A STUDY OF SOME HAEMATOLOGICAL CHANGES IN THE GOLDFISH (*Carassius auratus*) FOLLOWING THERMAL ACCLIMATION AND NON-LETHAL HEAT SHOCK

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ABSTRACT

The present investigation was undertaken in view of the conflicting reports in literature regarding the general haemotology of fishes, and the relationship of haematological variations to temperature changes and thermal acclimation.

Because it was readily available, easily maintained, and because there exists a considerable body of information concerning its thermal relationships, the goldfish was used throughout as an experimental animal.

In fishes acclimated to 20°C. (control group) and also in fishes transferred from this temperature to 30°C. (experimental group), determinations were made of haematocrit, red cell concentration, blood iron content, and leukocyte concentration. Using the information obtained, mean corpuscular iron content and mean corpuscular volume were calculated. An electrophoretic study of changes in the relative abundancies of haemoglobin polymorphs was also carried out on fishes acclimated to temperatures of 5°, 12°, 20°, and 30°C.

The results of this study indicated that red cell concentration, haematocrit, and mean corpuscular volume remain relatively constant over a 240 hour period following transfer of the animals to 30°C. Under the same circumstances, white cell concentration showed a slight trend towards an increase, while total blood iron and mean corpuscular iron content exhibited a reverse trend of small magnitude.

In fishes acclimated to 5°C. two haemoglobin polymorphs were

apparent: (1) a cathodal fraction (C_1), (2) a anodal fraction of lesser abundance and greater mobility (A_2). In the 12°, 20°, and 30°C. acclimated animals a second anodal fraction appeared (A_1). This fraction was of lesser abundance and lesser mobility than the two previously mentioned fractions.

The data suggest that metabolic compensation to higher temperatures is associated with hormonally-induced changes in branchial blood flow and blood pressure rather than the metabolically more costly process of increased red cell production and haemoglobin synthesis.

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INTRODUCTION

The effect of temperature upon fish has long been the object of numerous investigations. As early as 1895 thermal acclimation was a recognized phenomenon (Davenport and Castle, 1895), but it was not until recent years that a distinct pattern could be ascribed to the various avenues of research. Brett (1959b), in his review of the related literature, classified studies regarding the effect of temperature on fishes into two distinct groups:

(1) those in which temperature acted as a lethal factor;

(2) those in which temperature acted as a controlling factor.

Fry (1947) initiated investigation regarding the effect of temperature as a lethal factor, and observed that temperature acted through metabolism by governing the relative rates of temperature demand. Thus, fishes subjected to increased environmental temperature showed increased oxygen demand and heightened metabolism. Such changes would lead one to expect haematological variations facilitating increased oxygen uptake.

Previous studies regarding the general haematological picture of fishes, although quite numerous, have tended to be restricted in scope. Investigations regarding the effect of temperature upon the blood of fishes are scanty and often contradictory. It is interesting to note that Bondar (1957) and Spoor (1951), in studies on Notropis blennius and Carassius auratus respectively, observed an increase in

erythrocyte number with increased environmental temperature. Contrary to these authors, Anthony (1961) reported that the erythrocytes of the goldfish decreased in number with increased acclimation temperature.

In view of these facts, the present study was undertaken with the aim of: (1) describing the general haematological picture of the goldfish; (2) describing the immediate effects of thermal shock upon the blood, and adaptive responses of the blood to shock; (3) assessing the data obtained as possible criteria for determining rates of acclimation to temperature changes; (4) providing a basis for further haematological studies on the phenomena of thermal shock and acclimation in fishes.

Accordingly, determinations were made of the following: (1) haematocrit, (2) red cell concentration, (3) blood iron content, (4) white cell concentration. From these were calculated: (1) mean corpuscular iron content, (2) mean corpuscular volume. In addition to this, an electrophoretic study of the haemoglobin polymorphs was conducted to determine possible temperature-related changes in this protein.

LITERATURE REVIEW

1. Thermal Relationships of Fishes.

Prior to 1940, work done on the thermal relationships of fishes was of a fragmentary nature and difficult to draw into any generalized pattern (Brett, 1959). Brett (1956), in his review of the literature of that period, noted that upper and lower lethal temperatures were known to exist (Hathaway, 1927; Loeb and Wastenys, 1912), that acclimation was a recognized phenomenon (Davenport and Castle, 1895), and that deviations between the body temperature of the animals and the temperature of their external environment were small and transitory (Pearse and Hall, 1928; Neilson, 1938).

The movement towards a more systematic examination of the temperature relations of fishes was initiated by Doudoroff (1938). In his research on Girella nigricans (Ayres), Doudoroff studied temperature selection and the relationship of temperature selection to previous thermal history. In the same year, Sumner and Doudoroff (1938) carried out experiments on the relationship between oxygen consumption and temperature acclimation in Gillichthys mirabilis.

During the 1940's, much of the work on this general problem was centered around a description of the lethal effects of temperature, and the influence of thermal acclimation upon thermal tolerance. Among such investigations may be included those of Doudoroff (1938, 1942, 1945) on Girella nigricans, Fundulus paripinnis, and Atherinope affinis; of Sumner (1940, 1942), on a variety of species normally inhabiting warm

and cold springs; of Hart (1947), on various species of fish from the Toronto region; of Brett (1941, 1946), on the goldfish, Carassius auratus; and of Fry and his associates (Fry, Brett, and Clawson, 1942; Fry and Hart, 1946; Fry, Hart, and Walker, 1946), on Carassius auratus and Salvelinus fontinalis.

The work of Fry and his associates, referred to above, has been of particular significance in the description of thermal relationships in fishes. These studies, in general, followed the pattern established by Hathaway (1927). Fry, Brett and Clawson (1942), for example, on the basis of intensive studies made on the goldfish, determined upper and lower lethal temperatures (LD_{50}) for this species at a series of acclimation temperatures. Using these data they defined the limits of a thermal polygon. This polygon included all combinations of existing temperatures and acclimation temperatures in which temperature, acting alone, was not lethal to the animals. In later studies these concepts were extended to a variety of species (e.g., Fry, Hart and Walker, 1946).

On the basis of earlier studies, Fry (1947) presented a general review of the physiological effects of environmental variation upon animal activity. This study proved to be of great significance in the determination of lines of endeavour followed by later investigators. In this work a new approach to the organism-environment relationship was introduced, with environmental actions upon the organism being categorized as lethal, controlling, limiting, masking, accessory, and directive in nature. This classification provided clarity to the investigations followed by previous and succeeding researchers. Of prime importance

was the introduction of a tendency to pursue the topics of thermal acclimation and thermal tolerance in terms of metabolic functions and temperature-dependent activities.

Any discussion of the various avenues of research that have been followed would be unnecessarily repetitive as these have been admirably reviewed by several authors (e.g., Brett, 1956, 1959b; Bullock, 1955; Fry, 1958). Of considerably greater significance to the present study are the investigations directly pertaining to the effect of temperature on metabolism.

The extreme diversity of the various metabolic studies that have been carried out prohibits any strict classification of the research, but broad classification is possible under the following headings: (a) respiratory studies; (b) studies on fat metabolism; (c) endocrine studies; (d) studies on tissue metabolism; (e) haematological studies.

a. Respiratory Studies. One of the earlier experiments concerned with the relationship between temperature and respiration in fish was that of Edge and Krogh (1914), who studied temperature and respiratory exchange in fishes. Later research on thermal acclimation as related to oxygen consumption were undertaken by Fry and his associates (Fry, Hart and Walker, 1946; Fry and Hart, 1948) on the effect of environmental temperature and body temperature on oxygen consumption in Carassius auratus, and by Scholander (1953) on the oxygen consumption of tropical and arctic fishes at the temperature of their normal habitats and at various higher and lower experimental emperatures.

Other investigators have studied the effects of various oxygen and carbon dioxide tensions on thermal acclimation in fishes. Representative of this group were Fry (1947), who conducted studies on the relationship between temperature and various tensions of oxygen and carbon dioxide with respect to the asphyxiation of goldfish; Graham (1949), on the effects of oxygen tension and temperature on the metabolism of Salvelinus fontinalis; Downing and Merkins (1957), on the effect of low oxygen and temperature on the survival of several species of fish; and Shepard (1955), on the acclimation of Salvelinus fontinalis to low oxygen tensions. Kanungo and Prosser (1959), in their investigation of the physiological and biochemical adaptations of goldfish to various temperatures, measured the standard and active oxygen consumption of this animal while exposed to different acclimation temperatures.

b. Studies on Fat Metabolism. Another field of investigation has centered around fat metabolism and its association with temperature resistance and thermal acclimation. Of importance in this area of study was the work of Hoar and his associates (Hoar and Dorchester, 1949; Hoar and Cottle, 1952; Irvine, 1954; and Irvine, Newman and Hoar, 1957). Thus, Hoar and Dorchester (1949), and Hoar and Cottle (1952) investigated the relationship between dietary fats and thermal resistance in Carassius auratus. Irvine (1954) conducted research into cold resistance and the effect of various lipid diets on the same species. Irvine, Newman and Hoar (1957) extended these studies with an investigation on the effects of dietary phospholipids and cholesterol on temperature resistance in the goldfish.

c. Endocrine Studies. Endocrinological studies pertaining to thermal resistance and thermal acclimation have been a subject of considerable interest. Of particular note are investigations conducted by Hoar (1946, 1958), Hoar, Keenlyside and Goodall (1957), and Hoar and Robertson (1959) on the goldfish and Pacific salmon. This work was mainly concerned with the influence of photoperiod on thryoidal, gonadal, and adrenocortical activity, and the resultant effect on thermal acclimation and thermal resistance. Similar studies have been pursued by Northcote (1958), and Evans, Purdie and Hickman (1962), on the rainbow trout, Salmo gairdneri.

d. Studies on Tissue Metabolism. Recently, an increasing amount of attention has been devoted to the study of tissue metabolism and its relationship to thermal tolerance and thermal acclimation. Initially, interest was centered upon the respiratory metabolism of excised tissues (e.g., Battle, 1929; Peiss and Field, 1950; Exberg, 1957, 1958), but there has been an ever-increasing trend towards chemical analysis of the tissues, for example, the work of Hoar and Cottle (1952) on the relationship of the melting points of lipids to thermal resistance.

e. Haematological Studies. Studies of the cellular phenomena related to thermal tolerance and thermal acclimation have, to a large extent, been neglected until recent years. Of the relatively few investigations that have been reported, the majority have been confined to analysis of the blood. Spoor (1951) studied the erythrocyte counts of three

groups of goldfish acclimated to different temperatures, and Bondar (1957) conducted similar experiments on the River Shiner, Notropis blennius. Anthony (1956, 1960, 1961) carried out extensive research into the effects of the thermal environment upon the blood of Carassius auratus. The effects of cold shock upon the leucocytes of fishes have been pursued by Slicher and her associates (e.g., Slicher, Pickford and Ball, 1962).

2. Thermal Adaptation.

Fisher (1958) defined adaptation as the sum total of changes which occurred within the animal as a result of a change or changes in the external environment (e.g., environmental temperature changes). These alterations or responses on the part of the organism favour survival in the changed environment. Heart rate and blood pressure were cited as examples of specific characteristics or adaptates thus modified. Acclimation was considered to be composed of those adaptates possessing time-course characteristics measurable in terms of days or weeks.

Precht (1958) classified acclimation or short-term adaptation into two types: (a) capacity adaptation; (b) resistance adaptation. The first, capacity adaptation, was considered to be an adaptation occurring within the normal temperature range of the animal. Precht (1958) regarded adjustments within this category to be primarily concerned with the maintenance of a relatively constant metabolic rate. Resistance adaptation was classified as that associated with enhancing the animal's ability to tolerate extremes in temperature according to the direction of acclimation. For example, a cold acclimated animal would be able to

withstand lower environmental temperatures than would a warm acclimated animal.

a. Capacity Adeptation. The phenomena of capacity adaptation has been extensively treated by Precht (1958) and Prosser (1958). Precht (1958) used oxygen consumption as a measure of the metabolic rate of animals shifted from an acclimation temperature (T_1) to a higher or lower test temperature (T_2). It was noted that in experiencing such changes the animal may overcompensate or undercompensate immediately following transfer. Complete acclimation was said to have occurred if the rate of oxygen consumption at the test temperature (T_2) returned to that observed at the acclimation temperature (T_1). In all Precht (1958) noted five possibilities with respect to the steady state finally achieved at the test temperature:

i The rate function increased when the animal was transferred to T_2 and remained at this point with no apparent acclimation occurring

ii The rate function showed an initial increase when the animal was transferred to T_2, but returned to that equivalent to the rate at T_1 with complete acclimation occurring.

iii Partial acclimation occurred with the rate function reaching a steady state somewhat higher than that recorded at T_1

iv Hypocompensation occurred with the rate function at T_2 becoming slightly lower than that at T_1

v Hypercompensation occurred with the rate function reaching, and remaining at, a position considerably higher than that recorded for T_1.

Prosser (1958), in his review of the general field of physiological

adaptation, was of the opinion that information regarding the nature of compensatory adaptation might best be obtained by observing reaction rates (e.g., oxygen consumption) of warm and cold acclimated animals transferred to various temperatures above and below the acclimation temperature. In extending the theory presented by Precht (1958), Prosser reviewed the literature and came to the conclusion that nine possible types of rate-temperature relationships could be distinguished in animals transferred from an acclimation temperature to different test temperatures. These represented various combinational changes in the parameters of the classical Krogh and Belehradek temperature metabolism equations.

Of particular interest to the present study was the work of Kanungo and Prosser (1959) in which the standard and active oxygen consumptions of cold and warm acclimated goldfish were reported for various test temperatures. In this publication it was noted that the results obtained corresponded to a type 1VA rate-temperature relationship (see Prosser, 1958).

b. Resistance Adaptation. Resistance adaptation was illustrated by the work of Fry, Brett and Clawson (1942) on Carassius auratus. Upper and lower lethal temperatures (LD_{50}) were observed and represented graphically by a tolerance polygon. It was noted that upper and lower incipient lethals increased to maximum values with increase in acclimation temperature. The area bounded by the polygon was designated as the zone of thermal tolerance. This increase in upper and lower lethal temperatures represented resistance adaptation or adaptation as previously defined.

GENERAL HAEMATOLOGICAL PICTURE OF FISH

The general haematological situation of fishes will be discussed under the following headings:

1. Cell types
   a. erythrocytes
   b. leukocytes
2. Haematocrit
   a. general considerations
3. Haemoglobin
   a. haemoglobin content
   b. haemoglobin polymorphism
4. Blood oxygen combining capacity.

1. Cell Types

a. Erythrocytes. The erythrocytes of fishes have, in their mature form, been described by Jordan (1938) as elliptical discoid bodies with central nuclei. In the teleosts studied, the erythrocytes ranged in length from 6 to 13 microns. Varying numbers of immature erythrocytes, or large and small lymphoid haemocytoblasts, have been described in the blood vascular system of fishes (e.g., Dawson, 1933; Jordan, 1938; Duthie, 1939; Catton, 1951; Bondar, 1957). Jordan (1938) noted that the large lymphoid haemocytoblasts showed intravascular differentiation into erythrocytes, while the smaller lymphoid haemocyto-

blasts differentiated intravascularly into thrombocytes.

Several investigations on erythrocyte numbers and mean corpuscular volumes have been carried out in teleost species. The salient findings, with variations related to thermal conditions, have been recorded in Text Table I. It will be noted from this table that erythrocyte counts and mean corpuscular volume have been observed to vary with environmental temperature. Contrary to the findings of Spoor (1951) and Bondar (1957), Anthony (1961), in experiments on the goldfish, obtained results which indicated a decrease in the number of erythrocytes with increased acclimation temperature. This decrease in erythrocytes coincided with an increase in mean corpuscular volume. It is also of interest to note that Straub (1957, cited by Precht, 1958), who conducted experiments on the amphibian, Rana esculenta, found increases in erythrocyte counts and decreases in mean corpuscular volume with an increase in acclimation temperature.

b. Leucocytes. Considerable difficulty has been experienced in identifying certain leukocytes found in fishes. Jordan (1938) noted that lymphoid hemoblasts (stem cells), or large, small, and intermediate lymphocytes gave rise to granulocytes, monocytes, thrombocytes, and also erythrocytes. Intravascularly, the large and small lymphocytes differentiated into erythrocytes and thrombocytes respectively. Extravascularly, identical cells produced finely granular oxyphilic heterophils and coarsely granular eosinophils. Yuki (1957) cites the observation of Chuin (1920) who observed the presence of numerous lymphoid hemocytoblasts

TEXT TABLE I

ERYTHROCYTE NUMBERS AND MEAN CORPUSCULAR VOLUMES
IN VARIOUS SPECIES OF TELEOST FISHES,
INDICATING VARIATIONS RELATED TO
THERMAL CONDITIONS

SPECIES	TEMPERATURE °C	RBC (10^6)	MCV (u^3)	REFERENCE
Carassius auratus	5.0	2.0	172	Anthony (1961)
	6.0	2.1	181	
	26.0	1.7	219	
	30.0	1.8	176	
Pike (no species given)	----	1.7	175	
Roach (no species given)	----	1.9	238	
Notropis blennius	9.5	1.3		Bondar (1957)
	16.5 (after 21 days)	2.7		
Carassius auratus	13.9	1.6		Spoor (1951)
	30.0	2.2		
Carassius auratus	----	0.5		Baker & Kline (1932)
Scomber (no species given)	----	3.9		Kisch (1952)
Tautog (no species given)	----	2.1		Kisch (1952)
Cyprinus carpio	----	0.8	311	Field, Elvehjem, and Juday, (1943)
Salvelinus fontinalis	----	1.0	314	

in the circulatory system of fishes, and stated that such a situation resembled that of hemopoiesis at the beginning of the third stage of the foetus in higher vertebrates. The position of the lymphoid hemoblast in hemopoiesis was diagramatically represented by Catton (1951) and has been reproduced in Figure 1.

Due to the similarity of erythrocytic stem cells to lymphocytes, it has been virtually impossible to obtain accurate and consistent counts of the leukocytes of fish blood. Thus, recorded leucocyte counts made in fishes show significant differences. Lagler, Bardach, and Miller (1962), for example, noted that white cell counts as determined by various investigators showed a range of 20,000 to 150,000 per cubic millimeter in different groups of fishes. Yuki (1957) noted that the work of Onoda (1934) and Irie (1932) showed large discrepencies among the ratios of granulocytes, monocytes, and lymphocytes in the blood of the crucian carp examined in winter.

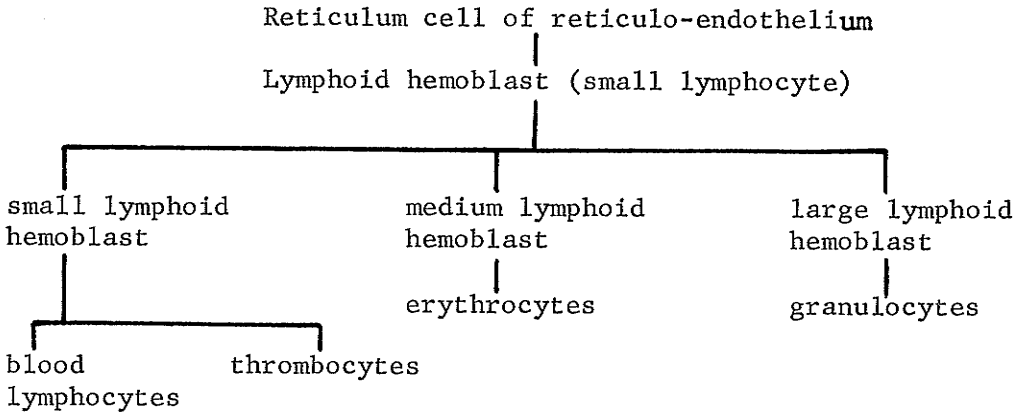
The effect of thermal environment upon the leucocytes of fishes has been largely neglected, however, Slicher and Pickford (1962) have studied the effect of cold shock on the white cell counts of fishes and observed a triphasic response elicited by immersion in ice water. This triphasic response was composed of an initial leucocytosis which occurred 15 minutes after immersion, a leucopenia reaching a maximum at one hour, and a leucocytosis at two hours.

Perhaps the most apparent difference between fish blood and human glood has been found to exist in the erythrocytes. As compared to the erythrocytes of fishes those of human blood have been described

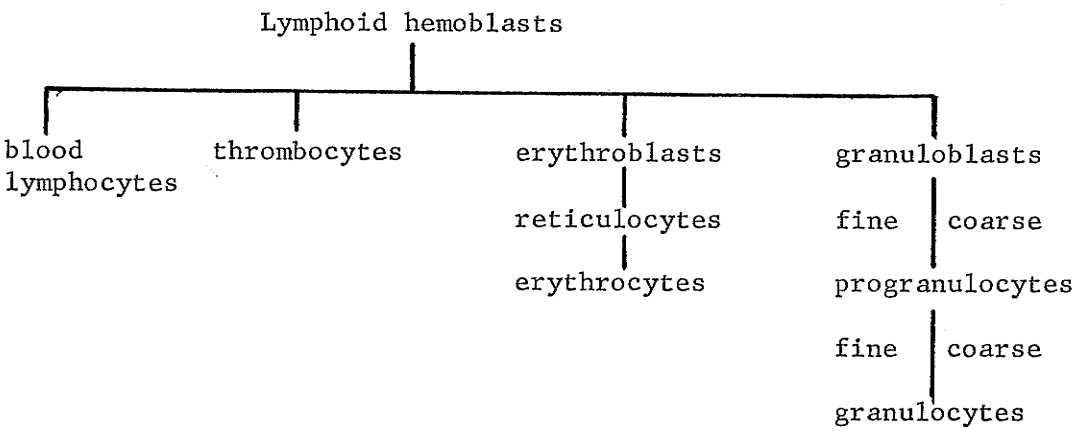
FIGURE 1

PROPOSED SCHEMES OF BLOOD CELL FORMATION IN FISHES

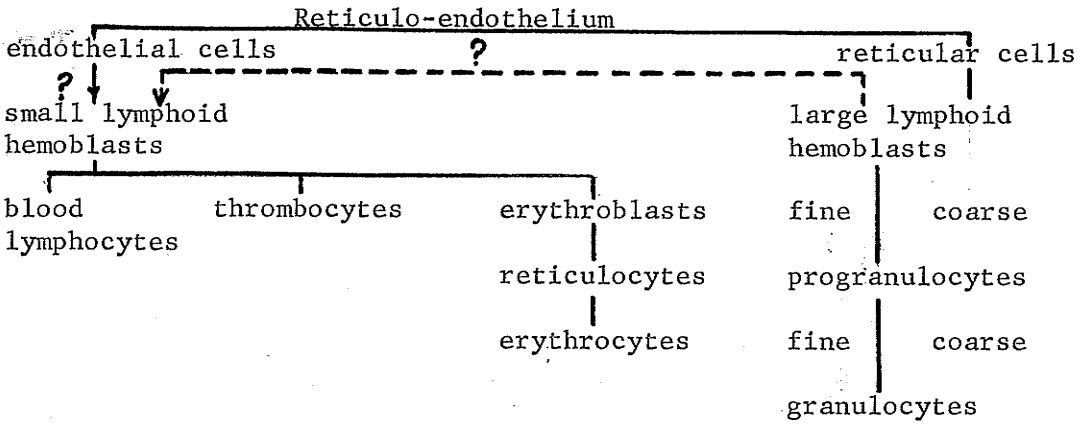
1. Jordan and Speidel (1923)



2. Duthie (1939)



3. Catton (1951)



by Dawson (1948) as flattened, biconcave discs without nuclei. Significant differences in the number of erythrocytes per cubic millimeter have been found in comparisons of fish and human blood. The human female usually has erythrocytes numbering 4.5 million per cubic millimeter, while the male has approximately 5.0 million per cubic millimeter.

Leucocytes of humans may show considerable variation in number according to the physiological condition of the body, but under normal conditions Dawson (1948) has reported them to number approximately 8,000 per cubic millimeter.

Comparisons in the size of the blood cells of humans and fishes have been tabulated in Text Table II. The data used in this case have been taken from Jordan (1938), Lowenthal (1931), and Dawson (1948). As will be noted from the table, variations in the comparative sizes are known to exist, but in general, considerable similarity is shown.

2. Haematocrit

a. General Considerations. Numerous investigators have carried out studies on the haematocrit values of fishes: Sniezko (1961) on rainbow trout, brown trout, and brook trout; Field, Elvehjem and Juday (1943) on carp and trout; Becker, Bird, Kelly, Shilling, Solomons, and Young (1958) on various species of marine teleosts. Anthony (1961) studied the relationship of haematocrit to environmental temperature in Carassius auratus. Characteristic values indicating variations as related to thermal conditions have been recorded in Table III.

TEXT TABLE II

COMPARATIVE SIZES OF THE BLOOD CELLS
IN FISHES AND HUMANS

CELL TYPE	SIZE IN MICRONS	
	Fish	Human
Erythrocyte	6.0-13.0	7.0- 8.8
Lymphocyte	6.0-11.0	6.0-12.0
Monocyte	9.0-16.0	12.0-15.0
Neutrophile	7.5-13.5	10.0-12.0
Basophile	5.0-12.0	8.0-10.0
Eosinophile	5.0-12.0	10.0-12.0

TEXT TABLE III

CHARACTERISTIC HAEMATOCRIT VALUES OF VARIOUS SPECIES OF TELEOST FISHES, INDICATING VARIATIONS RELATED TO THERMAL CONDITIONS

SPECIES	TEMPERATURE °C.	HAEMOCRIT (% RBC)	REFERENCE
Carassius auratus	5	34.2	Anthony (1961)
	6	36.0	
	26	37.5	
	30	30.0	
Rainbow trout (no species given)	Means of samples taken in winter and summer	47.8	Snieszko (1961)
Brook trout (no species given)		50.7	
Brown trout (no species given)		39.5	
Promicrops itaiara	----------	41.0	Becker et. al. (1958)
Thunnus thynnus	----------	41.0	
Mycteroperca venenosa	----------	24.0	
Scromberomorus maculatus	----------	38.0	
Mycteroperca bonaci	----------	28.0	
Cyprinus carpio	----------	31.3	Field, Elvehjem, and Juday (1943)
Salvelinus fontinalis	----------	27.2	

The packed cell volume or haematocrit of fishes compared very favourably with that of humans. Harper (1957) reported that under normal circumstances the cells comprised approximately 45 per cent of the total blood volume in human males and close to 41 per cent in females.

3. Haemoglobin

a. Haemoglobin Content. The haemoglobin content of fish has been determined by numerous investigators: Black (1954) on various species of freshwater fish; Klawe and his associates (Klawe, Barrett, Klawe, and Hillsdon, 1963) on six species of scromboid fishes; Bondar (1957) on Notropis blennius. Examples which serve to indicate the range encountered along with variations related to thermal conditions have been illustrated in Text Table IV.

b. Haemoglobin Polymorphism. Haemoglobin polymorphism has been a subject extensively pursued during the past decade. Prior to 1949, studies on the intraspecies differences of haemoglobin were mainly concerned with the properties of adult and foetal haemoglobin (Lemberg and Legge, 1949). Investigations regarding haemoglobin polymorphism in humans were similarly centered around the study of foetal and adult haemoglobins, haemoglobin F and A respectively. These haemoglobins were found to differ in their molecular forms with the structural variation residing in the globins (Huisman, 1963). With development, a shift from the foetal to the adult structure occurred, and in the adult, haemoglobin "A" was found to predominate. However, in certain types

TEXT TABLE IV

HAEMOGLOBIN CONTENT OF VARIOUS FISHES, INDICATING VARIATIONS RELATED TO THERMAL CONDITIONS

SPECIES	TEMPERATURE °C.	HAEMOGLOBIN gm%	REFERENCE
Notropis blennius	9.5 16.5 (after 21 days)	3.8 4.9	Bondar (1957)
Salmo gairdnerii kamloops		11.0±0.2	Black (1954)
Catostomas catostomas		9.4±0.6	
Cyprinus carpio		10.8±0.5	
Mylocheilus oregonensis		10.8±0.3	
Prychocheilus oregonensis		9.8±0.6	
Ameiurus melas melas		11.1±0.5	
Micropterus salmoides		8.1±0.4	
Scomber japonicus		8.0-14.8	Klawe and associates (1963)
Sarda chiliensis		8.3-14.8	
Thunnus albacares		15.8-18.9	
Katsuwonus pelamis		14.1-20.7	
Euthynnus lineatus		16.9-19.9	
Auxis rochei		17.8-21.2	

of anemia, haemoglobin "F" was found to persist when it had disappeared from normal individuals (Harper, 1957).

Itano and Pauling (1949) discovered the presence of an abnormal haemoglobin in individuals that were suffering from sickle celled anemia. Since that time, other abnormal haemoglobins have been identified (see, Ingram, 1963; Itano, 1956; Harper, 1957; Huisman, 1963).

The occurance of haemoglobin polymorphism in fishes has been demonstrated by various authors: Tsuyki and Gadd (1963) in members of the Salmonidae, Hashimoto and Matsuura (1959, 1960) in black bream, Mylio macrocephalus, dolphin fish, Coryphaena hippurus, mullet, Mugil cephalus, loach, Misgurnus anguillicaudatus, and carp, Cyprinus carpio; Chandrasekhar (1959) in fish belonging to the Cyprinidae, Ophicephalidae, Heteropneustidae, Claridae, and Cichlidae families; Becker, Bird, Kelly, Schilling, Solomon, and King (1958) and Huisman (1962) in several species of marine teleosts.

Changes in the relative abundancies of haemoglobin polymorphs have been known to occur in certain diseases (Fruton and Simmonds, 1958) and in the various developmental stages of the animal. Developmental changes in a particular species of a given animal have been observed in humans, as previously noted, in the change from haemoglobin "F" to haemoglobin A. A similar change was noted in the anurans Rana catesbeiana and Rana grylio during metamorphosis (Trader, Wortham, and Frieden, 1963). Developmental change have also been reported to occur in chicks (Wilt, 1962). Variations in the relative amount of haemoglobins have not been reported for animals undergoing thermal shock or during thermal acclimation,

but changes occurring during an animal's adaptation to a diseased state suggest that differences in oxygen uptake and a heightened oxygen demand might produce similar changes during thermal acclimation and thermal shock.

In humans, the oxygen affinity of haemoglobin F in the foetal circulation was found to be higher than the oxygen affinity of the adult haemoglobin in the adult circulatory system (Ingram, 1963). The advantage of such a system, with its lower loading and unloading tensions, becomes apparent when consideration is given to the fact that the foetus must derive its entire oxygen supply from the maternal blood (Guyer and Lane, 1964).

Research conducted on various animals has lead to the conclusion that different haemoglobin polymorphs possess varying oxygen affinities. Hashimoto and Matsuura (1959), in experiments conducted on the haemoglobin polymorphs of Oncorhynchus keta, noted that of the two components studied one showed a decrease in oxygen affinity with decrease in pH while the other maintained its oxygen affinity during variations in pH. Manwell and his associates (Manwell, Baker, Roslansky, and Foght, 1963) have identified different haemoglobins in the chick embryo and the adult chicken. The haemoglobin of the embryo differed from that of the adult in that it possessed a much greater affinity for oxygen and exhibited a Bohr effect of considerably less magnitude than the adult.

The haemoglobin content of human blood has, in most cases, been found to be somewhat higher than that of fishes. Under normal circumstances the haemoglobin content of human blood is in the range of 14-16 grams per 100 millilitres of blood (Harper, 1957).

As indicated by the previous discussion, haemoglobin polymorphism is found in both fish and humans; however, there has been infinitely greater progress towards a systematic nomenclature for those polymorphs found in humans (Gerald and Ingram, 1961).

4. Blood Oxygen Combining Capacity.

Numerous studies have been undertaken in relation to the oxygen combining capacity of the blood of different species of fish. Representative of such investigations are those of Burke and Woolcott (1957) on the black crappie, Pomoxis nigromaculatus, and the bluegill, Lepomis macrochirus; Black (1940) on the catfish, Ameiurus nebulosa, carp, Cyprinus carpio, bowfin, Amia calva, and common sucker, Catastomus commer sonnii; Haws and Goodnight (1962) on the brown bullhead, Ictalurus nebulosus (LeSueur), and the channel catfish, Ictalurus punctatus (Rafinseque); Irving, Black and Stafford (1941) on Salvelinus fontinalis, Trutta trutta, and Trutta iridea; Manwell (1958 a, 1958 b) on Squalis suckleyi and Polistrema stouti; and McCutcheon (1947) on various turtles and elasmobranchs. Of this group, the work of Irving, Black and Stafford (1941), describing the effect of temperature on the oxygen affinity of the blood, is particularly important. They observed that "in vitro" the oxygen combining capacity of the blood of the above mentioned species decreased with increasing temperature. However, Anthony (1961) commented that the blood of cold water species (e.g., trout) had a high haemoglobin content and low oxygen affinity, while warm-water species (e.g., bullhead) had low haemoglobin content and high affinity for oxygen.

MATERIALS AND METHODS

This portion of the investigation will be described under the following headings:

1. Experimental Animals
2. Conditions of Acclimation
    a. Temperature Control
    b. Photoperiod Control
    c. Feeding
    d. Aeration
3. Establishment of Control and Experimental Groups
4. Haematological Procedures
    a. Sampling Procedure
    b. Erythrocyte Counts
    c. Iron Determinations
    d. Haematocrit Determinations
    e. White Cell Counts
    f. Calculations
5. Electrophoresis
    a. Introduction
    b. General Method Employed
    c. Tests of Paper-Buffer Combinations
    d. Results Obtained
    e. Final Method

1. Experimental Animals

Family:	Cyprinidae
Genus:	Carassius
Species:	auratus
Common name:	the goldfish

The experimental animals used in this study were obtained from the Goldfish Supply Company, Stoufville, Ontario.

2. Conditions of Acclimation

The goldfish were housed in tanks constructed of wood, painted with a non-toxic epoxy-resin[1], and measuring 20 x 20 x 38 inches. These tanks were filled with distilled water to a capacity of approximately 50 Imperial gallons.

Water temperature, photoperiod, feeding, and aeration were all controlled.

a. Temperature Control. The tanks were housed in constant temperature rooms, the air temperature of which was maintained at a point somewhat lower than the desired tank temperature. Tank temperatures were then controlled by means of electronic relays[2] operating from thermistor probes. The temperature controlling relays were, in turn, connected to 500 watt, copper-coil heating elements. Each element was wrapped with

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[1]Fisher Scientific Company.

[2]Thermistemp Temperature Controller (Model 63). Yellow Springs Instrument Company Limited.

Teflon tape[3] to guard against the toxic effect of the metal. With this equipment, temperature variations from the set point averaged $\pm$0.5°C.

b. Photoperiod Control. The upper portions of all the tanks were enclosed by light-proof wooden covers. Two 40 watt incandescent lights[4] were mounted inside each cover. The dimensions of the covers were such that the light sources were situated some 15 inches from the surface of the water. Photoperiod was controlled by the use of an automatic timing device[5] and a schedule of 16 hours light and 8 hours darkness employed.

c. Feeding. Food was offered daily, with the time of feeding being restricted to the early morning. The food preparation given was prepared by, and obtained from, the Fisheries Branch, Department of Mines and Natural Resources, Manitoba. The amount of food offered was proportionate to the number of animals.

d. Aeration. The tanks were continuously aerated, and oxygen levels maintained close to saturation. Plastic aquarium filters, charged with glass wool and activated coconut charcoal, were used to purify the tank water.

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[3]Johnston Industrial Plastics Ltd.

[4]General Electric Lumiline 52. General Electric Company.

[5]Intermatic Time Switch (model T101). International Register Company.

3. Establishment of Control and Experimental Groups

Initially 50 fish were placed in each tank previously regulated to 20 ± 0.5°C. The animals were maintained at this temperature, according to the conditions described, for a period of 30 days. From the 15th to the 30th day, blood samples were taken every 24 hours from a sample of three fish. Red blood cell iron content, mean corpuscular iron content, haematocrit, erythrocyte number, and mean corpuscular volume were determined as outlined in the section on haematological procedures. The range of these determinations was noted, and mean, standard deviation and standard error calculated. Using these tests as criteria, it was concluded that a 20 day acclimation period would be sufficient to insure that accompanying physiological changes had taken place. The duration of the acclimation period thus exceeded that suggested by Brett (1946) and Anthony (1961) for this species.

After acclimation to 20 ± 0.5°C. the fish were shifted to a tank held at 30 ± 0.5°C. The animals were transferred in water in order to minimize the possibility of thermal shock resulting from sudden exposure to the air temperature.

After transfer to the 30°C. tank the animals were sampled, three at a time, every 12 hours for 96 hours, and every 24 hours thereafter until the total time of exposure to the test temperature was 240 hours. The previously mentioned determinations were carried out at each sampling period. The experiment was repeated three consecutive times with a total of nine fish being sampled at each interval.

At a later time, leukocyte counts were carried out on fish subjected to conditions identical to those described. In this case,

determinations were initiated 60 hours after the fish had been transferred to the 30°C. tank. Blood samples were taken every 12 hours from 60 to 96 hours and every 24 hours thereafter until the total time of exposure was 240 hours. Care was taken to establish a sampling program in which the blood could be taken at the same time on each day. By adhering to this program it was possible to avoid daily cyclical changes occurring in the number of leukocytes.

In all cases the remainder of the sample was centrifuged, and the centrifugate, consisting of red blood cells, frozen and stored for later electrophoretic studies of the haemoglobin.

4. Haematological Procedures

a. Sampling Procedure. The fish were anesthetized with tricane methanesulphonate[6] (MS 222, 300 mgm./L) as used by Shiffman and Fromm (1959).

Following anesthetization, the posterior portion of the body was scraped clean of scales, and the caudal peduncle transected. Blood from the caudal artery was collected in small polyethylene titration cups, which had been lightly coated with ethylendiamine tetraacetate (EDTA) to prevent coagulation. Contrary to Hesser (1960), this anticoagulant was found to be superior to heparin for use with this species.

b. Erythrocyte Counts. The technique used in mammalian

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[6] Sandoz Chemical Company.

erythrocyte counts was found generally applicable to fish blood. However, diluting solutions used in mammalian erythrocyte counts were found to be unsuitable for fish blood. Hendricks' diluting solution (Hendricks, 1952), used at a dilution of 1:200, gave acceptable results. The composition of this diluting solution has been shown in Text Table V.

Erythrocyte counts were made with Spencer "Bright Line" Haemacytometers[7] and accompanying diluting pipets. A detailed description of the procedure has been given by Hesser (1960).

c. Iron Determinations. Iron determinations were carried out by means of the total blood iron procedure as described by Natelson (1961). In this technique, the organic material was digested by heating in the presence of sulfuric acid and hydrogen peroxide. Potassium persulfate was used to oxidize the iron to the ferric state. Sodium thiocyanate (NaSCN) was then added and combination of this chemical with ferric iron resulted in the formation of ferric thiocyanate ($Fe(CNS)_6$). Addition of the thiocyanate was carried out only after cooling of the solution; therefore, decomposition of the thiocyanate and precipitation of the sulfur was avoided.

A blank, standard, and unknown were prepared for each analysis. The composition of these has been illustrated in Text Table VI. Electrophotometric analysis of the ferric thyocyanate complex was carried out

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[7]American Optical Company, Instrument Division.

TEXT TABLE V

COMPOSITION OF
HENDRICK'S DILUTING SOLUTION

Sodium sulfate	10.0 gm.
Sodium chloride	2.5 gm.
Sodium citrate	1.5 gm.
Glacial acetic acid	50.0 gm.
Water to	500.0 ml.

TEXT TABLE VI

PREPARATION OF BLANK,
STANDARD, AND UNKNOWN
USED IN IRON DETERMINATIONS

Material Added to Tube	BLANK	STANDARD	UNKNOWN
Distilled water	0.20 ml.	0.20 ml.	0.20 ml.
Iron standard	--------	0.02 ml.	--------
Whole blood	--------	--------	0.2 ml.
Sulfuric acid	0.2 ml.	0.2 ml.	0.2 ml.
Hydrogen peroxide	0.6 ml.	0.6 ml.	0.6 ml.
Distilled water	1.0 ml.	1.0 ml.	1.0 ml.
Potassium persulfate	0.2 ml.	0.2 ml.	0.2 ml.
Sodium thiocyanate	0.4 ml.	0.4 ml.	0.4 ml.
Distilled water	To 4.0 ml.	To 4.0 ml.	To 4.0 ml.

on a Fisher electrophotometer[8] used in conjunction with micro cylindrical absorption cells and a 525-B filter.

d. Haematocrit Determinations. The Strumia micro-haematocrit method was used for haematocrit determinations. The capillary tubes[9] employed measured 32 mm. in length and 0.8 mm. in diameter. In carrying out the procedure a capillary tube was touched to a drop of blood with the capillary action resulting in the formation of an erythrocyte-serum column within the tube. Following this, one end of the tube was sealed with a vinyl plastic putty[10]. Haematocrit values were determined by centrifuging the capillary tubes at 7,000 rpm. for ten minutes.

e. White Cell Counts. Due to the identification problems previously mentioned, considerable difficulty was experienced in obtaining accurate leukocyte counts. In order to simplify the problem, only cells positively identified as leukocytes were tabulated separately.

The method used was that outlined by Hesser (1960). Shaw's counting fluid (Shaw, 1930), was found to give satisfactory results with erythrocytes, thrombocytes, and leukocytes being readily differ-

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[8]Fisher Scientific Company.

[9]Capillary Tubes (Cat. No. 52495A). Scientific Products, Division of American Hospital Supply Corporation.

[10]Critoseal. Biological Research, Inc.

entiated. The composition of this counting fluid has been illustrated in Text Table VII. Solution A was prepared each day, while solution B was stable for several days.

f. Calculations. Calculations for most of the procedures were routine and references have been previously given. The methods of calculation of mean corpuscular volume and mean corpuscular iron content have been illustrated below.

Mean corpuscular volumes were determined by use of the formula given by Anthony (1960).

$$MCV = \frac{\%RBC \times 10}{\text{Millions of RBC/mm}^3} = u^3.$$

Values for mean corpuscular iron content were derived as follows:

$$MCIC = \frac{\text{Total blood iron (mg/100 ml.)}}{\text{RBC/100 ml.}}$$

$$= \frac{\text{Total blood iron (mg./100 ml.)}}{\text{RBC/mm.}^3 \times 10^5 \text{mm.}^3/100\text{ml.}} = \text{mg.}$$

Electrophoretic Procedures

a. Introduction. Prior to this study, conventional paper electrophoretic separation of the haemoglobin polymorphs of teleost fishes had met with little success. However, polymorphs had been demonstrated by starch gel, agar gel, and moving boundary electrophoresis, and also by ammonium sulphate precipitation (Chandrasekhar, 1959;

TEXT TABLE VII

COMPOSITION OF SHAW'S
DILUTING SOLUTION

Solution A

Neutral red	25.0 mg.
Sodium chloride	0.9 gm.
Distilled water	100.0 ml.

Solution B

Crystal violet	12.0 mg.
Sodium citrate	3.8 gm.
Formaldehyde	0.4 ml.
Distilled water	100.0 ml.

Hashimoto and Matsuura, 1959, 1960; Buhler and Shanks, 1959; Sick, 1961). Since these latter techniques are inconvenient, due to the time and preparation required, the advantage of a paper electrophoretic method suggested the desirability of its utilisation. In view of this, an investigation was undertaken to determine the efficiency of various paper-buffer combinations in the separation of the haemoglobin polymorphs of goldfish.

b. General Method Employed. An initial demonstration of haemoglobin polymorphism in Carrassius auratus was made by the vertical starch-gel electrophoresis method of Smithies (1959)[11]. Two fractions were observed and were notable for their low mobility relative to commercially prepared bovine haemoglobin samples.

In the manner previously described, blood was drawn from 15 animals ranging in weight from 8.2 - 19.2 gm. The samples were centrifuged and the plasma aspirated. The packed erythrocytes were then washed three times in 0.7% sodium chloride solution and haemolyzed in distilled water with repeated freezing and thawing. The resulting haemolyzate was a dark opalescent brown, and contrasted with the clear red-brown haemolyzate obtained from human blood treated by the same procedure.

Samples of 20 ul. of haemolyzate were applied centrally and submarginally to each of eight 2.5 x 30 cm. strips used in conjunction

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[11] I am deeply grateful to Mr. James C. Fenwick, Department of Zoology, University of Manitoba, for carrying out this procedure.

with a Spinco Model R electrophoretic system[12]. The duration of the electrophoretic period along with variations in the current and voltage have been recorded in Table VIII.

Following electrophoresis, the paper strips were oven dried at 110°F. and immediately scanned using a Photovolt Model 525 densitometer[13] in conjunction with a 595 mu. filter, and a self-balancing potentiometer recorder[14] set at a response of 10. The slit size of the light from the light source located in the densitometer was 10 x 1.5 millimeters.

Schleicher and Schuell 2043A, Whatman 3MM, and Hurlbut 934 - AH papers were stained in methanolic bromophenol blue, destained in dilute aqueous acetic acid, developed in ammonium hydroxide fumes and rescanned. Whatman DEAE cellulose and ecteola cellulose tended to take up the stain within the paper and were therefore not rescanned. Electrophoretograms obtained using this stain have been recorded in Figures 3 and 4.

c. Tests of Paper-Buffer Combinations. Five different paper types were used in combination with vernol and TRIS-EDTA buffers. The papers and composition of the buffers have been listed in Table VIII. Schleicher and Schuell 203A and Whatman 3MM are the most commonly used in paper electrophoresis. Hurlbut 934 -AH is a borosilicate glass fiber

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[12]Spinco Model R Electrophoretic System. Spinco Division, Beckman Instruments, Inc.

[13], [14]Densitometer and Recorder. Photovolt Corporation.

TEXT TABLE VIII

TEST SCHEDULE

BUFFER	PAPER TYPE	DURATION OF RUN, HR	CURRENT (mA)	VOLTAGE (v)
Veronal[1]	Schleicher & Schuell 2043A	16	3.5	78-79[4]
	Schleicher & Schuell 2043A	4	20.0	250-210
	Whatman 3MM	4	20.0	280-240
	Hurlbut 934-AH	3	20.0	152
	Whatman DEAE cellulose	4	20.0	382-280[5]-305
	Whatman ecteola cellulose	4	20.0	370-320-350
TRIS-EDTA[2]	Schleicher & Schuell 2043A	4	12.0[3]	500-320
	Whatman 3MM	4	20.0	382-280
	Hurlbut 934-AH	1.5	20.0	196-188
	Whatman DEAE cellulose	4	20.0	430-380-425
	Whatman ecteola cellulose	4	20.0	360-345-385

[1]Veronal buffer: diethylbarbituric acid -- 2.76 g/1, sodium diethylbarbiturate -- 15.4 g/1, pH -- 8.6.

[2]TRIS-EDTA buffer: trishydroxymethylaminomethane -- 78.65 g/1, ethylenediaminetetraacetic acid -- 7.80 g/1, boric acid -- 5.98 g/1,pH -- 8.9.

[3]Maximum current at 500 v limit of power supply.

[4]Initial and final voltages

[5]Minimum voltage reached during run.

paper, useful with substances tending to be absorbed by cellulose papers. Whatman diethylaminoethyl (DEAE) cellulose, frequently used in chromatographic separations of haemoglobins, is comparable to a tertiary amino acid resin in action. Whatman ecteola cellulose is a weakly basic tertiary amine exchanger of greater specificity than DEAE cellulose.

d. Results Obtained. The results obtained in the various test combinations have been recorded in Figures 2 and 3.

Schleicher and Schuell 203A, Whatman 3MM, and Whatman DEAE cellulose papers did not produce any separation when used in conjunction with vernol buffer. With Schleicher and Schuell 2043A, Whatman 3MM and Hurlbut 934 - AH papers, the haemoglobin tended to diffuse in a broad band about the point of application. Similar results were recorded by Rodnan and Ebaugh (1957), and Becker et al. (1957).

Partial separations occurred with Hurlbut 934 - AH paper in both vernol and TRIS-EDTA buffers, but because of numerous small peaks, pronounced migration, and considerable tailing, little significance could be ascribed to the results. Furthermore, the extreme fragility of the paper while wet made the staining process particularly difficult.

All of the following combinations showed partial separation, with a major peak migrating towards the cathode:

1. Schleicher and Schuell 2043A - TRIS-EDTA buffer
2. Whatman 3MM - TRIS-EDTA buffer
3. Ecteola cellulose - vernol buffer

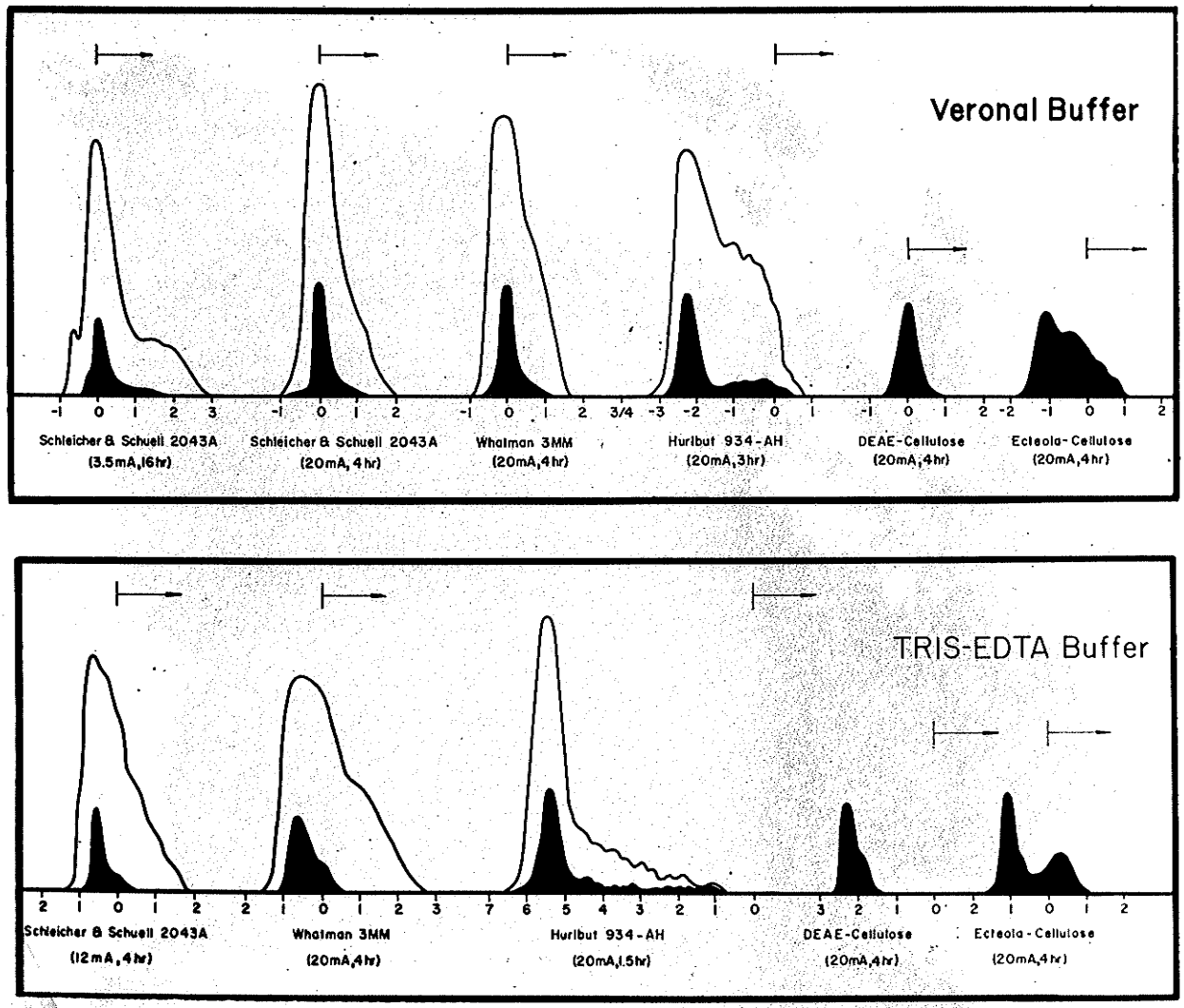

Fig. 2. Upper: Representative electrophaerograms of trials in veronal buffer, direction of anode indicated by arrow. Abscissa-cm. Ordinate-optical density.

Fig. 3. Lower: Representative electrophaerograms of trials in TRIS-EDTA buffer, direction of anode indicated by arrow. Abcissa-cm. Ordinate-optical density.

4. DEAE cellulose - TRIS-EDTA buffer.

Of all the combinations tested, the ecteola cellulose - TRIS-EDTA combination produced the most satisfactory separation. In this case, there was a sharply defined fraction, which sometimes appeared to be indistinctly separated into two components, migrating towards the cathode and a more diffuse fraction of lesser mobility migrating towards the anode.

e. Final Method. Although results obtained with the above method showed a reproducibility of replicate samples which was within 10% with respect to mobility and abundance, it was discovered in later studies that greater consistency could be obtained by carrying out the procedure at constant voltage and variable current. In this case the voltage was 500 V and the current varied from 10-40 mA.

In the final method, electrophoretic runs were carried out under constant voltage and varying amperage. A colored indicator[15] was placed at the point of origin on each strip, and bovine haemoglobin[16] was placed on one strip in each run. The indicator and the haemoglobin acted as controls whereby variations in the buffer could be detected.

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[14]RBY Reference Dye. Gelman Instrument Co.

[15]Mann Research Laboratories.

RESULTS

Haematological Investigation

The results obtained in the haematological study will be discussed under the following headings:

1. Erythrocyte Counts
2. Haematocrit Determinations
3. Mean Corpuscular Volume Estimations
4. Iron Determinations
5. Mean Corpuscular Iron Content Estimations
6. White Cell Counts.

The data obtained from the above determinations have been recorded, in full, in Appendix Tables I and II.

1. Erythrocyte Counts. The results obtained in the study of erythrocyte numbers have been graphically illustrated in Figure 4, and tabulated in Text Table IX. It would appear, from the slope of the regression line of erythrocyte numbers against time, that no significant change occurred during the 240 hours following transfer of the animals to 30°C. However, the results do suggest the possibility of a cycling phenomenon with a period of approximately 60 hours.

2. Haematocrit Determinations. Results obtained in the haematocrit determinations have been summarised in Text Table X and

Figure 5. The regression line indicates no significant change over the 240 hour period following transfer. However, the possibility of a cycling variation over 60 hour intervals again appears.

3. Mean Corpuscular Volume. The results of estimates of mean corpuscular volume have been recorded in Text Table XI and Figure 6. As indicated by the regression line, no significant change occurred over the 240 hour test period. However, cyclical variations again appeared, with the cycle period again being of approximately 60 hours duration.

4. Iron Determinations. Figure 7 and Text Table XII indicate the results obtained in determinations of the total blood iron. No significant change occurred in the total blood iron during the 240 hour test period, although the regression line drawn to suit the values obtained showed a slight decrease in the total blood iron. Once again, the previously mentioned cyclical variations appeared.

5. Mean Corpuscular Iron Content. Values obtained by calculation for mean corpuscular iron content are summarised in Text Table XIII and Figure 8. No significant variation occurred in mean corpuscular iron content over the 240 hour period. The regression line of mean corpuscular iron content against time, however, suggests that a slight decrease, comparable to that obtained in the total blood iron analysis, may have taken place.

6. White Cell Counts. As noted previously, leukocytes and haemocytoblasts were tabulated separately. The results obtained in the leukocyte counts are given in Text Table XIV and Figure 9, while those of the haemocytoblast numbers have been given in Text Table XV and Figure 10.

Although a trend towards an increase exists, no significant change occurred in leukocyte numbers during the 240 hour period following transfer. Contrary to the results obtained in the other haematological tests, there appeared to be little indication of any cycling phenomena.

The haemocytoblast number remained relatively constant, but did show a trend towards an increase and possible cycling was apparent.

TEXT TABLE IX

(RESULTS OBTAINED FROM)
ERYTHROCYTE COUNTS

GROUP	MEAN WEIGHT (gm.)	MEAN RBC/mm^3 (10^6)	STANDARD DEV.	STANDARD ERR.
20°C. Controls	10.3	2.12	0.11	0.02
30°C. Test 12 Hours	35.0	2.16	0.24	0.09
24 Hours	49.6	2.15	0.24	0.03
36 Hours	41.7	2.18	0.21	0.07
48 Hours	47.5	2.22	0.39	0.13
60 Hours	40.8	1.81	0.32	0.11
72 Hours	35.2	2.16	0.28	0.09
84 Hours	41.5	2.19	0.26	0.09
96 Hours	30.1	2.33	0.28	0.09
120 Hours	31.9	2.16	0.39	0.13
144 Hours	40.5	2.35	0.23	0.07
168 Hours	32.3	2.06	0.30	0.10
192 Hours	38.7	2.15	0.19	0.06
216 Hours	34.0	1.96	0.33	0.11
240 Hours	41.1	2.16	0.59	0.20

FIGURE 4. Erythrocyte number in millions/mm.^{3}, showing values obtained for fishes acclimated to 20°C. and fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation: small rectangle, standard error. Equation for regression line:

$$Y = 2.178 + 0.0002X$$

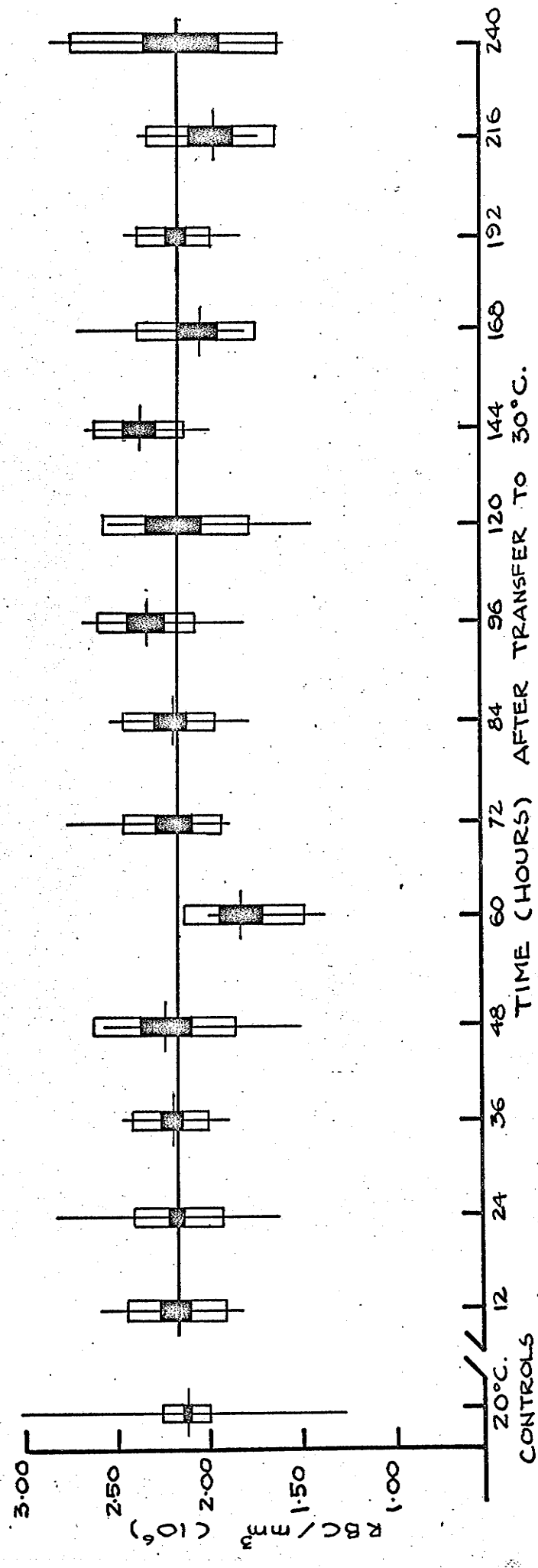
RBC/mm³ (10⁶)
3·00
2·50
2·00
1·50
1·00
20°C. CONTROLS
12
24
36
48
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30°C.

FIGURE 4

TEXT TABLE X

RESULTS OBTAINED FROM
HAEMATOCRIT DETERMINATIONS

GROUP	MEAN WEIGHT (gm.)	MEAN %RBC	STANDARD DEV.	STANDARD ERR.
20°C. Controls	10.3	29.6	5.9	1.0
30° Test				
12 Hours	35.0	32.2	5.5	1.8
24 Hours	49.6	28.4	2.3	0.8
36 Hours	41.7	31.8	3.5	1.2
48 Hours	47.5	30.4	5.3	1.8
60 Hours	40.8	25.1	3.8	1.3
72 Hours	35.2	29.0	6.7	2.2
84 Hours	41.5	36.1	7.8	2.6
96 Hours	30.1	34.5	3.3	1.1
120 Hours	31.9	27.1	4.4	1.5
144 Hours	40.5	26.7	4.4	1.5
168 Hours	32.3	26.9	4.0	1.3
192 Hours	38.7	30.0	4.4	1.5
216 Hours	34.0	25.5	3.3	1.1
240 Hours	41.1	34.6	5.4	1.8

FIGURE 5. Haematocrit expressed as percent red blood cells, showing values obtained for fishes acclimated to 20°C. and fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 30.4 - 0.005X$$

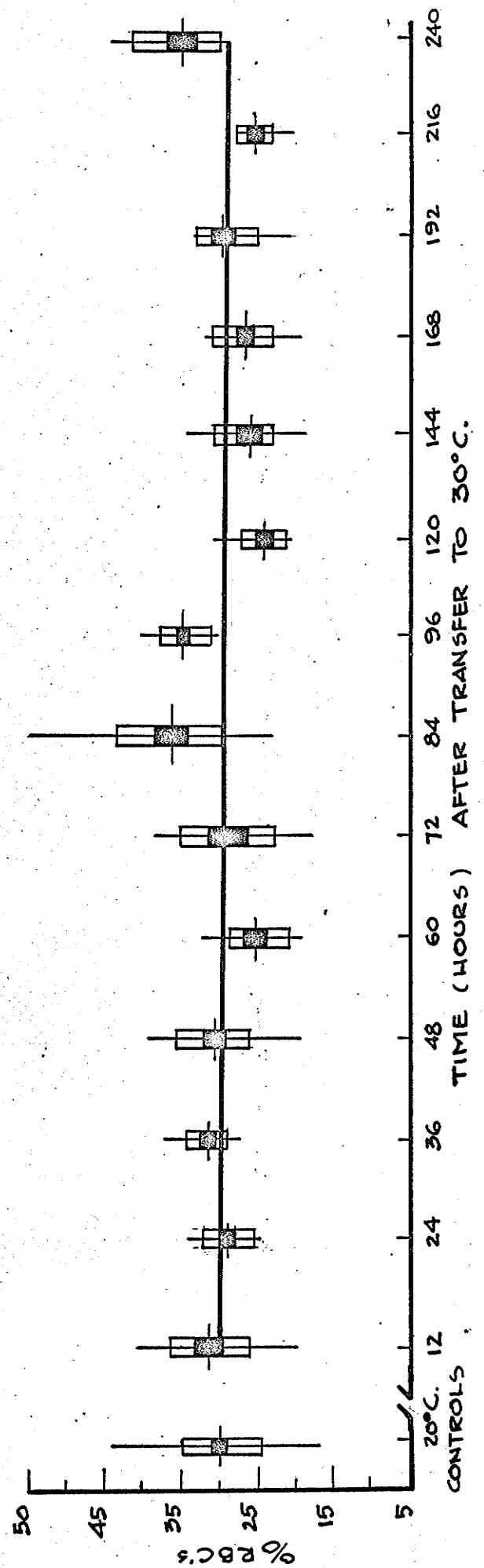
% RBC's
50
45
35
25
15
5
20°C. CONTROLS
12
24
36
48
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30°C.

FIGURE 5

TEXT TABLE XI

RESULTS OF MEAN CORPUSCULAR VOLUME DETERMINATIONS

GROUP	MEAN WEIGHT (gm.)	MEAN MCV (u^3)	STANDARD DEV.	STANDARD ERR.
20°C. Controls	10.3	162.7	29.8	5.8
30°C. Test				
12 Hours	35.0	148.0	18.3	6.2
24 Hours	49.6	133.0	15.2	5.1
36 Hours	41.7	146.0	10.0	3.3
48 Hours	47.5	135.0	9.1	3.0
60 Hours	40.8	138.0	10.7	3.6
72 Hours	35.2	134.0	27.5	9.2
84 Hours	41.5	156.0	12.8	4.3
96 Hours	30.1	149.0	29.4	9.8
120 Hours	31.9	125.5	8.9	3.2
144 Hours	40.5	114.0	18.7	6.2
168 Hours	32.3	130.0	6.4	2.1
192 Hours	38.7	147.0	28.7	9.6
216 Hours	34.0	134.0	13.9	4.6
240 Hours	41.1	166.0	25.1	8.4

FIGURE 6. Mean corpuscular erythrocyte volume expressed in cubic microns. Values shown are for fishes acclimated to 20°C. and for fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 138.2 + 0.0166X$$

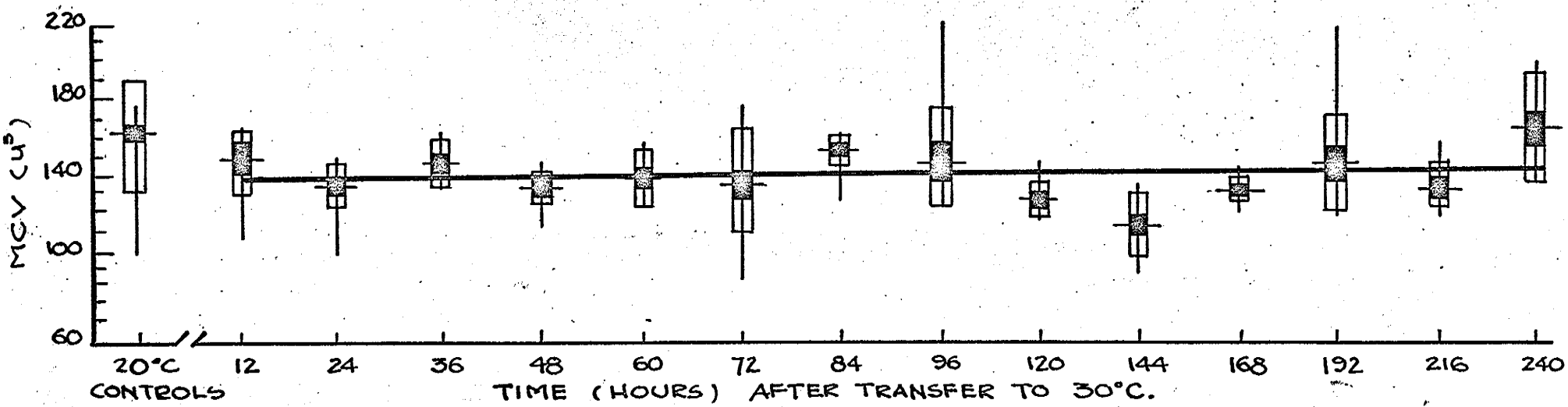
220
180
140
100
60
MCV (u³)
20°C
CONTROLS
12
24
36
48
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30°C.

FIGURE 6

TEXT TABLE XII

RESULTS OBTAINED FROM
IRON DETERMINATIONS

GROUP	MEAN WEIGHT (gm.)	MEAN TOTAL IRON (Mgm. %)	STANDARD DEV.	STANDARD ERR.
20°C. Controls	10.3	35.7	7.7	1.4
30°C. Test				
12 Hours	35.0	31.7	2.5	0.8
24 Hours	49.6	30.3	6.1	2.0
36 Hours	41.7	40.4	8.3	2.8
48 Hours	47.5	32.8	9.1	3.0
60 Hours	40.8	32.8	6.9	2.3
72 Hours	35.2	34.4	4.5	1.5
84 Hours	41.5	36.8	4.3	1.4
96 Hours	30.1	42.0	4.3	1.4
120 Hours	31.9	27.1	5.2	1.7
144 Hours	40.5	40.5	8.7	2.9
168 Hours	32.3	25.7	3.3	1.1
192 Hours	38.7	25.9	3.7	1.2
216 Hours	34.0	23.2	3.1	1.0
240 Hours	41.1	36.0	4.3	1.4

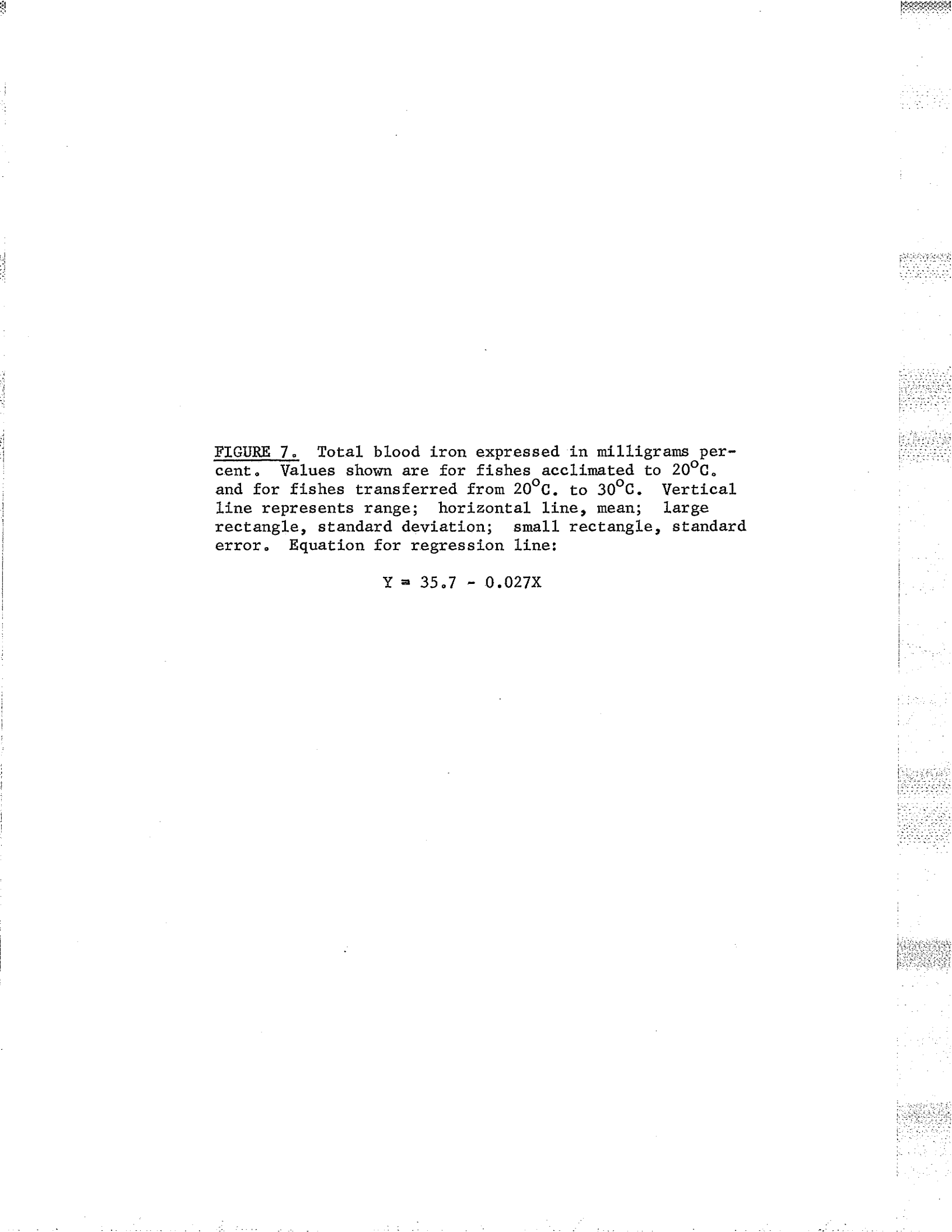

FIGURE 7. Total blood iron expressed in milligrams percent. Values shown are for fishes acclimated to 20°C. and for fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 35.7 - 0.027X$$

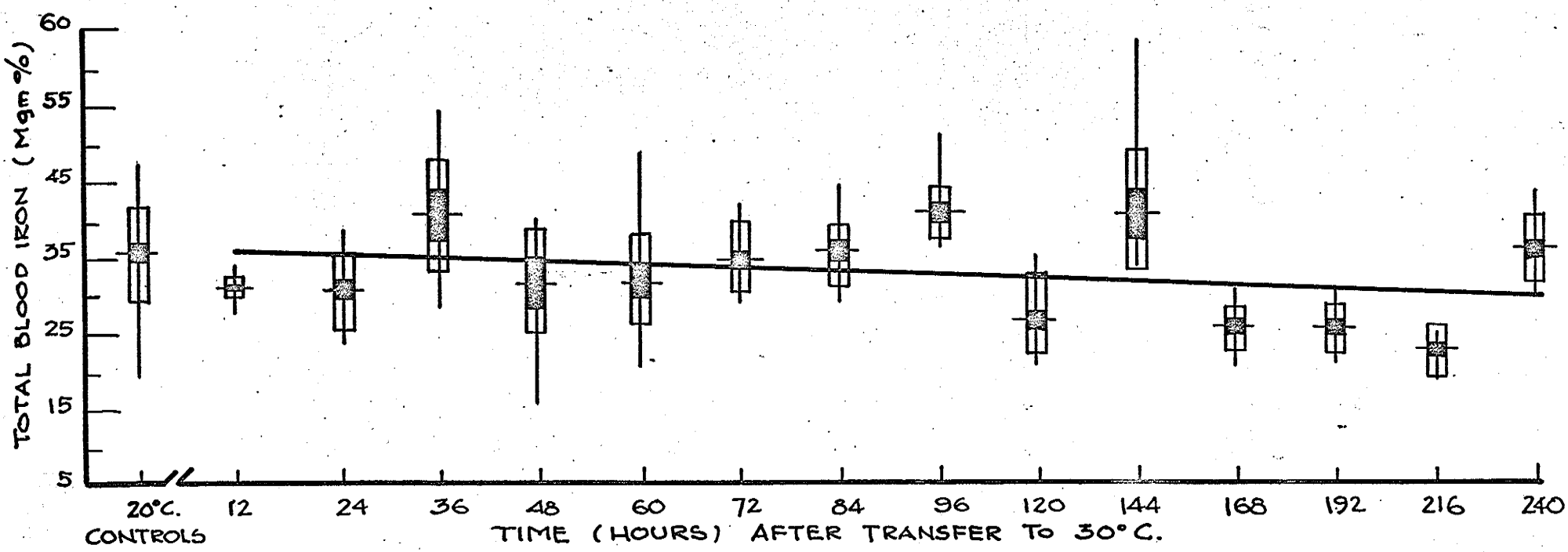
TOTAL BLOOD IRON (Mgm %)
60
55
45
35
25
15
5
20°C. CONTROLS
12
24
36
48
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30° C.

FIGURE 7

TEXT TABLE XIII

RESULTS OBTAINED FROM DETERMINATIONS
OF MEAN CORPUSCULAR IRON CONTENT

GROUP	MEAN WEIGHT (gm.)	MEAN MCIC ($Mgm.x10^{-11}$)	STANDARD DEV.	STANDARD ERR.
$20^{o}C.$ Controls	10.3	17.0	3.5	0.6
$30^{o}C.$ Test				
12 Hours	35.0	14.9	1.6	0.5
24 Hours	49.6	14.2	2.2	0.8
36 Hours	41.7	18.4	3.0	1.0
48 Hours	47.5	14.6	2.0	0.7
60 Hours	40.8	18.1	1.5	0.5
72 Hours	35.2	16.1	2.6	0.9
84 Hours	41.5	17.1	2.7	0.9
96 Hours	30.1	19.7	7.0	2.3
120 Hours	31.9	14.6	1.4	0.5
144 Hours	20.5	17.3	3.0	1.0
168 Hours	32.3	12.5	1.1	0.4
192 Hours	38.7	13.0	3.3	1.1
216 Hours	34.0	12.8	3.1	1.0
240 Hours	41.1	16.4	3.4	1.1

FIGURE 8. Mean corpuscular iron content expressed as milligram x 10^{-11}. Values shown are for fishes acclimated to 20°C. and for fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 16.4 - 0.0061X$$

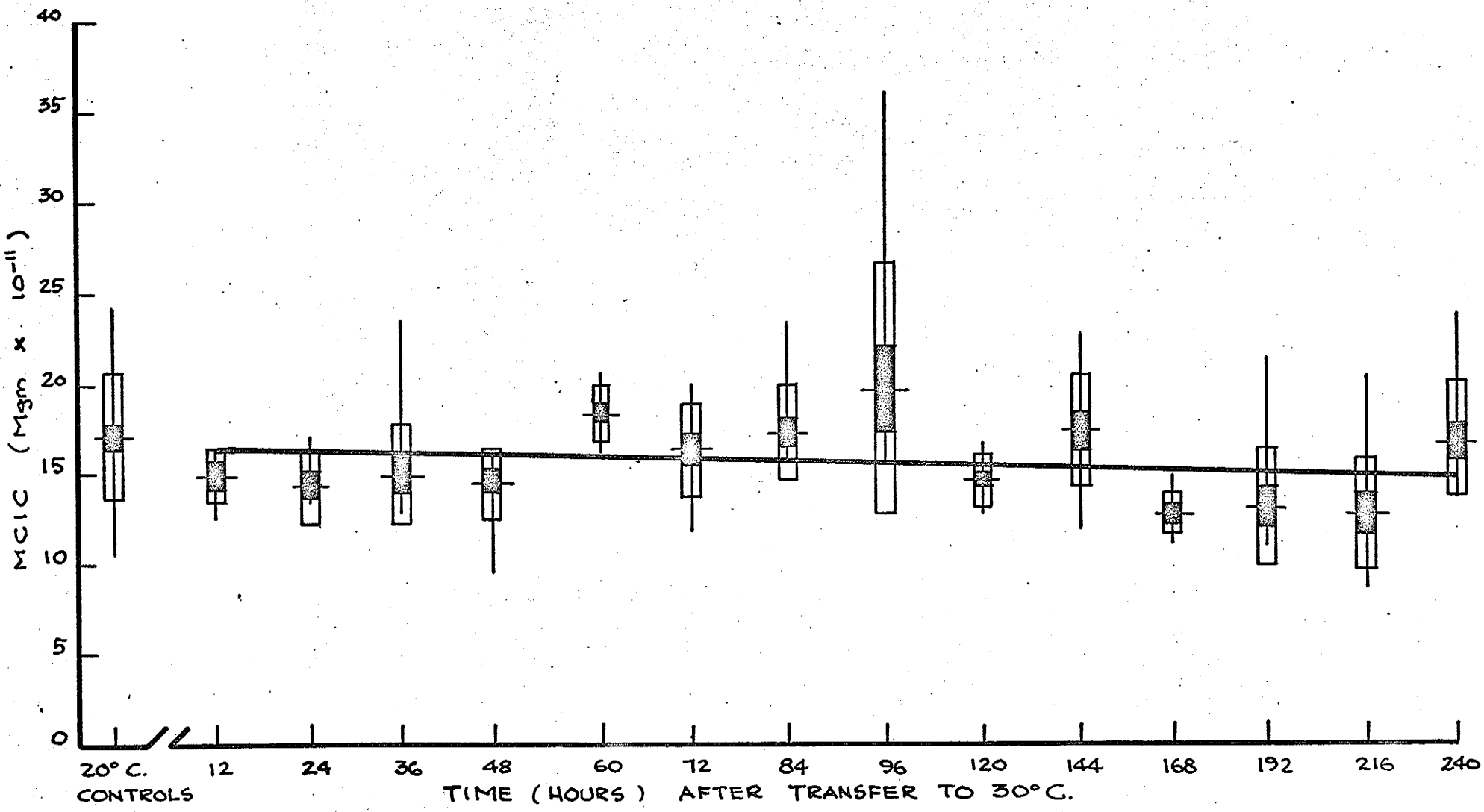
MCIC (Mgm x 10^{-11})
40
35
30
25
20
15
10
5
0
20° C.
CONTROLS
12
24
36
48
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30° C.

FIGURE 8

TEXT TABLE XIV

RESULTS OBTAINED FROM
LEUKOCYTE COUNTS

GROUP	MEAN WEIGHT (gm.)	MEAN LEUKOCYTES/mm^3	STANDARD DEV.	STANDARD ERR.
$20^{\circ}C.$ Controls	31.9	3,166	863	179
$30^{\circ}C.$ Test				
60 Hours	40.5	2,650	625	197
72 Hours	44.7	2,150	707	223
84 Hours	41.1	2,200	748	235
96 Hours	35.2	2,333	666	210
120 Hours	37.5	3,150	1,096	345
144 Hours	40.0	3,100	1,096	345
168 Hours	35.3	2,950	1,010	318
192 Hours	36.6	2,777	972	307
216 Hours	38.5	4,790	1,845	582
240 Hours	45.4	3,200	855	269

FIGURE 9. Leukocytes in thousands/mm.^{3}, showing values obtained for fish acclimated to 20°C. and fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 1774.6 + 8.3X$$

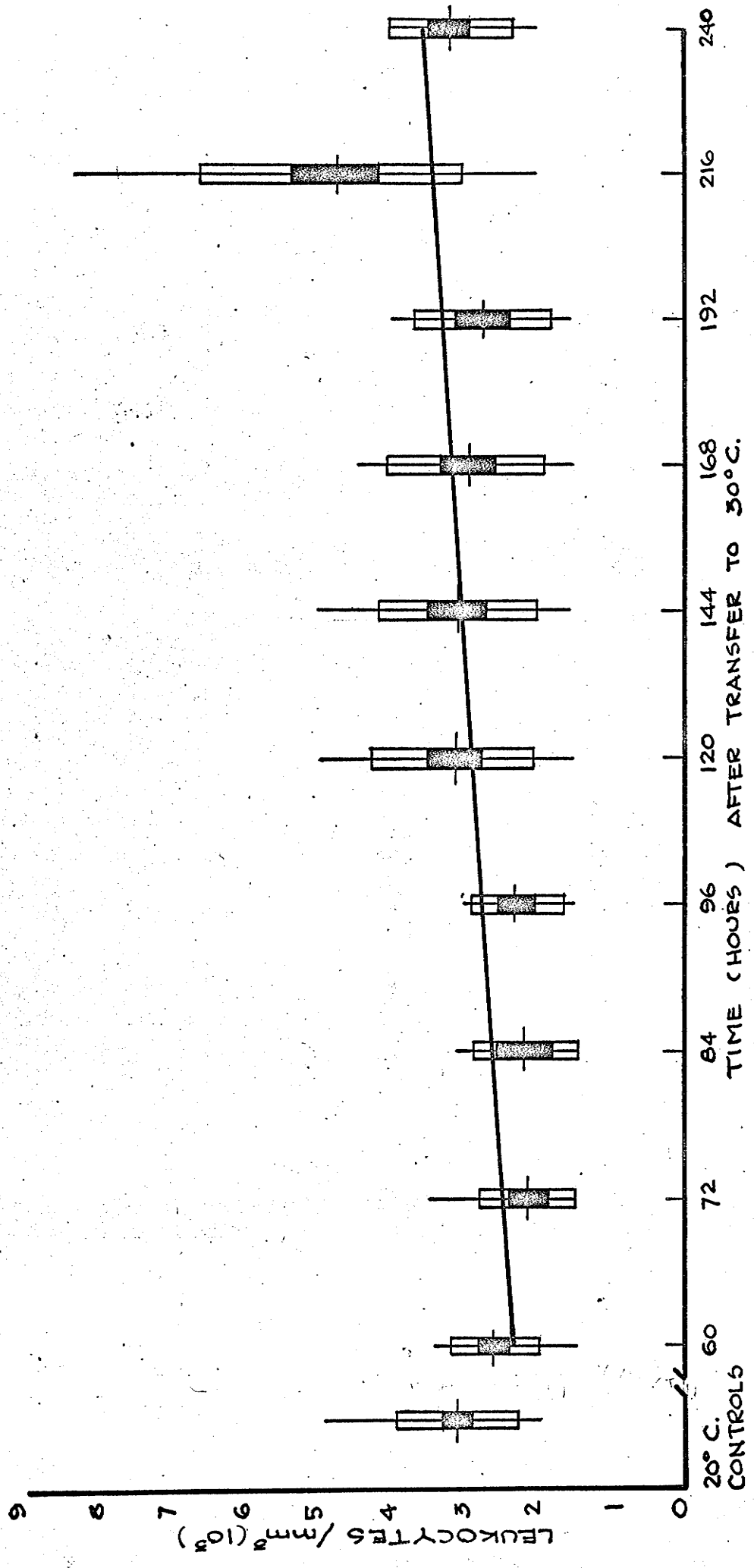
LEUKOCYTES /mm³ (10³)
0
1
2
3
4
5
6
7
8
9
20° C. CONTROLS
60
72
84
96
120
144
168
192
216
240
TIME (HOURS) AFTER TRANSFER TO 30° C.

FIGURE 9

TEXT TABLE XV

RESULTS OBTAINED FROM
HAEMOCYTOBLAST COUNTS

GROUP	MEAN WEIGHT (gm.)	HAEMOCYTOBLASTS /mm^3	STANDARD DEV.	STANDARD ERR.
20°C. Controls	31.9	41,975	9,100	1,895
30°C. Test				
60 Hours	40.5	43,600	9,400	2,965
72 Hours	44.7	34,700	9,540	3,009
84 Hours	41.1	39,500	9,500	1,996
96 Hours	35.2	33,833	4,470	1,410
120 Hours	37.5	45,850	10,000	3,154
144 Hours	40.0	49,200	11,420	3,602
168 Hours	35.3	42,250	6,600	2,100
192 Hours	36.6	48,866	13,600	4,533
216 Hours	38.5	48,450	14,400	4,542
240 Hours	45.4	46,350	5,910	1,864

FIGURE 10. Haemocytoblasts in thousands/$mm.^3$, showing values obtained for fishes acclimated to 20°C. and fishes transferred from 20°C. to 30°C. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error. Equation for regression line:

$$Y = 36,856 + 46X.$$

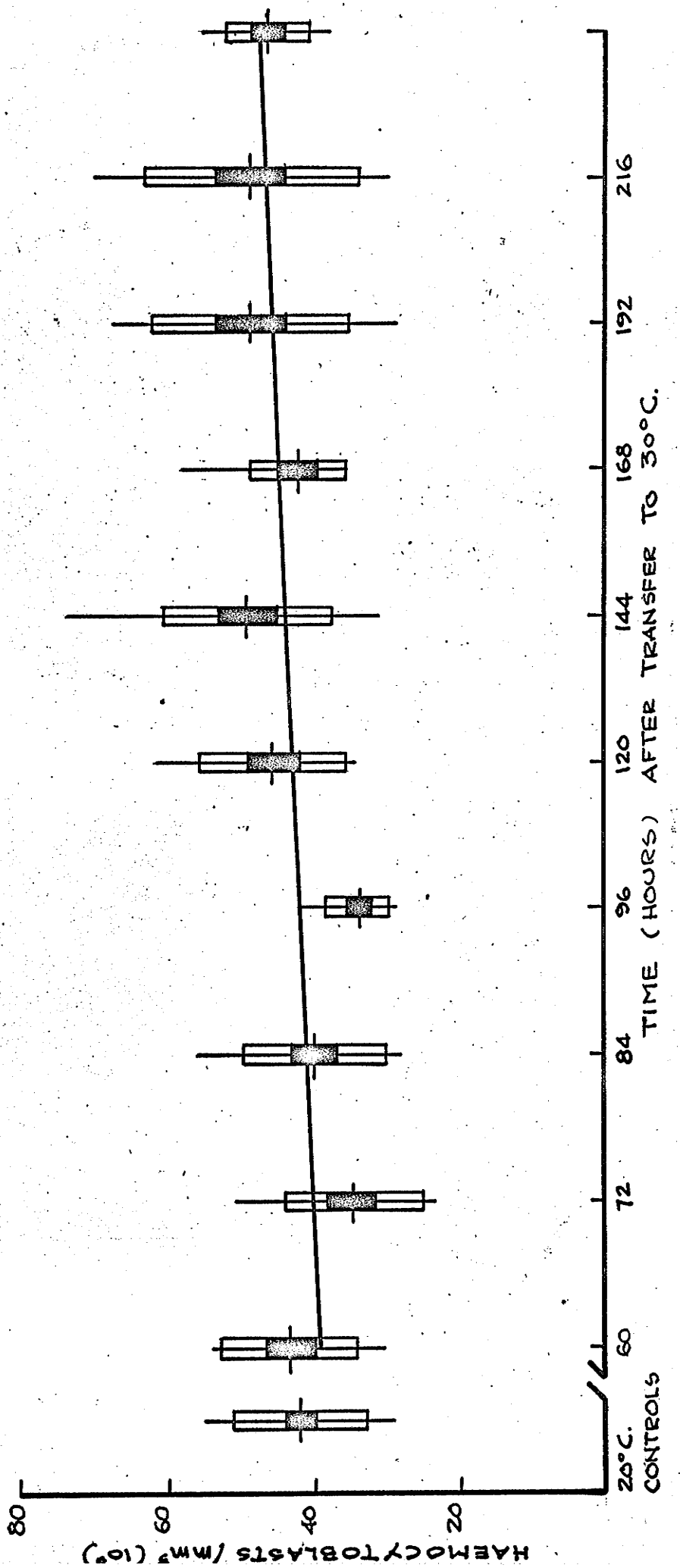
HAEMOCYTOBLASTS /mm³ (10⁶)
80
60
40
20
20°C. CONTROLS
60
72
84
96
120
144
168
192
216
TIME (HOURS) AFTER TRANSFER TO 30°C.

FIGURE 10

Results of Electrophoretic Procedures

In this portion of the investigation 21 fish were acclimated at each of four different temperatures, 5°, 12°, 20°, and 30°C. Following acclimation, electrophoretic studies were made on the haemoglobin of each group of animals.

Representative electrophoretograms are illustrated in Figure 11. The relative abundancies of the polymorphs have been given in Figure 12 and Text Table XVI, while migration distances of the polymorphs are shown in Figure 13 and Text Table XVI.

Fish acclimated to 5°C. had two haemoglobin polymorphs: a major peak (C_1) migrating toward the cathode; and, a minor peak (A_2), of greater mobility, migrating towards the anode.

The 12°, 20°, and 30°C. groups exhibited three fractions (C_1, A_1, A_2). The appearance of the third fraction (A_1) became progressively more apparent with increased acclimation temperature, and appeared to be composed of what was the minor peak of 5°C. group.

The major fraction (C_1) remained close to the same relative percentage throughout all the groups tested. The total percentage contribution of A_1 and A_2 to the whole in the 12°, 20°, and 30°C. animals was equivalent to that of the A fraction seen in the animals acclimated to 5°C.

The migration distance of C_1 remained fairly constant throughout all the groups. However, with the appearance of A_1 there was a subsequent decrease in the migration distance of A_2.

TEXT TABLE XVI

RESULTS OF ELECTROPHORESIS
SHOWING MIGRATION DISTANCE AND
RELATIVE ABUNDANCE OF HAEMOGLOBIN POLYMORPHS

ACCLIMATION TEMP. and MEAN WEIGHT (gm.)		MIGRATION DIST. (mm.) C_1	A_1	A_2	REL. ABUNDANCE (%) C_1	A_1	A_2
5°C. Group	Mean	12.0		16.0	59.8		37.2
39.0 Grams	Standard Deviation	1.7		1.9	7.2		6.7
	Standard Error	0.4		0.4	1.6		1.5
12°C. Group	Mean	11.0	9.0	15.0	64.2	10.5	25.7
40.7 Grams	Standard Deviation	2.0	1.1	1.3	5.4	2.3	4.7
	Standard Error	0.4	0.2	0.3	1.2	0.5	1.0
20°C. Group	Mean	11.0	7.0	8.0	63.0	16.6	20.4
33.6 Grams	Standard Deviation	3.0	2.2	4.4	10.5	5.6	8.7
	Standard Error	0.7	0.5	1.0	2.3	1.2	1.9
30°C. Group	Mean	11.0	11.0	15.0	54.5	13.6	31.9
38.7 Grams	Standard Deviation	3.7	2.4	2.6	8.9	6.8	8.9
	Standard Error	0.8	0.5	0.6	2.0	1.5	2.0

FIGURE 11. Representative electrophoretograms of haemoglobin from fishes acclimated to each of 5^{o}, 12^{o}, 20^{o}, and 30^{o}C. Acclimation temperature is given at the base of each electrophoretogram. Direction of anode is indicated by arrow. Point of application noted as "origin". C_1 represents cathodal fraction. A_1 and A_2 represent anodal fractions.

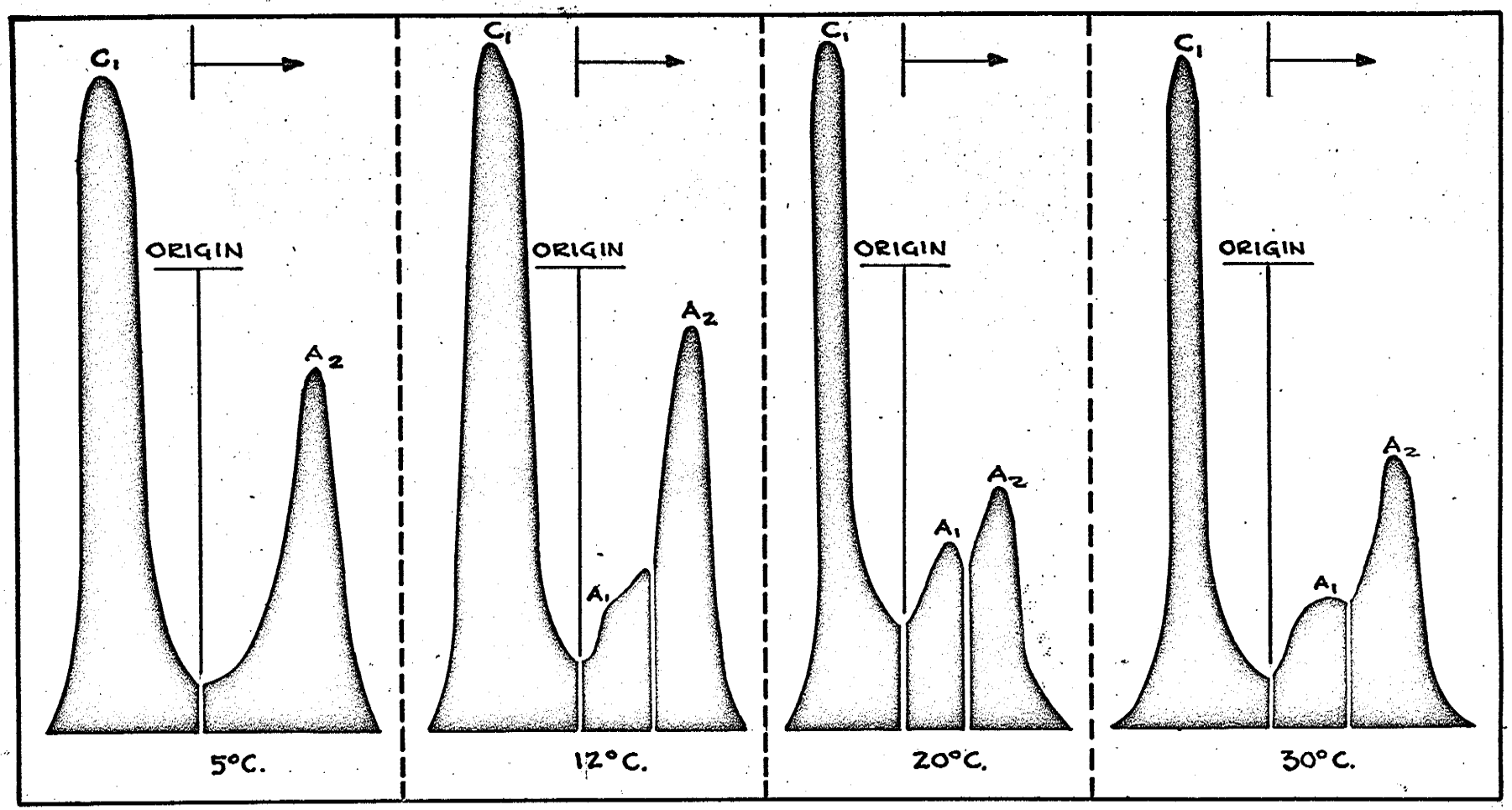
C1
ORIGIN
A2
5°C.
C1
ORIGIN
A1
A2
12°C.
C1
ORIGIN
A1
A2
20°C.
C1
ORIGIN
A1
A2
30°C.

FIGURE 11

FIGURE 12. Relative abundance of haemoglobin polymorphs of fishes acclimated to each of 5°, 12°, 20°, and 30°C. Broken lines separate acclimation groups. C_1 represents cathodal fraction. A_1 and A_2 represent anodal fractions. Vertical line represents range; horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error.

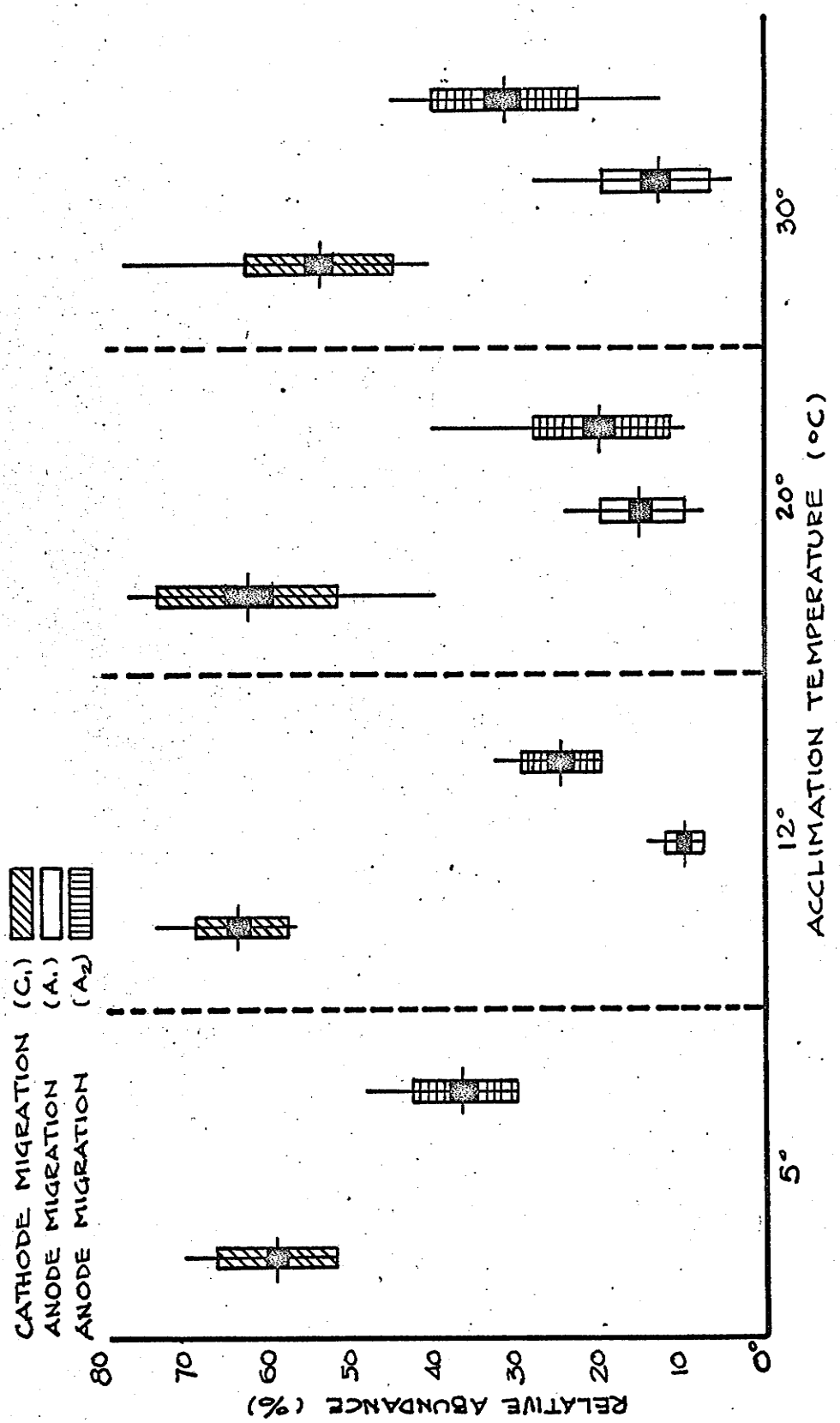
CATHODE MIGRATION (C1)
ANODE MIGRATION (A1)
ANODE MIGRATION (A2)
RELATIVE ABUNDANCE (%)
80
70
60
50
40
30
20
10
0°
5°
12°
20°
30°
ACCLIMATION TEMPERATURE (°C)

FIGURE 12

FIGURE 13. Migration distances of haemoglobin polymorphs of fishes acclimated to each of 5^{o}, 12^{o}, 20^{o}, and $30^{o}C$. Broken lines separate acclimation groups. C_1 represents cathodal fraction. A_1 and A_2 represent anodal fractions. Vertical line represents range, horizontal line, mean; large rectangle, standard deviation; small rectangle, standard error.

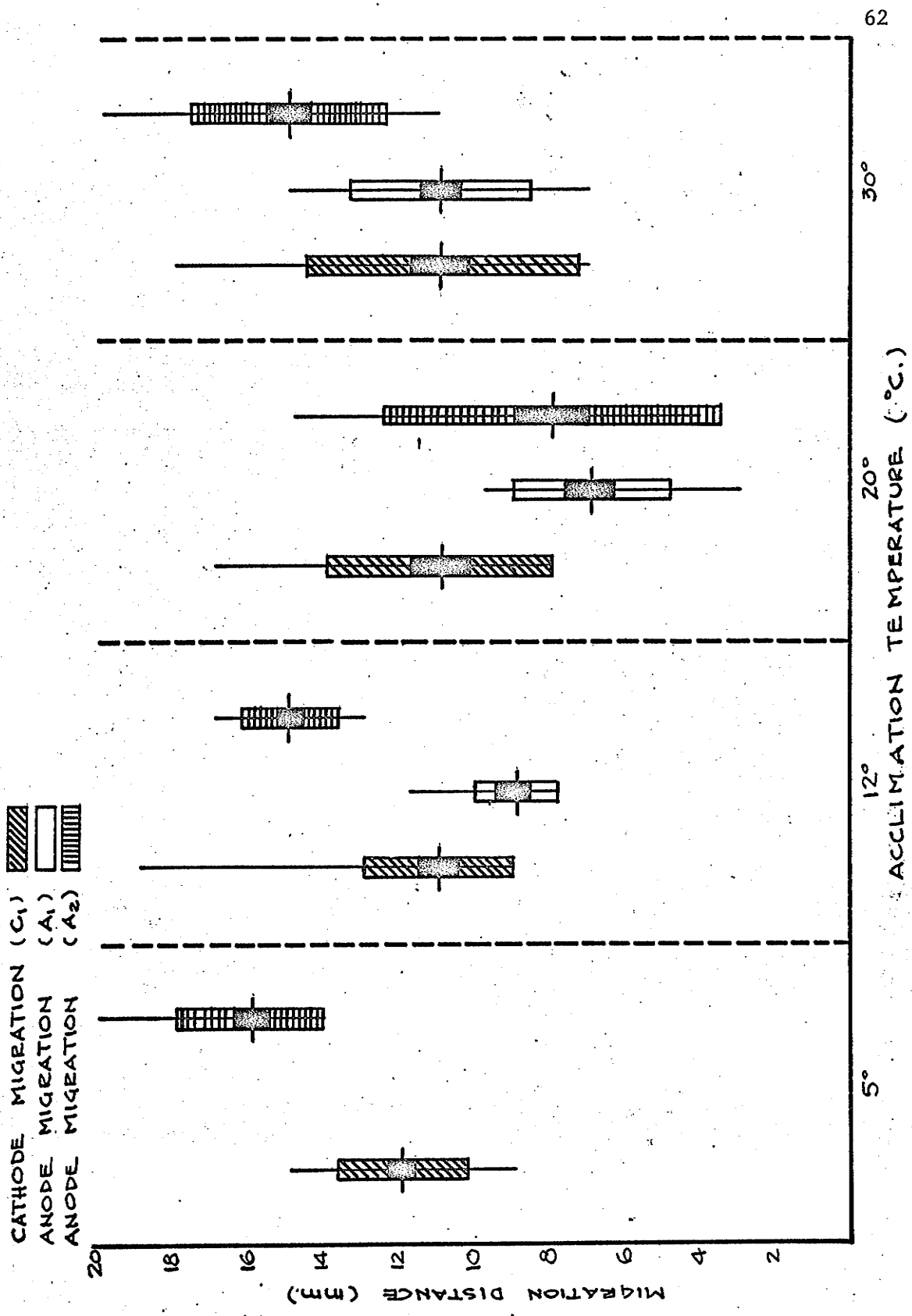
CATHODE MIGRATION (C_1)
ANODE MIGRATION (A_1)
ANODE MIGRATION (A_2)
MIGRATION DISTANCE (mm.)
20
18
16
14
12
10
8
6
4
2
5°
12°
20°
30°
ACCLIMATION TEMPERATURE (°C.)

FIGURE 13

DISCUSSION

1. Haematological Aspects

a. Erythrocyte Counts. Values obtained for erythrocyte in this study and those of other investigations may be compared by referring to Text Table I and Figure 4.

The erythrocyte counts of the 20°C. control group closely approximate the results obtained by Anthony (1961) and Spoor (1951) in goldfish acclimated to temperatures of 26° and 30°C. However, they are considerably higher than those reported by Baker and Kline (1932) for this species (T.T.I).

Species differences in erythrocyte counts are apparent when the results of the present study are compared with those recorded by Field, Elvehjem and Juday (1943) for carp and brook trout, by Kisch (1952a, 1952b), for the scomber and tautog, by Bondar (1957) for the river shiner, and by Anthony (1961) for pike and roach (T.T.I). The values observed in these earlier studies showed a range of 0.5 - 2.7 million/mm^3.

Although the erythrocyte counts carried out on the 30°C. animals are somewhat higher than those recorded by Anthony (1961) for the same species acclimated to this temperature, they compare well with those observed by Spoor (1951) in goldfish which had been tempered to a temperature of 30°C. by stepwise increases over a ten day period. The latter animals were maintained at a temperature of 30°C. for a period of 4 days prior to sampling.

Since the animals used by Anthony (1961) were probably fully acclimated to the various temperatures employed, and those of Spoor (1951) were, in all probability, only partially acclimated, it is difficult to make direct comparisons of the results. It is, however, interesting to note that both Spoor (1951) and Bondar (1957) observed increases in erythrocyte number with increased temperature (Spoor, 1.6 - 2.2 million/mm.^{3} or +26%: Bondar, 1.3 - 2.7 million/mm.^{3} or +52%). Anthony (1961), on the other hand, obtained results which indicated a decrease in erythrocyte number with increasing acclimation temperature (2.0 - 1.8 million/mm.^{3} or a 10% decrease in erythrocyte number).

The data obtained in this experiment, then, indicate that the temperature dependent changes reported by the above authors must take place at some period later than ten days following transfer. The reasoning behind this statement stems from the fact that no changes were observed over the 240 hour period in which the experimental animals of this study were subjected to 30°C.

In summary, the results obtained disagree with those reported by Bondar (1957) and Spoor (1951), but are in agreement with those of Anthony (1961).

b. Haematocrit Determinations. Values for haematocrit determinations (Figure 5) carried out on the 20°C. control group, although generally lower than those reported by Anthony (1961) for fish held close to this temperature, fall well within the range of values recorded for several species of teleost fishes (T.T.III, Figure 5).

In the present study, fish transferred to 30°C. show a slight decrease in haematocrit during the 240 hour interval. The values indicated by the regression line in Figure 5 are close to those noted by Anthony (1961) for fishes acclimated to the same temperature. These data suggest that no significant change in haematocrit could be expected even if the animals were maintained at the temperature for a period exceeding the 10 day test interval. The reasoning for this conclusion becomes apparent when consideration is given to the fact that Anthony (1961) noted a low of 31% in haematocrit determinations in fish fully acclimated to 30°C. In the present study this value is reached immediately following transfer of the animals to 30°C.

c. Mean Corpuscular Volume Estimations. A comparison of results obtained in this study and those of other investigations can be made by noting Text Table I and Figure 6.

As compared to the observations of Anthony (1961) and those of other researchers (see Text Table I), the estimations of the mean corpuscular volume for the 20°C. control group are rather low. The largest values are those obtained by Field, Elvehjem, and Juday (1943) on Cyprinus carpio (311 u^3) and Salvelinus fontinalis (314 u^3).

Mean corpuscular volume estimations for the 240 hour period in which the experimental animals were subjected to a temperature of 30°C. are, as indicated by the regression line, considerably lower than those of Anthony's 30°C. group (Anthony, 1961). Furthermore, in the present study no apparent difference was noted between the control and

experimental groups. This differs from the observation of Anthony (1961) who noted an increase in the mean corpuscular volume with increased acclimation temperature. The amount of this increase was slight with the values at 5°-6°C. (172-181 u^3) increasing only to 176-219 u^3 in fish acclimated to 26° and 30°C. The increase in mean corpuscular volume coincided with a decrease in erythrocyte number and resulted in no net change in total erythrocyte volume.

d. Total Blood Iron. Text Table IV and Figure 7 provide comparison between the results obtained in this study and those of previous investigations. Total blood iron, as recorded in Figure 7, may be converted to gm.% haemoglobin, assuming that the haemoglobin of fish has the same molecular weight as that of humans, by the following equation (Natelson, 1961):

Mg. Fe/100 ml. X 0.294 = Gm. % Haemoglobin

Using the above conversion factor, it will be noted that the results obtained for the 20°C. control animals fall within the expected range of values observed by Black (1954) and Klawe et. al. (1963). However, a considerable difference is seen when these values are compared with the results of Bondar (1957). In this latter study, the author recorded values of 3.8 - 4.9 gm. % haemoglobin.

As noted previously, the 30°C. experimental group tended to show a decrease in total blood iron. However, this decrease amounted to only 5 mgm.%(-14%). These observations contrast with those of Bondar (1957) who noted a progressive increase (3.8 - 4.9 gm.%) in haemoglobin

content for 21 days following transfer of the animals from 9.5°C. to 16.5°C. The trend toward decreased total iron, indicated in Figure 7, substantiates the results obtained by Anthony (1961) and is in agreement with the general conclusion of that author, i.e., cold water species (e.g., trout) and warm water species (e.g. bullhead) have differing levels of haemoglobin content. The haemoglobin content of warm water species is lower than that of cold water species.

e. Mean Corpuscular Iron Content. The author is aware of no comparable estimates of mean corpuscular iron content.

Since the iron determinations were made using the total blood iron bethod, one would expect a decrease in mean corpuscular iron content comparable to that recorded for total blood iron. As previously noted, this slight decrease does occur, but to such a small degree that no significant difference exists between the 20°C. control and the 30°C. experimental groups.

f. White Cell Counts. As illustrated by Figure 9, values for leukocyte numbers are comparable to those observed by Field, Elvehjem, and Juday (1943) in Cyprinus carpio and Salvelinus fontinalis. These authors noted a mean of 3.675 thousand/mm^3 for the former and 3.910 thousand/$mm.^3$ for the latter species. The extremely high white cell counts (20,000-150,000/mm^3) noted by Lagler, Baradach, and Miller (1962) may possibly be explained on the basis of leukocyte identification, since the sum of the leukocytes and haemocytoblasts at any particular

interval would fall within the range noted by these authors.

g. General Discussion of Haematological Aspects. The data obtained in this study indicate that little, if any change occurs in the various haematological parameters measured when fish were transferred from a lower to a higher temperature. Such observations would appear to be in disagreement with oft-made physiological observations that fish transferred to higher environmental temperatures undergo a substantial increase in oxygen consumption and metabolic rate. Thus one might expect blood changes to occur which would facilitate oxygen uptake. The apparent discrepancy of the results obtained in this study and that of Anthony (1961) may find explanation in the recent work of Steen and Kruysse (1964).

Steen and Kruysse (1964), in their study of the respiratory function of teleostean gills, noted that blood which entered the gill filament could follow three possible paths to the efferent artery: (1) by way of the respiratory lamellae, (2) by way of a non-respiratory central lymphatic compartment, (3) by direct route from the afferent to efferent artery. In the presence of adrenaline, blood passed solely through the respiratory lamellae, while in the presence of acetylcholine blood circulated through the non-respiratory central compartments and directly from the afferent to efferent artery at the tip of the filament. Thus, this latter route avoided the respiratory lamellae.

In further studying this phenomenon the authors injected adrenaline into the ventral aorta of the common eel, Anguilla vulgaris, and

examined the oxygen tension and oxygen content of the arterial and venous blood. The results obtained indicated a considerable increase in the oxygen tension and oxygen content of arterial blood, while the venous blood exhibited a decrease. Furthermore, it was noted that the arterial blood pressure increased upon administration of adrenaline. Thus it appears possible that oxygen uptake may be increased through hormonally controlled variations in circulation pattern and blood pressure.

Of particular bearing on this problem is the work of Mahon, Hoar, and Tabata (1962). These investigators carried out a study on histological variations occurring in the adrenal tissues of goldfish transferred to higher temperatures or injected with ACTH. The ("medullary?") chromaffin cells showed considerable change when the animals were subjected to either of the above experimental conditions. When the animals were injected with ACTH, the cells ". . . frequently appeared more numerous and were often grouped into large vacuolated spaces in the cytoplasm. In some cases, particulated chrome substance seemed to spill onto the adjacent tissues." It was also found that ACTH did not produce an exhausted picture in the interrenal (cortical) cells. However, exposure to high temperatures had a marked effect on the cytoplasm and nucleus of these cells.

The results of these investigations appear, therefore, to indicate that when fish are exposed to increased environmental temperatures there is an increase in the secretion of adrenaline. This increase in adrenaline would tend to stop the flow of blood through the non-respiratory lymphatic compartments and the direct path from afferent to efferent artery

through the filamentary tip. Blood flow would, therefore, be restricted to the respiratory lamellae. Thus, with no increase in red blood cell number or haemoglobin content the animal could, nevertheless, increase the rate of oxygen uptake to meet its heightened metabolic demand.

2. Haemoglobin Polymorphism.

The author is aware of no studies in which changes in haemoglobin polymorphs are related to changes in environmental temperature. Therefore, it is not possible to make a comparison of results obtained to those of other investigators.

The appearance of fraction A_1, as indicated by Figures 11, 12, and 13 and Text Table XV, is in all probability due either to a change in charge or structure of the globin part of the A_2 fraction obtained in the $5^{o}C$. group.

Since Steen and Kruysse (1964) observed that the pH of the blood increased upon injection of adrenaline, it may well be that there is a possible relationship between the pH level of the blood and the occurrence of fraction A_2.

Any theory regarding the functional appearance of fraction A_1 would be purely conjectural; however, the knowledge that different haemoglobin polymorphs possess different affinities for oxygen suggests a possible explanation.

Since adrenaline-controlled variations in blood pH are known to occur, and since variations in blood pH have a considerable effect on the oxygen combining capacities of some haemoglobin polymorphs and no

effect on others (Hashimoto and Matsuura, 1959) the following explanation is possible. With increased temperatures of acclimation there would occur an increase in osygen consumption and metabolic rate, along with an increased secretion of adrenaline. The pH of the blood would be slightly higher as a result of the greater amount of adrenaline secreted. Increased pH of the blood may tend to inhibit the formation of fraction A_2 and encourage the formation of A_1. The oxygen combining capacity of A_2 would tend to decrease with increasing pH, while that of A_1 would tend to increase.

In summary, the results of this study, while differing in detail, agree with those of Anthony (1961) in that they suggest no change in erythrocyte number or haemoglobin content following transfer of the animal to increased environmental temperature. The absence of such compensatory change in the blood picture appears strange in view of the heightened metabolic demand of these animals at higher environmental temperatures. The work of Steen and Kruysse (1964) along with that of Mahon, et. al. (1962) suggests an explanation of this apparent enigmatic situation, namely, that compensation is achieved by a hormonally induced increase in blood flow through the respiratory lamellae rather than by increased haemoglobin and erythrocyte number.

SUMMARY AND CONCLUSIONS

1. The blood of fish acclimated to 20°C. and transferred to 30°C., although exhibiting slight trends and possible cycling phenomena, showed little change in the various haematological parameters investigated (erythrocyte number, haematocrit, mean corpuscular volume, total blood iron, mean corpuscular iron content, and leukocyte number).

2. Since it is known that different haemoglobin polymorphs possess different affinities for oxygen, a possible adaptive response to temperature might be evident in the variation of the number of haemoglobin polymorphs. Two haemoglobin polymorphs were apparent in fishes acclimated to 5°C. while those acclimated to 12°, 20°, and 30°C. showed three distinct polymorphs. The appearance of the third polymorph (A_1) may be an adaptive response associated with increased oxygen uptake at the higher acclimation temperatures.

3. It is suggested that goldfish subjected to increased environmental temperature increase oxygen uptake through hormonally controlled variations of the circulatory pathway of blood through the gill.

4. Since little variation occurs, the haematological determinations carried out are not reliable criteria for determining rates of acclimation to temperature change.

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A P P E N D I X

ABBREVIATIONS USED IN THIS SECTION

FISH WT. (gm.): . . . . . fish weight in grams

RBC (10^6): . . . . . red blood cells in millions/$mm.^3$

MCV(u^3): . . . . . mean corpuscular erythrocyte volume in cubic microns

MCIC ($Mgm.x10^{-11}$): . . . . . mean corpuscular iron content in milligrams x 10^{-11}

C_1: . . . . . haemoglobin polymorph migrating towards cathode

A_1: . . . . . haemoglobin polymorph migrating shortest distance toward anode

A_2: . . . . . haemoglobin polymorph migrating greatest distance toward anode

St. Dev.: . . . . . Standard Deviation

St. Err.: . . . . . Standard Error

APPENDIX TABLE I

RAW DATA FROM HAEMATOLOGICAL STUDY

FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
20^oC. Control Group					
6.5	1.66	- - -	- - -	28.2	17.0
10.0	2.16	- - -	- - -	24.5	13.3
7.0	2.04	- - -	- - -	42.0	20.6
9.3	2.08	- - -	- - -	33.8	18.6
8.5	2.55	- - -	- - -	41.6	16.3
9.3	1.78	- - -	- - -	33.0	18.5
10.4	2.45	43.2	176.0	- - -	- - -
9.4	3.04	25.5	826.0	- - -	- - -
10.2	2.04	26.5	127.0	- - -	- - -
14.5	1.94	25.3	130.0	42.5	21.8
7.5	2.02	26.7	132.0	36.4	17.9
9.6	2.38	26.0	109.0	29.3	12.3
5.8	1.59	24.5	154.0	31.8	20.0
12.5	2.11	26.2	124.0	39.2	18.5
12.2	1.29	18.9	147.0	27.5	21.3
9.2	2.28	23.7	126.0	36.3	15.9
8.0	2.53	31.7	125.0	41.2	16.2
14.3	2.49	- - -	- - -	45.3	18.3
11.2	2.37	23.8	100.0	32.0	13.5
11.0	2.32	33.6	145.0	40.5	17.5
9.1	1.82	28.9	146.0	35.2	19.3
11.0	1.77	25.5	144.0	35.2	19.9
7.0	1.96	23.6	120.0	39.0	19.9
12.3	- - -	31.8	- - -	40.8	- - -
- - -	- - -	- - -	- - -	- - -	- - -
10.5	- - -	34.9	- - -	- - -	- - -
10.2	1.78	24.0	135.0	20.8	11.7
10.7	1.95	26.5	136.0	26.1	13.2
12.0	1.98	27.9	141.0	31.5	15.9
9.5	1.91	23.5	123.0	26.0	13.6
14.2	1.68	22.8	136.0	22.4	12.7
8.9	1.96	27.6	141.0	20.0	10.2
- - -	- - -	- - -	- - -	- - -	- - -
12.8	2.00	26.9	135.0	44.5	22.0
7.6	1.90	- - -	- - -	- - -	- - -
15.0	2.68	43.9	164.0	36.1	13.5
10.7	1.96	31.0	158.0	48.0	24.5
6.2	- - -	31.6	- - -	- - -	- - -

Cont'd. . . . . .

APPENDIX TABLE I (CONT'D.)

	FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
	9.5	- - -	31.9	- - -	- - -	- - -
	18.6	2.58	41.2	160.0	46.0	17.9
	12.8	2.32	35.5	153.0	40.3	17.3
	10.5	2.78	31.0	130.0	43.6	15.7
	8.6	- - -	23.4	- - -	- - -	- - -
MEAN	10.3	2.12	29.6	163.0	35.7	17.0
ST. DEV.		0.11	5.9	30.0	7.7	3.5
ST. ERR.		0.02	1.0	6.0	1.4	0.6
30°C. Test Group -- 12 Hours						
	61.0	2.46	32.5	132.0	30.1	12.6
	14.8	2.28	37.1	163.0	29.0	12.7
	19.7	2.62	40.7	155.0	34.4	13.1
	83.3	2.10	32.9	157.0	36.5	17.4
	56.6	1.83	19.5	105.0	28.8	15.7
	14.8	1.96	32.5	166.0	32.8	16.7
	20.2	2.05	29.5	142.0	30.3	14.8
	18.1	2.22	34.2	153.0	34.2	15.4
	26.7	1.92	31.0	161.0	29.4	15.3
MEAN	35.0	2.15	32.2	148.0	31.7	14.9
ST. DEV.		0.24	5.5	18.3	2.5	1.6
ST. ERR.		0.09	1.8	6.2	0.8	0.5
30°C. Test Group -- 24 Hours						
	30.6	1.85	25.0	135.0	24.2	13.1
	17.2	2.30	32.6	142.0	39.1	17.0
	21.0	2.54	33.8	131.0	38.8	15.3
	94.1	1.61	25.2	156.0	22.9	14.2
	89.8	1.76	24.3	138.0	24.5	13.9
	20.0	2.82	28.4	100.0	24.5	8.7
	28.1	2.01	25.2	125.0	31.1	15.5
	42.0	2.42	30.1	125.0	35.2	14.5
	54.2	2.10	31.2	148.0	32.0	15.2
MEAN	49.6	2.15	28.4	133.0	30.3	14.2
ST. DEV.		0.24	2.3	15.2	6.1	2.2
ST. ERR.		0.03	0.8	5.1	2.0	0.8

Cont'd. . . . . .

APPENDIX TABLE I (CONT'D.)

	FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
30°C. Test Group -- 36 Hours						
	64.0	1.89	30.8	163.0	39.2	20.6
	16.4	2.33	37.9	163.0	54.9	23.5
	17.0	2.47	34.3	139.0	45.0	18.2
	94.8	1.98	27.9	141.0	32.6	16.5
	80.5	1.91	28.0	147.0	34.5	18.0
	13.5	2.26	29.5	131.0	28.8	12.7
	17.4	2.07	29.0	140.0	33.4	16.0
	52.3	2.32	32.1	138.0	48.2	20.7
	19.8	2.41	36.4	150.0	47.0	19.5
MEAN	41.7	2.18	31.8	146.0	40.4	18.4
ST. DEV.		0.21	3.5	10.0	8.3	3.0
ST. ERR.		0.07	1.2	3.3	2.8	1.0
30°C. Test Group -- 48 Hours						
	62.0	2.57	33.9	132.0	41.0	16.0
	19.0	1.60	21.0	131.0	22.2	13.9
	18.0	2.86	35.3	123.0	41.0	14.3
	70.0	1.78	19.6	110.0	16.3	9.3
	17.5	2.47	38.8	141.0	39.6	16.0
	95.8	2.32	33.6	145.0	34.5	14.8
	81.6	2.05	29.5	144.0	33.2	16.2
	20.2	1.91	27.2	142.0	29.1	15.3
	44.1	2.41	34.6	144.0	38.2	15.7
MEAN	47.5	2.22	30.4	135.0	32.8	14.6
ST. DEV.		0.39	5.3	9.1	9.1	2.0
ST. ERR.		0.13	1.8	3.0	3.0	0.7
30°C. Test Group -- 60 Hours						
	69.5	1.65	25.9	157.0	32.1	19.5
	81.9	1.38	19.8	144.0	22.0	15.9
	17.0	2.52	33.0	131.0	48.0	19.0
	13.2	1.99	27.3	137.0	36.2	18.1
	16.8	1.71	22.3	131.0	32.1	18.9
	12.9	1.57	19.7	126.0	32.4	20.6
	82.0	1.85	28.1	152.0	31.6	17.1
	57.1	1.72	23.4	125.0	29.0	16.8
	17.3	1.89	26.7	141.0	32.6	17.3
MEAN	40.8	1.81	25.1	138.0	32.8	18.1
ST. DEV.		0.32	3.8	10.7	6.9	1.5
ST. ERR.		0.11	1.3	3.6	2.3	0.5

Cont'd. . . . . .

APPENDIX TABLE I (CONT'D.)

	FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
30°C. Test Group -- 72 Hours						
	65.5	2.24	38.0	170.0	37.0	16.5
	13.7	2.14	33.3	155.0	42.9	20.0
	19.2	1.99	35.0	176.0	33.6	17.0
	70.5	1.86	22.6	121.0	30.8	16.5
	87.0	1.88	24.0	128.0	30.8	16.3
	18.9	2.16	17.4	81.0	41.6	15.7
	14.0	1.97	26.9	136.0	31.0	18.9
	13.1	2.51	28.9	112.0	29.5	12.2
	15.5	2.75	35.8	130.0	32.1	11.7
MEAN	35.2	2.16	29.0	134.0	34.4	16.1
ST. DEV.		0.28	6.7	27.5	4.5	2.6
ST. ERR.		0.09	2.2	9.2	1.5	0.9
30°C. Test Group -- 84 Hours						
	10.6	1.79	23.0	128.0	33.1	19.6
	34.6	2.11	31.0	147.0	34.2	16.1
	50.1	2.39	51.4	152.0	35.3	14.8
	81.1	1.88	32.5	173.0	44.2	23.5
	72.3	2.49	39.8	164.0	38.0	15.3
	17.6	1.91	29.2	153.0	29.1	15.3
	22.2	2.52	41.6	166.0	41.0	16.3
	19.7	2.40	39.2	163.0	37.5	15.6
	65.6	2.24	37.0	165.0	39.2	17.5
MEAN	41.5	2.19	36.1	156.0	36.8	17.1
ST. DEV.		0.26	7.8	12.8	4.3	2.7
ST. ERR.		0.09	2.6	4.3	1.4	0.9
30°C. Test Group -- 96 Hours						
	64.0	1.80	40.0	222.0	52.1	28.0
	17.2	2.49	34.1	137.0	43.8	35.0
	13.7	2.44	30.5	125.0	38.0	15.6
	8.4	1.96	32.2	165.0	40.2	15.0
	14.4	2.70	37.2	138.0	45.6	16.9
	25.0	2.42	34.0	125.0	41.2	17.0
	76.4	2.38	29.2	123.0	37.6	15.8
	32.1	2.62	38.4	146.0	40.1	15.3
	20.0	2.21	35.1	159.0	39.1	17.7
MEAN	30.1	2.33	34.5	149.0	42.0	19.7
ST. DEV.		0.28	3.3	29.4	4.3	7.0
ST. ERR.		0.09	1.1	9.8	1.4	2.3

Cont'd. . . . . .

APPENDIX TABLE I (CONT'D.)

	FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
30°C. Test Group -- 120 Hours						
	64.7	2.13	25.0	117.0	34.3	16.1
	13.0	1.41	20.4	147.0	22.6	16.0
	16.4	2.28	27.7	123.0	28.6	12.6
	26.3	2.35	30.1	128.0	35.4	15.1
	13.6	2.39	28.1	118.0	130.1	12.7
	73.2	1.94	24.2	125.0	26.2	13.5
	18.6	2.52	32.1	127.0	35.1	13.5
	29.5	2.45	29.4	119.0	32.4	14.0
MEAN	31.9	2.16	27.1	125.5	27.1	14.6
ST. DEV.		0.39	4.4	8.9	5.2	1.4
ST. ERR.		0.13	1.5	3.2	1.7	0.5
30°C. Test Group -- 144 Hours						
	14.6	2.67	34.3	128.0	51.6	19.3
	16.5	2.56	20.4	81.0	58.9	22.8
	77.0	2.18	27.2	125.0	33.8	15.5
	12.1	2.24	29.7	133.0	37.7	16.9
	72.6	2.58	27.5	107.0	30.1	11.7
	41.0	1.98	26.5	134.0	40.2	20.3
	19.6	2.10	19.1	91.0	34.2	16.3
	65.1	2.32	30.2	130.0	36.1	15.6
	46.6	2.50	25.0	100.0	42.1	16.9
MEAN	40.5	2.35	26.7	114.0	40.5	17.3
ST. DEV.		0.23	4.4	18.7	8.7	3.0
ST. ERR.		0.07	1.5	6.4	2.9	1.0
30°C. Test Group -- 168 Hours						
	11.1	1.87	25.3	136.0	28.3	15.0
	17.5	2.07	29.3	142.0	25.2	12.1
	13.0	1.96	26.1	134.0	24.2	12.3
	74.5	2.42	31.2	129.0	30.1	12.3
	80.0	1.65	20.4	123.0	23.1	14.0
	31.2	2.01	25.2	125.0	25.2	12.5
	16.6	2.09	28.1	135.0	24.7	11.9
	17.1	2.70	34.0	127.0	31.2	11.5
	29.9	1.80	22.1	123.0	20.0	11.1
MEAN	32.3	2.06	26.9	130.0	25.7	12.5
ST. DEV.		0.30	4.0	6.4	3.3	1.1
ST. ERR.		0.10	1.3	2.1	1.1	0.4

APPENDIX TABLE I (CONT'D.)

	FISH WT. (gm.)	RBC(10^6)	HAEMATOCRIT	MCV(u^3)	TOTAL BLOOD Fe (Mgm. %)	MCIC (Mgm.x10^{-11})
30°C. Test Group -- 192 Hours						
	14.1	2.03	25.5	126.0	22.1	10.9
	16.4	2.24	32.5	145.0	30.2	13.5
	13.7	2.09	33.5	160.0	25.0	12.0
	80.2	1.92	22.1	115.0	23.2	12.1
	48.6	2.40	35.0	145.0	29.5	12.1
	35.2	2.21	33.1	220.0	31.2	14.1
	17.0	2.22	29.5	133.0	28.6	21.5
	75.1	2.43	35.0	144.0	22.3	9.3
	52.1	1.80	23.8	132.0	21.1	11.7
MEAN	38.7	2.15	30.0	147.0	25.9	13.0
ST. DEV.		0.19	4.4	28.7	3.7	3.3
ST. ERR.		0.06	1.5	9.6	1.2	1.1
30°C. Test Group -- 216 Hours						
	16.4	1.50	23.8	159.0	20.0	13.3
	15.1	2.20	25.8	118.0	23.1	10.5
	21.2	1.82	25.9	143.0	24.0	13.2
	64.5	2.10	28.2	134.0	26.2	12.5
	72.2	1.50	21.1	140.0	20.1	13.4
	19.9	2.38	30.0	148.0	29.8	12.5
	18.6	2.01	24.2	120.0	22.0	10.9
	50.1	2.45	29.9	122.0	19.9	8.3
	28.0	1.70	20.3	120.0	24.1	20.2
MEAN	34.0	2.16	25.5	134.0	23.2	12.8
ST. DEV.		0.59	3.3	13.9	3.1	3.1
ST. ERR.		0.20	1.1	4.6	1.0	1.0
30°C. Test Group -- 240 Hours						
	28.6	2.21	38.5	175.0	37.1	16.8
	37.9	3.19	45.0	141.0	43.2	13.6
	30.3	1.58	31.8	201.0	35.1	22.2
	76.6	1.62	32.1	198.0	29.0	17.9
	32.2	1.60	25.5	160.0	37.7	23.5
	49.5	2.81	35.4	125.0	37.2	13.2
	17.7	1.88	33.6	178.0	34.6	18.5
	15.0	2.85	39.2	138.0	40.1	14.1
	82.2	1.71	29.9	175.0	29.9	17.5
MEAN	41.1	2.16	34.6	166.0	36.0	16.4
ST. DEV.		.59	5.4	25.1	4.3	3.4
ST. ERR.		.20	1.8	8.4	1.4	1.1

APPENDIX TABLE II

RAW DATA FROM LEUKOCYTE AND
HAEMOCYTOBLAST COUNTS

FISH WT. (gm.)	LEUKOCYTES/mm^3	HAEMOCYTOBLASTS/mm^3
20°C. Control Group		
12.6	2,400	38,000
10.4	4,000	28,500
8.2	2,500	55,500
20.1	2,000	38,500
26.7	2,000	34,500
81.0	3,500	32,500
12.5	2,000	31,000
18.8	3,500	42,000
15.5	2,000	64,000
72.0	2,500	30,500
80.0	4,000	39,000
21.8	3,000	54,000
72.2	4,000	49,500
18.6	3,000	40,000
17.2	5,000	43,500
15.8	4,000	51,500
10.0	4,500	41,000
12.7	2,500	37,500
73.3	2,500	41,500
45.7	3,000	47,500
38.0	4,000	51,000
29.2	3,500	55,000
18.0	4,000	51,500
36.1	2,500	46,000
MEAN 31.9	3,166	41,975
ST. DEV.	863	9,100
ST. ERR.	179	1,895
30°C. Test Group -- 60 Hours		
12.9	1,500	41,500
69.5	2,000	52,500
81.9	2,500	33,500
17.0	3,000	55,000'
13.2	3,500	54,500
16.8	2,500	43,500
22.4	2,500	40,000
75.00	3,500	52,000

Cont'd. . . . . .

APPENDIX TABLE II (CONT'D.)

	FISH WT. (gm.)	LEUKOCYTES/mm^3	HAEMOCYTOBLASTS/mm^3
	33.6	3,000	33,500
	63.0	2,500	30,000
MEAN	40.5	2,650	43,600
ST. DEV.		625	9,400
ST. ERR.		197	2,965
$30^{\circ}C$. Test Group -- 72 Hours			
	65.5	2,000	31,500
	13.7	1,500	24,000
	19.2	2,500	42,500
	70.5	3,000	51,000
	87.0	1,500	23,500
	18.9	1,500	29,000
	24.4	1,500	25,000
	81.2	3,500	46,000
	46.7	2,000	33,000
	20.0	2,500	41,500
MEAN	44.7	2,150	34,700
ST. DEV.		707	9,540
ST. ERR.		223	3,009
$30^{\circ}C$. Test Group -- 84 Hours			
	10.6	2,000	34,000
	34.6	2,500	51,500
	50.1	1,500	29,500
	81.1	2,500	32,000
	24.0	2,000	45,000
	27.1	3,000	56,500
	55.4	2,500	44,000
	72.1	2,000	38,500
	20.2	2,500	36,000
	36.0	1,500	28,000
MEAN	41.1	2,200	39,500
ST. DEV.		748	9,500
ST. ERR.		235	1,996
$30^{\circ}C$. Test Group -- 96 Hours			
	17.2	2,500	42,500
	64.0	1,500	36,000
	13.7	1,500	38,500

Cont'd. . . . . .

APPENDIX TABLE II (CONT'D.)

	FISH WT. (gm.)	LEUKOCYTES/mm^3	HAEMOCYTOBLASTS/mm^3
	8.4	2,500	35,000
	17.2	3,000	28,500
	19.6	3,000	29,000
	48.1	1,500	34,000
	65.0	2,500	28,500
	64.2	3,000	32,500
	- - -	- - -	- - -
MEAN	35.2	2,333	33,833
ST. DEV.		666	4,470
ST. ERR.		210	1,410
30°C. Test Group -- 120 Hours			
	64.7	3,000	62,000
	13.0	3,000	58,000
	16.4	5,000	43,500
	26.3	3,500	35,500
	18.6	2,000	34,500
	19.0	1,500	35,000
	21.4	4,500	40,000
	72.6	4,000	45,000
	80.0	3,000	55,000
	43.3	2,000	50,000
MEAN	37.5	3,150	45,850
ST. DEV.		1,096	10,000
ST. ERR.		345	3,154
30°C. Test Group -- 144 Hours			
	14.6	2,500	74,000
	16.5	2,000	57,500
	77.0	1,500	35,500
	12.1	2,500	54,500
	14.2	4,000	46,000
	24.5	5,000	37,500
	43.4	4,500	40,000
	18.4	3,000	52,000
	73.2	3,500	40,000
	45.6	2,500	55,000
MEAN	40.0	3,100	49,200
ST. DEV.		1,096	11,420
ST. ERR.		345	3,602

Cont'd. . . . . .

APPENDIX TABLE II (CONT'D.)

FISH WT. (gm.)	LEUKOCYTES/mm^3	HAEMOCYTOBLASTS/mm^3
30^oC. Test Group -- 168 Hours		
13.0	2,000	38,500
11.1	4,000	42,000
17.5	4,500	36,500
74.5	2,500	41,000
18.6	2,500	58,500
71.4	1,500	50,000
32.5	3,000	40,000
19.6	3,500	35,000
67.5	4,000	45,000
28.1	2,000	36,000
MEAN 35.3	2,950	42,250
ST. DEV.	1,010	6,600
ST. ERR.	318	2,100
30^oC. Test Group -- 192 Hours		
14.1	4,000	59,000
16.4	3,000	67,500
13.7	3,000	56,000
80.2	2,500	44,500
48.6	1,500	27,500
21.5	1,500	33,500
16.7	2,000	40,000
72.1	3,500	50,000
46.6	4,000	60,000
- - -	- - -	- - -
MEAN 36.6	2,777	48,866
ST. DEV.	972	13,600
ST. ERR.	307	4,533
30^oC. Test Group -- 216 Hours		
16.4	4,000	31,000
15.1	5,000	31,500
21.2	3,500	57,500
64.5	5,000	65,000
72.3	8,400	50,000
45.1	2,000	29,500
63.2	5,000	70,000
21.4	4,500	55,000
46.3	7,000	45,000

Cont'd. . . . . .

APPENDIX TABLE II (CONT'D.)

	FISH WT. (gm.)	LEUKOCYTES/mm^3	HAEMOCYTOBLASTS/mm^3
	19.8	3,500	50,000
MEAN	38.5	4,790	48,450
ST. DEV.		1,845	14,400
ST. ERR.		582	4,542
$30^{\circ}C$. Test Group -- 240 Hours			
	28.6	3,000	40,500
	37.9	2,000	49,500
	30.3	2,500	50,000
	76.6	3,500	56,000
	29.2	3,000	54,000
	53.4	4,000	45,500
	26.3	3,500	45,000
	72.4	4,500	42,500
	37.7	2,000	43,000
	61.2	4,000	37,500
MEAN	45.4	3,200	46,350
ST. DEV.		855	5,910
ST. ERR.		269	1,864

APPENDIX TABLE III

RESULTS OF ELECTROPHORETIC STUDY,
SHOWING RELATIVE ABUNDANCE AND
MIGRATION DISTANCE OF HAEMOGLOBIN POLYMORPHS

	FISH WT. (gm.)	RELATIVE ABUNDANCE			MIGRATION DISTANCE		
		C_1	A_1	A_2	C_1	A_1	A_2
5°C. Acclimated Group							
	46.2	64.0		36.0	12.0		15.0
	29.0	57.5		42.5	11.0		15.0
	31.3	54.0		46.0	10.0		18.0
	48.2	53.0		47.0	12.0		18.0
	20.6	52.5		46.5	10.0		16.0
	71.0	68.2		31.8	10.0		14.0
	38.1	56.0		49.0	9.0		17.0
	29.8	71.0		29.0	15.0		14.0
	56.5	58.5		41.5	11.0		14.0
	80.0	71.3		28.7	12.0		14.0
	31.2	76.0		25.0	11.0		19.0
	18.6	62.2		37.8	10.0		18.0
	27.0	59.4		40.6	14.0		18.0
	15.9	62.8		37.2	12.0		19.0
	29.6	66.6		33.3	15.0		17.0
	82.1	70.2		29.8	13.0		20.0
	43.4	66.0		34.0	10.0		16.0
	36.0	60.8		39.2	12.0		18.0
	23.1	68.7		31.3	11.0		15.0
	42.6	56.7		42.3	12.0		18.0
	18.4	66.6		33.3	10.0		16.0
MEAN	39.0	59.8		37.2	12.0		16.0
ST. DEV.		7.2		6.7	1.7		1.9
ST. ERR.		1.6		1.5	0.4		0.4
12°C. Acclimated Group							
	18.6	61.7	10.9	27.4	10.0	10.0	15.0
	46.0	74.4	7.7	16.9	10.0	10.0	15.0
	21.2	72.5	7.9	19.8	10.0	11.0	17.0
	73.4	64.7	7.5	27.9	11.0	8.0	14.0
	80.0	61.5	14.6	23.9	12.0	10.0	16.0
	51.4	60.3	13.6	26.3	10.0	9.0	15.0
	16.6	57.6	8.3	34.3	10.0	10.0	17.0

Cont'd. . . . . .

APPENDIX TABLE III (CONT'D.)

	FISH WT. (gm.)	RELATIVE ABUNDANCE			MIGRATION DISTANCE		
		C_1	A_1	A_2	C_1	A_1	A_2
	18.0	59.1	13.2	27.7	10.0	8.0	13.0
	70.1	62.3	9.6	28.1	12.0	9.0	14.0
	65.4	65.5	10.2	24.3	13.0	10.0	17.0
	34.6	70.2	8.3	21.5	19.0	10.0	16.0
	21.0	64.2	11.0	24.8	11.0	8.0	15.0
	46.6	70.1	12.5	17.4	10.0	9.0	15.0
	32.8	58.6	13.0	28.4	12.0	9.0	13.0
	19.1	57.2	13.3	29.5	10.0	9.0	14.0
	22.0	64.7	14.1	21.2	11.0	8.0	16.0
	41.2	70.3	8.6	21.1	13.0	10.0	17.0
	17.8	72.0	9.2	18.8	9.0	12.0	15.0
	75.1	58.4	8.5	33.1	12.0	8.0	14.0
	54.0	60.3	10.3	29.4	10.0	8.0	15.0
	28.9	59.1	12.1	28.8	11.0	9.0	16.0
MEAN	40.7	64.2	10.5	25.7	11.0	9.0	15.0
ST. DEV.		5.4	2.3	4.7	2.0	1.1	1.3
ST. ERR.		1.2	0.5	1.0	0.4	0.2	0.3
20°C. Acclimated Group							
	12.6	71.0	15.9	13.3	16.0	10.0	15.0
	10.4	67.2	11.8	20.9	15.0	10.0	17.0
	8.2	68.0	10.9	21.2	14.0	8.0	15.0
	20.1	57.4	19.8	22.7	15.0	10.0	10.0
	26.7	41.4	26.0	32.5	14.0	8.0	14.0
	81.0	44.2	22.1	33.7	16.0	10.0	14.0
	12.5	40.4	18.7	41.0	17.0	10.0	13.0
	15.5	65.0	13.8	21.5	10.0	5.0	7.0
	72.0	78.0	9.6	12.0	10.0	7.0	7.0
	80.0	63.9	12.9	35.0	10.0	5.0	5.0
	- - -	- -	- -	- -	- -	- -	- -
	72.2	75.6	7.6	16.8	8.0	3.0	5.0
	18.6	68.2	14.4	17.5	12.0	8.0	8.0
	17.2	63.8	17.7	21.3	10.0	6.0	12.0
	15.8	75.5	11.6	13.0	10.0	5.0	5.0
	10.0	52.5	23.7	23.7	11.0	5.0	4.0
	12.7	65.5	25.3	9.4	9.0	5.0	4.0
	73.3	65.0	25.3	9.5	9.0	5.0	7.0

Cont'd. . . . . .

APPENDIX TABLE III (CONT'D.)

	FISH WT. (gm.)	RELATIVE ABUNDANCE			MIGRATION DISTANCE		
		C_1	A_1	A_2	C_1	A_1	A_2
	45.7	65.5	19.8	15.1	15.0	8.0	5.0
	38.0	64.5	19.5	16.1	13.0	6.0	5.0
	29.2	69.5	17.3	13.6	12.0	5.0	4.0
MEAN	33.6	63.0	16.6	20.4	11.0	7.0	8.0
ST. DEV.		10.5	5.6	8.7	3.0	2.2	4.4
ST. ERR.		2.3	1.2	1.9	0.7	0.5	1.0
30°C. Acclimated Group							
	12.6	78.5	8.6	12.8	12.0	10.0	15.0
	72.1	55.6	4.2	40.3	13.0	10.0	13.0
	8.7	46.2	9.3	44.6	13.0	12.0	15.0
	45.6	53.7	9.7	36.6	15.0	15.0	17.0
	62.2	68.5	6.9	24.7	15.0	10.0	12.0
	20.4	63.8	7.1	29.1	13.0	13.0	16.0
	25.6	60.1	4.6	35.1	14.0	14.0	17.0
	32.6	48.6	9.7	42.0	14.0	15.0	17.0
	74.1	49.5	13.9	36.5	13.0	13.0	17.0
	42.5	49.0	17.3	33.9	18.0	13.0	18.0
	61.4	41.2	12.4	46.0	15.0	10.0	18.0
	20.1	45.6	10.8	43.7	12.0	15.0	18.0
	41.0	61.0	13.3	25.8	18.0	10.0	15.0
	34.0	53.1	10.8	36.1	10.0	10.0	15.0
	34.5	- -	- -	- -	4.0	7.0	11.0
	24.1	48.8	26.2	24.8	7.0	7.0	15.0
	28.0	54.9	23.8	22.0	10.0	10.0	16.0
	48.6	49.1	20.6	30.3	8.0	10.0	20.0
	72.1	64.0	15.8	20.6	10.0	10.0	19.0
	33.4	54.9	19.5	25.7	7.0	14.0	20.0
	20.0	44.5	28.0	27.5	7.0	10.0	15.0
MEAN	38.7	54.5	13.6	31.9	11.0	11.0	15.0
ST. DEV.		8.9	6.8	8.9	3.7	2.4	2.6
ST. ERR.		2.0	1.5	2.0	0.8	0.5	0.6