SOME EFFECTS OF LETHAL AND SUBLETHAL THERMAL SHOCK ON BODY FLUID COMPOSITION AND DISTRIBUTION IN *Carassius auratus* L.

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by

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ABSTRACT

An investigation of variations in the composition and distribution of the body fluids of the goldfish, Carassius auratus L., previously acclimated to 20°C, during acclimation to 30°C, and following lethal heat shock (20°C to 36°C or 38°C) was undertaken. Fish exposed to abrupt sublethal thermal shock (20°C to 30°C) were killed at regular intervals up to 240 hours. Plasma and tissue samples were analysed for sodium, potassium and chloride content and the tissue only, for water content. Extracellular phase volume was estimated by the "chloride space" method, and the cellular phase volume and cellular potassium levels were determined. Fish exposed to lethal shock were killed at the "critical thermal maximum response point" and determinations of plasma chloride, tissue chloride, and tissue water content were made. Extracellular and cellular phase volumes were estimated.

Two effects of sublethal thermal shock were observed. The first, an increasing water permeability of the renal and branchial cell membranes, became apparent at the thirty-six hour sample period and continued until the 168 hour sample period. The second effect, an increased rate of active branchial and renal ion transport,

was not observed until the forty-eight hour period.

During acclimation of the fish to 30°C, both these effects were compensated. Active transport of chloride and sodium returned to control levels in the period between 48 and 84 hours exposure, and between 84 and 120 hours exposure respectively. The effect of heat shock on membrane permeability was of longer duration, and permeability did not return to the control level until the period between ten and twenty-one days.

With the exception of slight changes in plasma chloride, plasma potassium, and tissue sodium levels of the 30°C control group relative to those of the 20°C control group, acclimation could be classified as "complete compensation".

It was indicated that the imbalance observed within twenty-five minutes following lethal shock was the result of an increase in renal and branchial membrane permeability to water, and in part was an exaggeration of that found during the initial thirty-six hours following sublethal shock.

The changes in plasma chloride concentration following lethal shock were less than those withstood by steelhead trout during their adaptation to sea water; and the changes in tissue water content and distribution were less than those observed following sublethal shock in the present study. Water-electrolyte balance, therefore, did not appear to be the primary cause of heat death.

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While some of the biochemical changes accompanying acclimation are direct responses to temperature variation, other changes occur in response to variations in extracellular fluid composition resulting from variations in water-electrolyte metabolism induced by temperature change. However, relatively little is known of the effect of increased temperature upon extracellular fluid composition, although some of the effects of lethal and sublethal temperature changes have been investigated in goldfish and other species (Platner, 1950: Meyer, et al, 1956; Houston, 1962).

Accordingly, the present study was undertaken as an attempt to show variations in the body fluid distribution and composition of goldfish during acclimation to increased temperature, and during heat death. Goldfish, acclimated to 20°C, were subjected to sublethal temperature shock (20°C to 30°C) and sampled at regular intervals up to 240 hours after shock. Fish acclimated to 30°C were also sampled. Other goldfish, also acclimated to 20°C, were subjected to lethal thermal shock (20°C to 36°C-38°C) and sampled at the critical thermal response point.

Plasma and muscle tissue were analysed for chloride, sodium, and potassium concentration and muscle tissue water content. Extracellular and cellular phase volume and composition were estimated from the data thus obtained.

CHAPTER II

LITERATURE REVIEW

In this review thermal acclimation and ion regulation will be discussed.

I. THERMAL ACCLIMATION

1. Poikilothermic Regulation of Body Temperature

In general, while homeotherms tolerate a wide range of environmental temperature they can tolerate only a narrow range of internal temperature (Prosser, 1958). The ability of homeotherms to regulate body temperature within narrow limits is well established. Generally a body temperature of 37°C for mammals and 42°C for birds (Prosser and Brown, 1961) is maintained, regardless of seasonal or latitudinal temperature differences to which animals of the same or of different species are exposed (Scholander, et al, 1950; Krog and Monson, 1954). The range of environmental temperatures tolerated by a homeotherm is determined by the ability of the animal to maintain its body temperature constant when subjected to changing external temperatures. When the environmental temperature becomes so extreme that the animal can no longer regulate body temperature within narrow limits, it dies. However it is well established that the regulatory mechanisms

and thus the range of tolerated environmental temperature can be increased by the previous thermal experience of the animal (Sullivan, 1954; Prosser and Brown, 1961).

On the other hand, the body temperature of poikilotherms varies with the ambient temperature. As compared to homeotherms, poikilotherms tolerate a relatively narrow range of environmental temperature but a wider ranger of internal temperature (Prosser, 1958). Unlike homeotherms, poikilotherms do not survive extreme environmental temperatures because of the ability to regulate internal environmental temperature but, as will be discussed later, because of the ability to tolerate these extremes of external temperature. However, to a slight degree, poikilotherms do regulate body temperature. This regulation is of two types, behavioural and physiological, which will be discussed below.

A. Behavioural Regulation

Four aspects of behavioural temperature regulation will be discussed; construction of insulated abodes, absorption of radiant energy, preferred temperature selection, and control of environmental temperature.

(i) Construction of Insulated Abodes

Insulated abodes built by ants and termites prevent the escape of heat produced by the

activity of the animals. According to Schneirla (1954, cited in Prosser and Brown, 1961) temperature of a termite mound may exceed the outside temperature by 14 to 18 Centigrade degrees.

(ii) Absorption of Radiant Energy

The absorption of radiant energy enables some terrestrial poikilotherms to maintain a body temperature which is higher than that of the environment. The amount of energy absorbed is determined, in part, by the position of the body relative to the direction of the sun's rays; long axis of the body perpendicular for maximum absorption, and parallel for minimum absorption. Thus the extent to which body temperature exceeds environmental temperature can be somewhat controlled by the animal (Prosser and Brown, 1961). Animals which utilize this regulatory mechanism include the grasshopper Melanoplus (Pepper and Hastings, 1952), the desert locust Schistocerca (Fraenkel, 1929, cited in Prosser, 1950), and snakes and lizards (Cowles, 1947).

(iii) Selection of Preferred Environmental Temperature

Poikilotherms control body temperature by selecting a preferred environmental temperature when a range of temperature is available. Fry (1947) defines "preferred temperature" as the "region, in an infinite range of temperature, at which a given

population will congregate with more or less precision". Preferred temperature selection has been observed among bees, ants, locusts, fish (including Carassius auratus, the species presently under study), amphibians and reptiles (Prosser and Brown, 1961).

Temperatures, if acting alone, will determine the distribution of fish in a laboratory apparatus (Ferguson, 1958). However, factors such as diet, stage in life cycle, light, humidity and geographical location also affect distribution. For example, the preferred range of feeding housefly larvae is 15°C to 33°C while that of pupating larvae is 8°C to 20°C (Hafez, 1953). Diurnal species tend to select higher temperatures than do nocturnal species, and selected temperature tends to be higher in moist than in dry air (Prosser and Brown, 1961). Tribolium castaneum selects a higher temperature in a gradient than does T. confusum, a manifestation of the effect of geographical distribution on temperature preference (Graham, 1958). Temperature selection is also influenced by previous thermal experience. For example, ants which have been maintained at 3°C to 5°C select 23.5°C while those maintained at 25°C to 27°C select 32°C (Herter, 1924, cited in Prosser and Brown, 1961).

Fry (1947) also defines "final preferendum" as "a temperature around which all individuals will ultimately congregate regardless of their thermal

experience before being placed in the gradient." In the case of fish, the preferred temperature is generally higher than acclimation temperature, and it increases with an increase in acclimation temperature (Ferguson, 1958). As acclimation temperature increases, the difference between acclimation and preferred temperatures decreases until they concide at the final preferendum (Garside and Tait, 1958). This applies to goldfish Carassius auratus (Fry, 1947), Girella (Doudoroff, 1938) and carp Cyprinus carpio (Pitt, et al, 1956). The only known exception is the rainbow trout Salmo gairdneri richardson, for which the preferred temperature decreases as the acclimation temperature exceeds 10°C, and for which the final preferendum is 13°C (Garside and Tait, 1958).

(iv) Control of Environmental Temperature

Roxin and Mayer (1961) have trained goldfish to control body temperature through control of environmental temperature. When the tank becomes too warm the goldfish press a lever which allows cold water to flow into their container. Goldfish acclimated to 23°C can be trained to maintain a tank temperature of 33.5°C to 36.5°C.

Such temperature selection or control as discussed above indicates that fish discriminate between different temperatures. The nerve endings in the skin and the spinal nerves act as receptors, and, if the skin is cocanized, temperature selection

ceases (Prosser and Brown, 1961). Bardach and Bjorklund (1957) found that goldfish, Carassius auratus, respond to temperature increases or decreases as small as .05°C/min. when the changes are carried by convection currents and so are felt over the entire body surface. The smallest temperature increase to which goldfish trained for heat-food association react is .05°C and the smallest decrease is .1°C. However, when temperature changes are transmitted by a probe with an area of 2 sq mm, the area served by a unit nerve receptor, rather than by convection currents the smallest heat change to which goldfish react is ± 2°C. Heat sensitivity appears to exist over the entire surface of the fish. The low threshhold for perceiving temperature changes felt over the entire body surface and exciting many or all nerve receptors compared to the high threshhold for response to point heat stimulation is the result of areal summation in the performance of individual heat sensitive nerve endings in the skin. Bardach and Bjorklund also found that the number of unit receptors in the skin of Carassius auratus is comparable to that of other vertebrates with a similar heat discriminating capacity. The lateral line organ is not involved in temperature selection; cutting the lateral line nerve does not abolish aggregation in a temperature gradient (Prosser and Brown, 1961).

B. Physiological Regulation

Two aspects of physiological temperature regulation will be discussed, evaporative cooling and increased heat production.

(i) Evaporative Cooling

A body temperature lower than that of the environment can be achieved by some poikilotherms by means of evaporative cooling. The difference between body and environmental temperature increases with an increase in the evaporative rate, which is itself influenced by temperature and relative humidity. Poikilotherms in which this mechanism has been observed include the cockroaches Blatta and Periplaneta, the locust Schistocerca, the grasshopper Gastrimargus, the armadillum, and the toad (Prosser and Brown, 1961).

Another case of evaporative heat loss is that of Hymenoptra such as bees which spread water throughout the hive and fan it with their wings to aid evaporation (Prosser and Brown, 1961).

(ii) Increased Metabolic Heat Production

In some cases body temperature can be maintained above that of the environment by means of increased activity, with accompanying increased metabolic heat production. Heat production, a function of mass, increases as the cube of linear dimensions, while heat loss, a function of area, increases as the square. As the size of the animal increases the

capacity for heat production increases faster than the capacity for heat loss and under conditions of violent activity body temperature may exceed that of the environment. This has been observed in the case of the striped marlin Makaira mitsukurii (Morrow and Mauro, 1950).

Colonial species of Hymenoptera, such as bees, cluster in winter. The outer bees of the cluster, being more active, produce more heat than the inner bees. Outer and inner bees exchange positions frequently. The heat lost to the environment is that produced by the activity of the outer bees. Therefore loss of heat from the interior of the cluster is thus prevented, and the temperature of the cluster may well be above the temperature of the environment (Prosser and Brown, 1961).

2. Poikilothermic Tolerance of Environmental Temperature

With the exception of the instances cited above, poikilotherms do not normally regulate body temperature. Therefore, as stated previously, poikilotherms survive extreme environmental temperatures, not because of regulatory mechanisms as in the case of homeotherms, but because of tolerance of these extreme temperatures.

According to Odum (1959) the upper limits of temperature tolerance are more quickly critical

than lower limits despite the fact that many organisms appear to function more efficiently towards the upper limits of their tolerance ranges. Also, temperature tolerance of aquatic organisms is generally more limited than that of terrestrial poikilotherms since the temperature range is generally less in the water than on land. Tolerance to low temperatures is essential to terrestrial animals since most animals can find a shady microhabitat in even the most severe hot climate; but tolerance to high temperatures is important to aquatic poikilotherms since water temperature rarely falls below $0^{o}C$ (Fry, 1958). For instance, while all species of fish endemic to Canada and the United States, given adequate acclimation, can tolerate temperatures in the region of the freezing point of fresh water (Brett, 1959), the upper tolerance limits of these fish vary, and it is these limits which determine geographical distribution (Ferguson, 1958).

Temperature tolerance of a species is measured by the zone of tolerance, the area on a graph bounded by the upper and lower incipient lethal levals at increasing temperatures of acclimation (Hart, 1947). Brett (1956) defines the incipient lethal level as "the temperature which fifty percent of the population could withstand for an infinite time" while Hart (1947) defines it as "the level where temperature is just beginning to have a lethal effect". The incipient lethal temperature increases with increasing acclim-

ation temperature until the ultimate incipient level, which corresponds to the highest temperature to which the animal can be acclimated, is reached. The difference in ultimate lethal levels between species may be as great as 17°C. For example, that of Carassius auratus is 41°C while that of Onchorynchus gorbuscha is 24°C (Brett, 1959).

While both upper and lower incipient lethal temperatures increase with increasing acclimation temperature, the rate of increase of the lower incipient lethal temperature exceeds that of the upper, with the result that the range of tolerated temperatures is decreased (Hart, 1947).

A. Types of Adaptive Processes

Increase in temperature tolerance with an increase in acclimation temperature is a type of "adaptive process", a general term which refers to any alteration or response of an organism which favors survival in a changed environment (Fisher, 1958). Adaptive processes can be classified, according to the time required for their completion, into accommodation, acclimation, acclimatization, and adaptation. However, according to Fisher (1958) "To the degree that the several adaptive processes reflect, for example, similar changes in the colloidal structure of the protoplasm or its proteins, they are no

doubt inherently the same. Yet the very great differences in the time courses characteristic of each imply that there are fundamental differences in the mechanisms which are operative in the different instances."

(i) Accommodation

Accommodation is an adaptive process which is measured in fractions of a second. The increase in the threshhold of an excitable tissue in response to a gradual increase in the strength of the applied stimulus, and the decline in the response of a sense organ during the application of a constant stimulus are examples of accommodation (Fisher, 1958).

(ii) Acclimation

Acclimation involves compensatory changes in an organism in response to change of a single environmental factor (Prosser, 1950) and is measured in days (Fisher, 1958; Fry, 1958; Brattstrom and Lawrence, 1962), a time greater than that required for accommodation but short in comparison to the life span of the animal. Usually changes occur in the systemic and cellular organization of the whole animal (Fisher, 1958; Fry, 1958) and when the adaptive process is complete a measured rate function is the same under one environmental condition as under another. The present study, the effect of temperature

change on blood and tissue ion levels in goldfish, is one of acclimation. Another example is the change of lethal temperature which can often be produced by changing the temperature at which the animal is continuously living (Fisher, 1958). The time required for acclimation depends upon the size of the animal (Brattstrom and Lawrence, 1962) and on the temperature to which it is being acclimated, the latter arising because acclimation rate is itself a function of temperature. The rate of acclimation to high temperatures exceeds the rate of acclimation to low (Brett, 1946; Brattstrom and Lawrence, 1962). Thermal acclimation will be discussed in detail later.

(iii) Acclimatization[1]

Acclimatization involves physiological adjustments brought about in the lifetime of the organism by changes in climate. No one factor is entirely responsible for the adaptive change (Hart, 1957). Rather, the effects of the animal's thermal history act in conjunction with the conflicting or reinforcing effects of other environmental entities such as photoperiod, salinity, or humidity (Hart, 1957; Fry, 1958). For example diet, size, sex and season affect the thermal resistance of goldfish maintained

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1. Doudoroff(1942) refers to acclimation as acclimatization, and to acclimatization as adaptation.

at a constant temperature of 20°C (Hoar and Cottle, 1952; Hoar, 1955, 1956; Hoar and Robertson, 1959).

(iv) Adaptation

The process of adaptation involves perhaps millions of years and many generations (Fisher, 1958). The mechanisms for response to environmental stress become part of the genetic constitution of the animal (Fry, 1958). For example, the temperature sensitivity of a strain of Paramecium has been found to be due to a single recessive gene (Fry, 1958) and temperature resistance in the guppy, Lebistes reticulatus can be affected by selective breeding (Gibson, 1954). Arctic species, adapted over many generations to low temperatures, are in relatively the same region of temperature response from 5°C to 15°C as are tropical species in the range 20°C to 35°C (Scholander et al, 1953). Thus when fish and other poikilotherms, normally adapted to high temperatures, are acclimated to a given low temperature their metabolic rate drops to a level below that of species normally adapted to the given low temperature (Wolchlag, 1960).

B. Types of Thermal Acclimation

Precht (1958) differentiates between acclimation of changing and unchanging reaction systems. A changing system is one which alters with age, such

as development and growth processes, and little is known of their acclimation. The thermal acclimation of unchanging systems only will be discussed in this review.

Precht divides acclimation or short term adaptation[1] of unchanging reaction systems into capacity adaptation, acclimation within the noraml temperature range of the animal, and resistance adaptation, acclimation to extreme temperatures. Capacity adaptation enables the animal to maintain vital functions at a constant rate. As stated previously acclimation is considered complete when a measured metabolic rate is the same under one environmental condition as under another. Resistance adaptation permits survival in extreme temperatures.

Capacity and resistance acclimation will be discussed.

(i) Capacity Acclimation

a. Types of Capacity Acclimation - Precht (1958).

With reference to specific nonchanging reaction systems, such as oxygen consumption of a whole animal or of particular tissues, or such as measurement of enzymic activities, which can be readily compared to defined chemical reaction or

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1. 'Adaptation' as used by Precht refers to the adaptive process of acclimation. For the remainder of this discussion 'adaptation' and 'acclimation' are used synonymously.

chains of reactions, Precht (1958) outlines five types of capacity acclimation; no compensation, inverse compensation, complete compensation, partial compensation and supraoptimal compensation.

Precht states that the rate of a specific metabolic function of a poikilotherm acclimated to a low temperature t_1 increases with increasing experimental temperature up to a temperature t_2. After the animal is acclimated at t_2, the type of acclimation is determined by the rate of the metabolic function measured after acclimation at t_2 as compared to that initially found at experimental temperature t_2. The five types, as given by Precht, are outlined below.

No Compensation

In this case the metabolic rate measured after acclimation at temperature t_2 is the same as the rate initially found at t_2, and is higher than the rate found at acclimation temperature t_1. This is shown by the carbon dioxide production of the yeast Torulopsis kefyr under anaerobic conditions.

Inverse Compensation

In cases of inverse compensation, as shown by peroxidase activity of Torulopsis kefyr, the metabolic rate obtained after acclimation to temperature t_2 exceeds that initially obtained at experimental temperature t_2.

Complete Compensation

The metabolic rate obtained after acclimation to temperature t_2 is the same as that obtained after acclimation to temperature t_1. The 'inner clock' of animals, by which they know the time of day, is an example. This mechanism may be retarded or accelerated by an abrupt temperature change, but it rapidly returns to its original rate.

Partial Compensation

In cases of partial compensation the metabolic rates obtained after acclimation at t_2 are lower than those obtained initially at t_2, but they exceed those obtained after acclimation to t_1. This is the most common type of acclimation and is found in the case of membrane permeability of heart muscle and gastrocnemius of Rana temporaria, and oxygen consumption and succinodehydrogenase activity of muscle tissue.

Supraoptimal Compensation

The metabolic rate obtained after acclimation to temperature t_2 is lower than that at acclimation temperature t_1.

b. Types of Capacity Acclimation - Prosser, (1958)

In further classifying types of acclimation Prosser (1958) plotted the logarithm of the rate of a specific metabolic process against experimental

temperature at different temperatures of acclimation and described the relation between the curves thus obtained as continuous, translational, rotational, or translational and rotational.

These relationships, as outlined by Prosser, are discussed below.

Continuous Curves

According to Prosser, when the curves for the rate of a metabolic reaction measured at two or more temperatures of acclimation are continuous, there has been no change in the thermal characteristics of the reaction being measured. This applies to the oxygen consumption of various winter and summer Brazilian insects.

Translation of Curves

When the curves of a reaction rate measured at different acclimation temperatures form a series of parallel lines the relationship is translational. The lack of change in the Q_{10} of the reaction being measured is interpreted by Prosser to mean that there is no change in the activation energy of the enzymes involved, although there is a change in enzyme activity. In the case of the oxygen consumption of the crucian carp, the effect of a decrease in acclimation temperature is an upwards translation while the curve of the oxygen consumption of Alaskan fresh water

gammarids is translated downwards by a lowering of acclimation temperature.

Rotation of Curves

In this case the curves corresponding to different acclimation temperatures intersect. A change in acclimation temperature brings about a change in the activation energy of the enzymes involved, which in turn causes a change in the Q_{10} of the reaction, and thus a change in the slope of the curve. In response to a decrease in acclimation temperature the curve of oxygen consumption of the salamander Plethodon shows clockwise rotation, while that of the heart rate of frogs rotates counterclockwise.

Translation and Rotation of Curves

Translation of curves combined with rotation is the most common type of response to change in acclimation temperature. Since intersection of curves rarely occurs within the physiological temperature range, usually occurring by extrapolation and well outside the normal range of tolerated temperatures, Prosser states that the effects of translation outweigh those of rotation. In the case of the heart rate of the newt Triton, a lowering of acclimation temperatures causes clockwise rotation and upwards translation, while in the case of the oxygen consumption of

Gammarus it causes clockwise rotation but downwards translation. On the other hand cold acclimation causes counterclockwise rotation and upwards translation in the case of oxygen consumption of brain tissue of goldfish, and counterclockwise rotation but downwards translation for oxygen consumption of starved Eisenia.

c. Overshoot Reaction

Following a sudden change in the environmental temperature of a poikilotherm, a new steady metabolic rate may be reached quickly, or there may be an initial overshoot reaction (Grainger, 1958). Most poikilotherms show the overshoot reaction (Grainger, 1958). For example, after the transfer of Artemia salina from a low constant temperature to a higher one, the initial increase in oxygen consumption is followed an hour later by a decrease to a new intermediate steady level and after nine to twenty-six hours by a further decrease to a slightly lower steady level.

Overshoot is not due to changes in the activity of the animal as a result of stimulation of the temperature receptors, since it is observed in animals anaesthetized with ether (Grainger, 1958), and in the oxygen consumption of Carassius when the somatic musculature is immobilized by drugs (Baudin,

1932, cited in Grainger, 1958). Whether a temperature change is followed by overshoot or by a simple exponential change in the rate of a metabolic process of a unicellular organism is determined by the relative changes in the rates of the enzymic reactions involved (Burton, 1939, cited in Grainger, 1958). In the case of multicellular organisms, overshoot may be due to general cellular effects, as in yeast; it may involve neural or hormonal influences; or it may be due to antagonistic processes, each having a different time course in acclimation (Grainger, 1958).

(ii) Resistance Acclimation

As stated previously, poikilotherms are able to survive extreme environmental temperatures through resistance acclimation. In differentiating between acclimation to extreme high and to extreme low temperatures, Precht (1958) outlines the three general types of resistance adaptation discussed below.

Type 1

The first type of resistance acclimation is that in which the animal develops increased heat resistance and cold sensitivity in response to an increase in acclimation temperature, and increased cold resistance and heat sensitivity when acclimation temperature is lowered. This is shown by lobsters (Precht, 1958) and by fish such as Catostomus

commersonnii, Perca flavescens, Notropus cornutus, Salvelinus fortinalis and Carassius auratus (Hart, 1947; Brett, 1956).

Type 2

The second type is that in which poikilotherms adjust to only one temperature extreme, either the upper or the lower, and thus only one temperature tolerance limit depends on the acclimation temperature.

Type 3

The organism adapts to both temperature extremes but adaptation is reasonable in only one direction. For example, an increase in acclimation temperature may bring about an increase in both heat and cold resistance, but only the increase in heat resistance will be reasonable. Cold adapted plants show both increased heat and cold resistance, and an increased acclimation temperature effects an increase in both heat and cold resistance of the yeast Torulopsis kefyr (Precht, 1958).

C. Mechanisms of Thermal Acclimation

Mechanisms of capacity acclimatiom, by means of which relatively constant metabolic rates are maintained, and of resistance acclimation, by which thermal death is offset, will be discussed.

(i) Mechanisms of Capacity Acclimation

Fisher (1958) states that the site of thermal acclimation must be concerned with one or more of the processes and mechanisms involved in oxygen utilization. As evidence of this are the observations of Spoor (1946) and Vernberg (1953, 1954) that above the basal level of consumption, the oxygen consumption of a poikilotherm increases linearly with activity; and of Fry and Hart (1948) that the activity of fish at the temperature of acclimation increases as the acclimation temperature increases. Also Kanungo and Prosser (1959) have found that oxygen consumption of fish, measured at the acclimation temperature, increases with the acclimation temperature, and Freeman (1950) has found that both the metabolic activity of brain tissue of goldfish and their rate of opercular movements, as measured at the temperature of acclimation, also vary with that temperature.

Fisher (1958) lists the following five possible sites for oxygen utilization and thus for capacity acclimation.

1) cellular and hormonal factors determining the basal metabolism of cells.

2) cellular mechanisms determining the maximum rate of cellular oxidation.

3) vascularity of tissues and flow of blood through them.

4) operation of gills and gill irrigating mechanisms.

5) central nervous system which integrates all the carious aspects of respiration.

Factors involved in capacity acclimation will be discussed. These include hormonal and enzymic factors, gill mechanisms, and changes in the central nervous system.

a. Enzymic Factors

The translation effect due to a change in acclimation temperature (discussed previously) is explained by Prosser (1958) as indicative of a change in the activity of the enzymes involved in the reaction. This is in agreement with the report of Bullock (1955) that the temperature of maximum enzyme activity in tissue homogenates of acclimated poikilotherms generally varies with the acclimation temperature. Prosser attributes this change in enzyme activity to one or more of the following: a change in enzyme concentration; a change in ionic strength, acidity, or water content of the reaction medium; or a change in the proportion of the enzyme involved with respect to another enzyme in series or in parallel with it.

The rotation effect due to a change in acclimation temperature (also discussed previously) is interpreted by Prosser (1958) as indicative of a change in the activation energy of the enzyme or enzymes involved. This he attributes to one or more of: an

alteration of the enzyme protein; a shift in some co-factor involved in enzyme-substrate complexing; or a shift between alternate enzyme pathways, whose reactions have different Q_{10}'s. The hypothesis of pathway shift is supported by the work of Ekberg (1958), who found that the oxygen consumption of gills of fish adapted to 30°C is less sensitive to cyanide and more sensitive to iodoacetate than is that of fish acclimated to 10°C.

Prosser states that since the translation effect is a much more frequent result of temperature acclimation than is rotation, changes in enzyme activity probably play a larger role in acclimation than do changes in activation energy.

b. Hormonal Factors

Hormonal factors do not play as great a role in capacity acclimation as in resistance acclimation (their role in resistance acclimation will be discussed later). However some instances of hormonal change during capacity acclimation have been observed. Although an increase in acclimation temperature is accompanied by an increase in thyroid activity in two fish, Phoxinus phoxinus and Ameiurus nebulosus, there is no dependence of hormone secretion on surrounding water temperature in the case of other fish; and in the case of the eel and Salmon gairdnerii, an increase in acclimation temperature is accompanied

by a decrease in thyroid activity, (Precht, 1958). Precht (1958) states that the addition of thiourea (antithyroid factor) to water caused a disappearance of acclimation as measured by oxygen consumption of the whole animal in the case of Carassius, but in Leuciscus the acclimation effect was increased.

C. Gills and Gill-Irrigating Mechanisms

Although gills do not compensate metabolically to temperature change, they can meet the variations in oxygen demands effected by temperature change (Evans, 1962).

Gills, in conjunction with the circulatory system, play an important role in the transfer and distribution of heat (Davis, 1955). The body temperature of the living fish adjusts only slightly more slowly to a change in the temperature of the water flowing over the gills than to a change in the temperature of the water in which the fish is submerged (Davis, 1955).

d. Changes in Central Nervous System

Fisher (1958) suggests that during acclimation the activity of the thermal receptors in the skin is modified. This results in a change in the number of impulses which reach the pertinent integrating areas of the central nervous system at any given temperature. As a result of the increase in the number of impulses, synaptic pathways which were previously closed or

crossed only with difficulty (i.e. with very high impulse frequencies) become passable. This results in new temperature responses.

(ii) Mechanisms of Resistance Acclimation

The mechanisms of thermal resistance acclimation are those which offset thermal death. These include changes in cell proteins, in the lipid in cell membranes, in cell permeability, in the endoctrine system, and in the central nervous system. These will be discussed.

a. Changes in Cell Proteins

Upon exposure to heat, the outer cortex of the cell undergoes liquefaction (Thornton, 1935, cited in Heilbrun, 1943) while the inner protoplasm becomes more viscous, indicating protein coagulation (Heilbrun, 1943). This increase in viscosity is reversible at lower temperatures but becomes irreversible at higher temperatures and the cell dies. Therefore, the most obvious explanation of heat death is coagulation of cell proteins. However, while this may be the cause of death at high temperature, it is not the cause when death occurs in the range 16°C to 18°C. Therefore, modification of protein does not play a large role in thermal acclimation.

However, protein modification may play a minor role. For instance, the melting point of

gelatin increases if it is stored above room temperature, and the molecular weight is reduced (Precht, 1958). Also, the temperature of inactivation of some enzymes changes in response to a change of acclimation temperature. Edwards and Rettger (1837, cited in Heilbrun, 1943) correlated the temperature of inactivation of respiratory enzymes of Bacillus with the death temperature. However, they did not use pure enzyme preparations but measured the catalytic activity of the cells. Cessation of this activity might have been only a sympton rather than the cause of death. This is indicated by the work of Rahn and Schroeder (1941, cited in Heilbrun, 1943) who found no correlation between the temperature of heat death of Bacillus cereus and the temperature at which its actual anzymes were destroyed. On the other hand, the digestive enzyme pancreatin (Christophersen and Thiele, 1952, cited in Precht, 1958) and the proteolytic enzymes of the stomach juice of Helix pomata (Mews, 1957, cited in Precht, 1958) do adapt to a higher temperature. Precht (1958) suggests that the difference in the response of respiratory and digestive enzymes to a change in acclimation temperature may be due to interference of the intracellular environment in the acclimation process. While respiratory enzymes are subject to this interference, digestive enzymes are not.

b. Changes in Saturation of Lipids

The fats of plants and animals which live at lower temperatures have lower melting points than the fats of plants and animals accustomed to higher temperatures (Heilbrun, 1943). Also, the melting point of fat in the bone marrow and soft tissues of both herbivores and carnivores from arctic, temperate and tropical regions decreases with distance from the main body mass; that is, decreases with decreasing temperature (Irving, et al, 1957). Heilbrun (1924, cited in Heilbrun, 1943) first pointed out that differences in the sensitivity of various organisms towards heat are correlated with differences in the melting points of their lipids. Hoar and Cottle (1952) increased the saturation, and thus the melting point, of the lipids of goldfish by feeding them a diet of saturated natural fats. They observed that the thermal resistance of the goldfish increases with an increase in the melting point of the lipids[1]. Hoar and Cottle also found the reverse, that as the acclimation temperature rises the degree of saturation and thus the melting point of the lipids of goldfish increases. The relationship between thermal resistance and the degree of saturation of natural oils

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1. Irvine, et al, (1957) show there is no relation between temperature resistance and saturation of lipids.

suggests that the presence of double bonds may be important in the acclimation process.

According to Heilbrun (1943), the cell cortex is a combination of protein, lipid, and calcium ion. Heat favours the release of lipid from this complex and the effect is more pronounced when the fat is more fluid. This release of lipid is accompanied by the release of calcium ion. The release of calcium ion from the plasma membrane may be the cause of heat death. Heat coagulation of protoplasm is typically accompanied by vacuolization, the result of an internal surface precipitation which is dependent on the release of free calcium ion within the cell.

Heilbrun (1954) suggests that the released calcium ion also acts as a clotting agent, causing the protoplasm to coagulate. When the protoplasm coagulates it produces a clotting enzyme just as the enzyme thrombin is produced during blood coagulation. According to Heilbrun this enzyme, produced when cells are killed by heat (or other agents), is transported throughout the body by the blood and causes widespread severe clotting of protoplasm and cell death.

The increase in the degree of saturation of animal fat with an increase in temperature may explain the acclimation process, in that a more saturated fat

with a higher melting point stabilizes the protein-lipid-calcium complex of the cell membrane and makes a higher temperature necessary for the release of calcium ion into the cell interior.

c. Changes in Cell Permeability

Loeb and Wasteneys (1912) first postulated that a rise in temperature brings about death-producing changes in the permeability of the cells at the body surface. According to Heilbrun (1943) water and dissolved substances generally enter cells much more rapidly at high experimental temperatures than at low. Increased tissue water of goldfish at high experimental temperature may be due to two factors; increased production of metabolic water and increased entry of water into the cells. The increase in metabolic water production is insignificant when compared to the increased entry of water since the goldfish is hypertonic to its environment (Hoar and Cottle, 1952).

The heat resistance of the animal decreases as the water content of the cells increases. Baldwin (1954) found that, under conditions conducive to dessication, the heat resistance of the insect Dahlbominus fuscipernis is increased. Baldwin and House (1954) also found that, in the larvae of the sawflies Neodiprion lecontei and N.sertifer, increased thermal resistance is accompanied by a significant

increased in the osmotic pressure of the haemolymph. This creates a hypertonic environment for the body cells, and may thus increase the resistance of the organism to high temperatures by dessication of the cell protoplasm.

The observation that the addition of calcium and magnesium ions to water raises the lethal temperature of fish (Halsbad, 1953, cited in Fisher, 1958) is further evidence for the complicity of permeability changes in heat death. These ions reduce cell membrane permeability. When the permeability of the cell membrane, and thus the water content of the cells, is decreased, heat resistance is increased.

Although the water content of the cells of goldfish increases with increasing experimental temperature, neither tissue water nor cell water content increases with an increased acclimation temperature (Irvine, et al, 1957; "Results" in this thesis)[1,2]. Therefore Hoar and Cottle (1952) suggest that permeability properties of cell membranes alter during thermal acclimation. As the acclimation temperature increases the lipid content and cholesterol to phospholipid ratio of goldfish decreased while the

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1. Hoar and Cottle (1952) found that tissue water content of goldfish varies directly with acclimation temperature.

2. Roberts (1957) found that muscle tissue water content of crab Pachygrapsus crassipes increases with acclimation temperature.

cholesterol to fatty acid ratio increases (Hoar and Cottle, 1952). These changes may control the permeability of the cell and the accumulation of water. Also, Hoar and Cottle suggest that the increase in saturation of natural fats in response to an increase in acclimation temperature contributes to the stability of the plasma membrane and thus increases control over membrane permeability.

While Irvine, et al, (1957) found no correlation between temperature resistance and total lipid content, saturation of total lipids, and cholesterol to phospholipid ratio, they did find that temperature resistance can be increased by the addition of cholesterol or phospholipid to the basic diet; and Mussachia and Clark (1957) found that as the temperature increases the esterified fraction of the cholesterol content of the liver of the arctic sculpin increases, and the phospholipid of the myotomic muscle tissue is decreased. This also suggests that the ratio of cholesterol to fatty acids and to phospholipid may be indicative of the ability of tissues to imbibe or hold water.

d. Changes in the Endocrine System

Studies of the effect of the endocrine glands on thermal acclimation have been mainly centred on the thyroid gland. Adler (1916, cited in Precht,

1958) observed an atrophy of the tadpole thyroid gland in warmth, and a clear activation in cold. An increase in thyroid activity, with the resulting increase in metabolic rate, would tend to offset the effects of extreme cold. However, the response of thyroid activity to temperature is offset by the temperature-independent seasonal variations in activity, the poikilotherm thyroid gland being typically active in summer and atrophied in winter (Fisher, 1958). According to Hoar (1955), although thermal resistance of goldfish can be increased by diet, the protective effect of dietary fat supplements is less in winter, when thyroid activity is low, than in summer, when activity is high.

Thermal resistance, like thyroid activity, varies with season. Goldfish maintained at constant temperature and fed a standard diet are relatively more resistant to cold in winter and heat in summer (Hoar, 1956). The thermal resistance of goldfish also varies with photoperiod (Hoar, 1956), as does the metabolic rate of all goldfish tissue except the gills (Evans, et al, 1962). It is suggested, therefore, that seasonal changes in the resistance of goldfish to temperature are governed by the activity of the pituitary gland, which in turn is controlled by the length of photoperiod, which varies seasonally

(Hoar, 1955; Irvine, 1957; Evans, et al, 1962).

e. Changes in the Central Nervous System

Sullivan and Fisher (1954) found that trout will avoid an illuminated area in a trough, even if the lighted area conicides with the preferred temperature in a gradient. At high light intensity the effect of light is greater than the effect of heat on behavior, but at low or intermediate light intensities the response to temperature prevails, and the animal selects temperature in the normal way. Fisher (1958) concludes that since the effects of light are mediated through the central nervous system, the effects of heat must also be.

Evidence points to the central nervous system as the site of thermal acclimation. Battle (1929, cited in Fisher, 1958) working on skates and flounders observed that functions involving synapses such as the pacemaker mechanisms of the heart, conduction at myoneural junctions, and peristalsis in the smooth muscle of the intestine fail at or below the lethal temperature of the animal, while somatic muscle, smooth muscle, and cardiac muscle continue to function at temperatures above the lethal point. Battle also found that at the lethal temperature of Raja radiata and R. erinacea, muscle contraction could still be

elicted by direct stimulation of the nerves but not by stimulation of the spinal cord. From this, Fisher (1958) concludes that heat death results primarily from a failure of some coordinating mechanism in the central nervous system, and that resistance acclimation involves some modification of the nervous system which permits it to perform normally at a higher or lower temperature.

Further evidence that thermal death is due to failure of a coordinating mechanism of the central nervous system, and that it is this mechanism which undergoes temperature acclimation, includes the two observations of Orr (1955); that frogs succumbing to heat death resemble frogs which have just been pithed, and that the order of heat death in the adult animal is first the animal as a normally functioning, coordinating organism, followed by the muscle system, heart muscle, and nervous system in that order. Further evidence is given by Becht (1908, cited in Orr, (1955) who found that in certain poikilotherms such as the frog an internal temperature of 34°C results in a condition resembling motor paralysis. This paralysis is confined to the central nervous system; the myoneural junction remains functional.

According to Cerf, et al, (1958) delayed heat paralysis occurs in free swimming goldfish at 37°C.

When the brain temperature of experimental goldfish is raised to 37°C by surrounding the brain in hot paraffin oil the waves from the vagal lobe become irregular and electrical activity ultimately stops. Upon immediate cooling vagal activity with well synchronized waves is recovered, but if the brain has been heated to 39°C, 2°C above the narcotizing point, the rhythm of the vagal lobe remains irregular upon cooling, indicating irreversible change. This is probably due to heat paralysis of the electrical activity of the nerve cell bodies. Cerf, et al suggest that temperature elevation acts on the central nerve cell bodies in the same way it acts on peripheral fibers, by causing a critical amount of depolarization. Acclimation would involve increased ability of the central nerve cell bodies to function normally at raised temperatures.

Brett (1956) suggests that the cause of heat death may be inactivation of the respiratory center, followed by death from oxygen lack. He found that the respiratory movements of the yellow perch Perca flavescens cease entirely with the approach of heat death.

There is also metabolic evidence that heat death and thermal acclimation involve the central nervous system. Freeman (cited in Brett, 1956) found that the metabolic activity of goldfish brain tissue

approaches zero at a temperature approximating the ultimate upper lethal temperature reported for this species by Fry (1942).

D. Effects of Thermal Acclimation

The effects of thermal acclimation on activity, on temperature preference, on metabolic rate and on thermal resistance of poikilotherms will be discussed.

(i) Effects on Activity

When measured at the temperature of acclimation, the activity of a fish acclimated to a higher temperature is greater than that of one acclimated to a low temperature (Fry and Hart, 1948). However, when activity is measured at an intermediate temperature, the cold acclimated fish is the more active, as is shown by the observation that at an intermediate temperature the oxygen consumption of a cold acclimated fish exceeds that of a warm acclimated fish (Wells 1935; Kanungo and Prosser, 1959) and that oxygen consumption above the basal level is linearly related to activity (Spoor, 1946, Vernberg, 1953, 1954).

Change in acclimation temperature also affects the temperature of maximum activity. Trout movement, at a given temperature, shows two peaks of frequency, one of which approximates the preferred temperature. Since the preferred temperature increases in response

to an increase in acclimation temperature (see next section; 'Effects on Preferred Temperature') it is likely that the temperature of the peak of activity also increases (Fisher, 1958).

(ii) Effects on Preferred Temperature

In the case of fish the preferred temperature is generally higher than, and increases with an increase in, theaacclimation temperature (Ferguson, 1958). However, as the acclimation temperature increases the difference between the temperature of acclimation and the preferred temperature decreases until they coincide at the final preferendum (Garside and Tait, 1958) (see previously discussion). An exception is the rainbow trout Salmo gairdneri (Richardson) for which the preferred temperature decreases as acclimation temperature exceeds 10°C and for which the final preferendum is 13°C (Garside and Tait, 1958).

(iii) Effects on Metabolic Rate

When measured at the acclimation temperature, both the rate of opercular movement and the metabolic rate of the brain tissue of goldfish increase as acclimation temperature increases between 4°C and 27°C, and the ratio of the two rates remains constant. Above 27°C the rate of respiratory rhythm does not increase significantly (Freeman, 1950). Kanungo and Prosser (1950) report that at the temper-

ature of acclimation oxygen consumption of fish also increases with increasing acclimation temperature. However, at an intermediate temperature, the oxygen consumption (Ekberg, 1958) and the respiratory activity (Roberts, 1957) of cold acclimated poikilotherms exceeds that of warm acclimated animals.

(iv) Effect on Temperature Resistance

In the case of poikilotherms an increase in acclimation temperature results in increased temperature resistance (Brett, 1946; Baldwin and Riordan, 1956). The upper incipient lethal temperature of goldfish increases linearly 1°C for every 3°C rise in acclimation temperature until the upper lethal temperature reaches 41°C at an acclimation temperature of 36.5°C, beyond which there is no increase. The lower incipient lethal temperature decreases 2°C for every 3°C decrease in acclimation temperature, reaching 0°C at an acclimation temperature of 17°C (Fry, et al, 1942). Hart (1947) states the characteristics of the effect of thermal acclimation on lethal temperatures of fish as follows:

1. The upper incipient lethal temperature increases linearly with rising acclimation temperature up to a certain level.

2. Above a certain acclimation temperature there is no further increase in incipient upper lethal temperature. This is the ultimate upper lethal temper-

ature and is the ultimate temperature to which the species can be acclimated.

3. The lower incipient lethal temperature also increases linearly with rising acclimation temperature. The slope is steeper than that for the upper lethal temperatures.

4. The lower lethal temperature of fish is below the freezing point of fresh water.

However, as an exception, changes in acclimation temperature do not change the upper incipient lethal temperature of the guppy Lebistes reticulatus (Gibson, 1954).

II. ION REGULATION

This discussion will treat body fluids, osmoregulation, and acid-base balance.

1. Body Fluids

Body fluids are divided into two main phases; the intracellular phase, which consists of the water and solutes within the body cells, and the extracellular phase, whose fluids form an aqueous environment for the cells. The two phases differ greatly in composition. The principal ionic solutes of the extracellular phase are sodium, chloride, bicarbonate, and a small amount of protein which is mainly confined to the plasma, while those of the intracellular phase are potassium, magnesium, phosphate and protein (Elkinton

and Danowski, 1955). According to Manery (1954) this high concentration of large colloidal protein particles within the cells constitutes one of the main differences between the phases.

Bernard (1859, cited in Manery, 1954) further subdivides the extracellular phase into the circulating fluids, the stationary fluids, and secretions. According to Bernard, circulating fluids consist of the blood and lymph; stationary fluids include interstitial fluid, cerebrospinal fluid, aqueous humor, vitreous humor, pleural fluid, peritonal fluid, and synovial fluid; and secretions include those of the salivary glands, stomach glands, intestinal glands, liver, pancreas, reproductive organs, sweat glands, and tear glands and also include urine. However, Bernard's anatomical extracellular phase does not have the properties of a single physiological entity. Therefore, according to Manery (1954) the physiological extracellular phase is defined as the plasma and the fluids into which ions and small molecules diffuse freely from the plasma.

Elkinton and Danowski (1955) subdivide the physiological extracellular phase into plasma, interstitial fluid, and connective tissue fluid. The main difference in composition between plasma and interstitial fluid is the high protein content of the plasma of approximately 50 g/kg (Conway, 1957). Because

electrolytes and water are almost freely diffusible across the vascular wall, the ion concentrations on the two sides of the capillary membrane differ only by the Gibbs-Donnan effect of the nondiffusible plasma protein (Elkinton and Danowski, 1955). Manery (1954) gives the ratios of serum ion concentration to interstitial ion concentration for chloride and bicarbonate to be .977 and .985 respectively and the interstitial concentration to serum concentration for sodium and potassium as .942 and .943.

A. Plasma - Interstitial Fluid Shift

As stated above the protein content of plasma is much higher than that of interstitial fluid. Therefore, the two main factors controlling the shift of fluid between the plasma and the interstitial phase are the hydrostatic pressure of the plasma, which forces fluid into the interstitial phase, and the plasma osmotic pressure which attracts water back into the capillaries. These two factors are modified by a low interstitial fluid osmotic pressure and by the interstitial hydrostatic pressure resulting from tissue elasticity (Starling, 1896, cited in Elkinton andDanowski, 1955). At the arterial end of the capillary plasma hydrostatic pressure exceeds plasma osmotic pressure, and there is a net movement of fluid from the plasma into the interstitial phase.

Plasma hydrostatic pressure decreases and osmotic pressure increases along the length of the capillary and at the venous end there is a net movement of fluid back into the capillaries.

The rate of fluid flow across the capillary membrane is approximately 2% of plasma flow. Electrolytes and crystalloids, such as urea and glucose, diffuse rapidly back and forth between the phases at rates of ten to eighty times their flow rate in plasma, according to their respective diffusion coefficients. However, as stated previously, the overall electrolyte distributioniis determined by the Gibbs-Donnan effect of the plasma protein. Normally proteins do not diffuse across the membrane because of the 'sieving effect'. It is only when the rate of plasma flow is slowed that appreciable quantities of protein appear in the interstitial fluid (Elkinton and Danowski, 1955), raising its osmotic pressure and so increasing interstitial fluid volume.

B. Interstitial Fluid - Intracellular Fluid Shift

Manery (1954) cites the observations of Fenn (1936), Gilman (1937), Steinbach (1944) and Eggleton (1951) which indicate that intracellular and interstitial fluids are isosmotic; and also cites the work of Opie (1949) which provides evidence that osmotic concentration varies between the two phases.

According to Manery (1954), it is likely that osmotic equilibrium is always approached, but probably rarely obtained. Therefore, distribution of fluid between the interstitial and intracellular phases would depend on the slight differences in osmotic pressure resulting from differences in solute concentration between the two phases, and from the factors which regulate these concentrations (Elkinton and Danowski, 1955).

(i) Ion Distribution Between Intracellular and Extracellular Fluids

Because of the high cellular concentration of colloidal protein, it would be expected that cell osmotic pressure would exceed cell hydrostatic pressure. Therefore, according to Manery (1954), the cell membrane must possess either real or functional impermeability which restricts sodium, chloride, and bicarbonate ions to the extracellular phase where they exert an osmotic pressure equal, or almost equal, to that of the intracellular colloids. Thus the swelling and rupture of the cells that would be caused by the entry of water is prevented.

There is no one explanation for the restriction of potassium ions and protein to the intracellular phase and of sodium, chloride, and bicarbonate ions to the extracellular phase. Although potassium is primarily situated intracellularly and chloride is found mainly in the extracellular phase, it is known that both ions readily

cross the cell membrane (Fenn and Cobb, 1935; Wilde, 1939). Sodium ion also passes through the cell membrane (Heppel, 1940; Levi and Ussing, 1948; Steinbach, 1951; Desmedt, 1953; Eppley, 1958, 1959). Therefore, it is obvious that, since potassium ion is retained within the cell and sodium ion is excluded, while both pass freely through the membrane, either potassium is being accumulated against a concentration gradient or sodium is being excluded.

a. Sodium Extrusion

Dean (1941, cited in Elkinton and Danowski, 1955) suggests that the ion distribution is due to the active transport of sodium out of the cell after its passive inwards diffusion. This seems a satisfactory explanation in view of the fact that, since the interior of the cell is negatively charged, positive ions would diffuse inwards passively and be maintained in high internal concentration in accordance with the electrochemical potential. Since this high internal concentration is realized for potassium but not for sodium, it follows that sodium is being transported back across the cell membrane against the gradient by an active process (Davson, 1959). Chloride and bicarbonate would then remain in the extracellular phase to maintain electroneutrality.

b. Linkage of Potassium Influx and Sodium Efflux

Active sodium transport does not completely explain ion distribution. There is evidence that sodium efflux is not entirely an active process and that potassium influx is not entirely passive. Also the two processes are thought to be linked. Harris and Maizels (1952), working with erythrocytes, showed that a decrease in potassium influx is accompanied by a reduction in sodium efflux. By demonstrating that both processes are affected by metabolic inhibitors such as dinitrophenol, azides, and cyanide, Hodgkin and Keynes (1955) showed not only that the processes are linked, but that active mechanisms are involved in both. Eppley (1958) found further evidence of energy expenditure in both porcesses and of linkage of the mechanisms. His ratio of sodium efflux to potassium influx of 2.3 indicates a loose coupling. Glynn (1956) found a one to one relationship.

c. Active Potassium Influx and Passive Sodium Efflux

Hodgkin and Keynes (1955) hayesshown that sodium extrusion is not entirely an active process. When the external potassium concentration is reduced to zero, sodium efflux is reduced to one-third its normal value, but does not stop as expected. Therefore, there must be some passive distribution of

sodium across the membrane.

Glynn (1956) suspending erythrocytes in glucose solutions of varying potassium concentration, demonstrated that potassium intake is not entirely passive. At low external potassium concentrations the influx rises sharply as concentration increases, indicating a dependence on metabolic processes. However, beyond a critical external concentration, the relationship between concentration and influx becomes linear, indicating that the potassium carriers are saturated and potassium ions are now entering by passive processes.

d. Potassium Accumulation as Primary Active Mechanism

There is evidence that the primary active mechanism involved in restriction of potassium ion to the intracellular and sodium ion to the extracellular phase might be potassium accumulation rather than sodium extrusion. Eppley (1959) demonstrated potassium influx in the absence of sodium efflux but could not show sodium efflux without potassium influx, and Huf and Doss (1959) found potassium is necessary for the transport of sodium and chloride across the skin of the frog.

Pulver and Verzar (1940) found that yeast accumulates potassium from a medium of glucose and potassium. During this process the medium becomes acidic,

indicating a hydrogen-potassium exchange. This suggests that potassium accumulation depends upon the metabolic production of acids, such as succinic acid, whose anion does not pass through the cell membrane.

The order in which cations are accumulated by this hydrogenmetallic cation exchange is potassium, rubidium and sodium and calcium and magnesium do not appear to be taken up at all by this mechanism (Rothstein and Demis, 1953, cited in Davson, 1959).

The observation that potassium accumulation is inhibited by cyanides (Hodgkin and Keynes, 1955), whose mode of action is to poison organic catalysts involving a heavy metal, supports the suggestion by Manery (1954) that active transport is linked with enzyme systems such as the cytochrome system. Conway and Downey (1950) suggest that potassium accumulation involves oxidation of the substrate in the peripheral region of the cell, followed by a reduction reaction within the cell. The free hydrogen ions in the periphery are than free to exchange with the potassium in the surrounding medium, and potassium is thus carried into the cytoplasm. Rothstein (1954, cited in Davson, 1959) has shown that glucose uptake is stimulated by external potassium, and that the enzymes involved in some of the glycolytic reactions are on the cell surface. This interdependence of potassium

accumulation and metabolism indicates that potassium accumulation is the primary process, with sodium extrusion dependent upon it.

2. Osmoregulation

The range of environmental osmotic conditions which animals tolerate is much broader than the range of internal osmotic concentrations (Prosser and Brown, 1961). An animal may respond to a change in environmental osmotic concentration in two ways. The body fluid concentration of poikilosmotic animals, or osmoconformers, changes with that of the environment, while homoiosmotic animals, or osmoregulators, maintain a relatively constant internal concentration in the face of a changing environment. In general, osmoconformers can withstand a wider variation in internal osmotic concentration, while osmoregulators tolerate a wider environmental range (Prosser and Brown, 1961).

A. Osmoregulation in Teleosts

Teleosts are osmoregulators. According to Black (1951), the osmotic concentration of sea water is equivalent to a depression in freezing point of 1.5 to 2.3 Centigrade degrees, and that of fresh water is equivalent to a depression of 0 Centigrade degrees. However, both marine and fresh water teleosts maintain an internal osmotic concentration

equivalent to a freezing point depression of .5 to .8 Centigrade degrees.

(i) Osmoregulation in Marine Teleosts

The osmoregulatory mechanisms of marine telosts are those which offset outwards diffusion of water and inwards diffusion of salts. Because the kidneys of marine teleosts are poorly developed, loss of water in the form of urine is limited (Marshall, 1943, cited in Prosser and Brown, 1961). For example, the sculpin and the toadfish have a urine production of only 2.5 to 4 ml/kg/day (Prosser and Brown, 1961). However, the urine that is produced is always more dilute than the blood. Lophius, for example, has a plasma concentration equivalent to a freezing point depression of .67 Centigrade degrees while that of the urine is equivalent to .57 Centigrade degrees (Forster and Berglund, 1956).

To offset water loss, marine teleosts swallow large quantities of water (Smith, 1953), as much as 40 to 200 ml/kg/day in the case of the sculpin (Prosser and Brown, 1961). However, as the sea water passes down the intestine, its osmotic concentration decreases, indicating that relatively more salt than water is absorbed (Smith, 1932).

Salts are excreted. Magesium and sulphate

ions are lost in the feces; magnesium, calcium, sulphate, and phosphate ions are eliminated in the urine; and most of the sodium, potassium and chloride ions are excreted extrarenally (Prosser and Brown, 1961). The 'chloride secretory cell' in the gills is thought to be the site of active transport of chloride against a concentration gradient (Threadgold and Houston, 1961).

(ii) Osmoregulation in Freshwater Teleosts

Fresh water teleosts, such as Carassius auratus, must offset the inward diffusion of water and outward movement of salts. The skin is relatively impermeable, but water enters through the gills and oral membranes (Black, 1951). Unlike marine teleosts, which drink large amounts of water to prevent dehydration, fresh water fish drink very little (Prosser and Brown, 1961). The nephron of the fresh water teleost kidney has a well developed glomerulus; therefore, filtration rate and urine output is high. For example, the catfish produces 300 ml urine/kg/day (Prosser and Brown, 1961), and the goldfish produces forty to 100 ml/kg/day (Wikgren, 1953). As the filtrate passes along the tabules, most of the salts are reabsorbed, and the resulting urine is dilute. The osmotic concentration of urine of Ameiurus nebulosus is equivalent to a freezing point depression

of .025 Centigrade degrees and that of Catostomus commersonii is equivalent to a depression of .094 Centigrade degrees. (Haywood and Clapp, 1942).

Although the urine is hypotonic to the body fluids, the salt loss is condiderable because of the high urine flow. The resulting salt requirement may be supplied either by the food or by active absorption from the medium. Beadle (1943) suggests that the food is the main source of salt but Wikgren (1953) believes that active absorption of salts from the medium is the essential mechanism, with food as a supplementary source. Salt is absorbed by secretory cells onto the cell membrane. Meyer (1948) found that the gills of Carassius auratus absorb chloride ion against a concentration gradient down to a threshhold external concentration of .05 mM/l. Meyer (1951) also found that the movement of sodium ion through the gills parallels that of chloride.

Although there is a net inward movement of salts across the gill membrane, the secretory cells are continually eliminating sodium, potassium, and chloride ions. In the case of the fresh-water stickleback Gasterosteus aculeatus, the rate of exchange of these ions between the body fluids and the environment is about one percent of the total amount present in the body per hour (Mullins, 1950). Chloride

exchange across the gill membrane of goldfish was demonstrated by Meyer (1948). Handling of goldfish initiates changes in the absorptive mechanism and there is a net loss of sodium and chloride through the gills (Meyer, 1948, 1951). Retention or excretion of 150 to 238 μEq of chloride ion by the goldfish is equivalent to retention or excretion of one ml water (Jorgensen and Rosenkilde, 1956).

Since salt uptake by the gills is performed against a concentration gradient, it must be an active process utilizing metabolic energy. Beadle (1931) found that osmoregulatory ability can be impaired by narcotics and other metabolic poisons. This was also shown by Huf (1935), who suggested that the energy necessary for osmoregulation comes from the breakdown of carbohydrates. Since freshwater fish absorb salt against a concentration gradient while marine fish do not, freshwater fish would be expected to have a higher metabolic rate to supply the necessary energy. Fox and Simmonds (1933) found that the oxygen consumption of permanent fresh water species is one and a half to three times greater than that of related marine species. Osmoregulation and metabolism were further linked by Veselov (1949, cited in Black, 1951), who demonstrated a direct relationship between the oxygen consumption and the swelling of tissues due to salt intake in the gold-

fish and carp. Hoar (1951) stated that the thyroid is involved in salt metabolism and osmoregulation in the goldfish. This is supported by Woodhead and Woodhead (1956), who found that the osmoregularity ability of the cod Gadus callarias varies with the activity of the endocrine glands.

a. Effect of Temperature Change on Osmoregulation

As shown above, osmoregulation and metabolism are closely related. As discussed previously, changes in both experimental and acclimation temperatures affect the metabolic rate. Therefore, temperature changes would be expected to affect osmoregulatory ability.

Many poikilotherms compensate for temperature changes by altered behaviour, metabolism, or fluid distribution (Prosser, 1955). Exposure to cold has been found to cause a progressive shift of fluids from the cellular to the extracellular phase (Houston, 1962). Cooling has been found to have little effect on the urine concentration of the lamprey (Wikgren, 1953). The ability of the tubule cells to absorb ions is markedly reduced, but this is compensated by a decrease in the amount of fluid passing through the kidney per unit time. Wikgren (1953) found that temperature depression lowers membrane permeability to water and thus lowers urine output as well. He suggests that

this adaptation to low temperature involves a change or reorganization of the cell membrane. Then, as discussed previously, adaptation to high temperatures would also involve membrane changes.

3. Acid - Base Balance

The main reference for this discussion of acid-base balance is Elkinton and Danowski (1955).

The hydrogen ion concentration of the body fluids closely conditions cellular function and therefore must be regulated. The buffer systems of the body are the primary regulators of hydrogen ion concentration. The main buffers are the bicarbonate, phosphate, and protein systems, which operate according to the law of mass action as expressed by the Henderson-Hasselbalch equation:

(1).....$pH = pK + \log \left[\frac{A^-}{HA}\right]$

where A^- = concentration of dissociated anion

HA = concentration of undissociated acid

The buffer systems, limited by the law of mass action, would become progressively ineffective if they were not modified by the kidneys and respiratory organs. Therefore, the regulation of hydrogen ion concentration depends on the activities of these organs.

A. Carbonic Acid-Bicarbonate Buffer System

The carbonic acid-bicarbonate system is the main buffer of the erythrocytes and extracellular fluid. The concentration of carbonic acid in the plasma is a function of the partial pressure of carbon dioxide. Changes in carbon dioxide concentration, caused either by changes in its metabolic production or by changes in its rate of excretion across the respiratory membranes, results in changes in the concentration of carbonic acid in the body fluids. The extracellular concentration of bicarbonate ion is determined by the excess of the sum of the fixed cations (sodium, calcium, potassium, and magnesium) over the sum of protein, phosphate, and fixed anions (chloride, sulphate, and organic anions). Any renal, gastrointestinal, or metabolic change which alters the relative concentration of these anions and cations may produce a change in bicarbonate concentration. Hydrogen ion concentration, determined by the ratio of bicarbonate to carbonic acid, is thus affected by respiratory elimination of carbon dioxide and renal excretion of fixed ions.

B. Protein and Phosphate Buffer Systems

The protein buffer system includes amphoteric protein of the erythrocytes (haemoglobin), of the plasma (albumin and globulin), and in the tissue cells. There is

almost no protein in the interstitial fluid, except in the lymph. In the extracellular fluid the phosphate buffer is almost insignificant in comparison to the carbonate or protein systems, but it plays a larger role within the cells. These two systems, in equilibrium with each other and with the carbonate - bicarbonate system, are secondarily modified by the action of the respiratory organs and kidneys.

C Acidosis and Alkalosis

If the buffer systems do not adequately control hydrogen ion concentration, a physiological condition of acidosis or alkalosis results. Acidosis is defined as an increase in hydrogen ion concentration in the body fluids; alkalosis as a decrease (Elkinton and Danowski, 1955).

The causes of these conditions may be either respiratory or metabolic. In respiratory acidosis the increased hydrogen ion concentration, or decreased pH, is due to the excess of carbon dioxide which results from interference in the excretion of carbon dioxide across the respiratory membranes. Conversely, the decreased hydrogen concentration, or increased pH, of respiratory alkalosis is due to increased elimination of carbon dioxide.

Metabolic acidosis is caused by a relative decrease of fixed cation with respect to fixed anion as a result of metabolic and renal processes;

metabolic alkalosis is caused by a relative increase of fixed cation with respect to fixed anion.

D. Regulation of fixed Cations and Anions

To prevent metabolic acisosis, fixed cations in the glomerular filtrate must be reabsorbed. This absorption mechanism operates either by the exchange or hydrogen ions for fixed cations across the tubule, or else by the excretion of the ammonium ion, which is produced in the tubular cells of the kidney and then is exchanged for fixed cations. If the level of fixed cation becomes too high (alkalosis), they are excreted as bicarbonate.

It has been found that in cases of metabolic acidosis, in which fixed cation level is low, tubular reabsorption of filtered bicarbonate is complete, indicating that the cation-hydrogen exchange mechanism is functioning to its full capacity. In the case of metabolic alkalosis, when fixed cation level is high, part of the filtered bicarbonate appears in the urine accompanying the fixed cations which are being excreted (Pitts, et al, 1949).

The acidity of body fluids is linked with the ions levels in the cells and in the extracellular fluid. Darrow (1948) found that intracellular potassium deficiency is associated with extracellular metabolic alkalosis. Therefore, intracellular fluid must also participate in ionic adjustments to acid-

base disturbances; total regulation of body pH must involve a series of linked extracellular, intracellular, respiratory, and renal exchanges (Elkinton and Danowski, 1955).

CHAPTER I

INTRODUCTION

The body temperature of poikilotherms varies with the ambient temperature. Therefore, in order to survive, the animal must be able to adjust to, or tolerate, a range of internal temperature. Such adjustment involves a modification of metabolic processes. Thermal acclimation, the adaptive process which involves changes in an organism in response to temperature change (Prosser and Brown, 1961), is of two types; capacity acclimation and resistance acclimation.

Capactiy acclimation, adjustment to temperatures within the normal range of the animal, involves modification of metabolic processes which may offset the tendency for metabolic rate to vary with temperature. Resistance acclimation, adjustment to temperatures outside the normal range of the animal, involves modification which affect the limits of thermal tolerance. The biochemical aspects of capacity and resistance acclimation have been studied by Heilbrun (1943,1954), Freeman (1950), Fry (1958), Fisher (1958), Grainger (1958), Precht (1958), and Prosser (1958).

CHAPTER III

MATERIALS AND METHODS

1. Source and Maintainance of Stocks

Goldfish, originally from the Goldfish Supply Co,, Stouffeville, Ontario, were obtained from the Reliable Bird Comapny, Winnipeg, Manitoba, and maintained in continuously aerated, demineralized and charcoal-filtered water in static tanks described below. They were fed once daily on a commercial dry cereal, (Heinz "Pablum", or "Victoria Food", McCabe Grain Company, St. Boniface, Manitoba). The animals remained in active and apparently healthy condition throughout the experimental period.

Wooden tanks, twenty inches wide by twenty inches high by thirty-eight inches long, and of eighty-liters capacity were used for acclimation and testing. In each tank a charcoal-fiberglass filter, replaced at least once a week, siphoned debris from the bottom.

Water temperature was maintained at $20^{\circ}C \pm 1^{\circ}C$, $30^{\circ}C \pm 1^{\circ}C$, or $36^{\circ}C \pm 1^{\circ}C$ by means of electric relays[1]

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1. Model 71 Thermistemp Temperature Controller, Yellow Springs Instrument Co.

used in conjunction with partial immersion probes[1]. The five hundred-watt copper sheathed resistance heating elements were wrapped in "Teflon" tape to prevent contamination of the water by copper ions.

Illimination was provided by two General Electric twenty-five watt "Lumiline" incandescent bulbs mounted in the hood of each tank. Twelve-hour light-dark cycle was maintained by means of a time switch[2]. This precaution was taken in view of observations by Hoar (1956) and Hoar and Robertson (1959) of photoperiodically induced variations in thermal resistance of goldfish.

2. Methods

A. Acclimation and Application of Thermal Shock

All animals were acclimated for a minimum period of twenty-one days at either 20°C or 30°C. The length of this initial acclimation period was based upon the observations of Brett (1946) upon the same species, and would seem to be sufficient for the completion of acclimatory process.

Sublethal shock was achieved by direct transfer of the animals from 20°C to 30°C. Fish were transferred in water with a minimum of handling and

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1. Model 631 Stainless steel partial immersion probe, Yellow Springs Instrument Co.

2. Model T 101 Intermatic Time Switch, Canadian Laboratory Supplies, Limited.

disturbance since it has been well established (Jorgenson and Rosenkilde, 1956; Meyer, 1948, 1951) that this may induce marked water-electrolyte imbalance ("laboratory diuresis") which, if severe, may result in death.

Followingsublethal shock animals were sampled every twelve hours for ninety-six hours, and thereafter at twenty-four hour intervals for up to 240 hours.

It was determined by preliminary study that a shift from 20°C to 36°C caused death in approximately two hours. It was also found that a direct transfer from 20°C to 38°C caused death in goldfish in less than twenty minutes.

Lethal shocks were therefore applied by transfer of the animals to water at either 36°C or 38°C. The same handling and precautions were employed as with sublethal shock. The fish were observed individually, and removed for sampling upon reaching the critical thermal maximum response point (Heath, 1963). This is defined as the point at which neuromotor coordination is lost, as evidenced by inability to maintain the normal swimming position.

B. Sampling

Four groups of fish were sampled;

1) animalsacclimated to 20°C for twenty-one days or more.

2) animals acclimated to 30°C for twenty-one days or more.

3) animals subjected to sublethal shock (20°C to 30°C) after twenty-one days or more at 20°C.

4) animals subjected to lethal shock (20°C to 36°C or 38°C) after twenty-one days or more at 20°C.

Fish, other than those subjected to lethal shock, were anaesthetized prior to sampling in an aqueous solution of tricainemethanesulfonate(MS - 222) (Shiffman and From, 1959; Eisler and Backiel, 1960). The concentration was modified as necessary to produce narcosis within two to five minutes. When the animals no longer responded to mechanical stimulation (caudal peduncle pinched with forceps) they were removed from the anaesthetizing solution, and lightly blotted dry with paper towelling. Dorsal, anal, and pelvic fins were removed, and the caudal peduncle scaled. The animal was then suspended vertically with a clamp around the head region, and the caudal peduncle transected immediately behind the anus.

Blood was collected in polythene cups or glass dishes previously dusted with dry dipotassium ethylenediaminetetracetate (EDTA) to prevent clotting (Becker, et al, 1958). Heparin, used by Anthony (1961) and Hesser (1960) and oxalate, used by Field, et al (1943) were found unsatisfactory in the prevention of clotting in this species. Samples were

immediately centrifuged for three minutes at 10,000 rpm, and the plasma pipetted off and stored frozen in coded polythene tubes until required for analysis.

Each animal was weighed, wrapped in paraflim, and frozen. Tissue samples (0.2 - 1.0 gm. wet weight) grossly dissected of scales, skin, bone and tissue were removed from the epaxial muscle mass of the frozen fish and placed in previously weighed test tubes. The tissue was dried to constant weight at 80°C, and the test tubes reweighed. The weight loss, representing tissue water, was expressed in grams per kilogram wet tissue (gm/kg). Individual tissue water determinations were made in quadruplicate, and agreed within 0.2%. The tubes were than closed with parafilm, and the dried tissue samples stored frozed until required for analysis.

C. Analysis

(i) Plasma chloride

Plasma chloride was determined on ten microliter samples by the mercurimetric technique of Schales and Schales (1941), as modified by Asper, Schales and Schales (1947), using a Natelson ultra-micro burette. The indicator, diphenyl carbazone, gives its most accurate titration end-point in solutions which, at the beginning of titration, have a pH of 4.5 - 6.0 (Asper, Schales and Schales, 1947).

Accordingly a citrate buffer (Smith, 1957) was used to establish a beginning pH within this range.

Determinations, performed in triplicate, agreed within 1%. Labtrol[1] having a chloride concentration of 103 mEq/l, was used as a reference standard.

Plasma chloride was expressed as milliequivalents per liter of plasma (mEq/l).

(ii) Tissue Chloride

Determination of tissue chloride was based on the Volhard argentimetric back titration (MacDonald, 1960) as adapted by Kolthoff and Sandell (1952), Shenk (1954), and Manery (1955).

Dried tissue samples of 0.3 - 1.0 gm wet weight were soaked overnight in aqueous 0.02N silver nitrate. The tissue was then digested in concentrated nitric acid, with hydrogen peroxide and potassium persulfate added to facilitate oxidation of organic materials. The test tubes were capped during digestion with glass spheres to prevent the loss of volatile chloride compounds formed during the digestion process (Shenk, 1954).

Tissue chloride was precipitated as silver chloride. The silver ions remaining in solution were then titrated against aqueous thiocyanate using ferric alum indicator.

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1. Fisher Scientific Company.

One disadvantage of the Volhard method for chloride determination is that the end-point is not sharp, tending to fade. This is due to the reaction:

$$AgCl + CNS^- \longrightarrow AgCNS + Cl^-$$

However, this reaction is slow. Addition of one milliliter nitrobenzene to the solution before titration sharpens the end-point as silver chloride concentrates at the water-nitrobenzene interfaces and is thus effectively withdrawn from reaction with thiocynate.

A second disadvantage is that the color change appears 0.7 to 1.0% before the equivalence point. This arises from the tendency for silver thiocyanate to absorb silver ions. Therefore, titration, accompanied by vigorous shaking, must be continued past the end-point until the color change no longer disappears on shaking.

Two alternate methods of possible use in tissue chloride estimation are the mercurimetric technique of Schales and Schales (1941), and the Mohr titration (Kolthoff and Sandell, 1952). Both, however, have distinct limitations for measuring tissue chloride concentration. The method of Schales and Schales, used in determination of plasma chloride concentration, could not be used as inconsistent and unreliable results are obtained at pH values of 4.0 and below (Asper, Schales and Schales, 1947). Since the tissue is digested in concentrated nitric

acid, the pH is well below 4.0. The Mohr titration, a titration of chloride ion against silver nitrate with potassium chromate indicator, is not applicable to tissue chloride determination since it requires a neutral or weakly alkaline solution or pH 7.0 - 10.5 (Kolthoff and Sandell, 1952).

Tissue chloride determination, performed either in duplicate or quadruplicate, agreed within 5%. Tissue chloride estimates have been expressed as milliequivalents per kilogram fresh tissue (mEq/kg).

(iii) Plasma Sodium and Potassium

Plasma samples of 100 microliter were diluted 1:200 for potassium ion determination and 1:100 for sodium ion determination with aqueous lithium chloride to a final lithium concentration of 300 ppm. Measurements of sodium and potassium ion concentrations were made on an internal standard flame spectrophotometer[1] against bracketing standard solutions. Labtrol[2], having a sodium ion concentration of 140 mEq/l and a potassium ion concentration of 5.1 mEq/l, used as a reference standard.

Plasma sodium and potassium values were expressed as milliequivalents per liter plasma (mEq/l).

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1. Advanced Internal Standard Flame Spectrophotometer
2. Fisher Scientific Company.

(iv) Tissue Sodium and Potassium

Dried tissue samples of 0.2-1.5 gm wet weight were digested in concentrated nitric acid. The digest was diluted to two liters for potassium ion determination, and to ten milliliters for sodium ion determination with aqueous lithium chloride to a final lithium concentration of 300 ppm. As for plasma sodium and potassium, measurements were made on a flame spectrophotometer using labrtol as a reference standard.

Tissue sodium and potassium concentrations were expressed as milliequivalents per kilogram fresh tissue (mEq/kg).

D. Indirect Calculations

(i) Extracellular Space Volume ECSV

Extracellular space volume gives an estimate of extracellular phase volume. Extracellular phase volume is the volume occupied by physiological extracellular water. This is defined by Manery (1954) as the plasma and the fluids into which ions and small molecules diffuse freely from the plasma. Extracellular space refers to the volume of distribution of a solute believed to be confined to the extracellular phase. There is no known chemical confined entirely to the extracellular phase, and so the measured extracellular space is not an exact estimate of extracellular phase volume.

Solutes useful for measuring extracellular volume fall into two classes. The first class includes electrolytes which resemble sodium and chloride ions in distribution, and the second, nonelectrolytes which cross capillary walls but not cell membranes. Inulin and sucrose belong to the nonelectrolyte group. Neither gives a true measure of extracellular phase volume. Both inulin sucrose are distributed throughout the extracellular phase slowly (Cotlove, 1954; White and Rolf, 1956), and prior to complete distribution both underestimate extracellular phase volume. According to Cotlove, (1954) both inulin and sucrose spaces measure only ninety-three percent of chloride space fifteen hours after injection. Another explanation for discrepancies between inulin space and extracellular phase volume has been given by Cotlove (1954) and Nichols, et al, (1953, cited in Elkinton and Danowski, 1955) who suggest that the extracellular phase apart from the plasma in the vascular system should be divided into two subphases: the interstitial fluid which is a plasma ultrafiltrate, and connective tissue fluid. Inulin space is believed by the authors to measure only plasma plus interstitial fluid volume, and so underestimates extracellular phase volume by the volume of connective tissue fluid. If time for complete distribution is allowed inulin space estimates exceed chloride space

value, due to the metabolism and subsequent entry of inulin into the cells (White and Rolf, 1956). For these reasons nonelectrolytes were not considered suitable for measurement of the extracellular phase volume.

The electrolyte group includes thiocyanate, iodide, bromide, sulfate, thiosulfate and the radioactive isotopes of chloride, sulfur, and sodium. Sodium and chloride isotopes give better estimates of extracellular volume than do nonelectrolytes, because they diffuse rapidly through both interstitial and connective tissue fluids (Elkinton and Danowski, 1955). However, because sodium and chloride and their isotopes do situate intracellularly to some extent (Manery and Hastings, 1939; Manery, 1954; Conway, 1957), sodium and chloride spaces tend to overestimate extracellular phase volume. This is generally true of all materials of the electrolyte group. Bone exchange sodium with the extracellular fluid (Elkinton and Danowski, 1955), and this constitutes another source of error in determination of extracellular phase volume. Thiocyanate becomes bound to plasma albumin and sulfate becomes a part of sulfur-containing compounds such as chondroitin sulfuric acid (Sheatz and Wilde, 1950, cited in Manery, 1954). It is thus apparent that the volume of distribution of solutes of both electrolytic and

nonelectrolytic nature do not give true estimates of extracellular phase volume.

However, the volume of distribution of naturally present sodium or chloride ions is preferred for determining extracellular volume is fish. This has the advantage that the animal is not subjected to the injection of foreign substances and to other handling procedures which affect salt-water balance (Jorgenson and Rosenkilde, 1956; Meyer, 1948, 1951) as previously noted. In this study it has been assumed that sodium and chloride are entirely extracellular (Manery, 1954). According to Manery (loc. cit.) the intracellular fraction of sodium is greater than that of chloride, and therefore chloride space gives a better estimate of extracellular phase volume than does sodium space. Manery and Haege (1941) state that chloride space exceeds extracellular volume by one to three percent. Extracellular space volume was calculated using the following equation from Manery (1954)

(1). . . . . $ESCV = \frac{(Cl_t^-)\ (H_2O_p)\ (rCl^-)}{Cl_p^-}$

Where: Cl_t^- = tissue chloride (mEq/kg)

Cl_p^- = plasma chloride (mEq/l)

rCl^- = Gibbs-Donnan distribution ratio .977

H_2O_p = plasma water = 950 gm/kg (Conway, 1957).

ECSV is expressed as grams per kilogram fresh tissue (gm/kg)

Extracellular space volume for each sample group was calculated using the mean Cl^-_t and mean Cl^-_p (see E, Statistical analysis) values of that group. Therefore, the mean and 95 per cent confidence limits for the Cl^-_t/Cl^-_p ratio of each group were calculated using the formula given by Goldstein (1964,p.187). Cl^-_t and Cl^-_p were treated as independent observations as it was not always possible to obtain paired observations, i.e. to obtain both a Cl^-_t and Cl^-_p value for each fish. The mean and confidence limits thus obtained were than multiplied by the constant, (H_2O_p) (rCl^-) to obtain the mean extracellular space volume and its 95 per cent confidence limits.

Sample means were considered to be significantly different at the .05 level when their 95% confidence limits did not overlap.

(ii) Intracellular Space Volume ICSV

ICSV is taken as the difference between tissue water and ECSV.

(2) . . . . . $ICSV = H_2O_t - ECSV$

where: H_2O_t = tissue water (gm/kg)

ICSV is expressed as grams per kilogram fresh tissue (gm/kg)

(iii) Cell Solids

Cell solids were determined as the difference between the weight of the tissue and the tissue water.

(3) . . . . . Cell Solids 1000 gm/kg - tissue water (gm/kg). Cell solids were expressed as grams per kilogram fresh tissue (gm/kg).

(iv) Extravascular Ion Concentration.

The concentrations of sodium, potassium, and chloride ions in the extracellular fluid were derived from the concentration of the ions in the plasma, and from the Gibbs-Donnan distribution ratio (r). The following equations from Manery (1954) were used.

(4) . . . . . $$[A]_E = \frac{A_p\ (1000)}{rA\ (H_2O)_p}$$

(5) . . . . . $$[B]_E = \frac{B_p(_rB)\ (1000)}{H_2O_p}$$

where: $[A]_E$ and $[B]_E$ = anion and cation concentrations respectively (mEq/kg extracellular water)

A_p and B_p = anion and cation concentrations (mEq/l plasma)

H_2O_p = Plasma water = 950 gm/kg

rCl = .977

rNa = .942

rK = .943

(v) Extravascular Cation Content

The following equation from Manery (1954) was used:

(6) . . . . . $B_{ECS} = ECS^{Cl}_{H_2O} \times [B]_E$

where; B_{ECS} = amount of cation in extracellular space (mEq/kg tissue)

$ECS^{Cl}_{H_2O}$ = extracellular space (kg/kg tissue)

$[B]_E$ = cation extracellular water concentration (mEq/kg extracellular water)

(vi) Intracellular Cation Content

The following equation from Elkinton and Danowski (1955) was used:

(7) . . . . . $B_{ICS} \equiv B_t - B_{ECS}$

where B_{ICS} = amount of cation in the intracellular space (mEq/kg tissue)

B_t = amount cation in tissue (mEq/kg tissue)

(vii) Cellular Cation Concentration

The following equation was used:

(8) . . . . . $[B]_c = \frac{B_{ICS}(1000)}{ICSV}$

Where: $[B]_c$ = concentration of cation in intracellular water (mEq/l cell water)

(viii) Membrane Potential

The existence of potential gradients across cell membranes is well established (Hodgkin and Huxley, 1945; Graham and Gerard, 1946). This can be qualitatively explained in terms of a Gibbs-Donnan equilibrium. Because the concentration of nondiffusible anions is much higher in the cellular than in the interstitial fluid, (Elkinton and Danowski, 1955) the concentration of diffusible cations is also greater in the cellular fluid, while the concentration of diffusible anions is lower. This is true in the case of potassium and chloride which are freely diffusible across the cell membrane (Fenn and Cobb, 1935; Wilde, 1939) but it is not so for sodium, as is discussed in the Literature Review. The movement of these diffusible ions across the cell membrane is influenced by two opposing forces: a tendency to achieve equal concentration of individual diffusible ions in the cellular and interstitial fluid, and a tendency to achieve electroneutrality throughout the system (Davson, 1959). The tendency for potassium and chloride to achieve equal concentrations in the extracellular and intracellular fluid results in an uneven distribution of charges across the membrane, the inside of the membrane being negatively charged to the outside. The electrical gradient then opposes further diffusion of the ions. At equilibrium the

distribution of ions is such that the system as a whole is electrically neutral but there is an accumulation of negative charge on the outside. The distribution of ions between the intracellular and extracellular fluids is discussed in the Literature Review. The membrane potential is measured by the Nernst equation (Davson 1959):

$$(9) \quad M.P. = \frac{RT}{zF} \times 2.3(1000) \times \log_{10} \frac{[K]_c}{[K]_E}$$

where M.P = membrane potential in millivolts

R = gas constant (8.3 joules $degree^{-1} mole^{-1}$)

z = valency of ion

F = Faraday's constant (96,5000 coulombs)

$[K]_c$ = cellular potassium concentration

$[K]_E$ = extravascular potassium concentration

(ix) Plasma Sodium to Chloride Ratio

The plasma sodium to chloride ratio was determined by dividing the plasma sodium concentration by the plasma chloride concentration: $(Na^+_p)/(Cl^-_p)$

where: Na_p^+ = plasma sodium (mEq/l)

Cl_p^- = plasma chloride (mEq/l)

This ratio is an indirect estimate of plasma bicarbonate ion concentration and so can be used to establish, indirectly, the condition of acidosis or alkalosis.

E. Statistical Analysis

In the case of tissue water content and the chloride, sodium, and potassium concentrations of muscle and plasma the mean, variance, standard deviation, and standard error were calculated for each sample group (Wilks, 1951)

It was assumed that the observations followed a Gaussian distribution and therefore all observations differing from the mean by more than two standard deviations were regarded as spurious and were discarded. The mean, variance,standard deviation and standard error were recalculated on the basis of the remaining data.

Graphs were drawn to show the mean, standard deviation and standard error of each sample group.

One sided tolerance limits were calculated for plasma and tissue chloride concentration and tissue water content of the 20°C control group (Goldstein, 1964; p49)

The data of sets of two sample groups were compared:

1. The variance estimates of the two sample groups were compared by the variance ratio or F-test (Goldstein, 1964: p52).

2a. If the difference between the two sample variances was found to be not significant at the .05 level, the means of the two samples were compared by the Fisher extension of the Student t-test (Johnson and Jackson, 1959; p161). The t value obtained was compared with the tabled value of t for the corresponding degree of freedom at the .05 level.

b. If the difference between the two sample variances was found to be significant at the .05 level the means of the two sample groups were compared at the .05 level by the Cochran and Cox test (Johnson and Jackson, 1959; p163).

c. If the difference between the two sample means was found to be significant at the .05 level it was concluded that the samples were not drawn from the same population, i.e. that they differ from each other significantly (Goldstein, 1964; p53).

CHAPTER IV

RESULTS

1. Sublethal Shock

A. Plasma Chloride

Changes in plasma chloride levels following abrupt transfer of goldfish from 20°C to 30°C are shown in Fig. 1. As compared to the mean concentration of the 20°C control group, the decrease in mean concentration after twenty-four hours exposure and the subsequent increase after thirty-six hours were insignificant at the .05 level of significance (see Materials and Methods). After this initial lag period of thirty-six hours mean values rose sharply to a value of 104 ± 1.2 mEq/l after forty-eight hours exposure. This value was obtained by sampling two separate groups of fish. Chloride concentration fell after forty-eight hours exposure, returning to the levels characteristic of the 20°C control group by sixty-hours after transfer. After 120 hours exposure the mean chloride levels had fallen to values significantly lower than those of the 20°C control group and levelled off in the range $76.4 \pm .2$ to 79.2 ± 1.4 mEq/l. The mean level of the 30°C control group (78.5 ± 1.5 mEq/l) was significantly lower than that of the 20°C control group ($82.0 \pm .8$ mEq/l) and was comparable to the mean

levels obtained after 120 to 240 hours exposure.

Thus, the effect of thermal shock in this instance was seen as in increase in plasma chloride, following an initial lag period, and succeeded by a depression of significant magnitude.

B. Tissue Chloride

Changes in tissue chloride levels following abrupt transfer of goldfish from 20°C to 30°C are shown in Fig. 2. The mean values obtained after twelve and twenty-four hours exposure to 30°C did not differ significantly (See Materials and Methods) from that of the 20°C control group. At the thirty-six hours sample period the mean concentration was seen to have decreased significantly from the mean of 19.3±1.4 mEq/kg of the 20°C control group, to a mean of 14.5±1.4 mEq/kg. Values rose immediately and remained comparable to those of the 20°C controls from forty-eight to seventy-two hours after transfer. Again at eighty-four hours, mean tissue chloride concentration decreased to 11.6±1.0 mEq/kg, a value significantly lower than that of the 20°C control group. This second decrease was also followed by an immediate return to control values; a mean concentration of 22.1±4.3 mEq/kg was observed at ninety-six hours exposure to 30°C.

Fig. 1. Variations in plasma chloride concentration following abrupt transfer from 20°C to 30°C.

Fig. 2. Variations in muscle tissue chloride concentration following abrupt transfer from 20°C to 30°C.

Fig. 3. Variations in plasma potassium concentration following abrupt transfer from 20°C to 30°C.

Fig. 1-3. Horizontal line indicates sample mean. Vertical line indicates standard deviation. Solid box indicates standard error of the mean.

Fig. 1

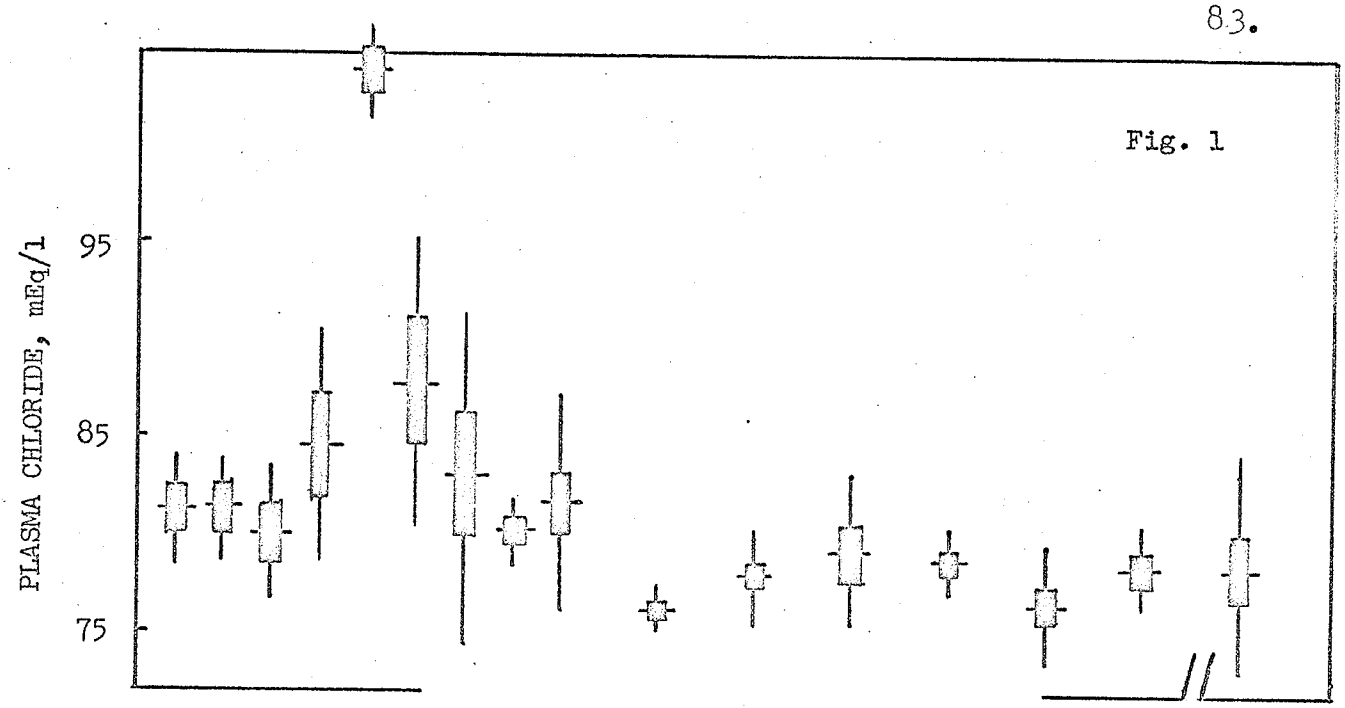


Fig. 2

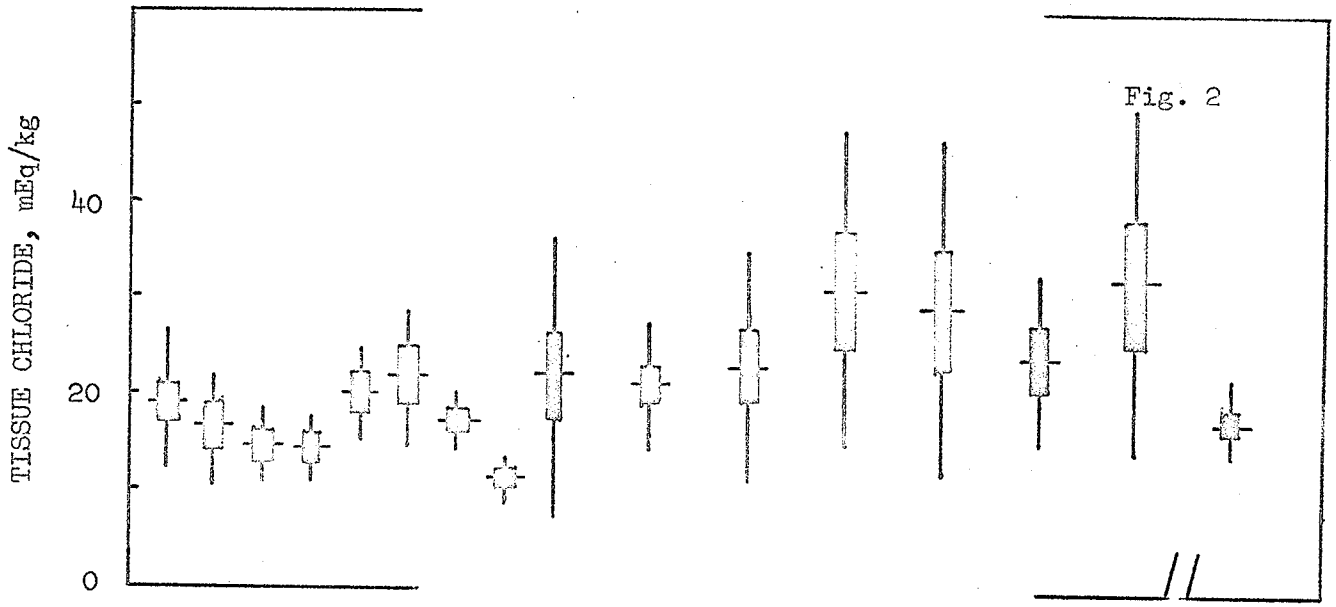
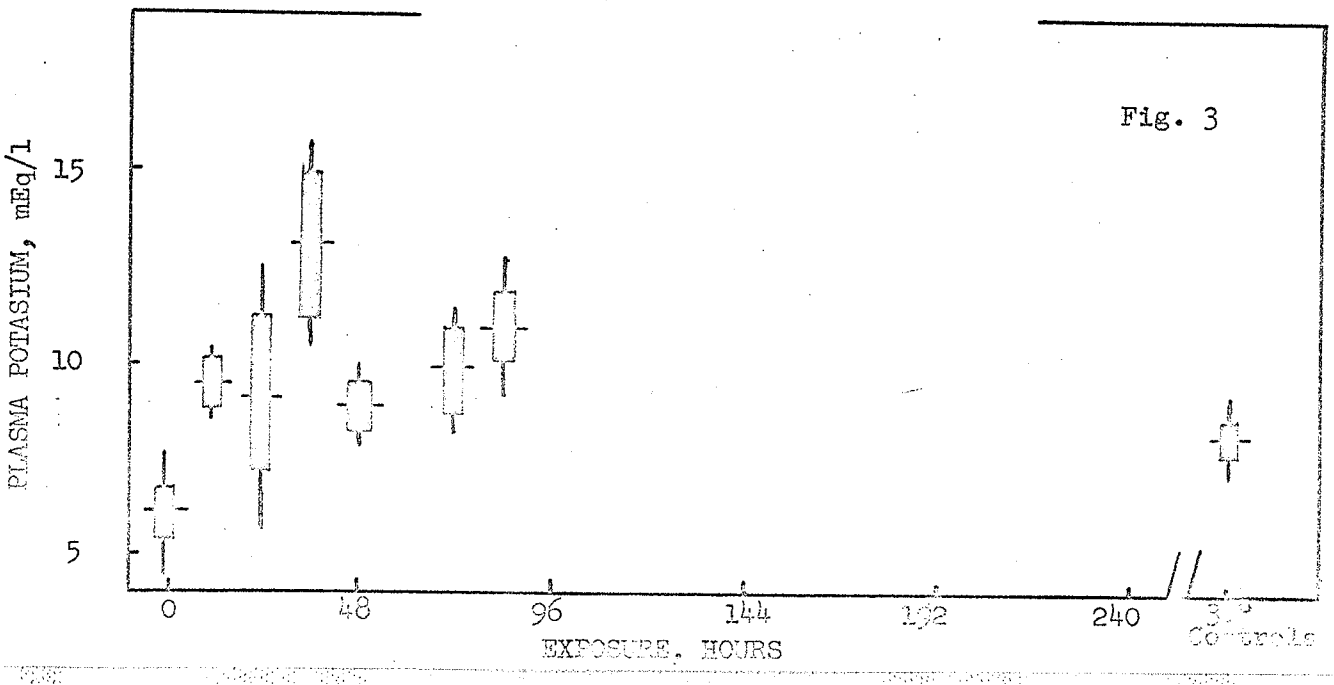


Fig. 3



Thereafter, mean chloride levels, ranging from 21.2±2.1 mEq/kg to 32.0±6.4 mEq/kg, did not differ significantly from the mean of the 20°C control group. The difference between the mean of the 30°C control group (17.4±.9 mEq/kg) and that of the 20°C control group (19.3±1.4 mEq/kg) also was not significant.

The overall effect of thermal shock on tissue chloride levels is thus seen as an initial lag period followed by a decrease after thirty-six hours exposure, a return to control values, and a second decrease after eighty-four hours exposure. Thereafter mean values remained comparable to those of the 20°C controls.

C. Plasma Potassium

Changes in plasma potassium concentration following abrupt shock are shown in Fig. 3. The immediate significant increase in mean potassium levels from 6.1±.6 mEq/l to 13.2±1.9 mEq/l at thirty-six hours was followed by a decrease to 8.9±.7 mEq/l at forty-eight hours. This intermediate value differed significantly from that of the 20°C control group at the .05 level of significance. Potassium concentrations rose again to a mean of 11.0±.9 mEq/l at eighty-four hours. Because of the small size of the goldfish, plasma volumes sufficient for potassium determination could not be obtained at sample periods from

96 to 240 hours. The steady state had not been reached at the end of the eighty-four hour sample period and between eighty-four hours and twenty-one days after transfer, plasma potassium concentration decreased to 8.3±.3 mEq/l. The mean of the 30°C control group was significantly greater than that of the 20°C control group.

The effect of thermal shock was seen in this instance as an immediate increase in plasma potassium concentration followed by a decrease and a secondary increase. The potassium concentration in goldfish acclimated to 30°C was significantly greater than in fish acclimated to 20°C.

D. Tissue Potassium

Tissue potassium changes are shown in Fig. 4. Following an initial lag period the mean level after thirty-six hours exposure was 123±6 mEq/kg, a value significantly greater than the mean (104±mEq/kg) of the 20°C control group. Potassium levels decreased immediately, and although mean values ranged from 105± 10 to 121±8 mEq/kg between 48 and 120 hours exposure, they remained comparable to the mean of the 20°C controls. A second significant increase (relative to the values of the 20°C control group) to a mean of 133±8 mEq/kg after 144 hours exposure was followed by a decrease, also significant, to a minimum of

73 ± 22 mEq/kg after 192 hours. The mean of 98 ± 11 mEq/kg of the 240 hour sample period was comparable to the mean of 115 ± 6 mEq/kg of the 30°C control group. Neither the mean value of the 240 hour sample group nor that of the 30°C control group differed significantly from that of the 20°C control group at the .05 level.

Thus, the effect of thermal shock in this instance is seen as a lag period followed by an increase and subsequent return to values comparable to those of the 20°C control group. This was followed by an increase after 144 hours, a decrease after 192 hours and an increase and achievement of a steady state level, comparable to that of the 20°C control group, by 240 hours.

E. Plasma Sodium

Plasma sodium values are shown in Fig. 5. Mean values did not differ significantly from the mean of 175 ± 8.6 mEq/l of the 20°C control group during the first thirty-six hours but increased significantly thereafter to a mean of 221 ± 10.7 mEq/l at forty-eight hours and to a mean of 255 ± 28.0 mEq/l at seventy-two hours. While both these values are significantly greater than the mean of the 20°C control group, they do not differ significantly from each other.

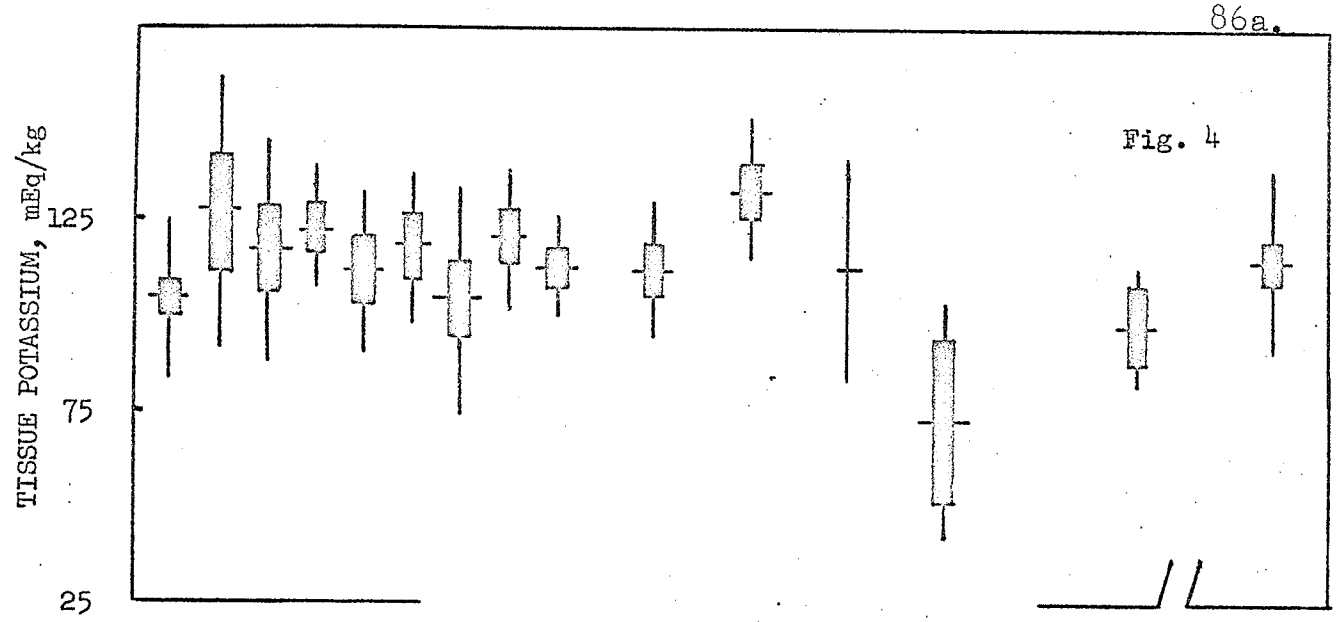


Fig. 4

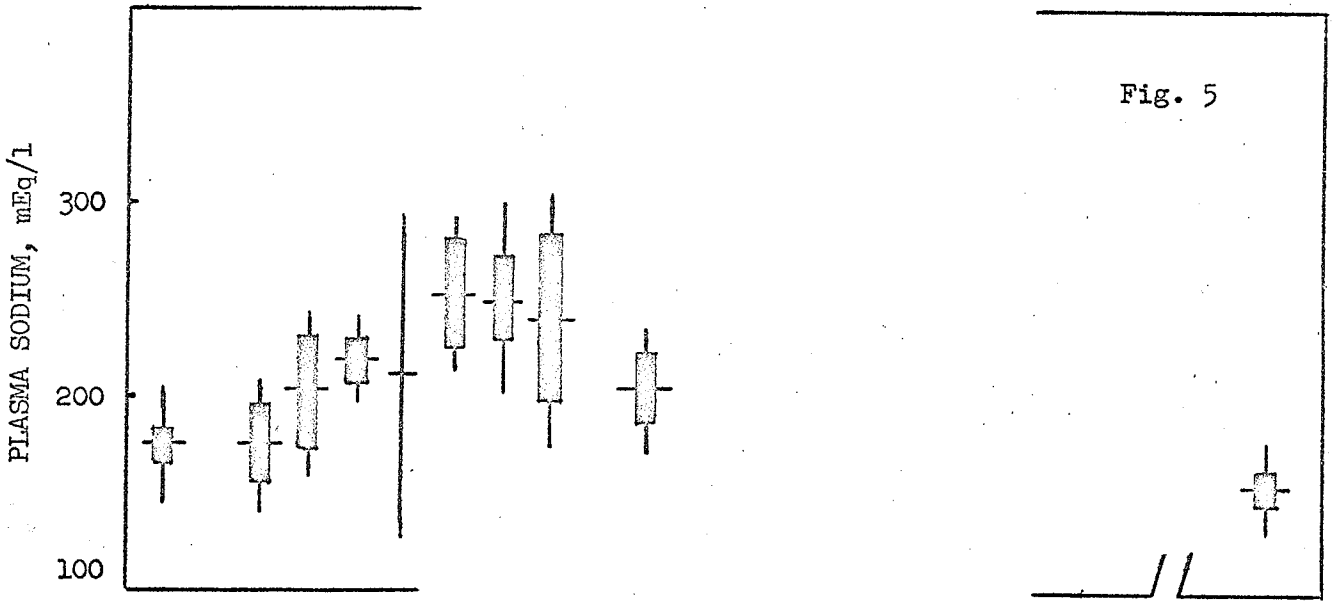


Fig. 5

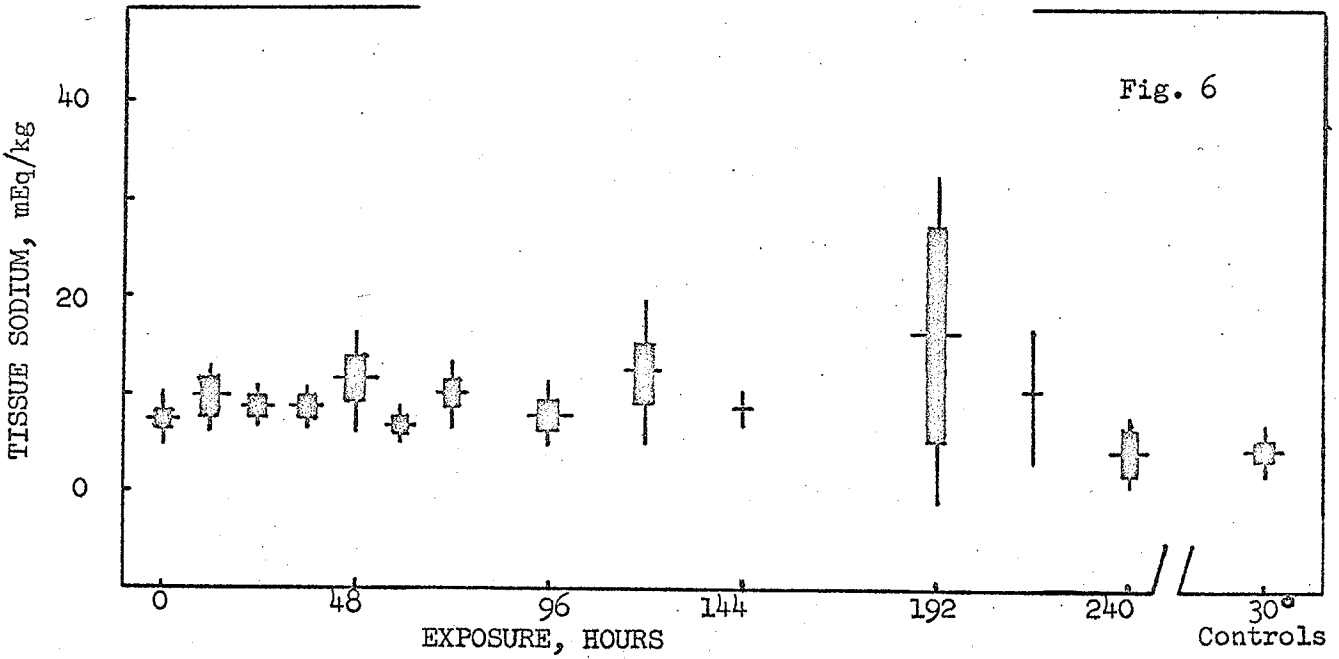


Fig. 6

Mean values then decreased and after 120 hours were again comparable to that of the 20°C control group. Plasma volumes sufficient for sodium determination could not be obtained at sample periods from 144 to 240 hours. At the .05 level of significance the mean level of 155±7.6 mEq/l recorded for the 30°C control group was not significantly different from that of the 20°C control group.

Thus the response of plasma sodium concentration to sublethal thermal shock was an initial lag period followed by an increase and then a decrease to the original level. In this instance the steady state was reached after 120 hours exposure, with the sodium level of fish acclimated to 30°C being comparable to that of fish acclimated to 20°C.

F. Tissue Sodium

Tissue sodium concentrations following abrupt transfer from 20°C to 30°C are shown in Fig. 6. The mean levels, ranging from 4.5±2.6 mEq/kg to 16.3±11.7 mEq/kg, obtained for sample periods from 12 to 240 hours did not vary significantly from the mean, 7.7±.5 mEq/kg, of the 20°C control group at the .05 level. However the mean of the 30°C controls, 4.7±.8 mEq/kg, differed significantly from that of the 20°C controls.

G. Summary of Tissue and Plasma Chloride, Sodium, and Potassium

The effect of sublethal thermal shock on mean concentrations of plasma sodium and chloride was seen as an initial lag period of thirty-six hours, followed by an increase and subsequent decrease to values similar to (in the case of sodium) or lower than (in the case of chloride) those of the 20°C control group. In both cases a steady state was reached after 120 hours exposure to 30°C.

There was no lag period in the case of plasma potassium. The immediate increase in concentration was followed by a decrease and secondary increase. The mean concentration of the 30°C control group was significantly higher than that of the 20°C controls.

Mean tissue chloride concentrations, with the exception of the decreases seen at thirty-six and eighty-four hours exposure, showed no significant variations from the mean of the 20°C controls. The mean of the 30°C control group was similar to that of the 20°C controls.

Mean tissue sodium levels showed no significant variations, relative to the mean of the 20°C controls, throughout the 240 hour sample period but the mean of the 30°C control group was significantly lower.

Variations, however, were apparent in the concentrations of tissue potassium. The increase after thirty-six hours exposure was followed by a decrease and secondary increase. A steady state, with a mean concentration similar to that of the 20°C controls, was achieved after 240 hours exposure.

H. Plasma Sodium to Chloride Ratio

Changes in the ratio of plasma $\frac{\text{sodium}}{\text{chloride}}$ are shown in Fig. 7. The initial lag period of thirty-six hours corresponded to the lag in the response of both plasma sodium and chloride to thermal shock. The ratios rose from 2.13, the value of the 20°C control group, to 3.14 at eighty-four hours and decreased between 84 and 120 hours, at which exposure time acclimation to 30°C had been completed in the case of both plasma sodium and plasma chloride. The slight decrease in the ratio of the 30°C control group as compared to that of the 20°C control group was due to a nonsignificant decrease in plasma sodium concentration.

Thus, the effect of thermal shock on the plasma sodium to chloride ratio, was seen as an initial lag period, an increase to a maximum after eighty-four hours exposure, and a decrease to the original value as acclimation to 30°C was completed.

The increase in the sodium to chloride ratio

suggests an increase in plasma bicarbonate concentration and the possibility of the establishment of a condition of alkalosis, which reached a maximum at eighty-four hours but decreased as acclimation was completed.

1. Tissue Water

Changes in tissue water content are shown in Fig. 8. The mean values observed after twelve and twenty-four hours exposure were not significantly different from the mean, 807±2 g/kg, of the 20°C controls. Mean values then increased to 821±5 g/kg and 836±7 g/kg (an increase of 3.5% over control value) at thirty-six and forty-eight hours, respectively. While the mean values of the thirty-six and forty-eight hour sample groups both differed significantly from that of the 20°C control group, they did not differ significantly from each other. Tissue water content then decreased and the mean value, 813±5 g/kg, obtained after eighty-four hours exposure was again comparable to the mean of the 20°C controls. Mean tissue water content then exhibited a second and greater increase of 9.5 per cent with values reaching a maximum mean of 885±16 g/kg at 168 hours after transfer. Between 168 and 240 hours, water levels remained in the range 855±13 to 885±16 g/kg. The mean water content of the 30°C control group(808±4 g/kg) was similar to that of the 20°C control group (807±2 g/kg).

Fig. 7

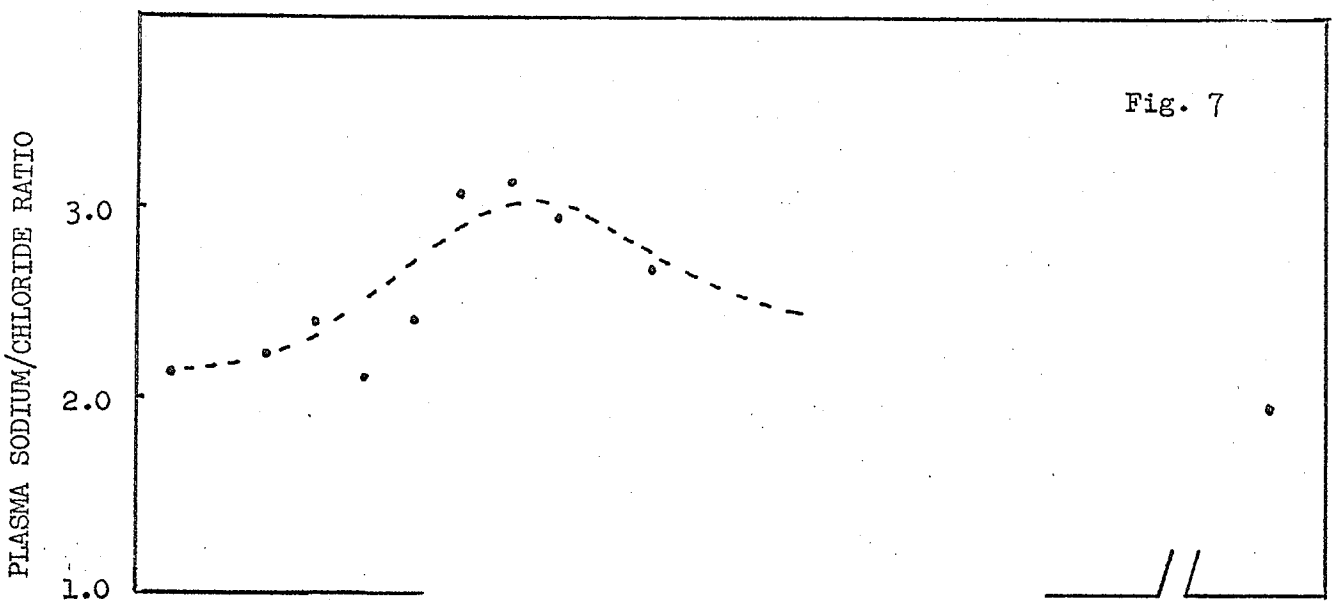


Fig. 8

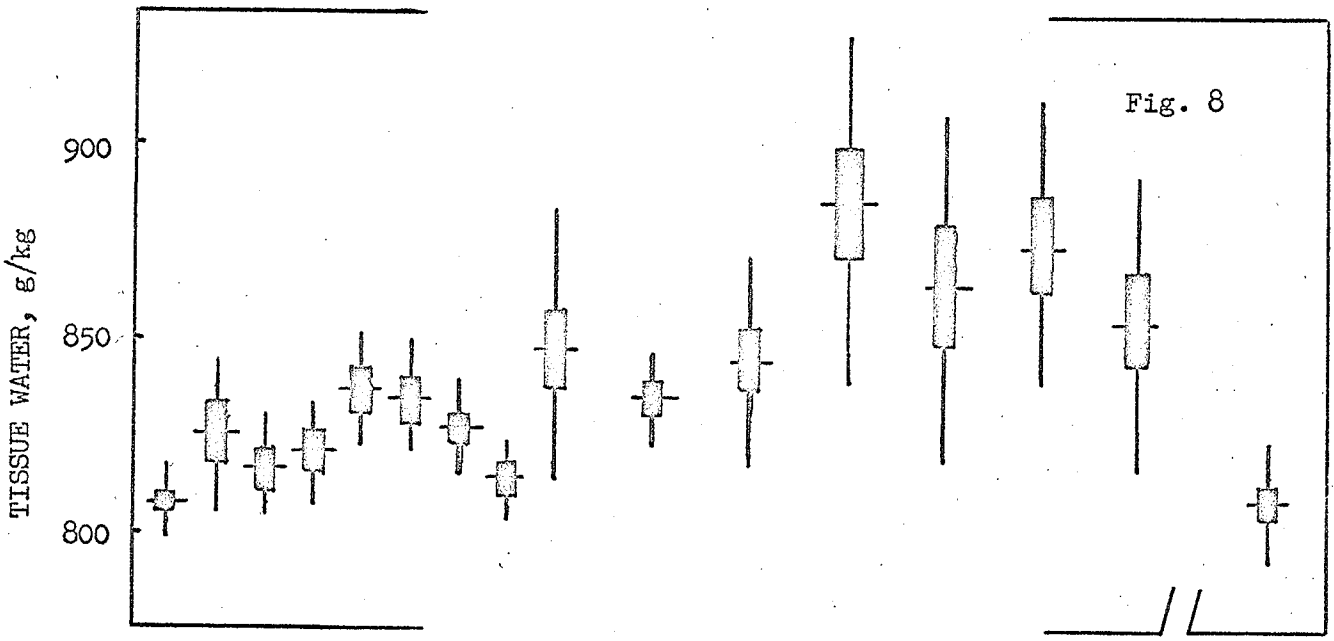
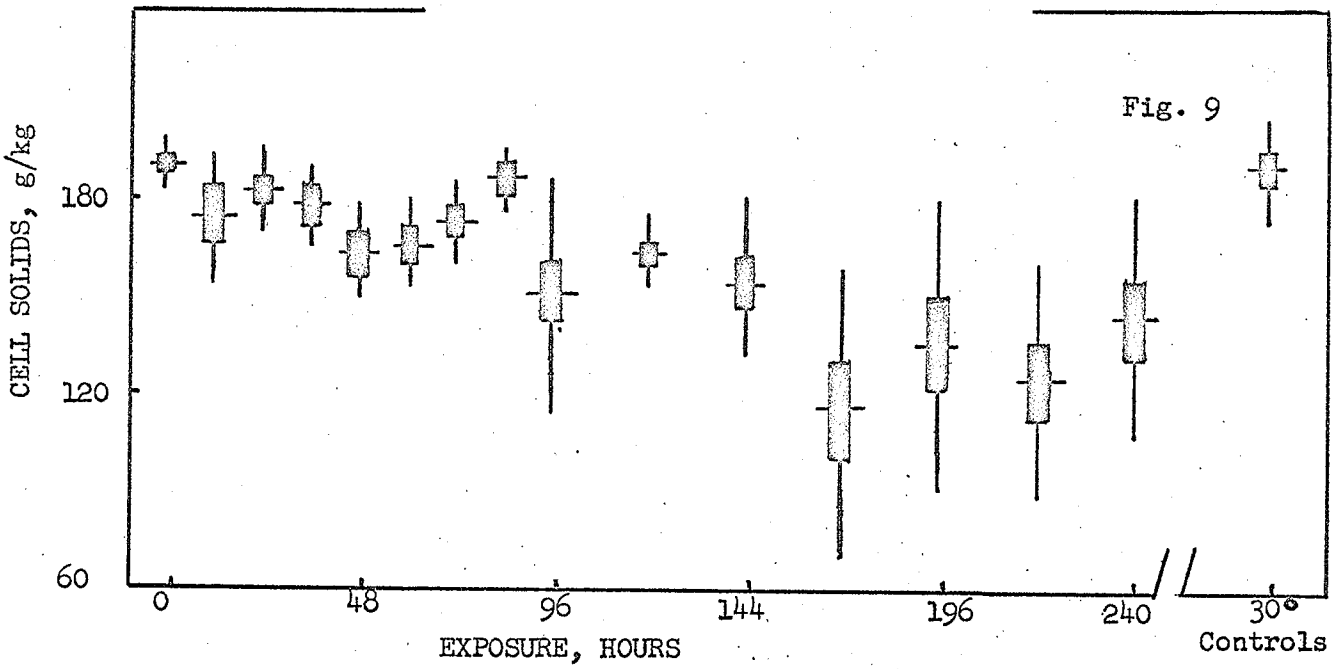


Fig. 9



Thus, after an initial lag period the effect of thermal shock in this instance was seen as an increase of 3.5 per cent followed by a return to the level of the 20°C control group. A second increase of 9.5 per cent then took place. Acclimation to 30°C was apparently completed between 240 hours and twenty-one days, with values returning to the original level of the 20°C controls.

J. Cell Solids

Changes in cell solids, shown in Fig.9, were found to be the reverse of changes in tissue water content. Acclimation was completed between 240 hours and twenty-one days, with the mean, 192 ± 4 g/kg, of the 30°C control group comparable to the mean, 193 ± 2 g/kg, of the 20°C control group.

K. Extracellular Space Volume

Variations in chloride space (extracellular space volume) are shown in Fig. 10. The mean chloride space, 134.5 g/kg (95% confidence interval of 105.0 to 164.0 g/kg), which was obtained after eighty-four hours exposure was significantly lower than the mean, 218.5 g/kg (95% confidence interval of 182.5 to 254.5 g/kg), of the 20°C controls; and the mean, 378.3 g/kg (95% confidence interval of 285.9 to 470.6 g/kg), obtained after 240 hours was significantly greater. Other values did not vary significantly

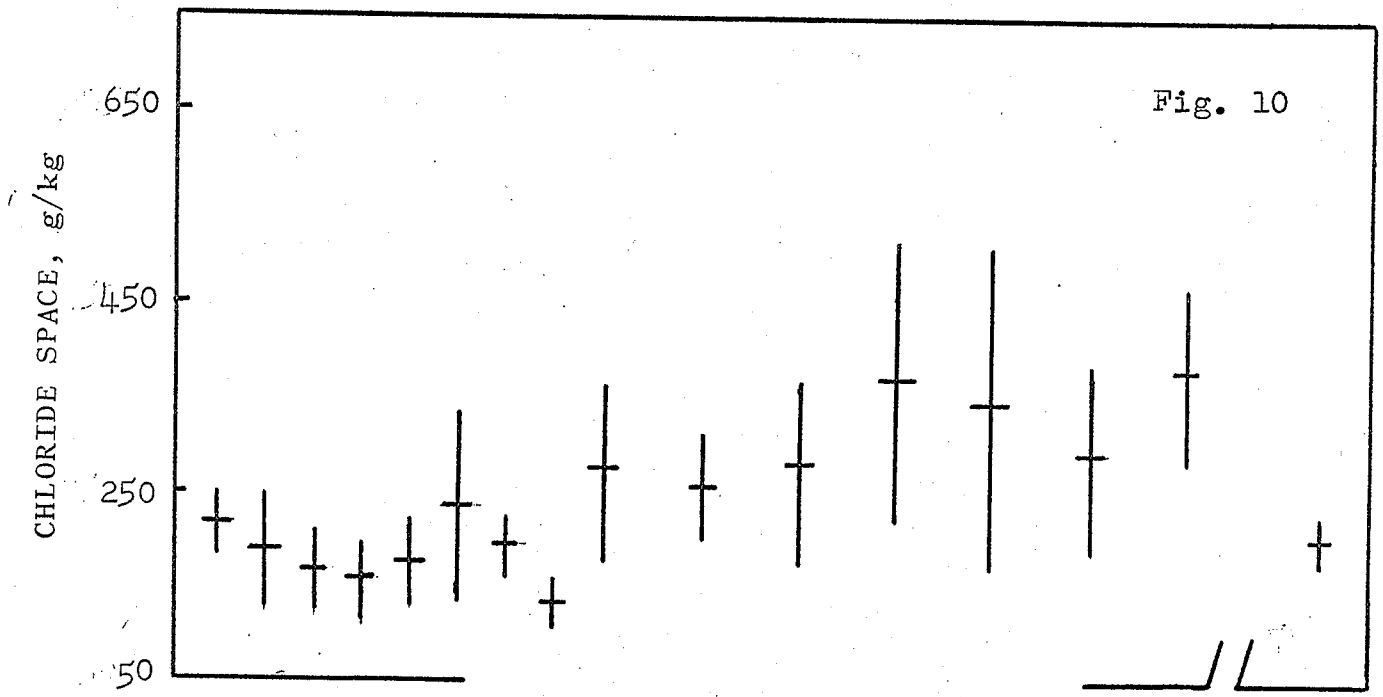

650
450
250
50
CHLORIDE SPACE, g/kg

Fig. 10

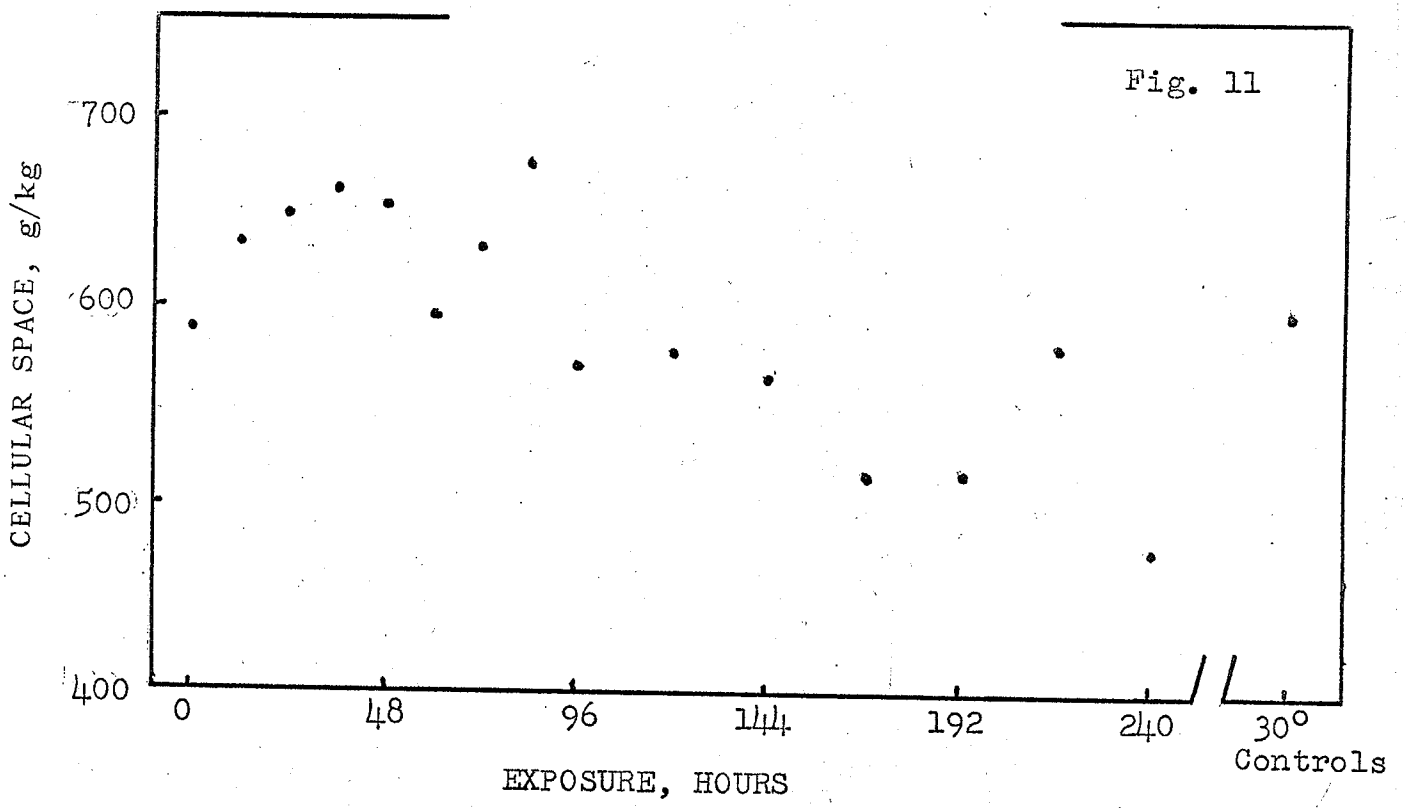

700
600
500
400
CELLULAR SPACE, g/kg
0
48
96
144
192
240
30°
Controls
EXPOSURE, HOURS

Fig. 11

from the 20°C control values. The mean, 206.1 g/kg, and 95 per cent confidence limits, 182.3 to 229.8 g/kg, of the 30°C control group were within the 95 per cent confidence limits, 182.5 to 254.5 g/kg of the 20°C control group.

L. Intracellular Space Volume

Changes in intracellular volume are shown in Fig. 11. The increase from the mean of 588.9 g/kg of the 20°C controls to 660.5 g/kg at thirty-six hours corresponds to the significant increase in tissue water content at that time. After sixty-hours exposure the mean cellular volume was found to be 598.6 g/kg. The second increase, relative to the mean of the 20°C control group, to a mean of 678.5 g/kg at eighty-four hours resulted from a significant decrease in chloride space relative to the mean of the 20°C controls. The mean cellular volumes found between 96 and 216 hours exposure, 517.0 to 584.1 g/kg, were slightly less than the mean value, 588.9 g/kg, of the 20°C controls. However since mean chloride spaces were found not to differ significantly from the mean of the 20°C controls during this time, and mean tissue water contents were found to be significantly greater, this observed decrease in cellular volume would seem to be insignificant. The decrease in cellular volume to 476.4 g/kg at 240 hours corre-

sponds to a mean tissue water content and mean chloride space both significantly greater than the means of the 20°C control group. The cellular volume of the 30°C control group was found to be 602.3 g/kg.

M. Summary of Tissue Water, Chloride Space, and the Intracellular Volume

The mean tissue water content and mean chloride space found for the 30°C control group did not differ from the means of the 20°C control group at the .05 level of significance. The mean intracellular volumes of the two control groups also appeared similar. In the case of tissue water content and chloride space acclimation did not appear to be complete at the end of the 240 hour sample period.

Tissue water levels showed a significant increase of 3.5% after thirty-six hours exposure and a decrease to the original level after eighty-four hours. Water content again increased to a mean value 9.5 per cent greater than that of the 20°C controls after 168 hours exposure and levelled out.

Chloride space was seen to decrease at eighty-four hours and increase at 240 hours exposure relative to the mean of the 20°C controls. Intracellular space, relative to the mean of the 20°C controls, showed increases after thirty-six and eighty-four hours exposure.

N. Intracellular Potassium Concentration

Changes in intracellular potassium concentration are shown in Fig. 12. The initial increase from the 20°C control value of 175 mEq/l to 197 mEq/l after twelve hours exposure was followed by a decrease to 163 mEq/l at seventy-two hours and a subsequent increase to 175 mEq/l, the mean value of the 20°C controls, at eighty-four hours. The mean concentration of the 30°C control group was found to be 188 mEq/l.

O. Membrane Potential

Changes in membrane potentials calculated from potassium values are shown in Fig. 13. The membrane potentials decreased from a mean value of 84.6 mV for the 20°C control group, to a mean of 72.4 mV at eighty-four hours. Potentials were not calculated for sampling periods from 96 to 240 hours because of the absence of plasma potassium values for that interval. The mean membrane potential of the 30°C control group was found to be 81.6 mV, a value similar to the potential of 84.6 mV of the 20°C control group.

2. Lethal Shock

A. Plasma Chloride

Changes in plasma chloride following exposure to temperatures of 36°C to 38°C are shown in Fig. 14.

Fig. 12. Variation in cellular potassium concentration following abrupt transfer 20°C to 30°C. Darkened circle indicates sample mean.

Fig. 13. Variation in membrane potential, as calculated from potassium distribution values, following abrupt transfer from 20°C to 30°C. Trend line is fitted by eye.

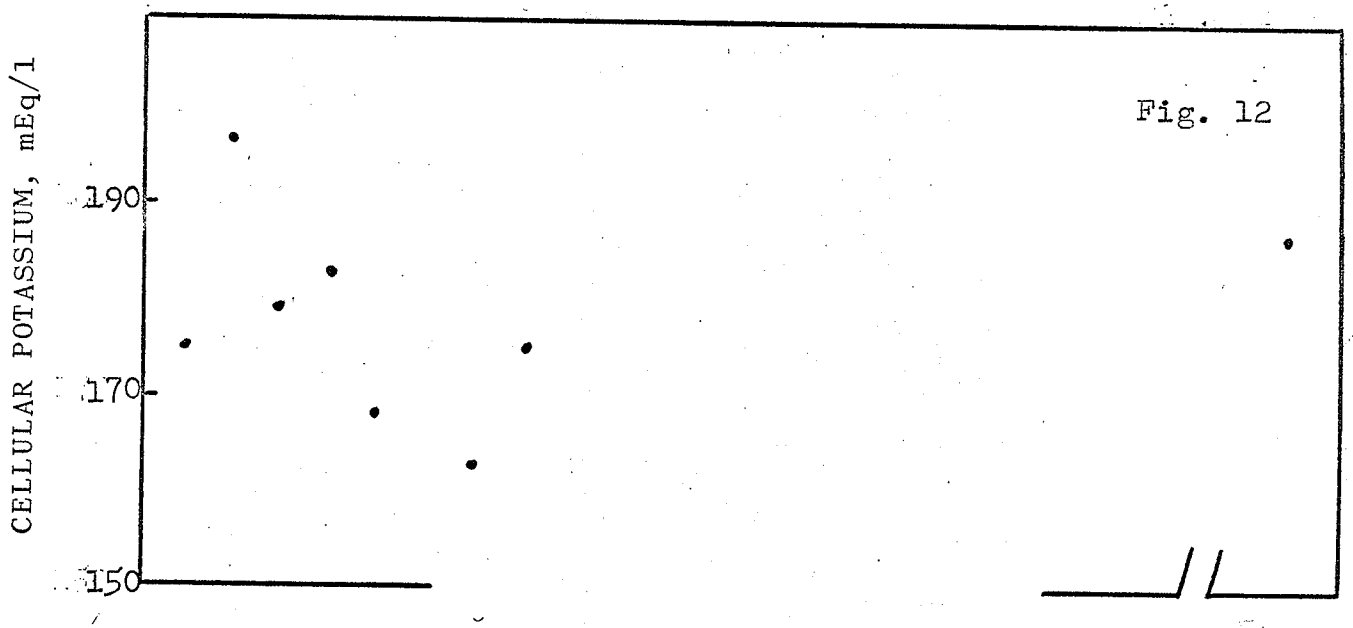

CELLULAR POTASSIUM, mEq/1
190
170
150

Fig. 12

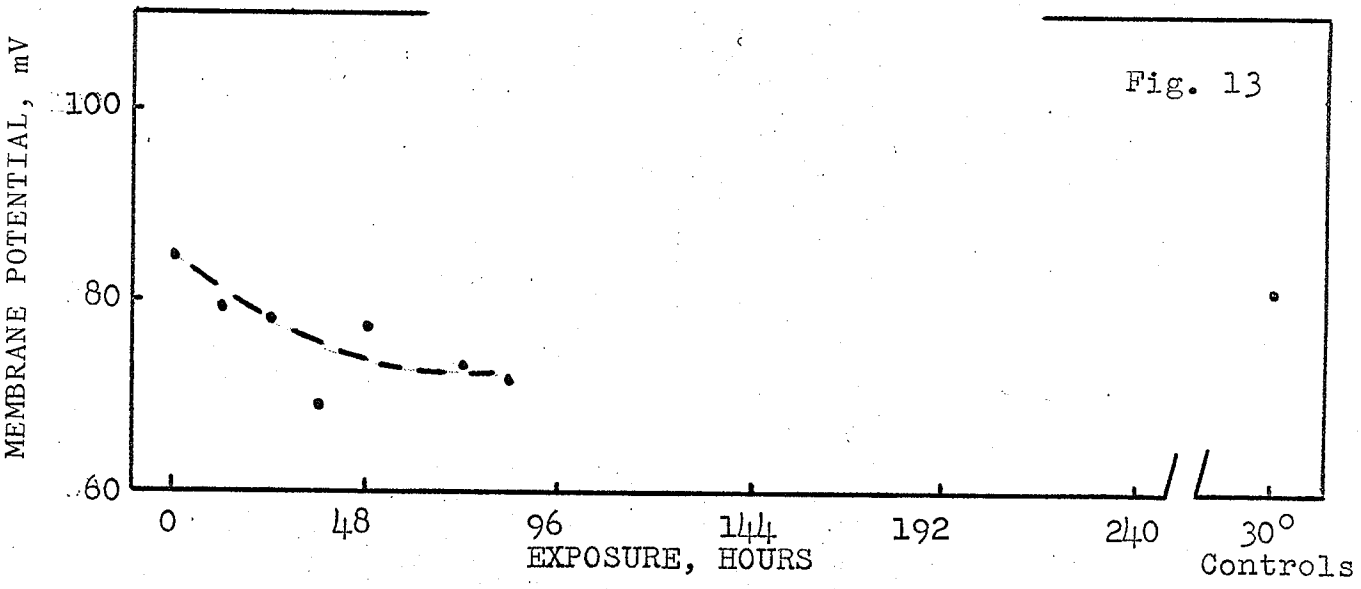

MEMBRANE POTENTIAL, mV
100
80
60
0
48
96
144
192
240
30°
Controls
EXPOSURE, HOURS

Fig. 13

At the .01 level of significance at least 95 per cent of the plasma chloride values of the population represented by the 20°C control group will be greater than the one-sided tolerance limit of 71.2 mEq/l (Materials and Methods, Appendix). Reference to Fig. 14 shows that following exposure to temperatures of 36°C or 38°C plasma chloride concentrations fell below this tolerance limit, 71.2 mEq/l, within ten minutes and reached a value of 56 mEq/l (a decrease of 32 per cent) after twenty-five minutes. Thereafter, values remained within the range 56 to 65 mEq/l with a mean of 59.5 mEq/l (a decrease of 27 per-cent). All the fish died within 135 minutes of exposure to lethal temperatures.

B. Tissue Chloride

Variations in tissue chloride levels following lethal thermal shock are shown in Fig. 15. At the .01 level at least 95 per cent of the tissue chloride concentrations of the population represented by the 20°C control group will be greater than the lower one-sided tolerance limit of .8 mEq/kg and at least 95 per cent will be smaller than the upper one-sided tolerance limit of 37.6 g/kg (Materials and Methods, Appendix). As shown by Fig. 15, tissue chloride values remained within these one sided tolerance limits following lethal shock.

Fig. 14. Variation in plasma chloride concentration following abrupt transfer from 20°C to 36-38°C.

Fig. 15. Variation in muscle tissue chloride concentration following abrupt transfer from 20°C to 36-38°C.

Fig. 16. Variation in muscle tissue water content following abrupt transfer from 20°C to 36-38°C.

Fig. 14-16 Horizontal line indicates mean of 20°C control group. Vertical line indicates lower (Figs. 14, 15) and upper (Figs. 15,16) 95% tolerance limit of 20°C control group.

Fig. 14

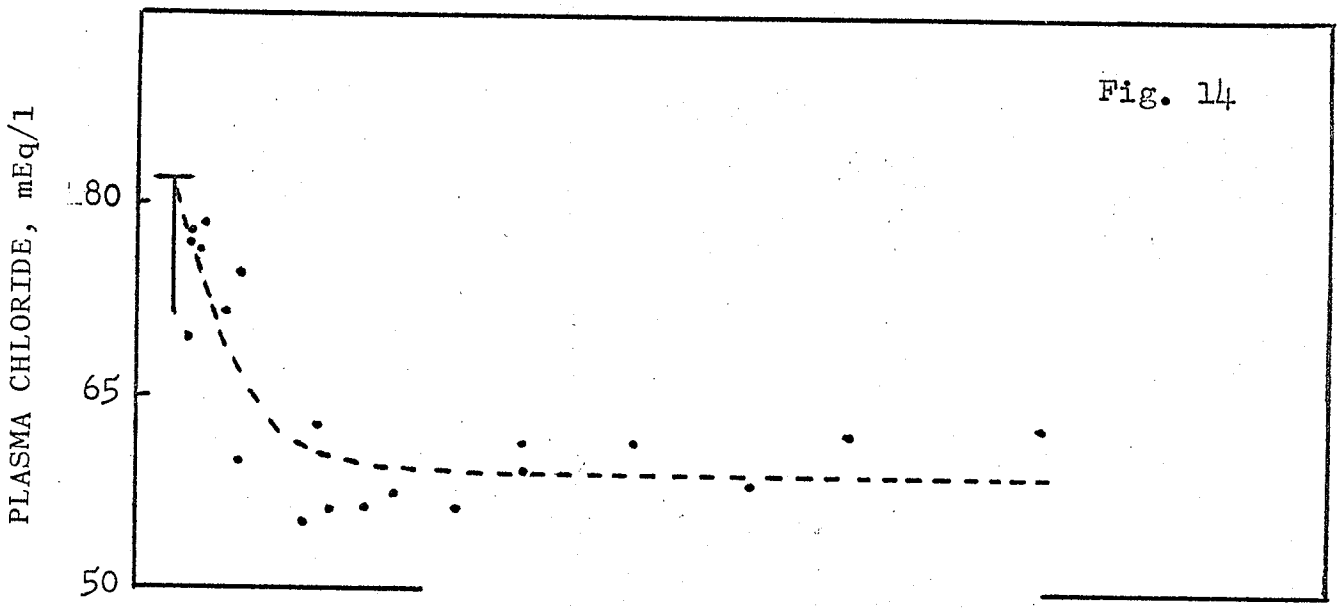


Fig. 15

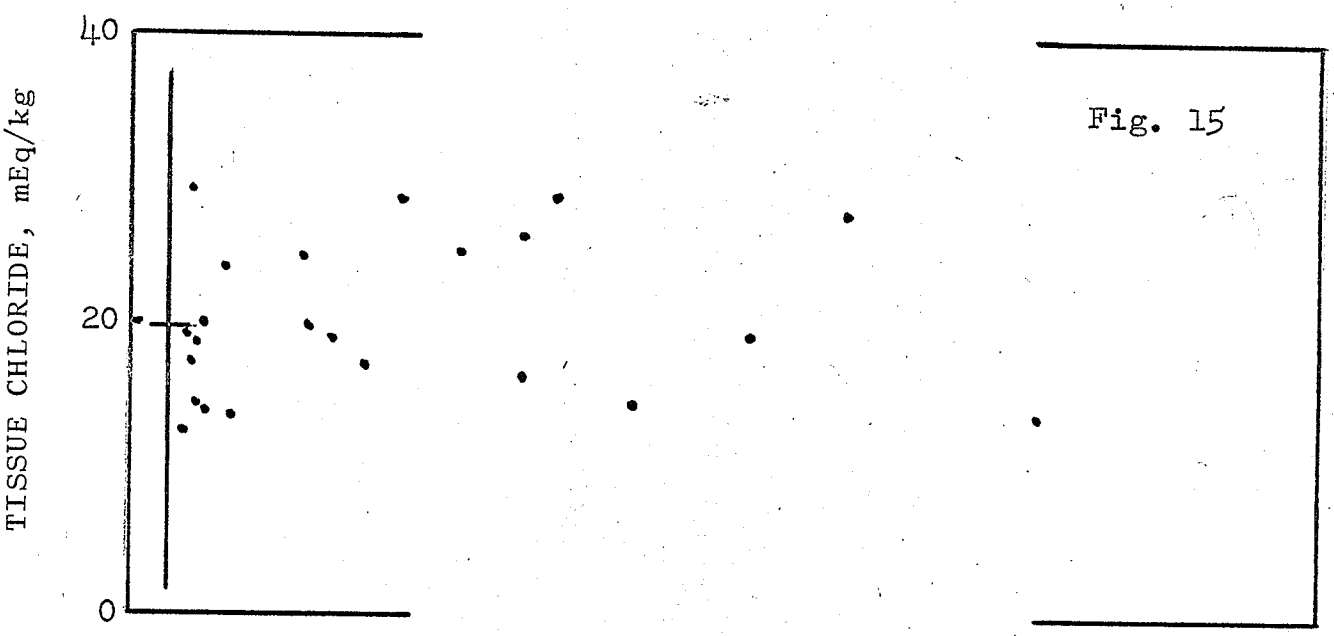
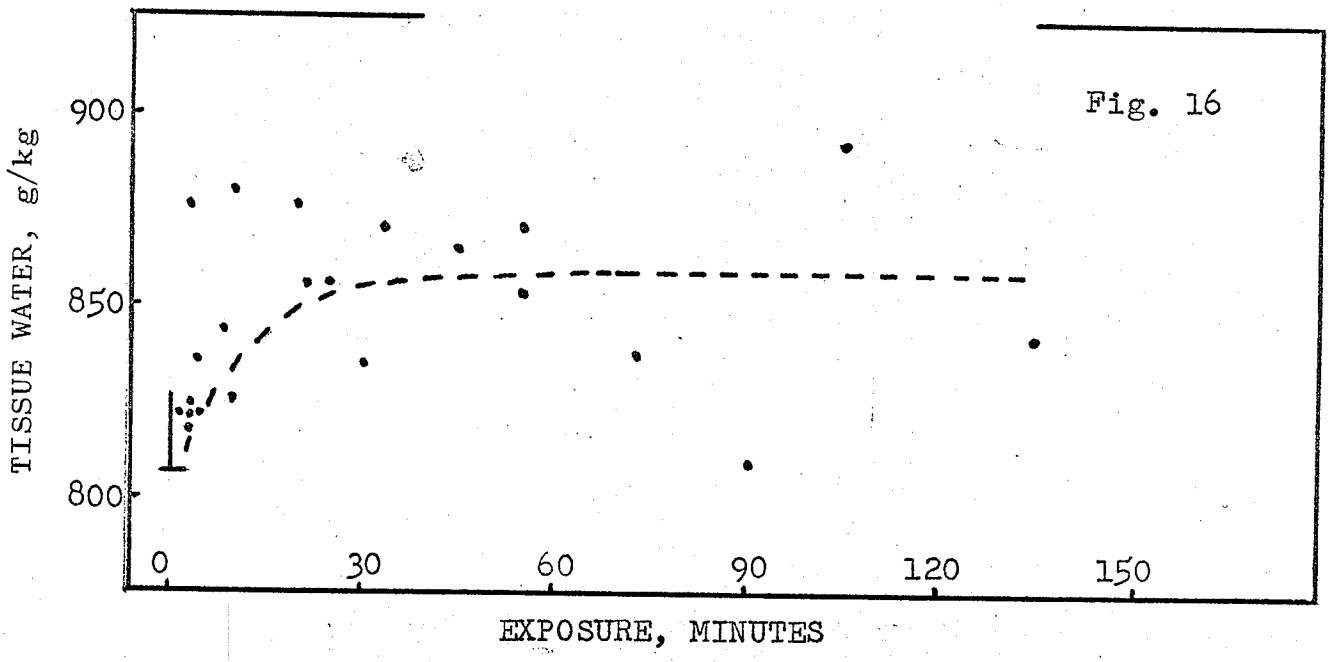


Fig. 16



C. Tissue Water

Changes in tissue water content are shown in Fig. 16. At the .01 level of significance at least 95 per cent of the tissue water levels of the population represented by the 20°C control group will be smaller than the upper one-sided tolerance limit, 827.5 g/kg. As shown by Fig. 16, tissue water content values rose immediately following lethal shock, exceeding the upper one-sided tolerance limit, 827.5 g/kg, within five minutes and reaching a level of approximately 860 g/kg after twenty-five minutes. Thereafter values remained within the range 810 to 893 g/kg, with a mean of 855 g/kg (an increase of 6 per cent over the mean tissue water level, 807 g/kg, of the 20°C control group).

D. Cell Solids

Changes in cell solids, shown in Fig. 17, mirrored the changes in tissue water content. Values fell below 172.5 g/kg, the level corresponding to the upper tolerance limit of the mean tissue water content of the 20°C controls, within five minutes and decreased to 140 g/kg after twenty-five minutes. Thereafter values remained within the range 107 to 190 g/kg with a mean of 145 g/kg.

Fig. 17. Variation in muscle tissue cell solids following abrupt transfer from 20° to 36-38°C.. Horizontal line, solid box, and vertical line indicate mean, standard error of the mean, and standard deviation of the 20°C control group.

Fig. 18. Variation in extracellular phase volume, or chloride space, following abrupt transfer from 20°C to 36-38°C. Horizontal and vertical lines indicate the mean and the 95% confidence limits of the mean of the 20°C control group.

Fig. 19. Variation in cellular phase volume following abrupt transfer from 20°C to 36-38°C. Horizontal line represents the mean of the 20°C control group.

Fig. 17-19. Darkened circles indicate values calculated for individual fish.
Trend lines are fitted by eye.

Fig. 17

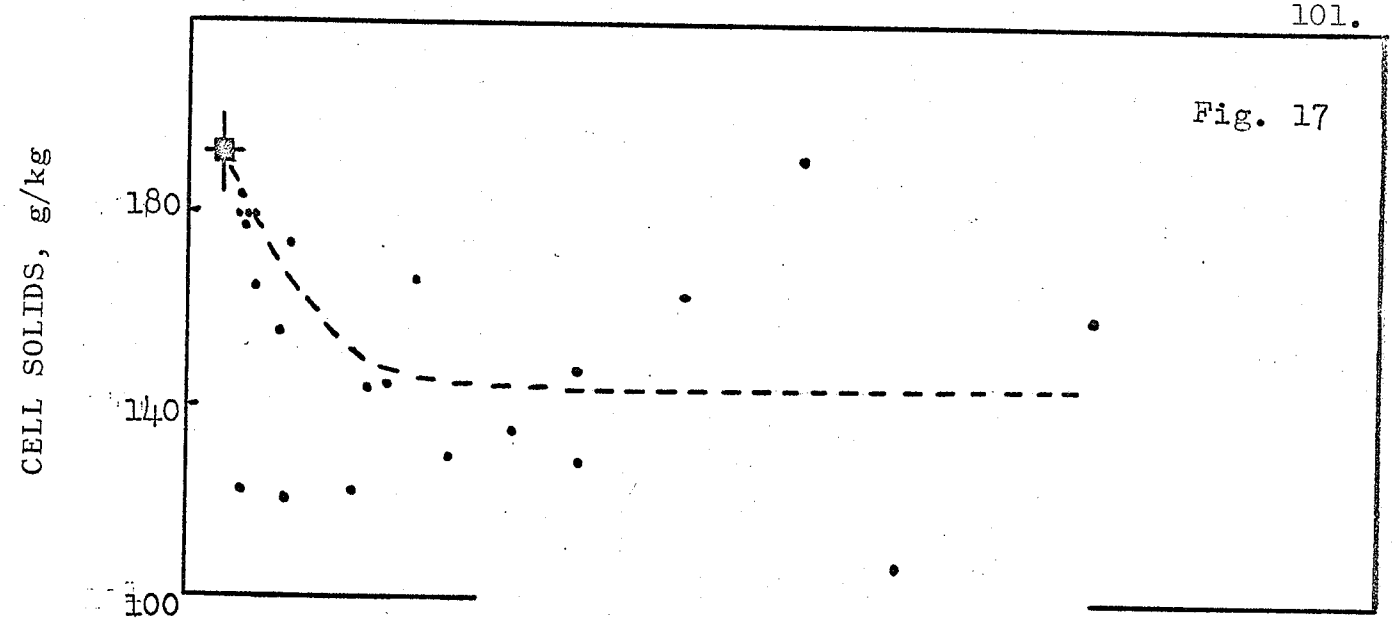


Fig. 18

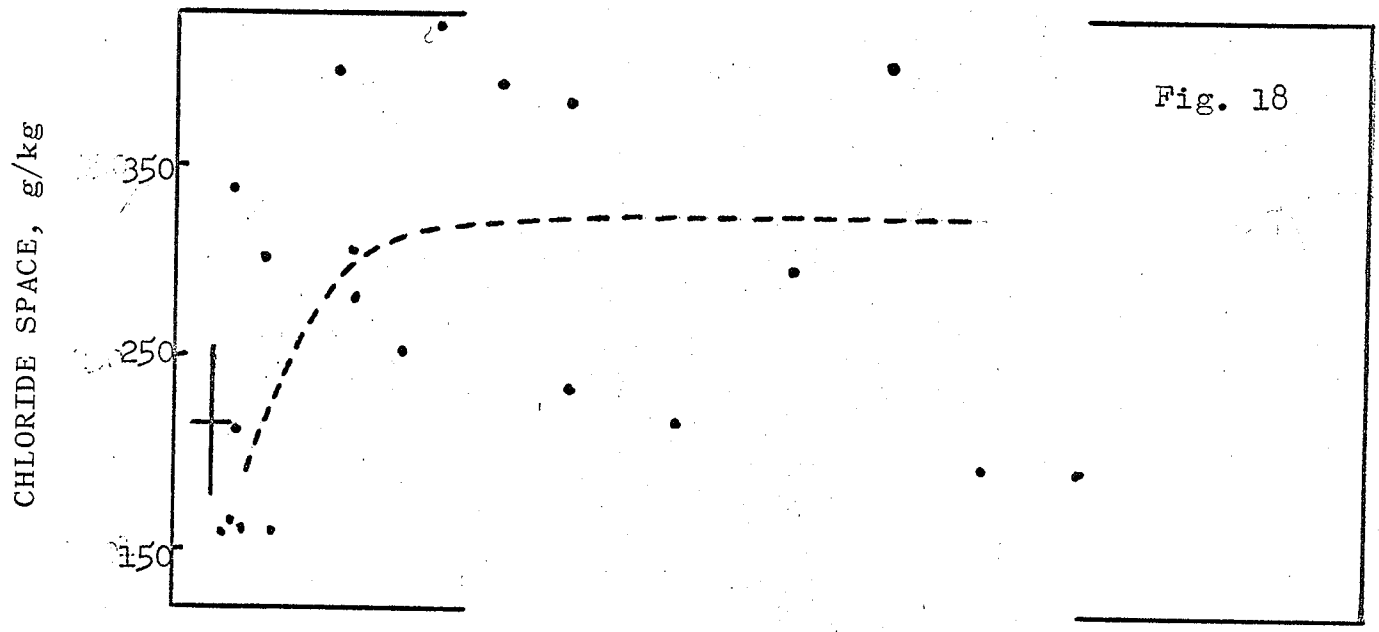
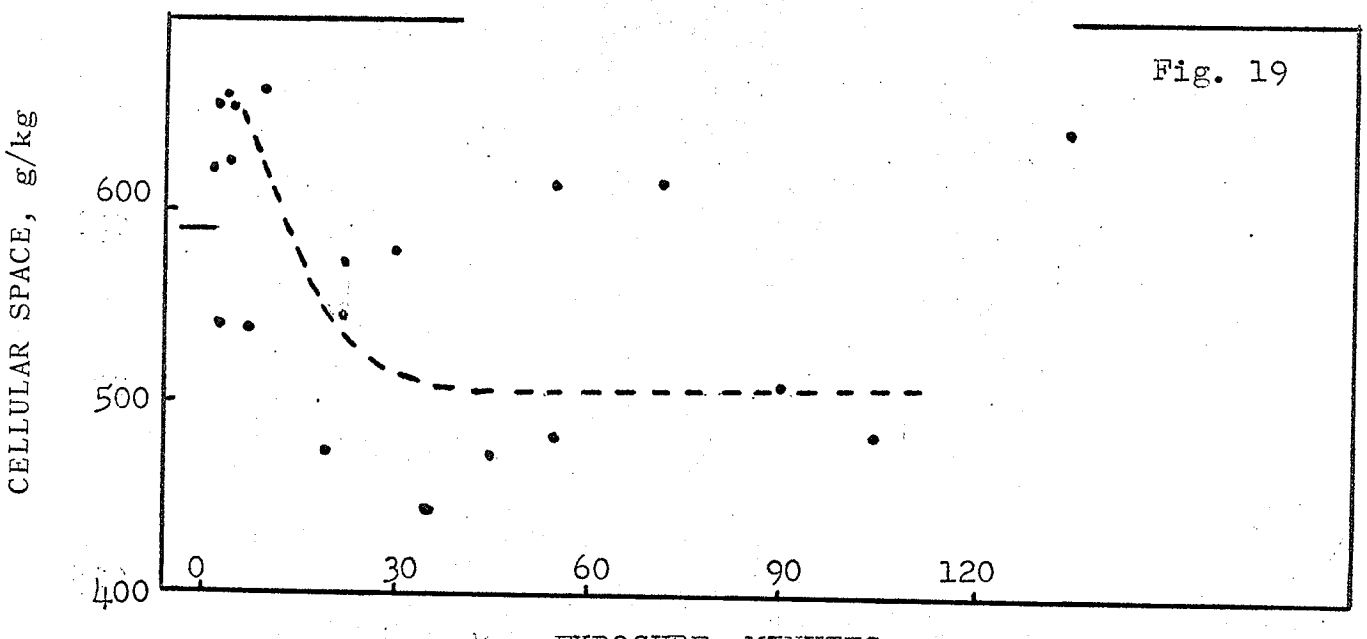


Fig. 19



E. Extracellular Space Volume

Changes in chloride space (extracellular space volume) are shown in Fig. 18. Four values, representing exposure times of two, four, five and ten minutes were found to be smaller than the lower 95 per cent confidence limit (182.5 g/kg) of the mean of the 20°C control group. Also, within the first ten minutes exposure to lethal temperature, one value (210 g/kg at four minutes exposure) was found to be within the 95 per cent confidence limits (218.5±36.0 g/kg) of the mean of the 20°C control group and two values were found to exceed the upper 95 per cent confidence limit (254.5 g/kg). After ten minutes exposure values exceeded the upper confidence limit and ranged between 197 and 448 g/kg.

E. Intracellular Space Volume

Changes in intracellular space volume are shown in Fig. 19. Although tissue water levels increased following lethal shock, the changes in cellular volume mirrored the changes in chloride space. From the mean level of 588.9 of the 20°C control group, values reached as high as 665 g/kg (after ten minutes exposure) within the first ten minutes. However, within the same exposure time cell volume also decreased as low as 541 g/kg (after three and eight minutes). After ten minutes exposure values ranged between 423 and 645 g/kg.

CHAPTER V

DISCUSSION

1. Plasma and Tissue Composition of Control (20°C-acclimated) Goldfish

Plasma chloride concentration found in 20°C-acclimated goldfish in the present study were somewhat lower than those observed earlier by Houston (1962), for the same species, and also lower than the values reported for a variety of freshwater adapted salmonid species (Gordon, 1957; Houston, 1959). However, the values for tissue chloride concentrations observed in this investigation were comparable to those recorded for this species (Houston, 1962) and for several species of salmon and trout (Gordon, 1957; Houston, 1959). Because of the relatively low values for plasma chloride, the values for extracellular phase volume (calculated on the basis of the volume of distribution of tissue chloride) found in this investigation were somewhat greater than those reported by Houston (1962) for goldfish. Those of the cellular phase volume were then slightly lower, as the the tissue water contents obtained for 20°C-acclimated goldfish in the two investigations were similar. In general, however, estimates of both

cellular and extracellular phase volumes fell within the ranges observed for steelhead and brown trout, and Atlantic salmon (Gordon, 1957; Houston, 1959; Houston and Threadgold, 1963), and in a variety of other freshwater species (Thorson, 1961).

The basis of the discrepancy seen between the plasma chloride values observed in the present study (range, 75 to 85 mEq/l) and those in the earlier investigation by Houston (1962) on the same species (range, 120 to 130 mEq/l) is not obvious. The animals were obtained from the same source[1] and were acclimated for approximately the same periods of time at the same temperature. Anesthetization procedures were common to both studies, and involved use of the compound tricaine methane sulphonate (MS-222).

Blood was sampled by slightly different procedures. In the present study samples were obtained by caudal transection, while in the earlier investigation direct cardiac puncture was employed. The possibility exists that the former treatment, being more traumatic, might have initiated a condition of "laboratory diuresis" (Meyer, 1948; Forster and Berglund, 1956; Jorgensen and Rosenkilde, 1956). Several factors, however, mediate against this. The animals were anesthetized throughout the procedure,

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1. Goldfish Supply Company, Stouffville, Ontario.

and were out of water. Moreover, no differences in tissue chloride concentration were observed between the control groups of the two studies, as might be expected had "laboratory diuresis" been initiated in the present investigation.

In both studies, plasma chloride values were obtained by the same analytical procedures, the mercurimetric titration of Asper, Schales and Schales (1947). In the earlier study sample volumesoof 0.2 ml were employed, while in the present investigation a micromodification requiring only 0.01 ml of plasma was used. Although some decrease in accuracy may have resulted from the reduction in sample volume, since the error in end-point detection was therefore magnified, all determinations were made against standardized plasma[1], and triplicate determinations of unknowns usually gave values agreeing within 1%.

Thus, one is led to the conclusion that the discrepancies may represent the effects of an uncontrolled variable such as diet, or may involve an innate seasonal variation in blood composition of the type reported in amphibians (Harris, 1960) and trout (Hickman, personnal communication).

The plasma and tissue potassium levels found in the present investigation were comparable to those

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1. Labtrol, Canadian Laboratory Supplies Company.

recorded for the arctic char, Salvelinus alpinus (Gordon, 1957), and plasma sodium levels were found similar to those reported for Salmo trutta and S. gairdneri (Houston, 1959). Since the sodium concentration of plasma is normally in excess of the chloride (mean value of 175±8.6 mEq/l as compared to 82±.8 mEq/l for the 20°C control group in the present investigation) tissue sodium concentration will exceed chloride (Manery, 1954). However, the mean 20°C control value obtained for tissue sodium (7.7±.5 mEq/kg) was considerably less than that found for tissue chloride (19.3±1.4 mEq/kg). In spite of the low degree of tissue dilution (approximately 1 g digested in 5 ml concentrated nitric acid and made up to 10 ml) the sodium concentration of the solution to be measured was in the extreme lower range of the flame spectrophotmeter[1]. Some inaccuracy of measurement may have resulted from this and from interference due to chloride ion in the tissue and nitrate ion used in digestion.

2. Water - Electrolyte Metabolism Following Sublethal Thermal Shock.

In considering the effects of sublethal thermal shock upon the water - electrolyte metabolism of

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1. Advanced Internal Standard Flame Spectrophotometer.

the animals it has been found convenient to divide the response pattern into five periods:

A. Initial 48 hours
B. Period from 48 to 84 hours
C. Period from 84 to 168 hours
D. Period from 168 to 240 hours
E. Period from 10 to 21 days

A. Initial Forty - eight hours

Reference to Figures 1 to 6 indicates that while plasma potassium values showed an almost immediate increase, the major blood electrolytes, sodium and chloride, remained stable for approximately thirty-six hours.

The lag period preceding the response of sodium and chloride cannot be attributed to time required for an increase in tissue and blood temperature, for Davis (1955) has shown that the body temperature of fish responds rapidly to environmental temperature variations. This rapidity of response might be expected in view of the extensive branchial circulation and consequent large surface area for heat equilibration in the gills (Brett, 1956).

Following this lag period, plasma chloride and sodium concentrations rose, reaching values which were 26.8 and 26.3 per cent, respectively, above control levels forty-eight hours after transfer. As

might be expected, the plasma sodium to chloride ratio remained approximately constant during this period.

After a twenty-four hour lag period, tissue chloride levels showed a decrease at thirty-six hours while tissue potassium levels rose after the same length of exposure. Tissue sodium levels did not vary significantly throughout this period.

The increase in plasma sodium and chloride may be attributable to one or a combination of sereral factors including:

a. increased absorption of ions through the gills; i.e. increased rate of activity of ion transport systems.
b. decreased dfifusive loss of ions through the gills; i.e. decreased branchial ionic permeability
c. increased tubular reabsorption of ions; i.e. increased rate of activity of ion transport systems in the nephrons
d. decreased water reabsorption in the kidney tubles; i.e. decreased tubular water permeability and consequent reduction in obligatory water reabsorption.
e. a cellular to extracellular phase shift of ions
f. an extracellular to cellular phase shift of water

The possibility of decreased water reabsorption in the kidney tubules, or increased urine flow, with consequent concentration of sodium and chloride through dehydration is unlikely, since the effect of increased temperature is usually to increase cell permeability (Heilbrun, 1943). Also, such a decrease in water reabsorption would then be expected to result in a decrease in tissue water. However, in this investigation the tissue water content was found to actually increase. It should, however, be noted that Wikgren (1953) observed that a temperature decrease caused a decrease in urine flow in the lamprey, and so a temperature increase might be expected to increase urine flow. In Wikgren's study the basis of change in the rate of urine flow was not specified, and it may have been a function of changes in renal plasma flow, blood pressure, and glomerular filtration rate as well as changes in active ion transport or permeability.

A decreased diffusive loss of ions through the gills is also unlikely, since branchial ionic permeability would likely increase with an increase in temperature (Heilbrun, 1943). Also, decreased loss of sodium and chloride would be expected to result in an increase in the tissue concentration of these ions as well as in the plasma levels. It was found that tissue sodium concentration did not

vary and that tissue chloride concentration actually showed a significant decrease after thirty-six hours exposure.

Although the cell membrane is normally effectively impermeable to chloride ions (Manery, 1954) the lowering of the membrane potential as calculated from potassium distribution values during the first forty-eight hours suggests the possibility of an anion shift from the cellular to extracellular phase. However, in this study all chloride is assumed to be extracellular (Manery, 1954). Therefore any such movement would be undetected. Concentration and movement of other anions were not investigated in the present study.

An increase in the rate of activity of branchial and renal ion transport systems might reasonably be expected, since oxygen consumption and metabolic rate of poikilotherms increases with increased temperature (Meuwis and Heuts, 1957; Prosser and Brown, 1961). An increase in renal ionic absorption would cause an increase in obligatory water reabsorption and thus effect an increased tissue water content, as was found in this investigation.

The movements of sodium and chloride ions through the gills of goldfish have been shown to be parallel (Meyer, 1951) and after forty-eight hours exposure, plasma sodium and chloride were found to

have increased 26.3 and 26.8 per cent respectively over control values. The constant ratio of plasma sodium to chloride suggests that the relative rates of renal and branchial absorption for the two ions remained constant. Wikgren (1953) observed that a decrease in temperature reduced the capacity of the lamprey kidney to absorb chloride, but that this was offset by a decrease in the amount of fluid flowing through the kidneys per unit time. It follows that an increase in temperature would increase the chloride absorbing capacity of the renal tubule, but that this might be offset by increased urine flow. However, the results of the present investigation do not suggest increased urine flow, and so increased branchial and renal ion absorption remain a possibility in accounting for the increased plasma chloride and sodium concentrations. It should be noted that tissue sodium levels did not vary significantly within the first forty-eight hours exposure, and tissue chloride levels decreased. Thus, it would seem that the effects of increased branchial and renal ion absorption were partially masked by other factors.

Variations in tissue water content and distributions became apparent at the thirty-six hour sample period. At that time water content and cellular volume increased. The increase in tissue water content may be attributable to:

a. increased osmotic concentration of the extracellular fluids due to increased branchial and renal absorption of ions.
b. increased tubular and branchial water permeability with consequent increased water absorption.
c. increased metabolic production of water.

An increase in tissue water content of goldfish in response to increased experimental temperature has also been observed by Hoar and Cottle (1952) who attributed it to increased cell permeability and to increased metabolic production of water. According to Hoar and Cottle (loc. cit.), increases in metabolic water production would be insignificant when compared to the increased entry of water.

While tissue water content showed a significant increase thirty-six hours after temperature shock, plasma concentrations of sodium and chloride did not show a significant increase over control values until forty-eight hours after transfer. Since chlorides account for approximately 80% of the osmotic concentration of extracellular fluids in fish (Scholander, et al, 1957), it seems unlikely that increased osmotic concentration was responsible for the increase in tissue water content observed after thirty-six hours exposure, However, plasma and

tissue potassium both showed a significant increase, and so may have contributed to endosmosis.

The increased renal absorption of sodium and chloride ions indicated above would also have increased obligatory water reabsorption. However this, too, would not have occured during the first thirty-six hours of exposure.

The increase in the activity of the branchial and renal ion transport systems, and the consequent increase in extracellular osmotic concentration, proposed for the forty-eight hour sample period suggest that endosmosis and increased obligatory water reabsorption were responsible for the increased tissue water content (relative to that of the 20°C control group) seen forty-eight hours after transfer. It was found, however, that while the increase in mean plasma chloride concentration from 84.5 mEq/l at thirty-six hours to 104.0 mEq/l at forty-eight hours was significant at the .05 level (Materials and Methods, Appendix), both the increase in tissue water content from a mean of 820.5 to 835.9 g/kg and the increase in plasma sodium concentration from 202.3 mEq/l at thirty-six hours to 220.6 mEq/l at forty-eight hours were not. It would seem, then, that the increase in plasma chloride between the thirty-six and forty-eight hour sample periods did

not effect a further significant increase in tissue water. However, it probably contributed to the maintainance of the water content level established after thirty-six hours exposure. The mean tissue water contents obtained at thirty-six and forty-eight hours were both significantly greater than that of the 20°C controls.

Thus it would seem that the increased tissue water content was due primarily to increased branchial and tubular water permeability, and secondarily to increased activity of the branchial and renal ion transport systems.

It was of interest that the water thus taken up by the tissue after thirty-six hours exposure appeared to increase the volume of the cellular phase without producing an equivalent increase in extracellular volume. It is therefore indicated that in some fashion an osmotic inequality was set up between the cellular and extracellular fluids, favouring transfer of water into the former phase. This may have been attributable to an increase in the cellular concentration of inorganic electrolytes or organic solutes. In this regard it should be noted that the cellular potassium content of the tissue rose by some 18 mEq/kg tissue (from 103 to 121 mEq/kg tissue). In addition it may have been possible that the temperature increase uncoupled or otherwise modified cellular metabolism in such a way that degradation of

macromolecules into smaller molecules was favoured. This might thereby have increased the contribution of organic solutes to the total cellular osmotic pressure.

The difference in the rate of response of potassium and sodium to temperature change, indicates that the system for active transport of sodium is different from that for potassium. It should be noted, however, that evidence for the linkage of sodium efflux and potassium influx across cell membranes has been recorded (Harris and Maizels, 1952; Hodgkin and Keynes, 1955; Eppley, 1958).

The response during the first forty-eight hours of exposure may, then, have been characterized by first, an increase in branchial and renal water permeability, and second, an increase in renal and branchial active ion absorption. Localization of water in the cellular phase occurred.

B. Period from Forty-eight to Eighty-four Hours

During this period, as indicated by reference to Figures 1 to 8, plasma chloride concentration and tissue water content returned to control values. Plasma potassium and sodium concentrations remained significantly greater than control values, with potassium decreasing to a level intermediate between the control mean value and the maximum mean seen at

forty-eight hours. While plasma sodium concentrations remained above control levels, the increase from a mean of 221 mEq/l at forty-eight hours to a mean of 255 mEq/l at seventy-two hours or to a mean of 252 mEq/l at eighty-four hours was not significant. Again during this period tissue sodium levels did not vary while tissue potassium had returned to control levels by sixty hours. Tissue chloride concentration, which had returned to control values at forty-eight hours, showed a second significant decrease at eighty-four hours.

The decrease in plasma chloride could be attributable to one or more of the following:

a) decreased branchial chloride absorption; i.e. decreased rate of activity of the active chloride transport system
b) increased diffusive loss of ions through the gills
c) decreased tubular reabsorption; i.e. decreased rate of activity of the active chloride transport system in the nephrons
d) increased water reabsorption in the kidney tubules; increased tubular water permeability and consequent increase in obligatory water reabsorption
e) shift of ions from extracellular to cellular phase

f) shift of water from cellular to extracellular phase

Increased tubular water reabsorption and consequent lowering of plasma chloride concentration by dilution is unlikely, as during this period tissue water content was seen to decrease.

Any shift of chloride ions from the extracellular to cellular phase could not be detected as the calculations used in this study were based on the assumption that all chloride is extracellular (Manery, 1954).

An increased diffusive loss of chloride ion through the gills would seem probable since the rate of diffusion, which is a passive process, would depend on the chloride concentration gradient between the fluids of the body and the environment. This gradient was seen to increase at forty-eight hours. However, it should be noted that Meyer (1948) found that an increase in chloride concentration in body fluids of goldfish tended, not to increase the rate of excretion through the gills, as would be expected, but to retard the rate of absorption.

Thus, regulatory responses such as decreased renal and branchial absorption seem likely. This would be due to a regulatory response rather than to a decrease in metabolic rate, since the metabolic rate of goldfish increases with increasing acclimation temperature

when measured at the temperature of acclimation (Freeman, 1950; Kanungo and Prosser, 1950). A decrease in renal chloride reabsorption would cause a decrease in obligatory water reabsorption, and tissue water would therefore decrease, as was found in this investigation. The decrease in tissue chloride concentration at eighty-four hours is further evidence of diffusive chloride loss and of decreased branchial and renal absorption.

As shown by Figures 1 and 5, the variations in plasma sodium and chloride concentrations during this period were no longer comparable; plasma sodium concentration remained above control levels while plasma chloride decreased. As a consequence of the unbalanced rates of activity of the sodium and chloride transport systems, the ratio of plasma sodium to chloride increased to a maximum at eighty-four hours. Assuming the rate of renal sodium absorption to be unchanged from the increased rate indicated at forty-eight hours, and that of renal chloride absorption to be decreased, it is probable that renal bicarbonate reabsorption was increased to maintain electroneutrality. This would result in a condition of alkalosis.

The decrease in tissue water content observed during this period could be attributed to one or more of the following:

a) decreased osmotic concentration of the extracellular fluids due to decreased

branchial and renal absorption of ions

b) decreased tubular and branchial water permeability with consequent decreased water absorption

c) decreased metabolic production of water

Decreased metabolic water production is not likely to occur, since metabolic rate has been found to increase with increased temperature (Freeman, 1950; Kanungo and Prosser, 1950). Moreover, changes in tissue water content due to changes in metabolic water production would be insignificant relative to the changes due to to variations in tubular and branchial water permeability (Hoar and Cottle, 1952) and due to variations in the osmotic concentration of the extracellular fluid.

A decrease in renal and branchial water permeability is a possible explanation of decreased tissue water content. Hoar and Cottle (1952) suggest that, as the acclimation temperature increases, the stability of the cell membrane of goldfish is increased by the increase in saturation of the lipids in the membrane, and permeability is thus reduced.

The decrease in plasma chloride concentration to control values, possibly caused by increased diffusive loss and decreased active branchial and renal chloride transport, would result in a decreased osmotic concentration of the extracellular fluids. Endosmosis

and obligatory water reabsorption would be decreased accordingly.

The significant decrease (relative to the 20°C control mean) in chloride space after eighty-four hours exposure suggests a decreased osmotic concentration of the extracellular fluids or an increased osmotic concentration of the cellular fluid. As stated above, the decrease in plasma chloride concentration during this period would result in decreased osmotic concentration of the extracellular fluids. Cellular potassium concentrations did not vary during this period and variations in concentrations of cellular organic solutes were not investigated in this study.

During this period, then, plasma chloride concentration decreased, possibly due to diffusive loss and to decreased renal and branchial absorption; tissue water content decreased, probably due to either reduced tubular and branchial water permeability or to reduced osmotic concentration of the extracellular fluids, or to both. The exact factors determining the change in the rates of active ion transport cannot be determined on the basis of the data available in this study. However, it would seem that as a consequence of unbalanced changes in sodium and chloride transport, an unbalancing of the acid-base system occurred and a condition of alkalosis resulted.

C. Period From 84 To 168 Hours

During the interval between 84 and 168 hours after transfer, plasma sodium and chloride levels decreased. At 120 hours exposure sodium concentrations had returned to, while chloride levels had fallen below, control values. As would be expected, the ratio of plasma sodium to chloride decreased to a value intermediate between the control and the maximum found at 84 hours. Tissue chloride and tissue sodium concentrations remained comparable to control values throughout this period. Also, with the exception of an increase at 144 hours, tissue potassium levels did not vary from control values.

During this interval, tissue water content and distribution varied. The 9.5% increase in tissue water content at this time was of even greater magnitude than the 3.5% increase seen during the initial forty-eight hours exposure.

The factors to which the decrease in plasma chloride concentration might be attributable have been listed previously. (See page 116).

Increased branchial diffusion of ions during this interval is not probable. Chloride concentration fell below control values, and so the gradient would not have supported increased diffusion. On the other hand, the high plasma sodium concentration seen at eighty-four hours indicated a sodium gradient that could have supported such an increased diffusion.

However, since tissue ion levels showed no decrease it seems that decreased plasma sodium and chloride concentrations are not attributable to increased branchial diffusion.

Decreased branchial and renal absorption of soidum and chloride would result in lowered tissue ion levels, and in lowered tissue water content which would result from decreased obligatory water reabsorption. Thus, this regulatory mechanism, seen as a possible factor in the decrease of chloride described previously, did not function during this interval.

A movement of chloride and sodium ions from the extracellular to cellular phase would result in a decreased extracellular ion content of the tissue. Such a movement would not be detected in the present investigation (see A and B of this discussion). The observed increase in tissue water content would be a probable factor in the decreased plasma sodium and chloride concentrations.

Factors which might have contributed to increased tissue water content have been listed previously (see page III).

Plasma sodium and chloride concentrations decreased during this time. The resulting decrease in osmotic concentration of the extracellular fluids eliminates endosmosis as a cause of the increased

tissue water content. As before, increased metabolic water production would be insignificant. Structural changes in the cell membranes of branchial and renal tubule cells leading to increased water permeability remains as a possible cause of increased tissue water.

Decreased cell permeability and decreased plasma chloride concentration were suggested as factors leading to the decrease in tissue water content between forty-eight and eighty-four hours. In view of the above it would seem probable that the increase in renal and branchial water permeability, first indicated at the thirty-six hour sample period, continued uninterrupted until 168 hours after transfer; and that the decrease in tissue water content between forty-eight and eighty-four hours exposure was caused solely by the decrease in endosmosis and obligatory renal water reabsorption.

Thus, during the interval between 84 and 168 hours tissue water content increased, possibly due to increased branchial and renal permeability. A decrease in plasma sodium and chloride concentrations resulted.

D. Period From 168 To 240 Hours

As reference to Figures 1 to 12 suggests, tissue potassium concentration decreased at the 192 hour sampling period. Since plasma potassium concen-

trations were not determined for this interval, the factors responsible for this decrease are not obvious. After 240 hours exposure to 30°C, chloride space increased (relative to 20°C control values). As there is no evidence of increased osmotic concentration of the extracellular fluids at that time, a decrease in the osmotic concentration of the cellular fluid is indicated.

E. Period From 10 To 21 days

The acclimation process was not completed after 240 hours exposure. Between ten and twenty-one days, as indicated by Figures 1 to 12, tissue water content and fluid distribution returned to the level of the 20°C controls. Tissue sodium levels decreased non-significantly from 4.5 kEq/kg at 240 hours to a mean level 4.7 mEq/kg, which was, however, significantly lower than that of the 20°C controls.

Factors which may have contributed to the decrease in tissue water content, as noted previously, include decreased osmotic concentration of the body fluids (possibly due to decreased branchial and renal ion absorption or to a decrease in organic solute concentration), decreased permeability of branchial and tubule cells to water, and decreased production of metabolic water.

Any reduction in metabolic water production

as a result of acclimation processes would have been insignificant (Hoar and Cottle, 1952). There is not evidence of decreased branchial and renal chloride absorption, and organic solute concentration was not investigated in the present study.

Reduced branchial and tubular cell permeability, possibly due to an increased degree of saturation of the lipids in the membrane (Hoar and Cottle, 1952) would cause a decrease in tissue water.

The return of tissue fluid distribution to 20°C control values indicates that equality between osmotic concentrations of the cellular and extracellular fluids was restored. This is attributable either to a decrease in osmotic concentration of the extracellular fluids (due mainly to decreased electrolyte concentration) or to an increase in osmotic concentration of the cellular fluid (possibly due to increased organic solute or electrolyte concentration). The electrolyte concentration of the extracellular fluid was found not to decrease; the other factors were not determined in this investigation. However, since the earlier increase in chloride space was attributed to a decrease in cellular osmotic concentration, the decrease bewteen 10 and 21 days might be attributed to increased osmotic concentration of the cellular fluid.

Thus, between 10 and 21 days, tissue water

content decreased. This was possibly due to decreased tubular and branchial cell permeability. Fluid distribution also returned to that of 20°C controls, possibly due to the increased osmotic concentration of the cellular fluid.

F. Summary

The initial forty-eight hours exposure was characterized by

a) a 3.5 per cent increase in tissue water content thirty-six hours after transfer, possibly due to increased branchial and renal cell permeability to water,
b) by an increased branchial and renal absorption of sodium and chloride at forty-eight hours after transfer, and
c) by increased osmotic concentration of cellular fluid, due to an increased potassium and possibly increased organic solute concentration.

Observation of the interval between 48 and 84 hours indicate

a) diffusive loss of chloride through the gills and decreased renal and branchial chloride absorption
b) decreased tissue water, probably due to decreased osmotic concentration of the extracellular fluids and to decreased

obligatory water reabsorption

c) decreased chloride space, probably a result of decreased osmotic concentration of the extracellular fluids.

The decrease in plasma sodium and chloride seen during the interval between 84 and 168 hours was thought to be due to the increased tissue water content. It was indicated that the increase in tissue water content was due to increased renal and branchial cell permeability to water.

The decrease in tissue water content seen between ten and twenty-one days was attributed to a decrease in renal and branchial cell permeability to water.

It would seem that the increases in water content seen at 36 hours and between 84 and 168 hours were both due to increased renal and branchial permeability. It was suggested that the decrease in water content seen between 48 and 84 hours was not the result of decreased renal and branchial water permeability but, rather, was the result of a decreased chloride concentration of the extracellular fluid.

Thus is appears that two regulatory mechanisms were operative. The increased rate of branchial and renal active ion absorption, induced by thermal shock, decreased between 48 and 120 hours exposure to 30°C;

and increased renal and branchial cell permeability to water, also induced by thermal shock, did not decrease until after ten days. Active ion absorption may be linked with enzyme systems such as the cytochrome system (Manery, 1954) or the enzymes involved in glycolytic reactions on the cell surface (Rothstein, 1954, cited in Davson, 1959). Changes in membrane permeability probably involve structural changes of the cell membrane (Hoar and Cottle, 1952).

Except for the decrease in plasma chloride and tissue sodium concentrations, and the increase in plasma potassium concentration after 21 days exposure to 30°C, the acclimation process observed could be classified as complete compensation according to the scheme of Precht (1958).

3. Water-Electrolyte Metabolism Following Lethal Thermal Shock

As indicated by Figures 14 to 19 the effect of lethal thermal shock on plasma and tissue chloride concentrations, and on tissue water content and fluid distribution was seen mainly within the first twenty-five minutes exposure. Plasma chloride concentration decreased while tissue chloride concentrations showed no significant change. Tissue water content and extracellular phase volume increased while cellular phase volume decreased.

Factors to which the decrease in plasma chloride concentration might be attributable have been listed previously (see page 116). Increased outward diffusion is not probable, as plasma chloride concentration was falling below normal control levels. Decreased branchial and renal absorption of chloride is also not probable, as no significant change in tissue chloride concentration was observed. A movement of chloride ions from the extracellular to cellular phase would not be detected as all chloride is assumed to be extracellular (Manery, 1954).

Thus, an increase in water reabsorption in the kidney tubules, which would also contribute to the observed increase in tissue water content, and an increase in extracellular phase volume remain as the two most probable causes of the decreased plasma chloride concentration.

Tissue water content was found to increase. Any increase in metabolic water production would have been insignificant, and there was no indication of increased branchial or renal chloride absorption. Thus, increased branchial and renal water permeability remains as the most probable explanation.

During the first ten minutes exposure, the increased tissue water seems to have localized in the cellular phase in the case of some fish and in the

extracellular phase in the case of others. Obviously, the osmotic equilibrium of the 20°C controls was disturbed by the thermal shock, and a new equilibrium had not yet been established within the first ten minutes. Thereafter the water taken up by the tissue appears to have increased the extracellular phase volume without having caused the expected equivalent increase in cellular phase volume. This indicates that an inequality of osmotic concentration between the two phases resulted from thermal shock. The inequality could be due to one or both of:

a) increased osmotic concentration of extracellular fluid, or

b) decreased osmotic concentration of the cellular fluid.

As there is no evidence of increased osmotic concentration of the extracellular fluids it would seem that decreased solute concentration of the cellular fluid was the main factor. This would be caused by a decrease in either electrolyte or organic solute concentration. Neither were determined in the present investigation.

Thus lethal thermal shock was characterized by an uptake of water, possibly due to increased renal and branchial water permeability, and by the localization of this water in the extracellular phase, possibly as a result of decreased osmotic concentration of the cellular fluid.

These changes may be a magnification of the changes observed during the initial thirty-six hours following sublethal shock. However, following sublethal shock, cellular volume increased, due probably to increased potassium concentration of the cellular fluid.

The extent of the water-electrolyte disturbance thus observed during heat death would not have been sufficient to have alone caused death. The effects of heat shock occurred during the first twenty-five minutes exposure, yet some of the goldfish survived as long as 135 minutes.

The maximum decrease in plasma chloride concentration, relative to the level of the 20°C controls, was 32 per cent, and after twenty-five minutes exposure the average value represented a decrease of 27 per cent. Tissue water increased 6 per cent, and chloride space increased 51 per cent. These changes are less than those withstood by the steelhead trout, Salmo gairdneri during their adaptation to sea water (Houston, 1959).

In this study the maximum increase in tissue water content following lethal shock was 6 per cent over control values, while that following sublethal shock was 9.5 per cent. The average increase in extracellular phase volume following lethal shock was 51 per cent, while the maximum following sublethal shock represented an increase of 72 per cent over control values.

Thus it would seem that thermal death observed in this investigation is attributable to factors other than water-electrolyte imbalance.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Thermal shock, both lethal and sublethal, induced variations in the water-electrolyte balance of goldfish, Carassius auratus L.

2. The effects of sublethal shock (20°C to 30°C) were found to include:

a. An initial increase in tissue water content during the first forty-eight hours exposure to 30°C, and a second increase between 84 and 168 hours. Both were attributed to an increase in renal and branchial membrane permeability to water. The decreased chloride concentration of the extracellular fluids, due to diffussive loss and decreased branchial and renal active chloride transport, was thought to have caused the decrease in tissue water between forty-eight and eighty-four hours through a decrease in endosmosis.

b. The increase in plasma concentrations of sodium and chloride observed at the forty-eight hour sample period were found to indicate an increase in the active renal and branchial transport of these ions. The decrease in plasma chloride concentration between 48 and 84 hours found was found to be the result of diffusive chloride loss and a decrease in active renal

and branchial chloride transport. The further decrease in plasma sodium and chloride levels seen between 84 and 120 hours was thought to result from the increased tissue water content.

3. It appears that two regulatory mechanisms were effective. The increased active branchial and renal chloride transport seen at 48 hours exposure, probably resulting from an increased metabolic rate induced by thermal shock, decreased between forty-eight and eighty-four hours as acclimation to the higher temperature proceeded; and plasma chloride concentration returned to control values. Thus, this would seem to be an example of "complete compensation" (Precht, 1958). On the other hand, the increased branchial and renal cell permeability to water, first observed at thirty-six hours and found to increase up to 168 hours, did not decrease until after ten days exposure.

4. Analysis of samples taken after 240 hours exposure to 30°C, and compared to analysis of samples taken from control fish (which were exposed to 30°C for twenty-one days and were believed to be fully acclimated (Brett, 1946) indicated that the acclimation process as shown by tissue water content, body fluid distribution and tissue sodium concentration, had not been completed within 240 hours. However, in the case of tissue chloride concentration a steady state was achieved within forty-eight hours exposure to 30°C, and in the case of plasma chloride and sodium concentrations it was achieved within 120

hours. (In terms of active chloride transport a steady state was reached at eighty-four hours exposure; a dilution due to water uptake occured between 84 and 120 hours). In the case of tissue potassium the steady state appeared to have been achieved at 240 hours.

5. With the exception of a slight increase in plasma potassium, and the slight decreases in plasma chloride and tissue sodium levels of the 30°C control group relative to the 20°C control group, acclimation to 30°C expressed in terms of body fluid composition and distribution could be classified as "complete compensation" according to the scheme outlined by Precht (1958).

6. The effects of lethal shock were observed within 25 minutes exposure. The increase in tissue water content was attributed to increased branchial and renal membrane permeability, as was the initial increase following sublethal shock. However, following lethal shock the water thus taken up by the tissue localized in the extracellular phase. This was attributed to a possible decrease in osmotic concentration of the cellular fluid.

7. The changes in water-electrolyte balance following lethal shock were not believed to be the primary cause of death.

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APPENDICES

APPENDIX I

Observational Data

Observation	Unit
Plasma ion concentration	mEq/l
Tissue ion concentration	mEq/kg
Tissue water content	g/kg

Abbreviations

M	=	mean
SD	=	standard deviation
SE	=	standard error

Numbering of fish

example:	30-12h(d)-2	
where	30	= temperature to which fish is exposed
	12h(d)	= length of exposure in hours (days)
example	2	= individual fish
example:	38-3	=
where	38	= temperature to which fish is exposed
	3	= individual fish

1. Sublethal Shock

Fish number	Plasma Na^+	Plasma K^+	Plasma Cl^-	Tissue Na^+	Tissue K^+	Tissue Cl^-	Tissue Water
30-12h-1	-	9.2	79.4	10.0	102	13.8	814
30-12h-2	-	-	85.2	-	188	17.9	812
30-12h-3	-	-	80.8	-	139	16.6	808
30-12h-4	-	10.6	84.0	7.1	103	12.6	819
30-12h-5	-	8.8	79.3	14.0	95	12.1	860
30-12h-6	-	-	78.5	6.7	133	26.5	836
M	-	9.5	81.2	9.5	127	16.6	825
SD	-	.95	2.8	3.4	35	5.4	20
SE	-	.67	1.2	1.9	16	2.4	9
30-24h-1	198	9.8	84.7	11.4	104	12.8	802
30-24h-2	-	-	77.6	7.1	158	14.2	810
30-24h-3	-	-	-	7.3	154	17.6	802
30-24h-4	213	12.3	81.0	8.5	96	7.5	836
30-24h-5	162	10.5	79.6	10.0	97	16.2	827
30-24h-6	-	-	82.0	-	130	20.0	825
30-24h-7	134	4.1	75.6	-	85	13.5	817
M	177	9.2	80.1	8.8	118	14.5	817
SD	35.6	3.5	3.2	1.8	30	4.0	13
SE	20.6	2.1	1.5	.9	12	1.6	5
30-36h-1	247	16.3	82.0	12.5	122	9.8	838
30-36h-2	-	-	86.4	8.7	123	14.6	826
30-36h-3	-	-	-	8.2	140	17.1	802
30-36h-4	-	-	95.5	8.6	141	15.8	810
30-36h-5	160	11.8	80.0	5.9	109	12.7	813
30-36h-6	200	11.5	79.6	8.9	99	11.7	821
30-36h-7	-	-	83.4	-	125	19.9	834
M	202	13.2	84.5	8.8	123	14.5	821
SD	43.6	2.7	5.9	2.1	15	3.4	13
SE	30.8	1.9	2.7	1.0	6	1.4	5
30-48h-1	254	8.8	103.1	-	101	14.3	820
30-48h-2	196	-	105.1	18.0	111	24.6	849
30-48h-3	-	-	108.8	7.2	139	19.4	826
30-48h-4	210	8.0	105.1	9.6	92	15.4	822
30-48h-5	222	-	101.9	-	134	26.0	843
30-48h-6	221	9.9	102.2	12.6	92	22.3	856
M	221	8.9	104.0	11.8	112	20.3	836
SD	21.4	.95	2.6	4.7	21	4.8	15
SE	10.7	.67	1.2	2.7	9	2.2	7
30-60h-1	272	-	92.9	-	105	15.2	814
30-60h-2	153	-	97.2	8.0	106	13.4	831
30-60h-3	-	-	90.9	6.5	96	28.4	820
30-60h-4	-	-	77.3	-	146	30.7	845

Fish number	Plasma Na^+	Plasma K^+	Plasma Cl^-	Tissue Na^+	Tissue K^+	Tissue Cl^-	Tissue Water
30-60h-5	-	-	82.0	6.0	120	17.9	845
30-60h-6	-	-	86.5	8.4	136	29.8	843
M	213	-	87.8	7.2	118	22.1	833
SD	84.2	-	7.3	1.2	19	7.3	14
SE	84.2	-	3.3	.7	9	3.0	6
30-72h-1	280	11.0	-	8.3	106	13.0	817
30-72h-2	-	-	96.8	8.1	134	19.0	824
30-72h-3	-	-	88.9	4.7	122	14.2	814
30-72h-4	209	10.6	84.2	14.6	79	17.8	845
30-72h-5	275	8.0	81.6	12.3	113	14.7	819
30-72h-6	-	-	78.7	-	100	21.1	826
30-72h-7	-	-	84.2	7.3	146	20.0	839
30-72h-8	-	-	83.3	18.6	48	-	-
30-72h-9	-	-	67.4	7.2	96	18.8	831
M	255	9.9	83.1	10.1	105	17.3	827
SD	39.6	1.6	8.4	4.6	29	3.0	11
SE	28.0	1.2	3.2	1.8	10	1.1	4
30-84h-1	-	-	78.0	-	-	-	-
30-84h-2	302	-	80.5	-	108	9.5	828
30-84h-3	270	9.7	80.3	-	107	12.7	801
30-84h-4	208	13.5	78.0	-	110	13.1	804
30-84h-5	222	9.3	82.0	-	144	9.6	816
30-84h-6	313	10.5	81.9	-	140	14.8	820
30-84h-7	195	12.1	80.1	-	115	10.1	810
M	252	11.0	80.1	-	121	11.6	813
SD	50.3	1.8	1.6	-	17	2.2	10
SE	22.5	.9	.7	-	8	1.0	5
30-96h-1	312	-	89.5	9.0	106	13.2	820
30-96h-2	-	-	92.3	5.0	125	16.6	824
30-96h-3	-	-	90.9	-	128	16.2	811
30-96h-4	-	-	76.4	-	-	-	890
30-96h-5	-	-	77.2	6.2	107	24.6	829
30-96h-6	-	-	77.0	-	-	-	823
30-96h-7	-	-	76.0	6.9	115	15.0	836
30-96h-8	228	-	76,4	7.8	111	13.8	821
30-96h-9	185	-	76.4	14.1	91	19.4	850
30-96h-10	-	-	81.4	-	-	49.7	928
30-96h-11	-	-	80.4	-	-	19.9	825
30-96h-12	-	-	82.1	-	-	49.4	890
30-96h-13	-	-	80.3	-	-	-	-
30-96h-14	-	-	82.7	-	-	26.8	837
30-96h-15	-	-	85.1	-	-	26.1	881
M	242	-	81.6	8.2	112	22.1	848
SD	64.6	-	5.6	3.2	13	14.2	35
SE	45.7	-	1.5	1.4	5	4.3	10

Fish number	Plasma Na^+	Plasma K^+	Plasma Cl^-	Tissue Na^+	Tissue K^+	Tissue Cl^-	Tissue Water
30-120h-1	242	-	-	10.3	112	10.0	808
30-120h-2	-	-	-	6.8	130	30.7	841
30-120h-3	162	-	75.8	26.4	85	31.7	851
30-120h-4	210	-	76.4	10.2	102	16.5	835
30-120h-5	206	-	76.6	7.3	114	15.7	831
30-120h-6	-	-	76.7	-	126	15.6	831
30-120h-7	-	-	77.5	-	-	24.7	840
30-120h-8	-	-	76.2	-	-	19.4	837
30-120h-9	-	-	75.0	-	-	25.8	832
30-120h-10	-	-	76.8	-	-	20.5	828
30-120h-11	-	-	76.0	-	-	-	-
30-120h-12	-	-	77.1	-	-	22.9	847
M	205	-	76.4	12.5	112	21.2	835
SD	32.9	-	.7	7.3	17	6.7	11
SE	19.0	-	.2	3.3	7	2.1	4
30-144h-1	-	-	-	8.1	146	26.2	827
30-144h-2	-	-	76.3	-	136	7.8	821
30-144h-3	-	-	75.3	9.8	104	8.4	820
30-144h-4	-	-	76.7	-	128	18.0	830
30-144h-5	-	-	77.9	-	126	17.7	840
30-144h-6	-	-	78.4	-	155	23.8	837
30-144h-7	-	-	77.3	-	-	26.1	862
30-144h-8	-	-	-	-	-	48.3	889
30-144h-9	-	-	75.2	-	-	-	-
30-144h-10	-	-	79.5	-	-	38.9	899
30-144h-11	-	-	81.4	-	-	15.2	833
30-144h-12	-	-	81.8	-	-	24.8	835
M	-	-	78.0	8.9	133	23.2	845
SD	-	-	2.3	1.2	18	12.2	27
SE	-	-	.8	1.2	8	3.8	8
30-168h-1	-	-	76.3	-	-	58.2	921
30-168h-2	-	-	76.0	-	92	13.1	834
30-168h-3	-	-	76.3	-	133	14.2	836
30-168h-4	-	-	81.1	-	-	22.4	862
30-168h-5	-	-	86.1	-	-	43.6	910
30-168h-6	-	-	82.4	-	-	36.3	946
30-168h-7	-	-	78.2	-	-	50.2	933
30-168h-8	-	-	74.7	-	-	16.9	834
30-168h-9	-	-	82.1	-	-	26.9	886
M	-	-	79.2	-	112	31.3	885
SD	-	-	3.8	-	29	16.5	45
SE	-	-	1.4	-	29	5.8	16

Fish number	Plasma			Tissue			Tissue
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Water
30-192h-1	-	-	78.4	35.4	39	54.2	919
30-192h-2	-	-	79.1	7.8	96	16.0	838
30-192h-3	-	-	78.8	5.6	85	18.8	817
30-192h-4	-	-	76.7	-	-	21.2	837
30-192h-5	-	-	81.1	-	-	25.2	851
30-192h-6	-	-	-	-	-	59.3	924
30-192h-7	-	-	78.1	-	-	11.2	825
30-192h-8	-	-	80.1	-	-	19.5	840
30-192h-9	-	-	78.9	-	-	38.2	920
M	-	-	78.9	16.3	73	29.3	863
SD	-	-	1.3	16.6	30	17.3	44
SE	-	-	.5	11.7	22	6.1	16
30-216h-1	-	-	73.7	-	-	-	835
30-216h-2	-	-	78.2	5.8	-	14.0	834
30-216h-3	-	-	76.0	15.4	-	21.2	848
30-216h-4	-	-	76.7	-	-	24.4	882
30-216h-5	-	-	80.2	-	-	-	925
30-216h-6	-	-	81.1	-	-	19.2	842
30-216h-7	-	-	77.0	-	-	19.0	880
30-216h-8	-	-	71.7	-	-	28.2	915
30-216h-9	-	-	74.2	-	-	41.1	910
M	-	-	76.5	10.6	-	23.9	875
SD	-	-	3.1	6.8	-	8.8	36
SE	-	-	1.1	6.8	-	3.6	13
30-240h-1	-	-	77.8	8.6	86	16.7	845
30-240h-2	-	-	79.6	3.2	93	11.5	832
30-240h-3	-	-	79.6	1.7	115	11.0	811
30-240h-4	-	-	81.9	-	-	38.9	858
30-240h-5	-	-	79.1	-	-	45.0	897
30-240h-6	-	-	78.1	-	-	44.0	873
30-240h-7	-	-	74.7	-	-	43.6	925
30-240h-8	-	-	-	-	-	17.4	826
30-240h-9	-	-	77.6	-	-	60.2	825
M	-	-	78.6	4.5	98	32.0	855
SD	-	-	2.1	3.7	15	18.0	38
SE	-	-	.8	2.6	11	6.4	13
20-18d-1	-	-	84.1	-	150	12.6	812
20-18d-2	-	-	86.8	-	130	11.7	803
20-18d-3	-	-	84.2	6.3	137	19.4	812
20-18d-4	-	-	83.4	4.8	141	21.7	801
20-18d-5	-	-	86.9	-	-	21.7	805
20-19d-1	182	8.7	85.6	12.2	103	17.5	821
20-19d-2	-	-	84.2	-	97	14.4	803
20-19d-3	234	6.1	81.1	-	106	15.4	818
20-19d-4	227	4.8	-	-	101	11.8	815

Fish number	Plasma Na^+	Plasma K^+	Plasma Cl^-	Tissue Na^+	Tissue K^+	Tissue Cl^-	Tissue Water
20-25d-1	-	-	74.5	11.6	102	16.9	829
20-25d-2	-	-	77.4	7.7	86	9.4	810
20-25d-3	-	-	76.7	10.4	93	14.0	821
20-25d-4	-	-	79.5	-	130	20.1	-
20-25d-5	181	-	-	6.2	98	24.1	805
20-25d-6	160	-	79.2	5.9	83	23.8	801
20-25d-7	138	8.0	88.0	-	87	34.8	804
20-25d-8	150	7.9	83.0	8.5	84	31.6	804
20-22d-1	166	5.1	76.6	2.9	100	14.0	802
20-22d-2	185	3.0	78.7	7.1	149	9.5	810
20-22d-3	-	-	78.9	7.5	92	14.3	806
20-22d-4	185	-	77.1	5.4	98	14.0	804
20-24d-1	-	-	89.5	5.5	-	19.2	816
20-24d-2	-	-	83.9	7.3	91	21.6	798
20-24d-3	-	-	-	12.1	88	24.9	805
20-24d-4	-	-	-	8.1	96	30.3	814
20-26d-1	173	6.7	85.0	8.7	89	24.9	793
20-26d-2	-	-	78.1	6.9	98	-	799
20-26d-3	143	4.5	82.3	6.8	89	27.6	797
20-26d-4	145	5.8	85.0	9.0	86	30.6	802
M	175	6.1	82.0	7.7	104	19.3	807
SD	29.9	1.8	4.1	2.4	21	7.2	8
SE	8.6	.6	.8	.5	4	1.4	2
30-16d-1	-	-	80.3	6.3	95	16.2	819
30-16d-2	-	-	79.5	8.8	67	20.8	-
30-16d-3	-	-	78.1	12.3	82	23.1	844
30-16d-4	-	-	85.2	-	70	19.4	845
30-21d-1	174	7.1	76.4	3.7	120	21.2	800
30-21d-2	-	-	90.4	1.5	117	11.8	803
30-21d-3	137	10.6	79.2	-	110	15.4	810
30-21d-4	129	8.7	79.6	4.5	127	14.2	804
30-21d-5	166	8.5	75.9	3.6	129	18.8	805
30-21d-6	189	8.2	79.1	3.6	130	14.4	794
30-22d-1	122	7.2	67.2	3.1	147	19.2	805
30-22d-2	185	8.2	78.5	4.5	136	21.1	800
30-22d-3	178	8.2	-	3.0	140	12.6	795
30-22d-4	140	7.3	70.0	4.1	127	20.4	796
30-22d-5	144	8.7	83.6	2.7	122	13.5	806
30-22d-6	140	-	74.5	4.6	118	16.3	802
M	155	8.3	78.5	4.7	115	17.4	808
SD	23.9	1.0	5.6	2.8	24	3.5	16
SE	7.6	.3	1.5	.8	6	.9	4

2. Lethal Shock

Fish number	Exposure (Min.)	Plasma Cl	Tissue Cl	Tissue Water
38-1	5	78.3	14.2	821
38-2	8	71.1	24.1	845
38-3	10	75.1	13.6	827
38-4	2	69.3	12.3	817
38-5	3	76.4	-	-
38-6	2	-	19.6	821
38-7	4	76.8	13.7	821
38-8	4	78.0	18.2	836
37-1	3	78.5	29.3	877
37-2	3	-	17.0	823
36-1	35	57.4	28.6	871
36-2	20	55.8	24.8	877
36-3	25	56.1	19.2	856
36-4	45	56.3	24.5	866
36-5	10	57.1	-	880
36-6	30	56.4	15.9	835
36-7	90	58.3	19.4	810
36-8	105	62.0	27.9	893
36-9	22	63.1	19.7	856
36-10	55	59.4	25.5	872
36-11	55	61.2	16.1	853
36-12	72	61.8	15.1	838
36-13	135	63.0	13.8	842

APPENDIX II

Calculated Data

Calculation	Unit
Cell Solids	g/kg
ECSV	g/kg
ICSV	g/kg
Extravascular ion concentration	mEq/l extracellular water
Tissue extracellular ion content	mEq/kg tissue
Cellular ion concentration	mEq/l cell water
Cellular ion content	mEq/kg tissue
Membrane potential	mV

Abbreviations

M	=	mean
SD	=	standard deviation
SE	=	standard error
ECSV	=	extracellular space volume
ICSV	=	intracellular space volume
M P	=	membrane potential
CL	=	confidence limit

1. Sublethal Shock

The data listed below were calculated (see Chapter III, Materials and Methods) on the basis of the mean plasma and tissue sodium, potassium and chloride concentrations and mean tissue water content obtained at each sampling period following sublethal temperature shock (see Appendix I).

GROUP	CELL SOLIDS M	SD	SE	ECSV M	upper 95%CL	lower 95%CL
30-12h	175	20	9	190.0	254.0	126.0
30-24h	183	13	5	168.3	215.5	120.8
30-36h	180	13	5	160.0	198.6	120.7
30-48h	164	15	7	181.5	228.6	134.3
30-60h	167	14	6	235.5	341.7	129.3
30-72h	173	11	4	194.7	228.7	160.8
30-84h	187	10	5	134.5	164.0	105.0
30-96h	153	35	10	276.0	368.8	183.3
30-120h	165	11	4	257.6	313.8	201.3
30-144h	155	27	9	276.1	374.7	177.6
30-168h	115	45	16	367.6	518.1	217.0
30-192h	137	44	16	344.7	520.5	168.9
30-216h	125	36	13	290.5	389.3	191.6
30-240h	145	38	14	378.3	470.6	285.9
20°C Controls	193	8	2	218.5	254.5	182.5
30°C Controls	192	16	4	206.1	229.8	182.3

Group	ICSV M	Sodium Extravascular Concentration	Chloride Extravascular Concentration
30-12h	634.7	-	87.5
30-24h	648.6	175	86.3
30-36h	660.5	201	91.0
30-48h	654.4	219	112.1
30-60h	597.6	211	94.6
30-72h	632.1	253	89.5
30-84h	678.5	250	86.3
30-96h	571.5	240	87.9
30-120h	577.2	203	82.3
30-144h	568.7	-	84.0
30-168h	517.0	-	85.3
30-192h	518.7	-	85.0
30-216h	584.1	-	82.4
30-240h	476.4	-	84.7
20°C controls	588.9	173	88.4
30°C controls	602.3	154	84.6

POTASSIUM

Group	Extra-vascular Conc.	Tissue Extra-cellular Content	Cellular Content	Cellular Conc.
30-12h	9.4	1.8	125	197
30-24h	9.1	1.5	116	179
30-36h	13.1	2.1	121	183
30-48h	8.8	1.6	110	168
30-60h	-	-	-	-
30-72h	9.8	1.9	103	163
30-84h	10.9	1.5	119	175
30-96h	-	-	-	-
30-120h	-	-	-	-
20°C controls	6.1	1.3	103	175
30°C controls	8.2	1.7	113	188

Group	Plasma Na/Cl Ratio	Membrane Potentials
30-12h	-	79.5
30-24h	2.21	77.7
30-36h	2.39	68.8
30-48h	2.11	77.0
30-60h	2.42	-
30-72h	3.07	73.4
30-84h	3.14	72.4
30-96h	2.96	-
30-120h	2.68	-
20°C controls	2.13	84.6
30°C controls	1.98	81.6

2. Lethal Shock

Data listed below were calculated on the basis of plasma and tissue chloride concentrations and tissue water content obtained for each fish following lethal temperature shock (See Appendix I).

Fish No.	Exposure (Min.)	Cells Solids	ECSV	ICSV	Chloride Extra-vascular Conc.
38-1	5	179	163	658	84.5
38-2	8	155	304	541	76.7
38-3	10	173	162	665	80.8
38-4	2	183	160	657	74.6
38-5	3	-	-	-	82.2
38-6	2	179	-	-	-
38-7	4	179	161	660	82.7
38-8	4	164	210	626	84.2
37-1	3	123	336	541	84.5
37-2	3	177	-	-	-
36-1	35	129	448	423	61.8
36-2	20	123	400	477	60.2
36-3	25	144	308	548	60.5
36-4	45	134	392	474	60.8
36-5	10	120	-	-	61.5
36-6	30	165	254	581	60.8
36-7	90	190	299	511	62.7
36-8	105	107	405	488	66.7
36-9	22	144	281	575	68.0
36-10	55	128	387	485	64.0
36-11	55	147	236	617	65.8
36-12	72	162	220	618	66.5
36-13	135	158	197	645	67.8

3. Comparison of Sample Groups

Tests applied:

F - test: A comparison of variance estimates of the two sample groups.

t-test, Cochran and Cox test: A comparison of the means of the two sample groups.

Results of tests:

S- Significant difference at the .05 level.

NS- Non significant difference at the .05 level.

PLASMA CHLORIDE CONCENTRATION

Groups Compared	F-Test	t-Test	Cochran and Cox Test
20-control, 30-24h	NS	NS	
20-control, 30-36h	NS	NS	
20-control, 30-48h	NS	S	
20-control, 30-60h	S		NS
20-control, 30-84h	S		NS
20-control, 30-120h	S		S
20-control, 30-control	NS	S	
30-36h, 30-48h	S		S
30-48h, 30-84h	NS	S	

TISSUE CHLORIDE CONCENTRATION

20-control, 30-12h	NS	NS	
20-control, 30-24h	NS	NS	
20-control, 30-36h	S		S
20-control, 30-60h	NS	NS	
20-control, 30-72h	S		NS
20-control, 30-84h	S		S
20-control, 30-144h	S		NS
20-control, 30-168h	S		NS
20-control, 30-240h	S		NS
20-control, 30-control	S		NS

PLASMA SODIUM CONCENTRATIONS

20-control, 30-36h	NS	NS	
20-control, 30-48h	NS	S	
20-control, 30-72h	NS	S	
20-control, 30-120h	NS	NS	
20-control, 30-control	NS	NS	
30-36h, 30-48h	NS	NS	
30-48h, 30-72h	NS	NS	
30-48h, 30-84h	NS	NS	

TISSUE SODIUM CONCENTRATIONS

20-control, 30-12h	NS	NS	
20-control, 30-48h	S		NS
20-control, 30-60h	S		NS
20-control, 30-72h	S		NS
20-control, 30-120h	S		NS
20-control, 30-192h	S		NS
20-control, 30-240h	NS	NS	
20-control, 30-control	NS	S	
30-240h, 30-control	NS	NS	

PLASMA POTASSIUM CONCENTRATIONS

Groups Compared	F-Test	t-Test	Cochran and Cox Test
20-controls, 30-36h	NS	S	
20-controls, 30-48h	NS	S	
20-controls, 30-controls	NS	S	

TISSUE POTASSIUM CONCENTRATIONS

20-controls, 30-12h	S		NS
20-controls, 30-36h	NS	S	
20-controls, 30-60h	NS	NS	
20-controls, 30-84h	NS	NS	
20-controls, 30-144h	NS	S	
20-controls, 30-192h	NS	S	
20-controls, 30-240h	NS	NS	
20-controls, 30-controls	NS	NS	

TISSUE WATER CONTENT

20-controls, 30-12h	S		NS
20-controls, 30-24h	S		NS
20-controls, 30-36h	S		S
20-controls, 30-48h	S		S
20-controls, 30-84h	NS	NS	
20-controls, 30-168h	S		S
20-controls, 30-240h	S		S
20-controls, 30-controls	NS	NS	
30-36h, 30-48h	NS	NS	

4. One Sided Tolerance Limits

Plasma chloride, 20°C control group: At the .01 level, at least 95% of the observations from this population will be greater than 82.0-10.8 = 71.2 mEq/l.

Tissue chloride, 20°C control group: At the .01 level, at least 95% of the observations from this population will be greater than 19.2-18.4 = .8 mEq/kg; and at least 95% of the observations from the population will be smaller than 19.2+18.4 = 37.6 mEq/kg.

Tissue water content, 20°C control group: At the .01 level at least 95% of the observations from this population will be smaller than 807+20.5 = 827.5 g/kg.