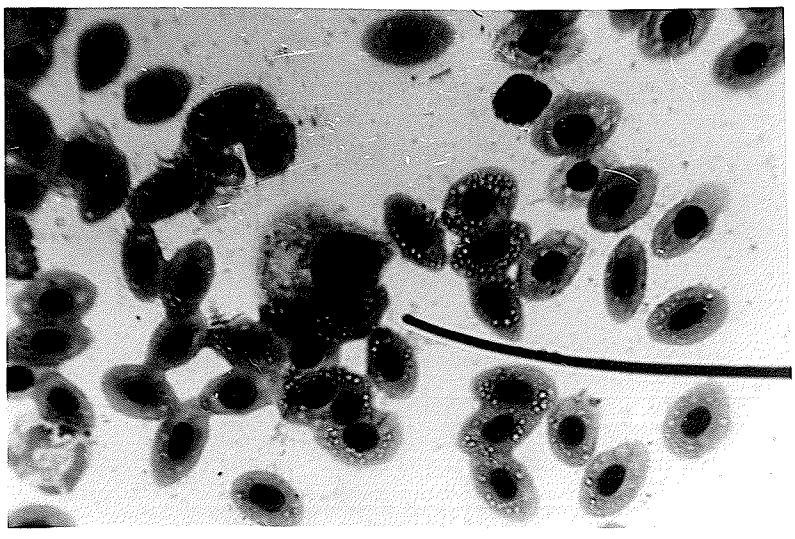

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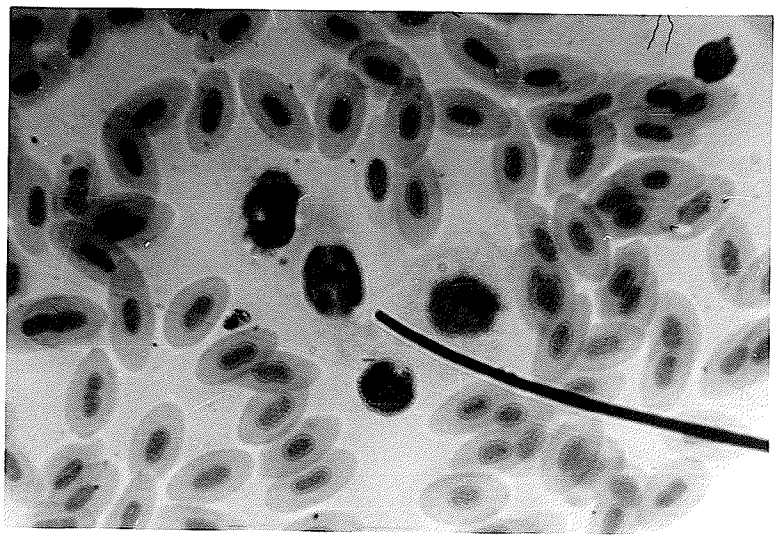
Figure IX... Large lymphocytes... X 1200.

Figure X...Lymphocyte and eosinophil... X1200.



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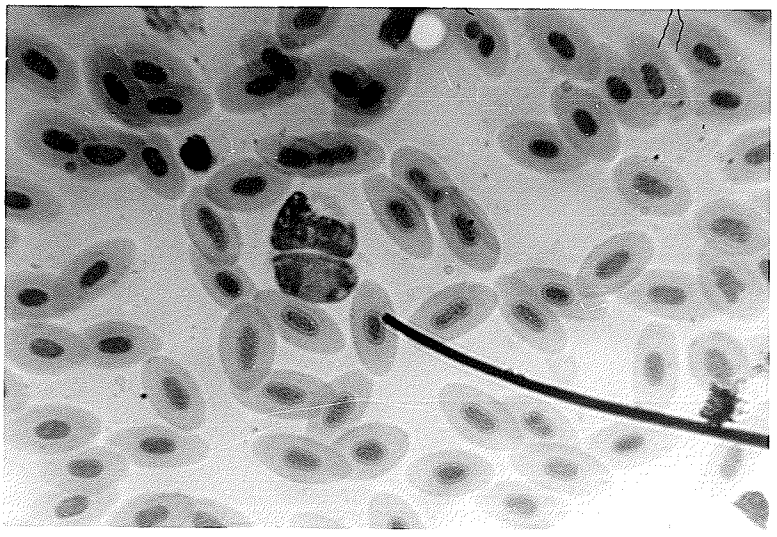

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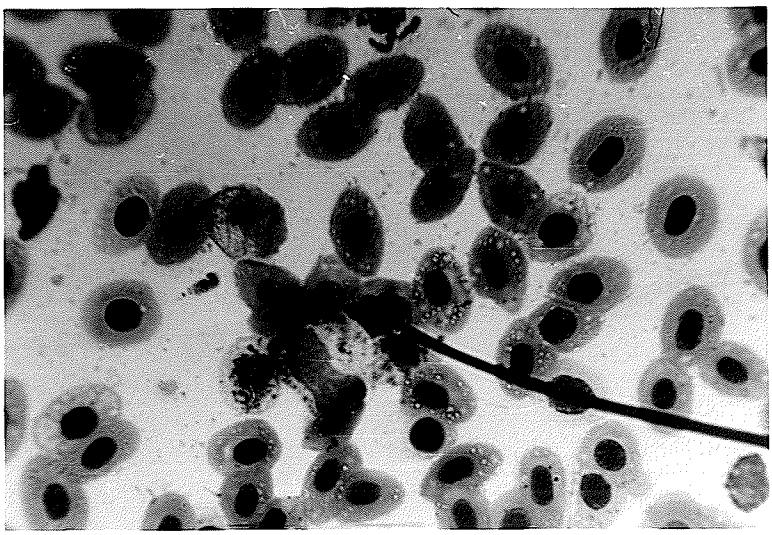
Figure XI...Lymphocyte and a monocyte... X1200.

Figure XII...A variety of blood cells... X 1200.



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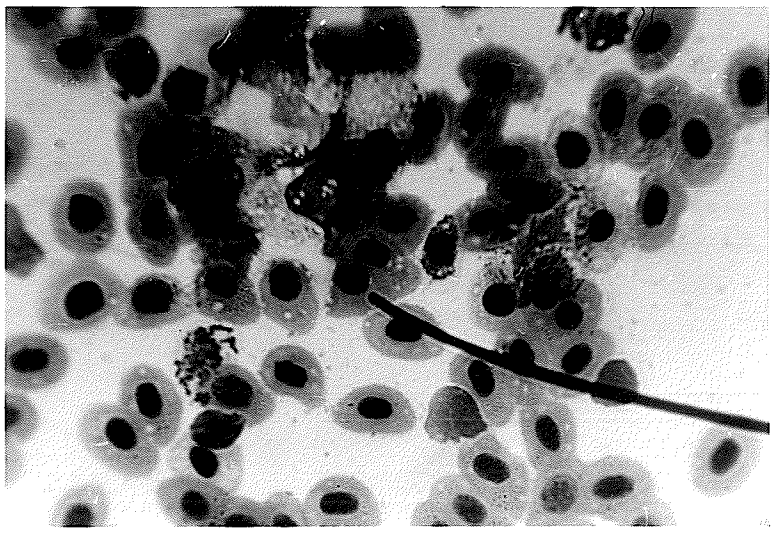

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(1) Blood of Notropis + sodium citrate
↓
calcium chloride
↓
fibrin formation
↓
Coagulation---→ clot formation

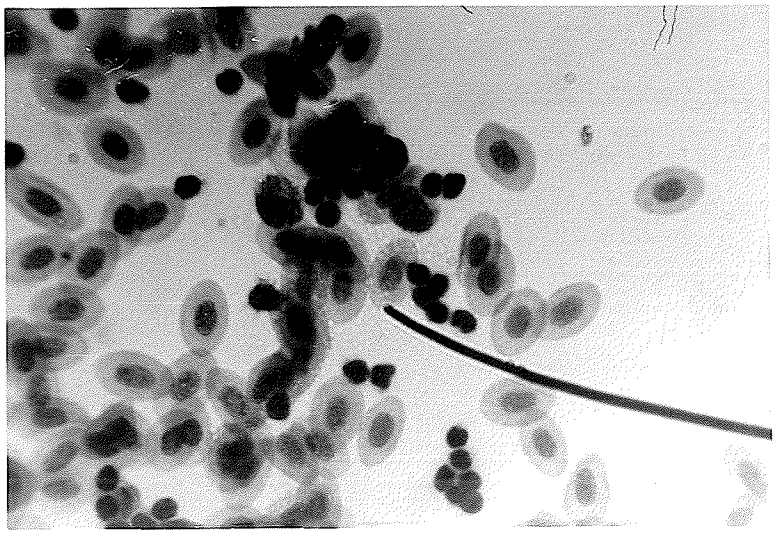
(2) Blood of Notropis + heparin
↓
thrombin
↓
fibrin formation
↓
Coagulation---→ clot formation

Due to the small volume of blood obtainable, the experiments of blood coagulation were limited. The blood reacted similarly to human blood, with the citrate, heparin, calcium and thrombin. Several slides were smeared just as the fibrin threads were being formed in the coagulating gel. These slides were stained similarly to the differential smears. Microscopic examination showed an abundance of platelets concentrated around the edges of blood drops. The platelets were approximately half the size of the erythrocytes with a dark dense nucleus and a faint appearance of cytoplasm surrounding this nucleus. Photomicrographs illustrate the size and appearance of the platelets or thrombocytes. Figures XIII and XIV.

Numerous fish, of approximately 3.5 to 4 grams, were experimented with to determine the blood coagulating time by the technique referred to in Chapter IV, Techniques Used for

Figure XIII...Thrombocytes... X 1200.

Figure XIV...Thrombocytes...X 1200.



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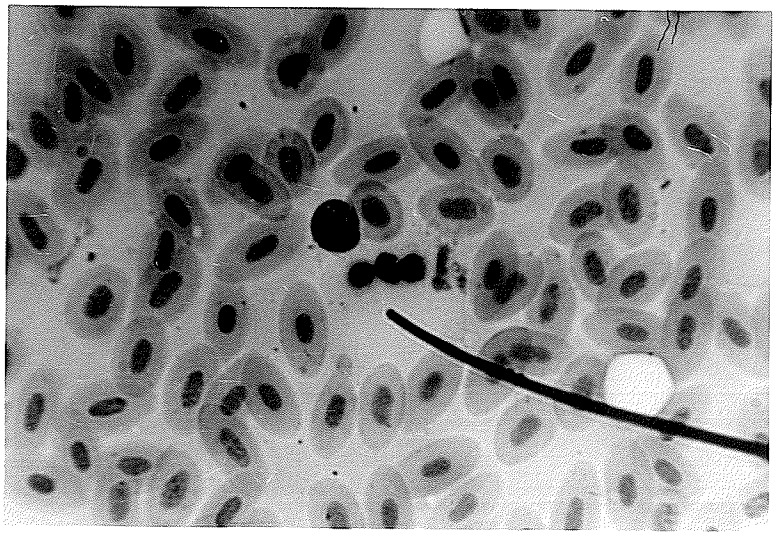

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the Determination of the Blood Clotting Time. At 15° centigrade the results were as follows:

An average of 15.2 ± 2 seconds elapsed before the first fibrin thread was drawn out. At an average of 26.4 ± 2 seconds the blood was quite solid and microscopically no erythrocyte movement was observed. 15.2 ± 2 seconds did not refer to the gel process but to the fibrin thread formation. The gel process was timed at an average of 12.5 ± 2 seconds.

The coagulation time was unchanged by any appreciable amount with a temperature or diet change. Coagulating times were taken at the extremes of the experimental temperature and diet feeding and neither of these showed any appreciable change in the coagulating time.

C. RED BLOOD CELL COUNTS

The red blood cell count of Notropis, at the temperature of 15° C., was determined using the Neubauer counting chamber, to be 2.67 in millions per cu. mm.

D. HAEMOGLOBIN

The haemoglobin content using the Sahli hemoglobinometer, was read as 30.9% or 4.47 grams at the temperature of 15°C.

This covers the general blood picture of the genus Notropis. The small volume of blood limited a more complete

blood picture. It did serve a purpose in establishing a general haematological picture for the minnows.

CHAPTER VI

THE RESPONSE OF THE HAEMOPOIETIC TISSUES TO EXPERIMENTAL CONDITIONS

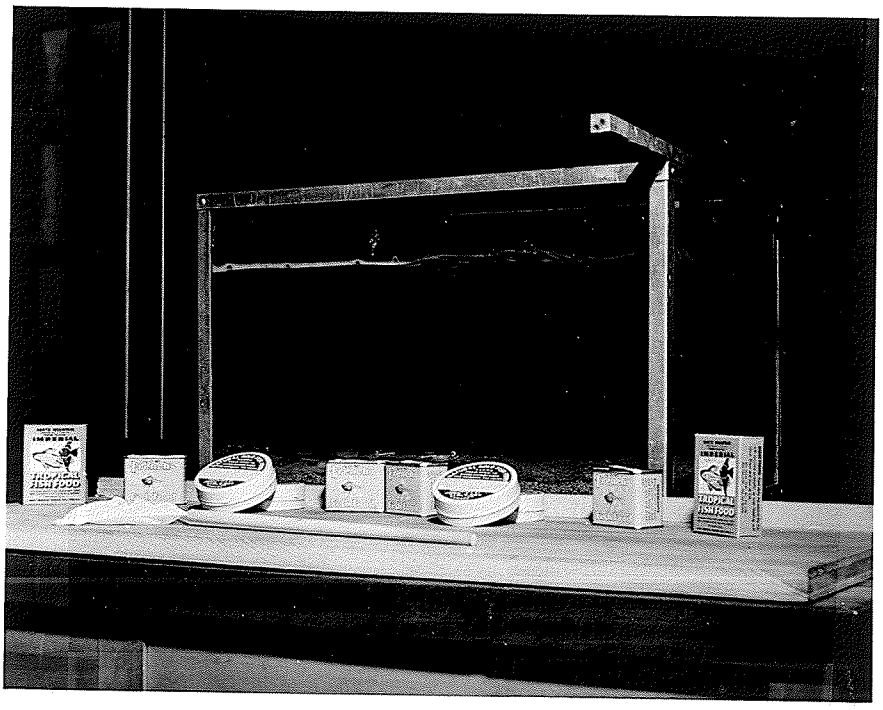
The genus Notropis showed excellent response to experimental conditions. The changes were determined by red blood cell counts, haemoglobin readings and differential white blood counts.

A. EXPERIMENT I - EFFECTS OF TEMPERATURE CHANGE

The procedure for this temperature change was as follows:

The control tank temperature had dropped to 9.5°C. about a month prior to this experiment. Five of these minnows were used to determine the red blood cell count, the haemoglobin reading and the differential count. Thirty-five minnows were transferred from the control tank to an experimental aquarium at 9.5°C. Figure XV. All these minnows were approximately 3.8 to 4.2 grams in weight. Over a twenty-four hour period the temperature of the experimental tank increased to 16.5°C. This experimental tank was adjusted similarly to the control aquarium with regards to aeration and water flow. The fish were fed the same type and amount of food as the control fish. The only

Figure XV... Experimental aquarium displaying the fish, the fish foods and the catching net ...


IMPERIAL
TROPICAL
FISH FOOD
IMPERIAL
TROPICAL
FISH FOOD

condition changed was the increased temperature from 9.5°C. to 16.5°C. The changes in the erythrocyte counts and the haemoglobin readings were tabulated over a period of three weeks and the results are shown graphically. Tables I, II, and Graphs 1, 2.

The tables and graphs show that the increase of the red blood cell counts and the haemoglobin readings are directly proportional to the increase in temperature. The graphs indicate that the greatest change occurred in the first twenty-four hours, followed by an increase until a stabilized reading was reached within 18 to 21 days.

Examination of the control fish sections (Chapter VII) did not reveal any appreciable blood reserves or large haematopoietic areas, but examination of experimental fish sections revealed extensive areas of blood cells in various stages of development in the haematopoietic tissues. Therefore it is concluded that the haematopoietic organs produced this increase within twenty-four hours in response to the stimulus of increased temperature.

B. EXPERIMENT II - EFFECTS OF DIETARY CHANGE

This experiment was conducted similarly to Experiment I with the addition of a dietary change. Twenty-five minnows were transferred from the control tank at 9.5°C. to an experimental aquarium at 9.5°C. Over a twenty-four hour

TABLE I

THE RESPONSE OF THE BLOOD OF NOTROPIS TO TEMPERATURE CHANGE

Specimen	Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
* 1	30	9.5°	1.29	26.0%	3.77
2	30	9.5°	1.28	25.8%	3.74
3	30	9.5°	1.30	26.1%	3.78
4	30	9.5°	1.28	25.9%	3.75
5	30	9.5°	1.29	26.2%	3.79
1	1	16.5°	1.92	27.9%	4.04
2	1	16.5°	1.99	28.1%	4.07
3	1	16.5°	1.89	27.0%	3.91
4	1	16.5°	1.90	27.5%	3.98
5	1	16.5°	1.95	28.0%	4.06
1	2	16.5°	1.99	27.9%	4.04
2	2	16.5°	2.10	28.5%	4.13
3	2	16.5°	2.10	28.2%	4.08
4	2	16.5°	2.98	28.6%	4.06
5	2	16.5°	2.10	28.3%	4.10
1	7	16.5°	2.39	30.5%	4.42
2	7	16.5°	2.40	30.9%	4.48
3	7	16.5°	2.38	30.9%	4.48
4	7	16.5°	2.40	30.5%	4.42
5	7	16.5°	2.32	30.8%	4.46
1	14	16.5°	2.65	30.0%	4.78
2	14	16.5°	2.70	33.5%	4.85
3	14	16.5°	2.72	33.5%	4.85
4	14	16.5°	2.69	33.4%	4.84
5	14	16.5°	2.75	33.5%	4.85
1	18	16.5°	2.72	34.2%	4.95
2	18	16.5°	2.74	33.6%	4.87
3	18	16.5°	2.72	33.8%	4.90
4	18	16.5°	2.73	34.0%	4.93
5	18	16.5°	2.74	33.9%	4.91
1	21	16.5°	2.74	34.4%	4.98
2	21	16.5°	2.75	34.2%	4.95
3	21	16.5°	2.73	34.0%	4.93
4	21	16.5°	2.74	34.2%	4.95
5	21	16.5°	2.76	34.0%	4.93

* The control readings.

TABLE II

THE RESPONSE OF THE BLOOD OF NOTROPIS TO TEMPERATURE CHANGE

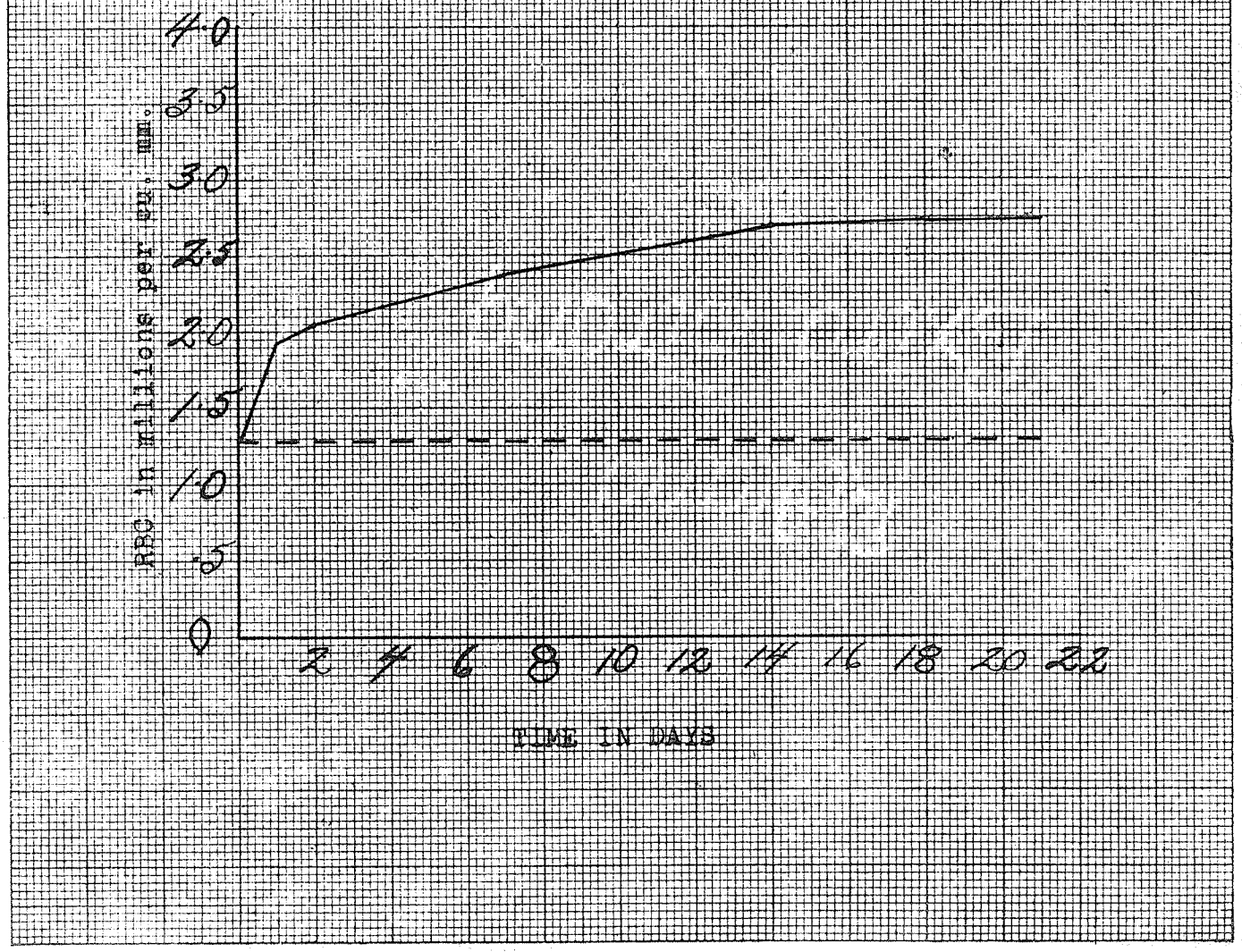
THE AVERAGE OF THE READINGS OF TABLE I USED FOR GRAPHING

Specimen Total	Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
* 5	30	9.5°	1.28	26.0%	3.76
5	1	16.5°	1.93	27.7%	4.01
5	2	16.5°	2.05	28.1%	4.08
5	7	16.5°	2.37	30.7%	4.45
5	14	16.5°	2.70	33.3%	4.83
5	18	16.5°	2.73	33.9%	4.91
5	21	16.5°	2.74	34.1%	4.94

* The control readings.

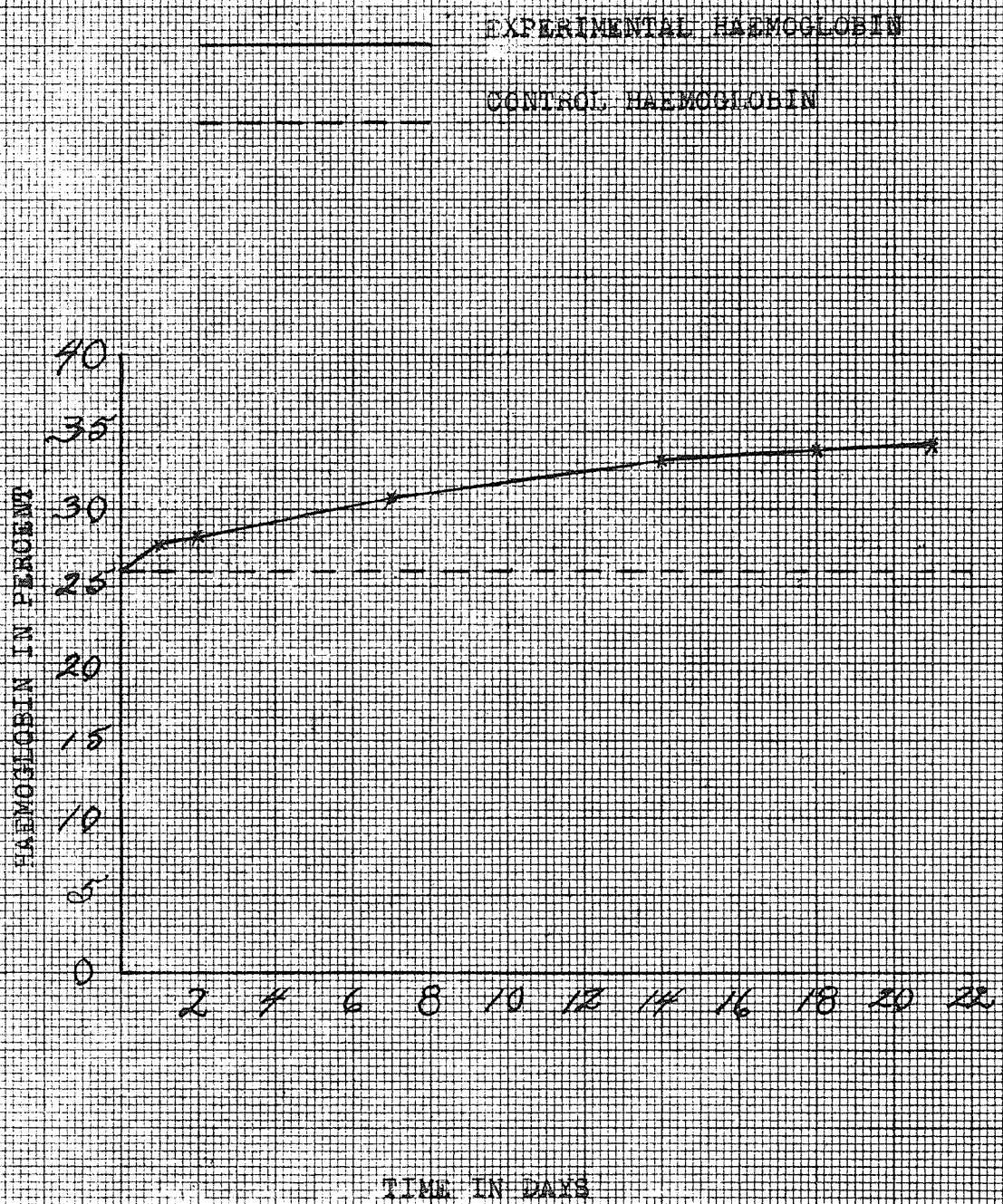
GRAPH 1

RESPONSE OF RED BLOOD COUNT TO TEMPERATURE INCREASE.



GRAPH 2

RESPONSE OF HAEMOGLOBIN TO TEMPERATURE INCREASE.



period the temperature increased to 16.5°C. The experimental tank was adjusted in a manner similar to the control tank with regards to aeration and water flow. These fish were fed a straight diet of pre-cooked liver which had been grated and frozen to facilitate the handling. The changes in the red blood cell counts and the haemoglobin readings were tabulated and graphed. Tables III, IV and Graphs 3, 4.

Graphs 5 and 6 show the differences of the red blood cell counts and the haemoglobin readings in response to the stimuli used. The Experiment II readings were consistently higher than the readings of Experiment I. The stabilized readings of Experiment II were .07 in millions per cu. mm. higher in the erythrocyte count and 1.5% higher in the haemoglobin. The stabilized reading was reached in 14 days in Experiment II, four days sooner than Experiment I which took 18 days. This comparison seems to indicate that there was a quicker response to the combined stimuli of temperature and liver-diet than to the stimulus of temperature alone. Since the liver is high in iron content, a rise in the haemoglobin was not surprising. A rise in the red blood cell counts seems to indicate that the liver is an excellent anti-anaemic factor.

TABLE III

THE RESPONSE OF THE BLOOD OF NOTROPIS TO DIETARY CHANGE AND TEMPERATURE INCREASE

Specimen	Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
* 1	30	9.5°	1.29	26.0%	3.77
2	30	9.5°	1.28	25.8%	3.74
3	30	9.5°	1.30	26.1%	3.78
4	30	9.5°	1.28	25.9%	3.75
5	30	9.5°	1.29	26.2%	3.79
1	1	16.5°	1.95	27.9%	4.04
2	1	16.5°	1.98	28.2%	4.08
3	1	16.5°	1.92	28.0%	4.06
4	1	16.5°	1.98	28.4%	4.11
5	1	16.5°	1.97	28.0%	4.06
1	2	16.5°	2.11	29.0%	4.20
2	2	16.5°	2.10	28.9%	4.19
3	2	16.5°	2.09	28.8%	4.17
4	2	16.5°	2.07	28.5%	4.13
5	2	16.5°	2.08	28.6%	4.14
1	7	16.5°	2.60	33.5%	4.85
2	7	16.5°	2.50	33.1%	4.79
3	7	16.5°	2.50	33.0%	4.79
4	7	16.5°	2.60	33.4%	4.84
5	7	16.5°	2.62	33.5%	4.85
1	14	16.5°	2.85	35.5%	5.14
2	14	16.5°	2.80	35.5%	5.14
3	14	16.5°	2.80	35.0%	5.07
4	14	16.5°	2.75	34.9%	5.06
5	14	16.5°	2.80	34.9%	5.06
1	21	16.5°	2.82	36.6%	5.16
2	21	16.5°	2.78	35.5%	5.14
3	21	16.5°	2.82	35.6%	5.16
4	21	16.5°	2.80	35.5%	5.14
5	21	16.5°	2.76	35.4%	5.13

* The control readings.

TABLE IV

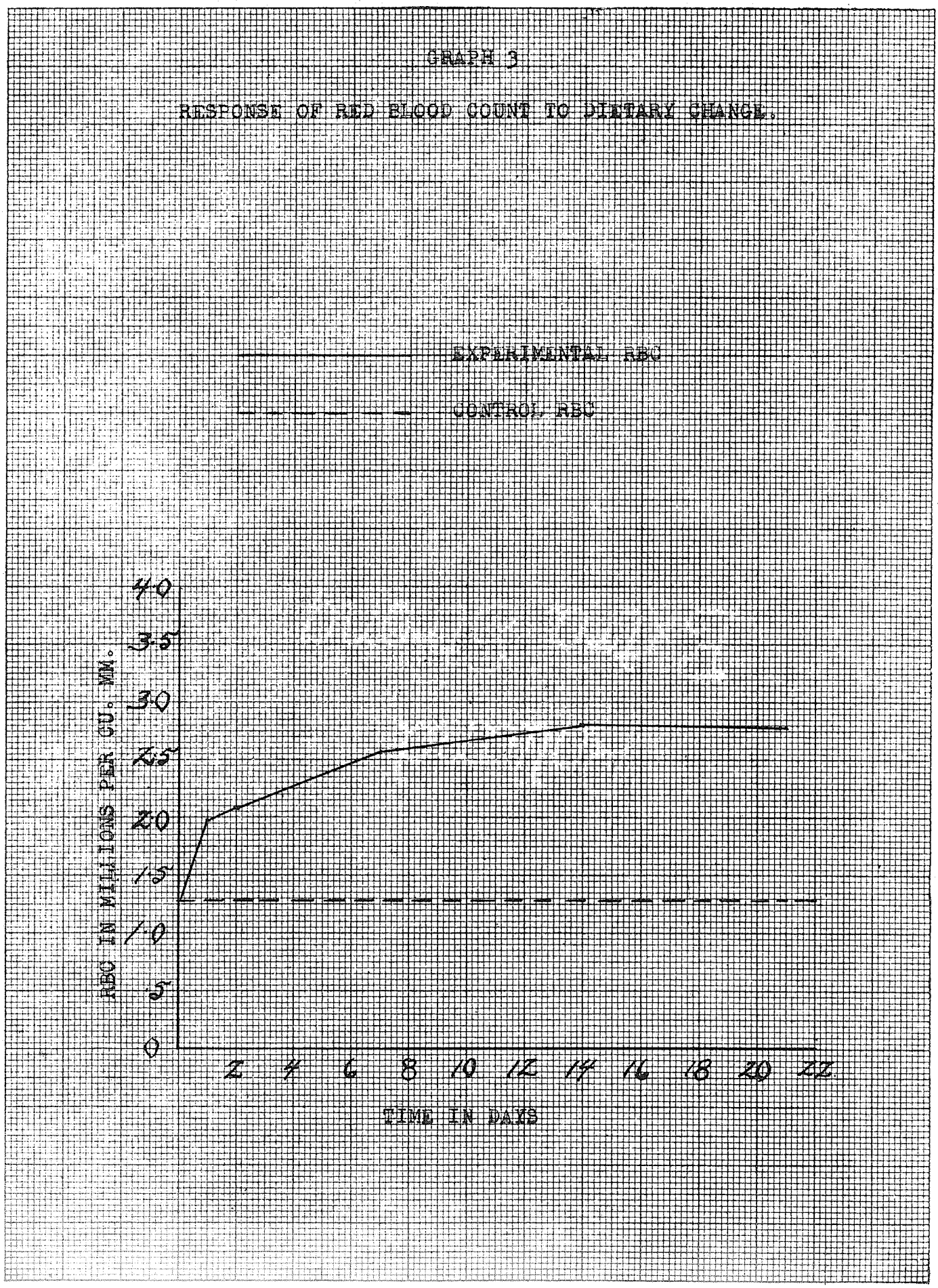
THE RESPONSE OF THE BLOOD OF NOTROPIS TO DIETARY CHANGE AND TEMPERATURE INCREASE

Specimen Total	Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
* 5	30	9.5°	1.28	26.0%	3.76
5	1	16.5°	1.96	28.1%	4.07
5	2	16.5°	2.09	28.7%	4.16
5	7	16.5°	2.56	33.3%	4.82
5	14	16.5°	2.80	35.1%	5.09
5	21	16.5°	2.79	35.4%	5.14

* The control readings.

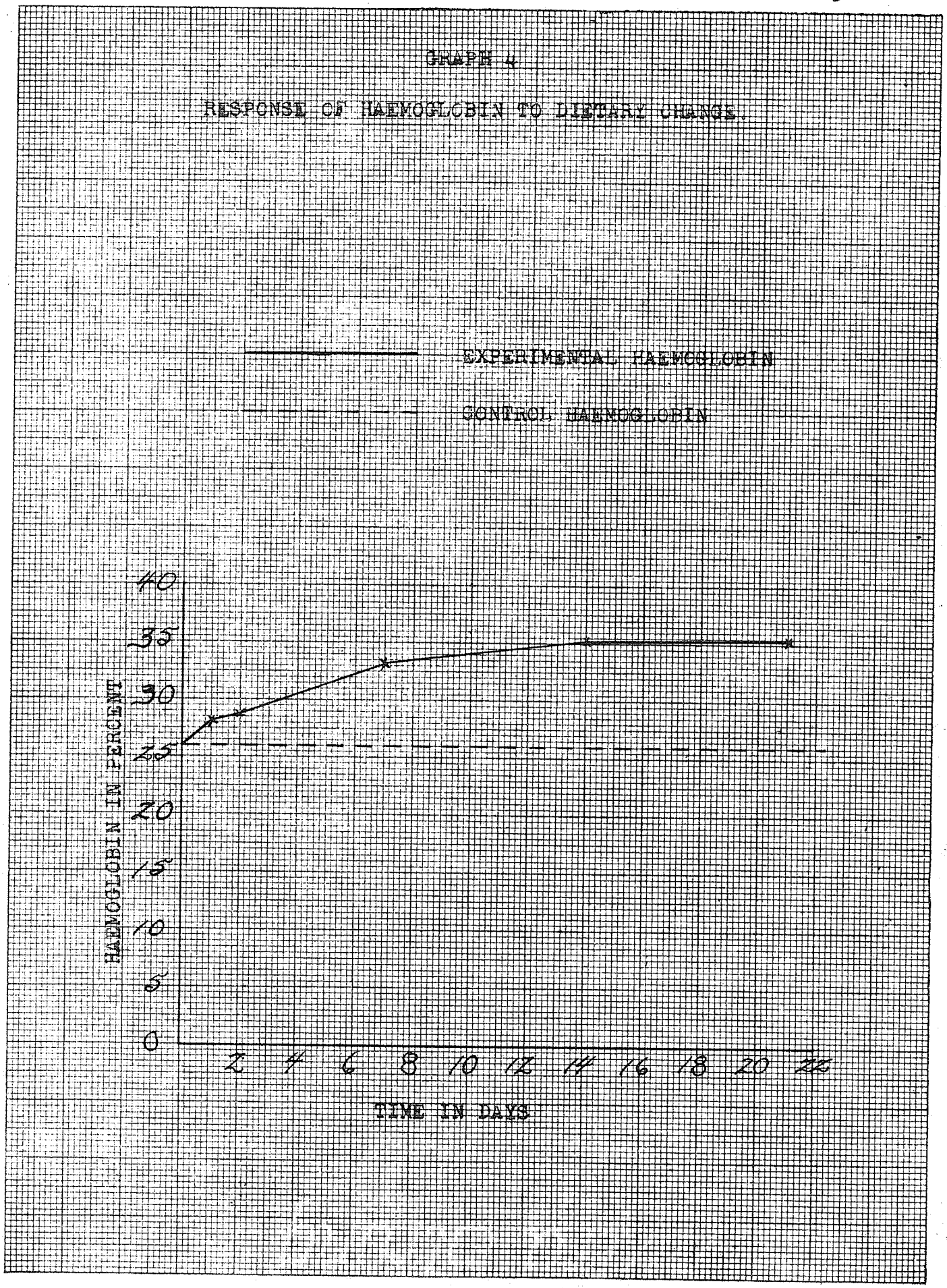
GRAPH 3

RESPONSE OF RED BLOOD COUNT TO DIETARY CHANGE.



GRAPH 4

RESPONSE OF HAEMOGLOBIN TO DIETARY CHANGE.

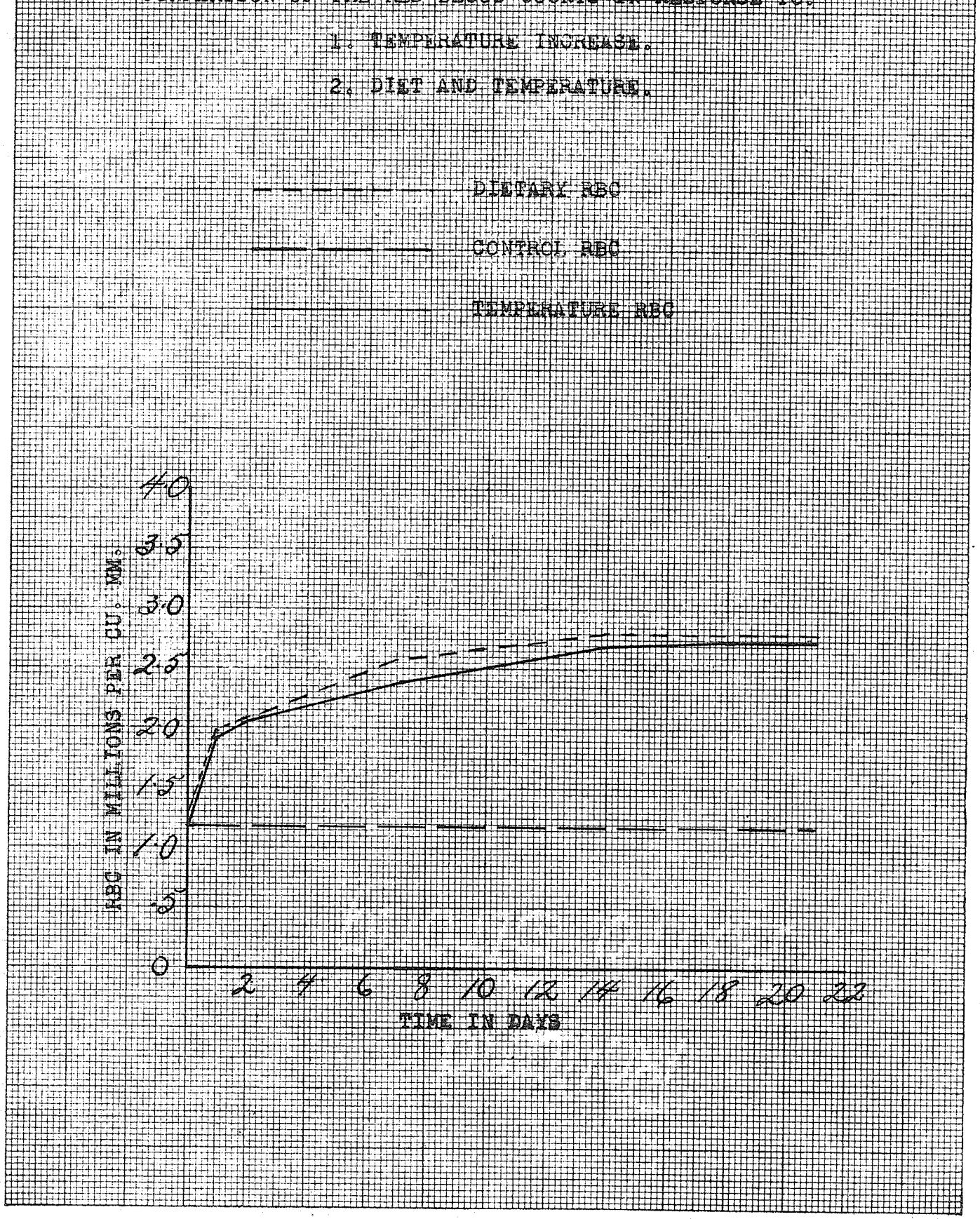


GRAPH 5

COMPARISON OF THE RED BLOOD COUNTS IN RESPONSE TO:

1. TEMPERATURE INCREASE.

2. DIET AND TEMPERATURE.



GRAPH 6

COMPARISON OF THE HAEMOGLOBIN IN RESPONSE TO:

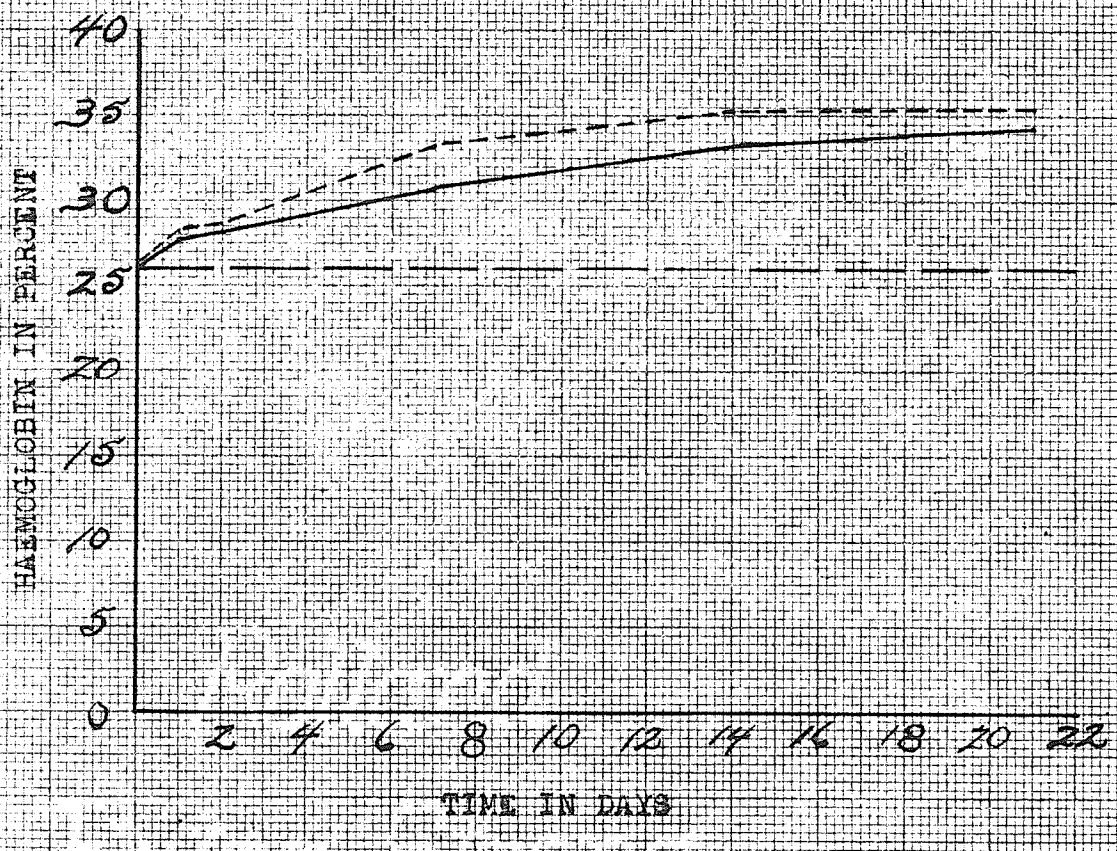
1. TEMPERATURE INCREASE.

2. DIET AND TEMPERATURE.

———— TEMPERATURE HAEMOGLOBIN

– – – – – – – – – – DIETARY HAEMOGLOBIN

———— ———— ———— CONTROL HAEMOGLOBIN



C. EXPERIMENT III - THE EFFECTS OF VITAMIN B_{12}

Thirty-five minnows of approximately 3.8 to 4 grams in weight were transferred from a control tank at 15°C. to a test aquarium under similar conditions of water change, aeration and temperature. The fish had been fed the food outlined in Chapter II, Feeding. The control fish haemoglobin and erythrocyte counts were tabulated. The fish in the experimental tank were fed the food outlined in Chapter II, Feeding, with the addition of a one and half capsules of vitamin B_{12} mixed in the food.

Pulvules-Trinsicon - Eli Lilly and Company.

Each Pulvules contained:

Vitamin B_{12} with Intrinsic factor Concentrate, C.S.D.	½ unit
Vitamin B_{12} (activity equivalent)	7.5 mcg.
Special Liver - Stomach concentrate, Lilly	150 mg.
Ferrous sulfate, Anhydrous	300 mg.
Ascorbic acid (Vitamin C)	75 mg.
Folic acid	1 mg.

This experiment was continued for 14 days, five minnows were used for each determination of the red blood cell counts and the haemoglobin readings. The behaviour of these experimental fish was outlined in Chapter II, Behaviour.

Graphs 7 and 8 show the rapid rise in the haemoglobin

TABLE V

THE RESPONSE OF THE BLOOD OF NOTROPIS TO VITAMIN B_{12}

Specimen		Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
*	1	30	15°	2.66	30.8%	4.46
	2	30	15°	2.67	30.7%	4.45
	3	30	15°	2.66	30.8%	4.46
	4	30	15°	2.68	30.2%	4.52
	5	30	15°	2.68	31.1%	4.50
	1	1	15°	3.06	31.1%	4.50
	2	1	15°	3.04	31.4%	4.55
	3	1	15°	3.08	31.3%	4.53
	4	1	15°	3.06	31.2%	4.52
	5	1	15°	3.04	30.9%	4.48
	1	2	15°	3.62	31.9%	4.62
	2	2	15°	3.59	31.6%	4.58
	3	2	15°	3.60	31.8%	4.61
	4	2	15°	3.58	31.6%	4.58
	5	2	15°	3.56	31.5%	4.56
	1	3	15°	3.66	32.0%	4.64
	2	3	15°	3.68	32.1%	4.65
	3	3	15°	3.65	31.9%	4.62
	4	3	15°	3.68	32.1%	4.65
	5	3	15°	5.10	33.8%	4.90
	1	8	15°	4.84	33.4%	4.84
	2	8	15°	4.96	33.2%	4.81
	3	8	15°	4.82	33.2%	4.81
	4	8	15°	4.82	33.2%	4.81
	5	8	15°	4.88	33.4%	4.84
	1	12	15°	3.92	33.2%	4.81
	2	12	15°	3.88	33.0%	4.78
	3	12	15°	3.92	33.2%	4.81
	4	12	15°	3.96	33.4%	4.84
	5	12	15°	3.88	33.0%	4.78
	1	14	15°	3.96	33.1%	4.79
	2	14	15°	3.88	32.8%	4.75
	3	14	15°	3.94	33.0%	4.78
	4	14	15°	3.88	32.7%	4.74
	5	14	15°	3.94	32.9%	4.77

* The control readings.

TABLE VI

THE RESPONSE OF THE BLOOD OF NOTROPIS TO VITAMIN B_{12}

THE AVERAGE OF THE READINGS OF TABLE V USED FOR GRAPHING

Specimen Total	Time (days)	Temp. (°C.)	RBC in millions per cu. mm.	Haemoglobin Percentage	Haemoglobin Grams
* 5	30	15°	2.67	30.9%	4.47
5	1	15°	3.05	31.1%	4.52
5	2	15°	3.59	31.6%	4.59
5	3	15°	3.66	32.0%	4.62
5	6	15°	5.03	33.6%	4.87
5	8	15°	4.86	33.2%	4.82
5	12	15°	3.91	33.1%	4.80
5	14	15°	3.90	32.9%	4.75

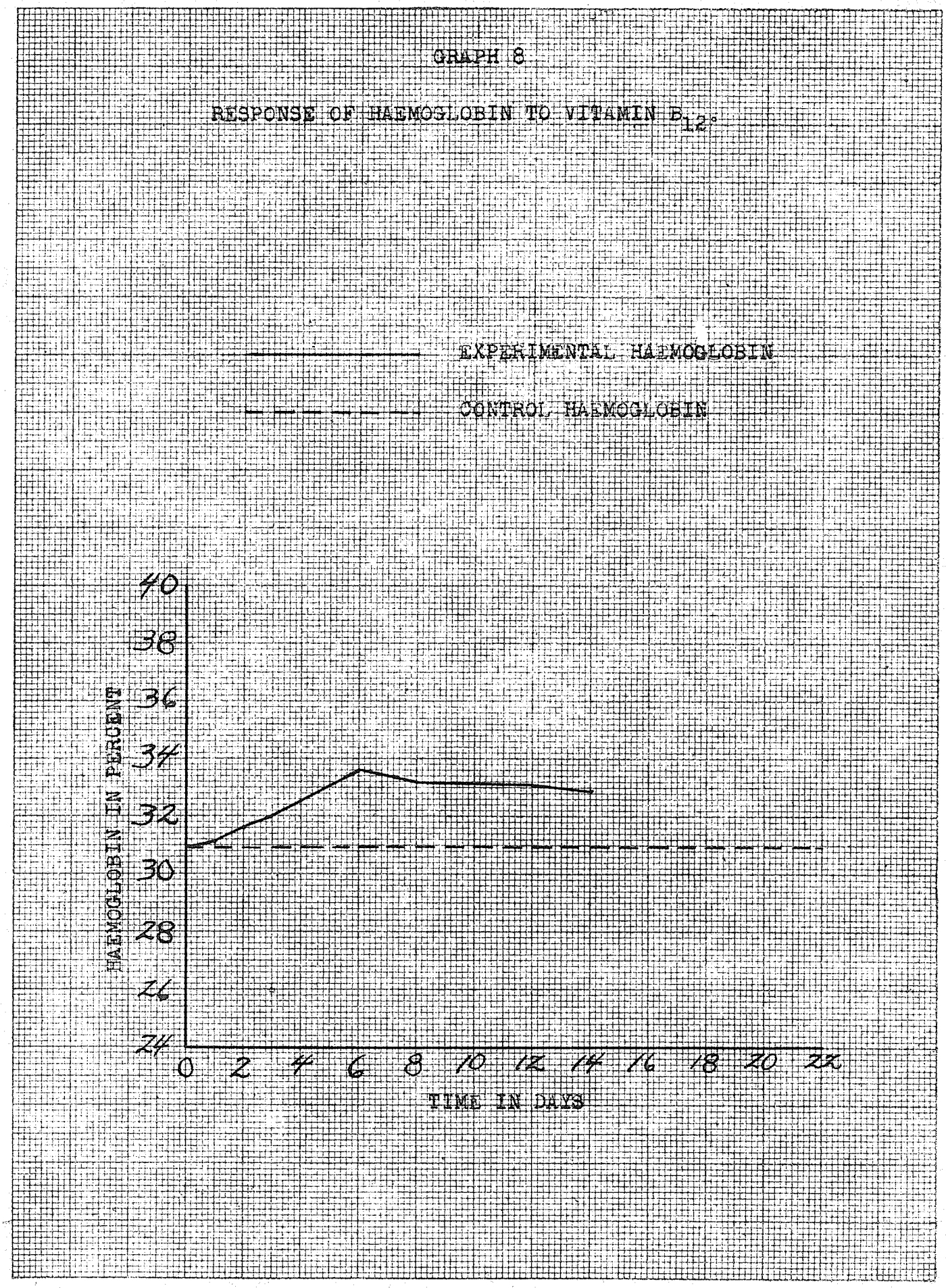
* The control readings.

GRAPH 7

RESPONSE OF RED BLOOD COUNT TO VITAMIN B_{12}.

GRAPH 8

RESPONSE OF HAEMOGLOBIN TO VITAMIN B_{12}.



readings and erythrocyte counts to a peak within six days. The curves were somewhat characteristic of growth curves with the control fish readings as an initial period, the six day gradual rise as the period of exponential multiplication, followed by a short period of stationary readings and then a slight drop to a new stationary reading.

The increases in the erythrocyte counts and the haemoglobin readings indicated that the vitamin B_{12} had an immediate effect, which reached its peak in 6 days and then declined to a lower stabilized rate within 12 to 14 days. Tables V and VI.

The sections of the experimental vitamin B_{12} fish showed extensive areas of haematopoietic activity. The experimental readings and histological sections seem to indicate that the rapid rise and slight decline to a stabilized rate were due to the initial strength of the vitamin B_{12} and to the lack of its components in the fish diet.

D. EFFECTS OF EXPERIMENTS I, II, and III ON THE WHITE BLOOD CELL COUNTS OF THE MINNOWS

The minnows which had been acclimatized to different temperatures but the same conditions of food and aeration for at least a month, showed a fairly stable count. Table VII. These counts were similar to the counts of the control fish at 15°C. referred to in Chapter V, Differential Blood Counts

TABLE VII

WHITE BLOOD CELL COUNTS OF NOTROPIS UNDER EXPERIMENTAL CONDITIONS

Time (days)	Temp. (°C.)	Diet	Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
30	9°	fish food	4%	5%	0%	67%	24%
30	10°	fish food	4%	6%	0%	64%	26%
30	15°	fish food	6%	6%	0%	64%	24%
30	16.5°	fish food	5%	6%	0%	65%	24%
7	16.5°	Exp. I fish food	6%	6%	0%	71%	17%
14	16.5°	Exp. I fish food	4%	5%	0%	73%	28%
21	16.5°	Exp. I fish food	5%	6%	0%	65%	24%
7	16.5°	liver-fed	4%	7%	0%	74%	15%
14	16.5°	liver-fed	5%	6%	0%	75%	14%
21	16.5°	liver-fed	4%	6%	0%	66%	24%
2	15°	B_{12} fish food	6%	10%	0%	54%	30%
6	15°	B_{12} fish food	4%	6%	0%	70%	20%
14	15°	B_{12} fish food	4%	5%	0%	64%	25%

for the Minnows. A control range for the white blood cell count was worked out from these 30 day readings. A reading in this range indicated that the minnows had acclimatized themselves to their new environment or adapted to their stimulus. The control range was made using the highest and lowest for the 30 day readings. Table VII.

Neutrophils	4 - 6%
Eosinophils	5 - 6%
Basophils	0%
Lymphocytes	64 - 67%
Monocytes	24 - 26%

In Experiment I, with sudden temperature increase, the white blood counts changed gradually. The lymphocyte counts increased, the monocyte counts decreased and the other cells remained in the control range. In a three week period the counts returned to the control range. This indicated that a three week period was necessary for the acclimatization of the Experiment I minnows, to the temperature increase.

The liver-fed minnows were under two stimuli, temperature and diet. Their lymphocyte counts were higher, and their monocyte counts lower than that of the fish in Experiment I. Again as in Experiment I the counts of these liver-fed fish returned to the control range within a three week period.

This indicated that the response to temperature change

alone, or a combination of temperature and liver diet still required a three week acclimatization period before the white cell counts were within the control range.

The minnows kept at a constant temperature with the supplement diet of vitamin B_{12} exhibited a varied white blood cell count. In a two day interval, the lymphocyte counts dropped, the monocyte and eosinophil counts increased slightly out of the control range. Within six days, the lymphocyte counts increased, the monocyte counts decreased, the eosinophil and neutrophil counts were in the control range. By the fourteenth day the white blood cell counts were all within the control range.

The white blood cells increased in response to experimental conditions of temperature and diet. The slides showing the increased lymphocyte count also showed the majority of the lymphocytes to be small cells. In the control fish slides, the majority of the lymphocytes were large mature cells. The blood smears of the control fish and experimental fish consisted of; nucleated erythrocytes, small and medium sized lymphocytes, monocytes, thrombocytes, coarse and fine granulocytes. Immature and senile erythrocytes were commonly seen. Slides were characterized by the presence of many more disintegrating cells and more cellular debris than are usually seen in films of mammalian blood. Most of this fragmentary material appeared to be derived from the breaking down of

erythrocytes and coarse granulocytes. Isolated disintegrating nuclei of erythrocytes, sometimes with part of the cytoplasm still attached, were scattered widely over the preparations. Figure VII to XIV.

In this chapter, on the response of the Notropis to experimental conditions, the tables and graphs indicate that the blood cells reacted to the stimuli within a twenty-four hour period and adapted to these stimuli within fourteen to twenty-one days. All the blood cells increased in response to the experimental stimuli used.

CHAPTER VII

THE HAEMATOPOIETIC ORGANS OF THE GENUS NOTROPIS

A series of sections and smears of the internal organs and tissues of the genus Notropis were made. The fixation, preparation and staining are outlined in Chapter IV, Methods and Techniques. Microscopic examination of all the sections and smears showed that the spleen, kidney, intestine, and the liver contained haematopoietic tissue in varying amounts. The sections and smears made of intestinal mesenterys, the air bladder, gonads and the tissues around the heart and gill region exhibited no haematopoietic areas, although mature red and white blood cells were found.

The haematopoietic organs of the control and experimental fish were the same, only the extent of the haematopoietic area varied. The experimental fish sections were made at the peak of red and white cell production. Refer to Chapter VI. They exhibited large areas of haematopoiesis while the control fish sections exhibited much smaller areas.

Each haematopoietic organ will be dealt with individually. The changes, if any, in the gross anatomy of the organs during the experimental conditions, are noted.

The chief haematopoietic sites in the minnows were

the kidney and spleen. The lesser sites were the intestinal mucosa and submucosa and the liver. The presence of haematopoietic tissue in the kidney and spleen may be detected macroscopically by the intense red color of the organs.

A. Spleen

The spleen in the control fish was usually small and tended toward a triangular shape. In the experimental fish, the spleen assumed an elongate sausage-like shape. These spleens were approximately twice the size of the control fish spleens.

The cytologic splenic smears of the control fish showed an abundance of macrophage cells, mature erythrocytes, reticulocytes, erythroblasts, thrombocytes, lymphocytes of various sizes, reticular cells and pulp cells.

The splenic sections of the experimental fish showed an increase in the erythroblasts, reticulocytes, lymphocytes, and thrombocytes and a decrease in the pulp and reticular cells.

The splenic capsule was extremely thin. There were no muscle cells or trabeculae observed. The splenic pulp consisted of a spongy cellular reticulum with no clear distinction between the red and white pulp. Erythroblasts with small dark nuclei were scattered throughout the pulp. Mature and disintegrating erythrocytes and lymphocytes were

evident. Macrophage cells, with ingested blood cells and pigment granules, were scattered throughout the section. Lymphocytopoiesis was evident in scattered areas. Mitotic and amitotic figures were observed. The reticular cells, pulp cells and occasional monocytes were noted.

In the experimental fish spleens, the complete spleens appear to be engorged with blood cells. The sections showed the spleen as one large area of erythrocytopoiesis with smaller areas of lymphocytopoiesis interspersed with macrophage cells, reticular and pulp cells. Figures XVI to XX.

B. Kidney

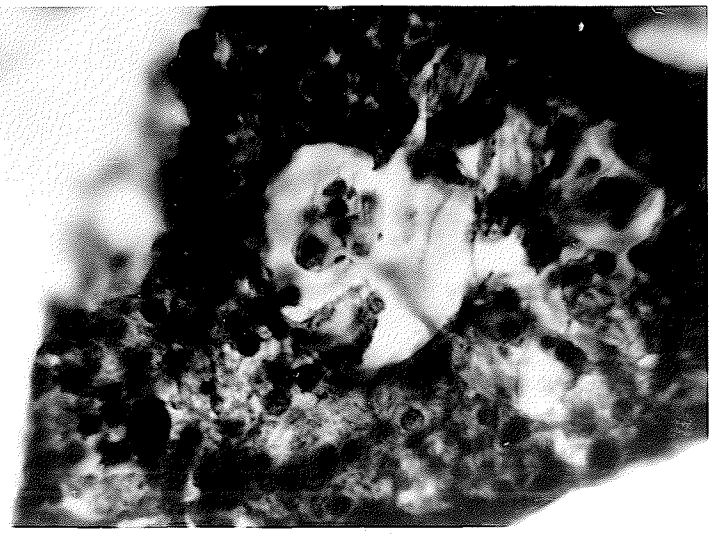
The kidneys of the minnows are long paired organs which are applied to the abdominal cavity on either side of the backbone. They are made up of two regions; the pronephros and mesonephros.

The haematopoietic tissues were found in the inter-tubular areas of the mesonephros. Figure XXII. The capsule or the head of the pronephros was mainly an erythrocyte storage area. Figure XXIII.

Microscopic examination revealed that the haematopoietic activity was concentrated in the renal intertubular tissue of the mesonephros. A capillary network surrounds each kidney tubule, these capillaries were recognized by the epithelial lining cells and the presence of blood cells within them. Reticular cells seem to form close meshes round the developing blood cells,

Figure XVI... Section of spleen showing erythroblasts... ... X 1200.

Figure XVI... Section of spleen showing erythropoietic tissue... X 1200.



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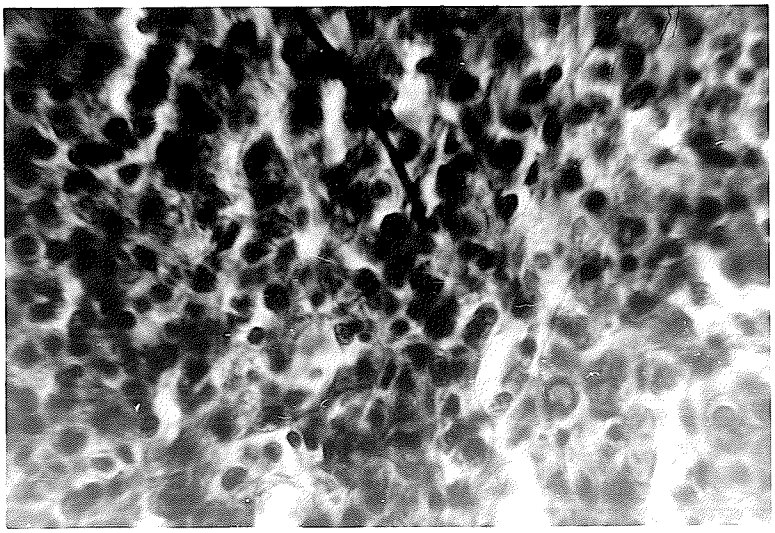

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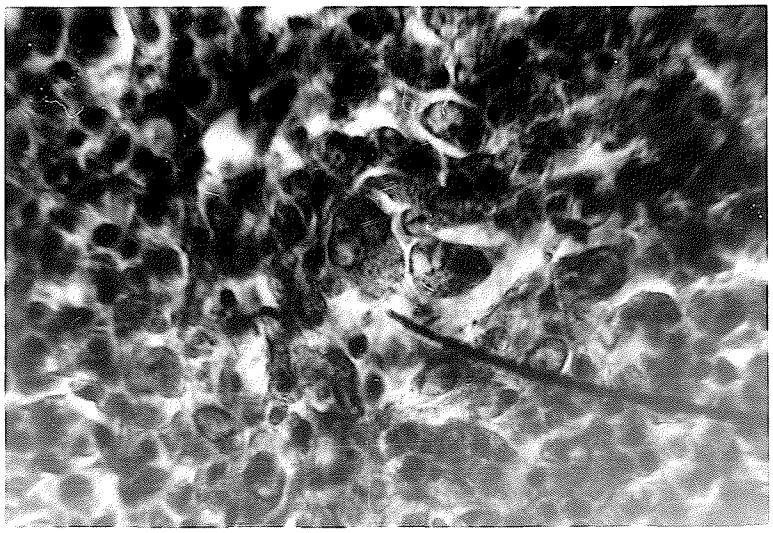
Figure XVIII...Section of the spleen. Note the hematopoietic tissue... X 1200.

Figures XIX...Section of the spleen. Note the small lymphoid center... X 1200.



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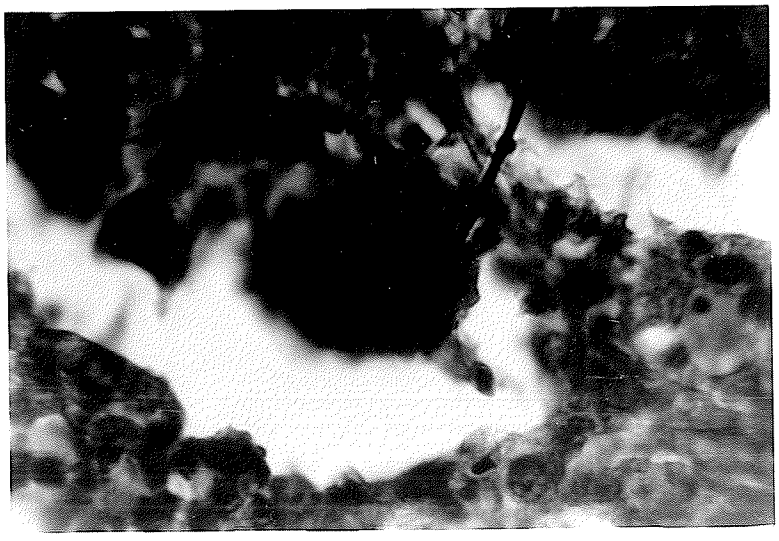

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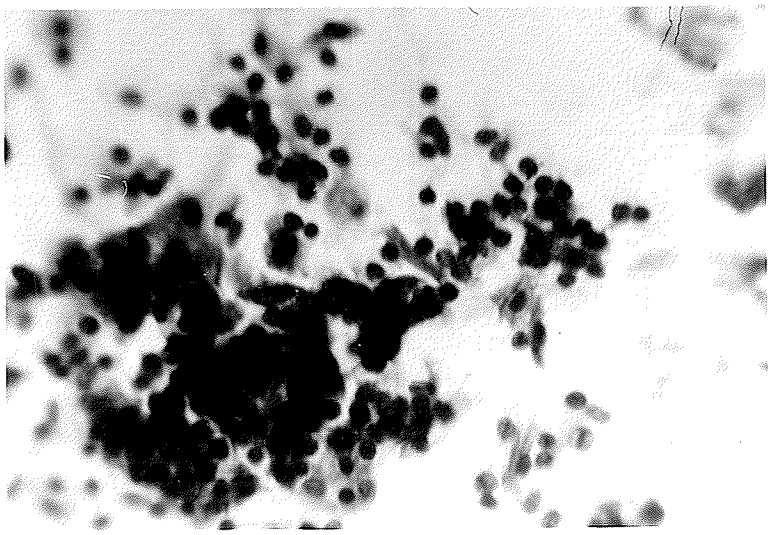
Figure XX...Section of the spleen. Note the erythropoietic tissue... X 1200.

Figure XXI...Section of the villi of the intestine. Note the eosinophils... X 1200.



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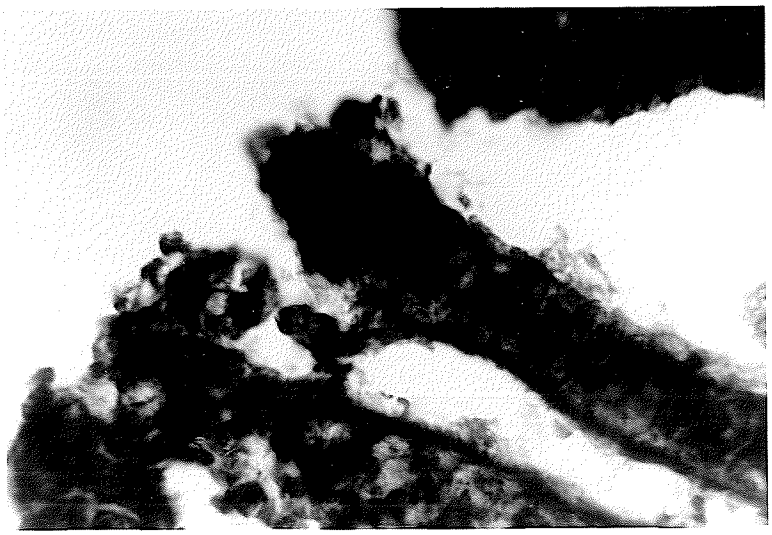

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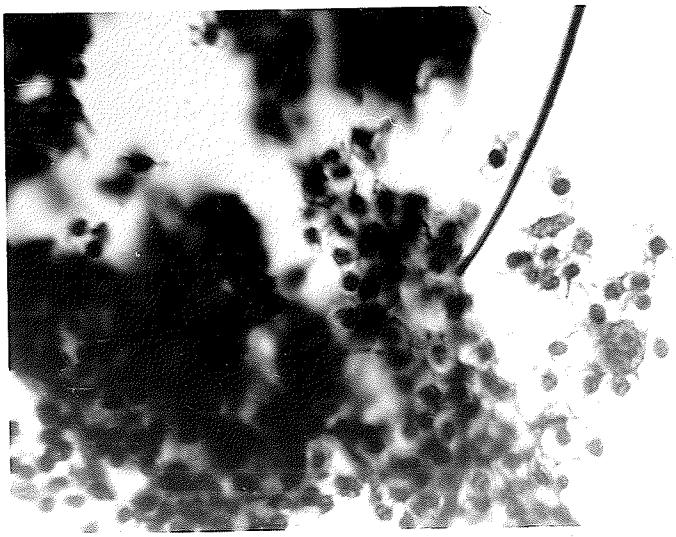
Figure XXII... Section of the kidney. Note the developing erythrocytes... X 1200.

Figure XXIII... Section of the pronephros (of the kidney). Note the storage of mature erythrocytes... X 1200.



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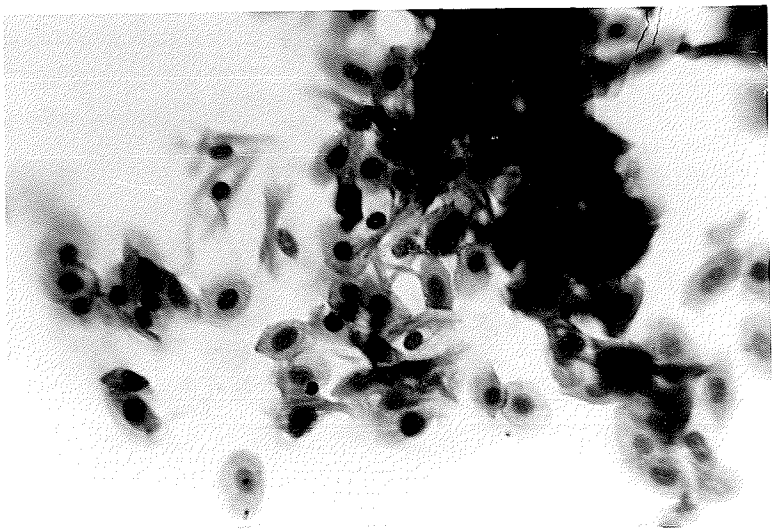

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with a "sponge work" of reticular cells extending between the peri-tubular capillary nets forming the supporting frame of haematopoietic tissue. The cords and sinuses of mesonephric myeloid tissue contained innumerable transition stages between lymphoid hemoblasts and definitive cells.

The segregation of the precursor cells of erythrocytes and granulocytes was not evident. They all seem to intermingle with each other quite closely. Erythroblasts and hemoblasts were seen in various developing stages. Figures XXIV and XXV. The between stage of erythrocyte development, the pro-erythroblast cannot be distinguished, only the erythroblasts and the reticulocytes were recognized. Mitosis were frequently seen in the erythrocytes and amitosis in the lymphocytes. It was difficult to distinguish blood lymphocytes and small lymphoid hemoblasts in the sections and smears of the kidney.

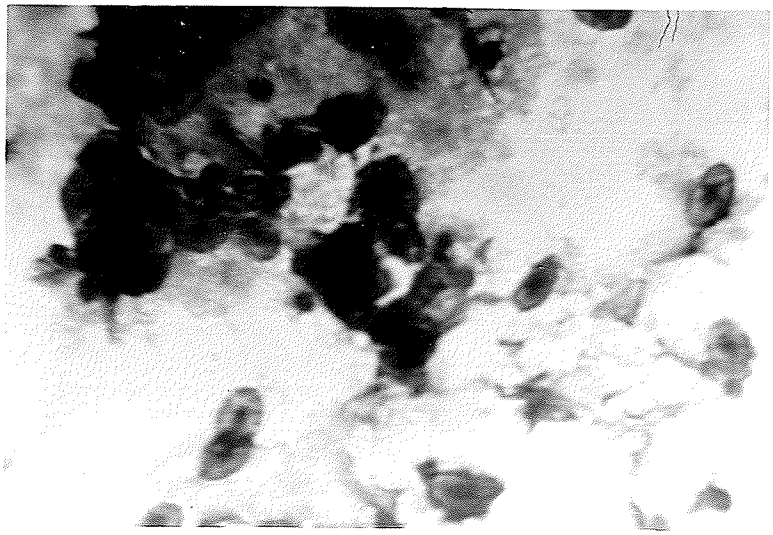
In the experimental fish, all the above mentioned cells were found in greater numbers. Among these, haemoblasts, lymphocytes, erythrocytes, monocytes and occasional neutrophils were noted in the shape usually found in the circulating blood. The haematopoietic area was notably increased under all of the experimental stimuli.

C. Liver

The multi-lobed liver was found as another site of

Figure XXIV...Section of the kidney. Note the developing erythrocytes... X 1200.

Figure XXV...Section of the intertubular hematopoietic tissue of the kidney. Note the lymphoid hemoblasts, erythroblasts and pigment granules... X 1200.



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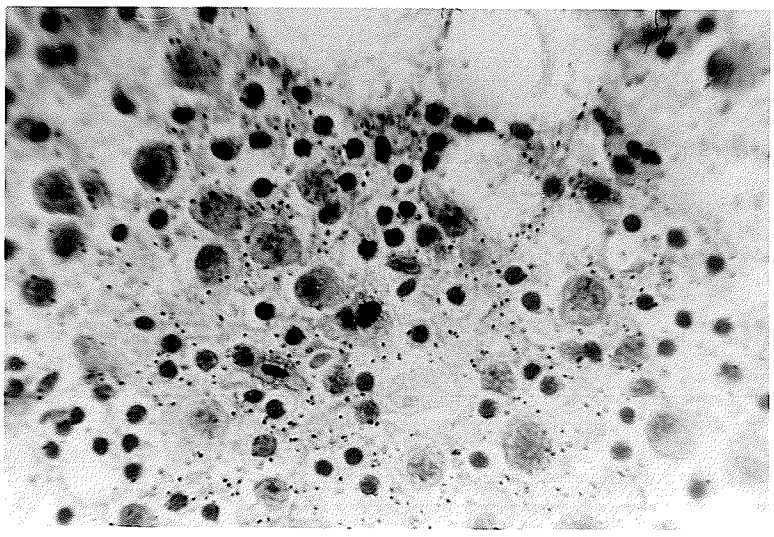

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blood cell production. Normally, the liver in the genus Notropis showed only traces of blood cell production, but in the experimental fish, erythrocytopoiesis and lymphocytopoiesis were much more evident. Clumps of erythroblasts and maturing erythrocytes were found amid the hepatic sinusoids. Figures XXVI and XXVII.

D. Intestines

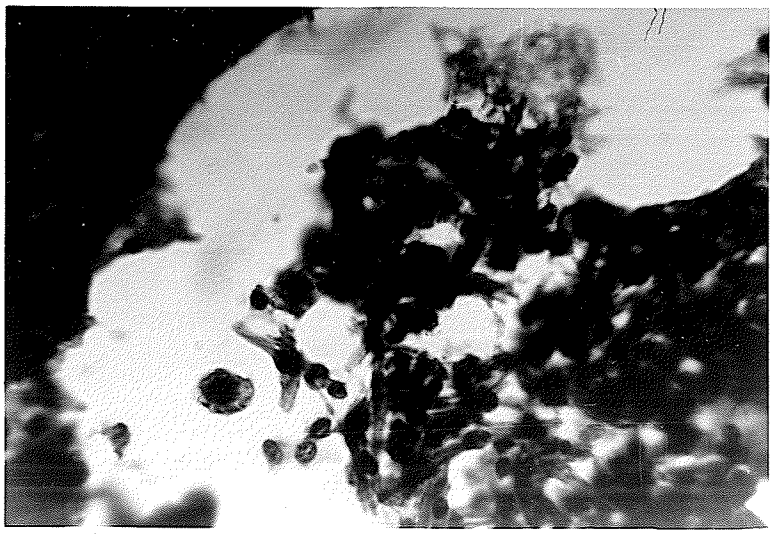
The eosinophils were found in the mucosa and submucosa of the intestine. They developed in the mucosa and submucosa connective tissue and were extruded into the blood stream or into the lumen of the intestine. Figure XXI, page 61.

The haematopoietic system of the genus Notropis was found to be quite distinct. The four organs described in detail were the sites of the haematopoietic activity. Table VIII.

The sections of the organs of experimental fish exhibited the same sites of blood production but with more extensive areas of haematopoiesis. Numerous smears and sections made of the intestinal mesentery, the air bladder, the gonads and the tissue around the gill and heart region showed no evidence of haematopoietic activity.

Figure XXVI...Section of the liver. Note the developing erythroblasts...X 1200.

Figure XXVII...Section of the liver. Note the erythroblasts...X 1200.



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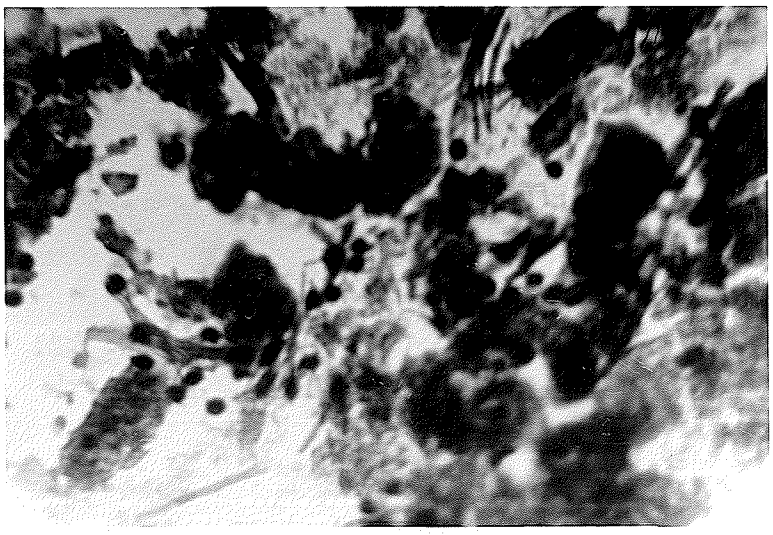

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TABLE VIII

LOCI OF BLOOD-FORMING ACTIVITY IN THE GENUS NOTROPIS

Genus	Erythro-cytopoiesis	Granulo-cytopoiesis	Lympho-cytopoiesis	Thrombo-cytopoiesis
Notropis	Spleen Mesonephros (of the Kidney) Liver	Mesonephros (of the Kidney) Intestine	Spleen Mesonephros (of the Kidney) Intestine	Spleen Mesonephros (of the Kidney) Circulation

E. Blood Cell Formation

Although there are no a priori grounds for denying that the blood stem cells may be of a different nature in fishes and mammals, representing extremes of the vertebrate scale, yet the commonly accepted view that the vertebrates form a compact phylogenetic group appears to render such a conception open to controversy, Catton.[2]

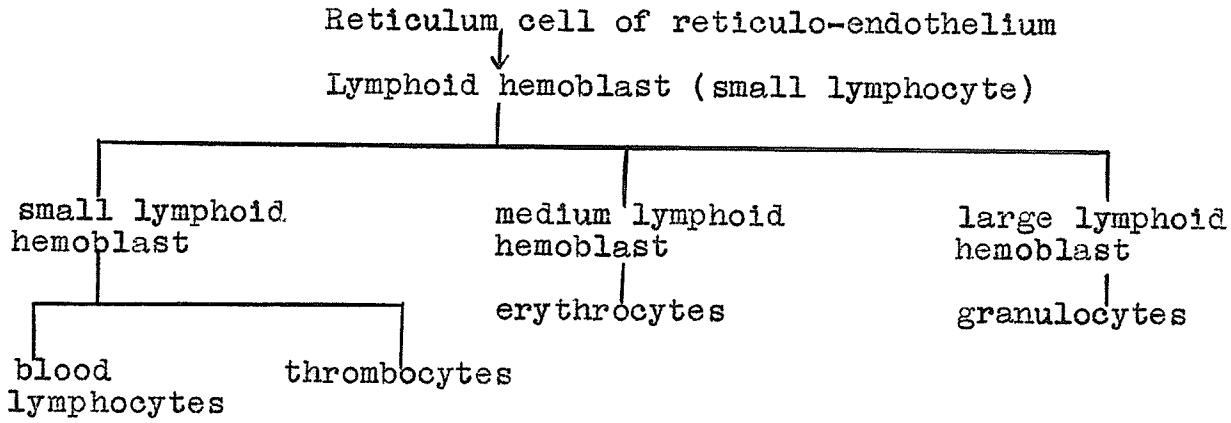
Downey,[5] Maximow,[17] Jordan and Speidel,[13] Duthie[6] and Yoffey[27] all adopted a monophyletic view on the origin of the fish blood cells. Although they did not agree on the subsequent lines of development, they did share the view that the parent cell was a "large lymphoid hemoblast," Table IX, (1) and (2). The monophyletic school assumes that there is only one type of stem cell; which is inherently pluripotential. The subsequent course of its development is dependent on the environment in which it arises. This was the first hypothesis of blood cell formation.

In the second hypothesis; the large lymphoid hemoblast, derived by transformation of reticular cells, is the sole precursor of the granulocytes. The small lymphoid hemoblast is to be derived from the endothelial cells and is the precursor of the erythrocytes and thrombocytes. In this case the large cell is to be compared with the "primitive white cell" of Doan, Cunningham and Sabin[4] and the small cell with the "megaloblast" of the same authors. On this theory the

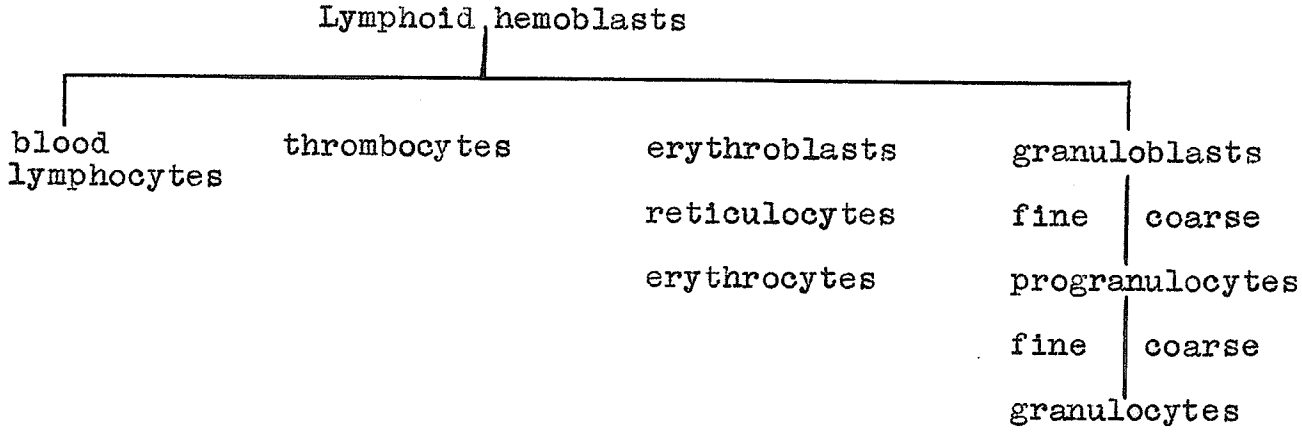
TABLE IX

PROPOSED SCHEMES OF THE BLOOD CELL FORMATION

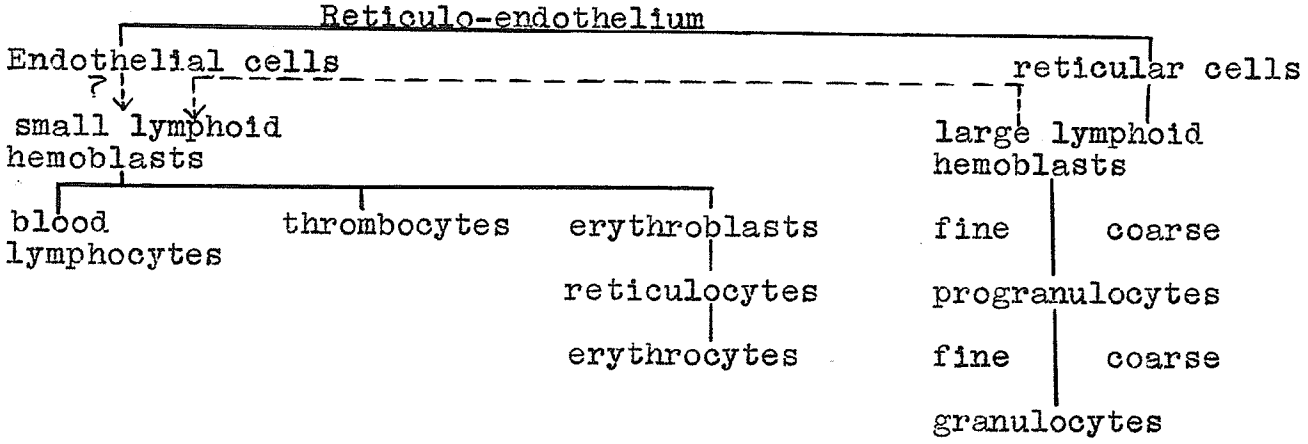
(1) Jordan and Speidel[14]



(2) Duthie[6]



(3) Catton[2]



erythrocyte would have an "intravascular" origin and the leukocyte an "extravascular origin." Such a theory is included in the general term "polyphyletic." No evidence, however, is available of the derivation of small hemoblasts from endothelial cells.

In the genus Notropis the renal intertubular tissue offered no evidence of formative pockets limited to one type of cell. The developing cells appeared to be closely intermingled, precursor cells of both the erythrocytes and granular leukocytic series lying along side each other. Small and large lymphoid hemoblasts were recognized. The large lymphoid hemoblasts gave rise to granulocytes directly. All other cells (including lymphocytes) arose from the small lymphoid hemoblast which appeared indirectly as the stem cell for erythrocytes, lymphocytes and thrombocytes. Therefore, this small hemoblast cannot be homologized with the hemocytoblast of the monophyletic school. It approaches more closely the neo-unitarian hypothesis.

The neo-unitarian school distinguishes two types of stem cells, the myeloblast and the precursor cell of the lymphocyte. Each cell under normal conditions has its own limited potentiality of development, the environment having no particular significance, Table IX (3), page 69.

The mode of blood cell formation in the genus Notropis approaches the proposed scheme of Catton[2] and follows closely the neo-unitarian hypothesis.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

1. The differential blood counts, the red blood cell counts and the haemoglobin were all determined for the genus Notropis at the acclimatized temperature of 15°C.

2. The reactions of the blood to sodium citrate, heparin, calcium chloride and thrombin indicated that the blood clotting mechanism of the genus Notropis were similar to that of higher mammals.

3. The tables and graphs of Experiment I, (Tables I, II, Graphs 1, 2, pages 35 to 38 inclusive) indicated that increases in the erythrocyte counts and the haemoglobin were directly proportional to the increases in temperature.

4. The tables and graphs of Experiment II, (Tables III, IV, Graphs 3 to 6, pages 40 to 45 inclusive) indicated a direct response in the red blood cells and the haemoglobin to the temperature increase. The additional stimulus of a liver diet increased the erythrocyte counts by .07 in millions per cu. mm. and the haemoglobin by 1.5%.

5. The Experiment III vitamin B_{12} diet exerted its greatest effect in six days as illustrated by Graphs 7 and 8, pages 49 and 50. The concentration of the vitamin B_{12} and the lack of its components in the fish diet accounted for the initial increase. The slight decline to the stabilized reading was observed as the fish adapted to the vitamin B_{12} supplement.

6. The minnows of Experiments I, II and III reacted to their stimuli within a twenty-four hour period. The final stabilized readings of the erythrocyte counts and haemoglobin were considerably higher than the control readings in all three experiments.

7. The white blood cell counts increased in response to the stimuli in Experiment I, II and III. Table VII, page 52.

8. The white blood cell counts returned to the control range in all three experiments within 14 to 21 days. Table VII, page 52.

9. A. The kidneys and the spleen were the main haematopoietic centers. Erythrocytopoiesis, lymphocytopoiesis, thrombocytopoiesis and a little granulocytopoiesis were found in the haematopoietic tissues of these organs.

B. The liver and the intestinal mucosa and submucosa were found to be lesser sites of haematopoiesis. Erythrocytopoiesis and lymphocytopoiesis observed in the liver and granulocytopoiesis in the intestine.

C. The sections of the experimental fish revealed the same sites of haematopoietic tissue. These sites were more extensive and more active as a result of the experimental stimuli. Table VIII, page 67.

10. The mode of blood cell formation in the genus Notropis approached the neo-unitarian hypothesis. Table IX (3), page 69.

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