# TEMPERATURE ACCLIMATION IN *TILAPIA SPARRMANII*: SOME EFFECTS OF THIOUREA

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## INTRODUCTION

*Tilapia* is a tropical genus, indigenous to Southern Africa. Most species will not survive for prolonged periods below about 10C. *T. sparrmanii* (Smith), the banded bream or vlei kurper, is unusually eurythermal and survives outdoors in places subject to cold winters (Harrison 1963). Thus *T. sparrmanii* would appear to be an excellent species for investigations into the processes of temperature acclimation.

It has frequently been demonstrated that alterations in a poikilotherm's environmental temperature result in compensatory modifications of metabolic rates, partially or completely overriding the physico-chemical effects of temperature change. This is particularly marked in aquatic animals and especially fishes (Bullock 1955). Temperature acclimation has often been demonstrated in terms of the respiration rate of the whole animal. Thus a hot acclimated animal respires more slowly than a cold acclimated one, when the rate is measured at the same temperature, and, as would be expected, the respiration rates of organs excised from acclimated animals have been shown to follow the same trend, although few measurements have been reported. For instance, Freeman (1950) found an inverse relationship between acclimation temperature of goldfish and the metabolic activity of excised, homogenised brains, though muscle did not show this relationship.

In homeotherms, the rate of tissue oxidation is controlled to a large extent by the endocrine glands, and the thyroid is of importance in modifying metabolic heat production during temperature acclimation. There is some controversy as to the role played by this gland in the temperature acclimation processes of fishes. Suhrmann (1955) reported that chemical thyroidectomy by means of the commonly used goitrogen thiourea abolishes temperature induced alterations in oxygen consumption of *Carassius vulgaris*. However, Klicka (1965) found no such observable changes in acclimation patterns of *C. auratus*. He also quotes further references demonstrating the conflicting nature of reports in this connection.

This work was undertaken in order to explore some of the processes of temperature acclimation in *T. sparrmanii*, and the effects of chemical thyroidectomy upon these processes. During the course of these experiments, measurements have been made of the endogenous respiratory rates of excised livers, liver water content, and the weight changes of various organs of fish after acclimation to two temperatures. These results have been compared with those obtained using fish subjected to prolonged pre-treatment with thiourea. Some effects of thiourea on thyroid morphology are also described.

## MATERIALS AND METHODS

### TREATMENTS

*T. sparrmanii* varying between 9–13 cms in length and 12–35 g in weight were obtained from Jonkershoek Hatchery, Stellenbosch. A determination of the product-moment correlation coefficient, r, for liver respiration and fish size between these extremes for 56 fish gave a figure of 0·04. After preliminary measurements of ventilation rates to determine approximately the rate of acclimation, these fish were randomly distributed in two groups, both maintained at 16C. One of these groups was treated for two months with 0·15% thiourea, dissolved in their water. After this, half of the fish from each group were transferred to tanks whose water temperature was then raised over a period of three hours from 16C to 29C. Thiourea treatment was maintained at both temperatures. The two temperatures were chosen so as to be well within the lethal temperature limits. Temperatures (±0·05C) were controlled by Tempunit thermostats; tanks were all the same size, vigorously stirred and aerated and the water was changed weekly; natural illumination and photoperiod was used; fish were fed twice daily on a proprietary brand of dried fish food.

In the experiment where the rate of change of the effects of thiourea on livers at 16C was measured, two fish stocks were used, both maintained at 16C. One stock was treated with thiourea as described above and the other stock acted as a control. At weekly intervals after commencement of treatment, six thiourea treated, and three control fish, were sacrificed for liver measurements.

### VENTILATION RATES

The mean number of ventilations per minute for 10 individuals was determined daily, after transfer from 16C to 29C. Tidal volumes were not measured, since only a rough estimate of the rate of acclimation was required. The rates of ventilation of fish at 16C acclimated to 16C were not measured, as opercular movements were irregular and frequently almost imperceptible.

### LIVER METABOLIC RATE

Fish were killed by spinal section and the entire liver rapidly excised, weighed and dropped into ice-cold buffer. Gall inhibits respiration, thus care was taken not to puncture the large, thin walled gall bladder. The liver was homogenised in 10% w/v of phosphate buffer (0·16M $KH_2PO_4$ + 0·12M $Na_2HPO_4$ adjusted to pH 7·4) using a hand operated Tenbruk homogeniser. Oxygen consumption of the homogenates was measured at 25C using duplicate Warburg respirometers and corrected to 0C and standard pressure, according to methods advocated by Umbreit *et al* (1959), ($Qo_2$= $\mu$l $0_2$/hour/mg. dry weight at 0C and standard pressure). The flasks were not gassed and $CO_2$ produced was removed by 0·2 ml of 10% KOH absorbed onto folded squares of filter paper in the centre well. Oxygen uptake was virtually linear for at least the first hour, and $Qo_2$ was calculated from readings taken during this period.

The dry weight of the liver in the flasks was determined by weighing the main chamber contents after overnight drying at 100C on watchglasses and subtracting the calculated dry weight of buffer.

Duplicate flasks usually agreed to within 5% of each other, and mean results are presented.

### LIVER WATER CONTENTS

These were calculated from the dry weights of the homogenates used in the Warburg flasks after correcting for the known dry weight of added salts.

ORGAN WEIGHTS

For this experiment fish were selected at random from a stock ranging in size from 14–28 g. Heart, liver, spleen and brain were excised from fish, in groups of eight, at various times after transfer from 16C to 29C; these were weighed after a quick blotting with absorbent paper. Weights were calculated in terms of percent total body weight. Heart weight includes the conus arteriosus, and the brain was severed at the level of the swelling of the medulla oblongata.

STATISTICAL ANALYSES

Differences between means were analysed, where necessary, using the students' "t" test, after application of Bessel's correction for numbers between fifteen and six.

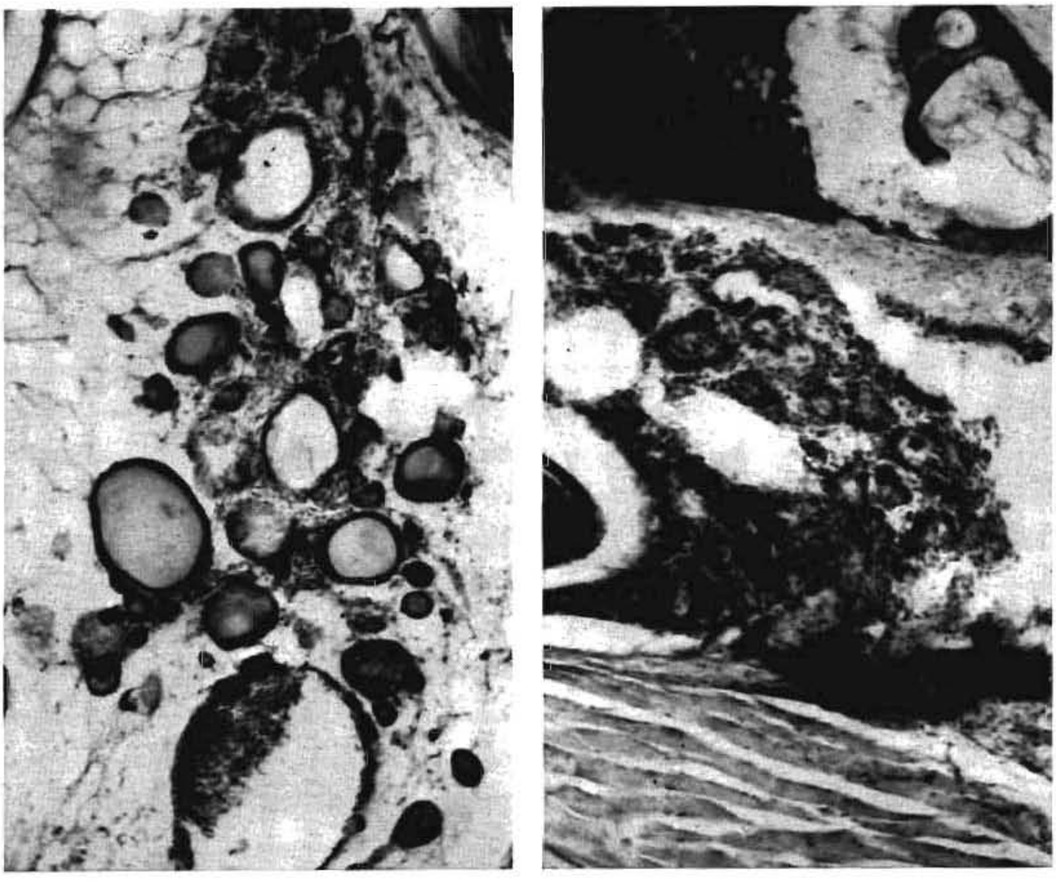

Figure 1. Sections of *T. sparrmanii* thyroid gland. On the left is a normal thyroid and on the right a hyperplastic thyroid, induced by two months of thiourea treatment. Tissues were fixed in 10% formalin and sections stained with haematoxylin and eosin. (Photographs: Mr. R. H. Simons.)

## RESULTS

### CLINICAL EFFETCS OF THIOUREA

After a month of thiourea treatment, the condition of the fish deteriorated. They became listless, dorsal fins were kept folded, the fins in general were ragged.

Towards the end of two months, appetites decreased, and a string of mucous often hung from the anus. Localised skin lesions developed, with scales dropping out in patches. Some fish kept the mouth perpetually half open.

Transfer to 29C accentuated the symptoms and 10–20% mortality occurred.

### MORPHOLOGICAL EFFECTS OF THIOUREA UPON THE THYROID

Fig. 1 shows a thyroid section cut from a fish which had undergone the initial thiourea pre-treatment at 16C, compared with a thyroid section from an untreated fish kept at the same temperature.

In normal *T. sparrmanii*, the thyroid consists of loosely associated follicles spread thinly on either side of the ventral aorta. Thiourea treatment results in increased gland size and density as a result of hypercellularisation. There is considerable reduction in total amount of colloid, which frequently shows peripheral scalloping. No large follicles remain. The epithelium lining the follicles has become distinctly columnar, whereas in the normal thyroid this is usually cubical to sub-cubical. No conspicuous increase in lymphoid tissue was observed, but there is a definite increase in total vascularisation.

### VENTILATION RATES

The results are presented in Figure 2. The rates are very scattered. However, the tendency towards a levelling off of the ventilation rates indicated that both normal and thiourea treated

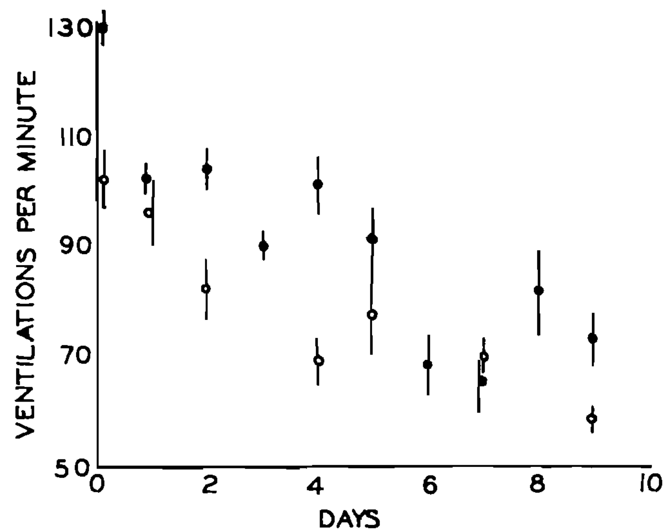


Figure 2. Change in ventilation rates of *T. sparrmanii* transferred from 16C to 29C, on day 0. Each coordinate represents the mean of ten readings, and vertical bars indicate one standard error on either side of the mean.

○ fish treated with thiourea
● untreated fish.

fish were almost acclimated to the new temperature within eight days. Allanson and Noble (1964), studying heat tolerance of *T. mossambica* found fairly similar acclimation rates. It was considered that metabolic acclimation must have been virtually complete after fourteen days, and the fish were therefore allowed this period of time at the higher temperatures before liver $Qo_2$ was measured.

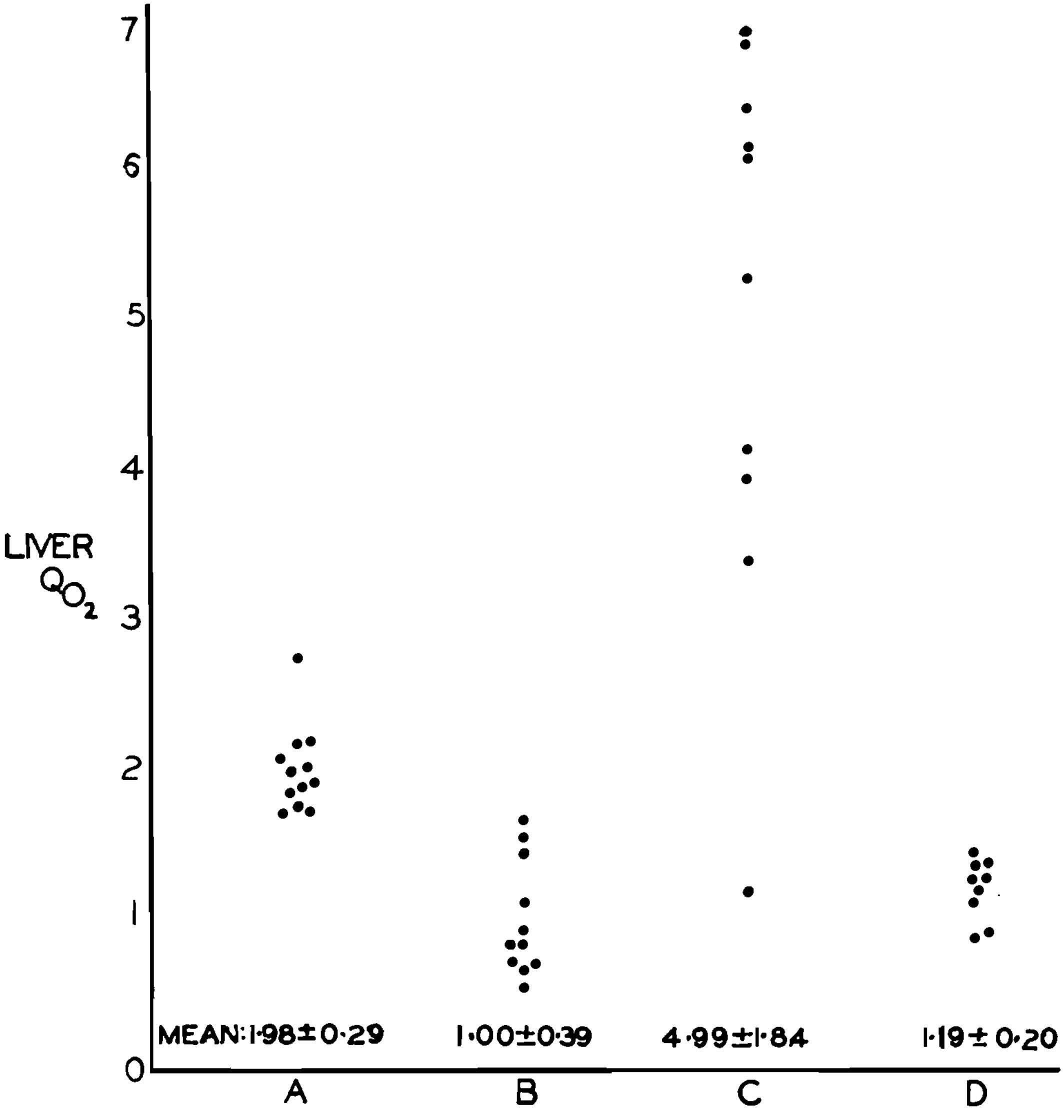


Figure 3. Scatter diagram showing effect of temperature acclimation upon the liver respiration of *T. sparrmanii.* Each point indicates the $Qo_2$ of the excised liver of one fish. A—acclimated to 16C; B—acclimated to 29C; C—acclimated to 16C + thiourea treatment; D—acclimated to 29C + thiourea treatment.

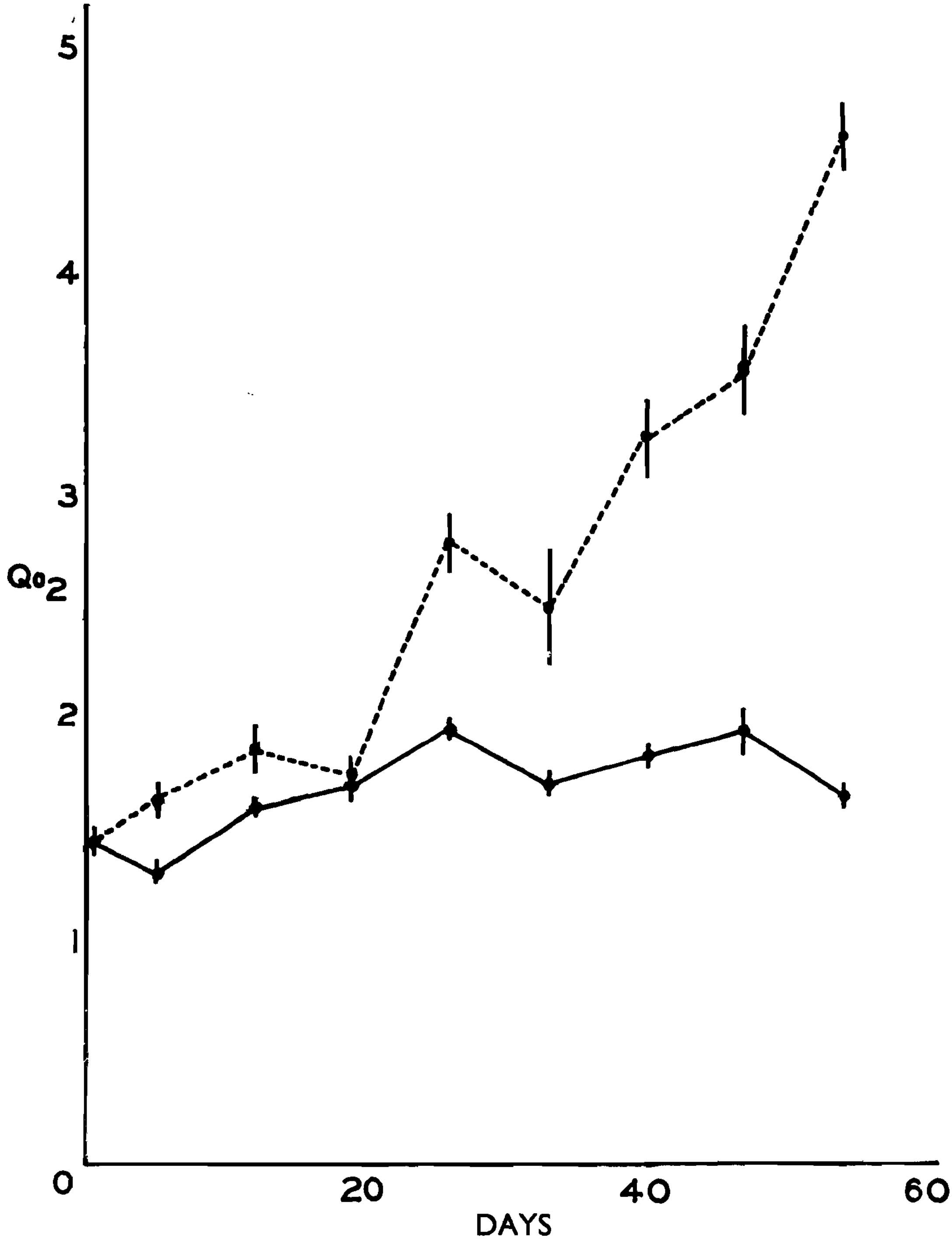


Figure 4. Change in *T. sparrmanii* liver $Qo_2$ during thiourea treatment. Vertical bars represent one standard error on either side of the mean. Broken line—treated with thiourea; continuous line—not treated with thiourea.

LIVER OXYGEN UPTAKE

The results are presented in the form of a scatter diagram (see Figure 3). Acclimation to 29C has nearly halved the $Qo_2$ at 25C when compared with 16C acclimated fish. The difference between the two rates is highly significant; the probability (P) of it being due to chance is less than 0·001.

Pretreatment with thiourea increased $Qo_2$ of 16C fish livers about 2·5 times the levels measured on untreated fish at 16C. These rates were very scattered, implying considerable

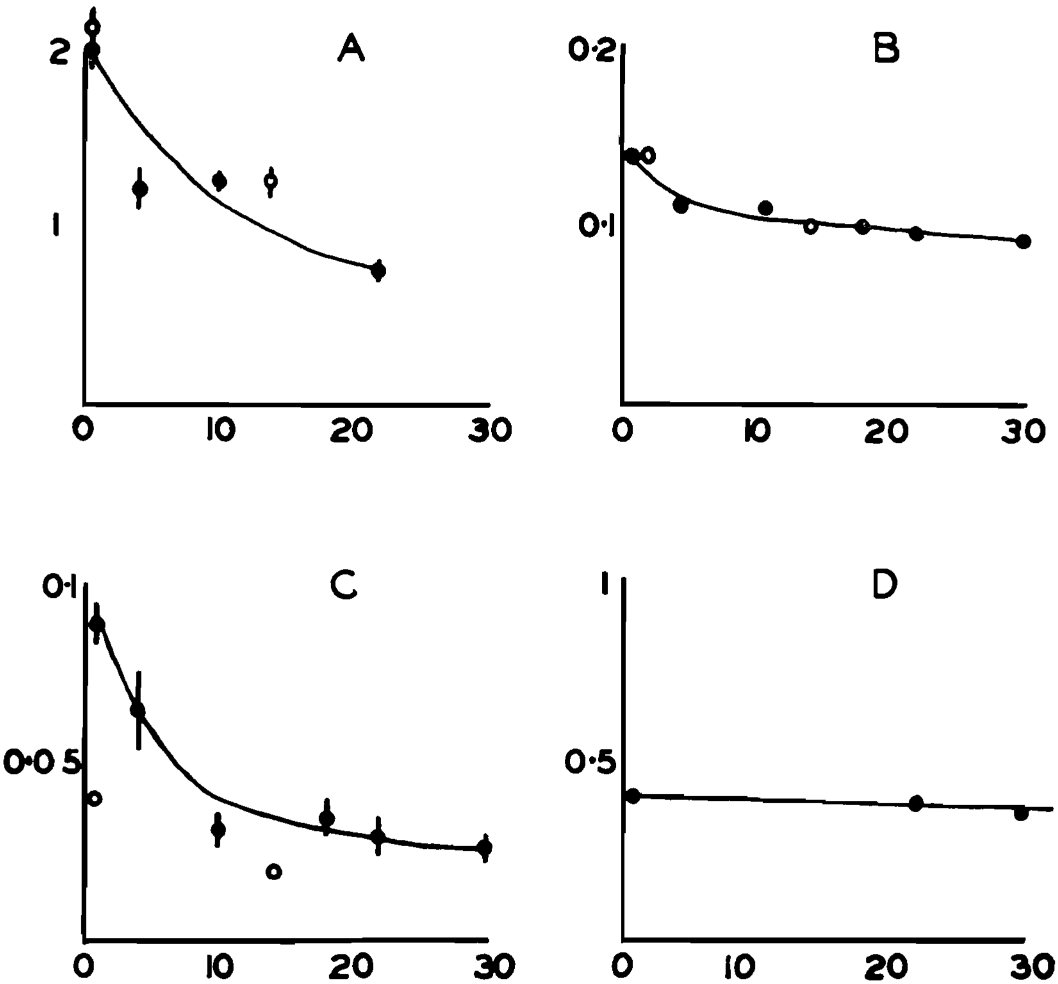


Figure 5. Changes in weight of various organs of *T. sparrmanii* after transfer from 16C to 29C at day 0. Ordinates—weight of organ as a percentage of body weight, abscissae—days after transfer. Each coordinate represents the mean of eight measurements and vertical bars represent one standard error on either side of the mean. In some cases the standard error is contained within the point. A—liver; B—heart; C—spleen; D—brain.

● untreated fish.
○ fish treated with thiourea.

variation in response to the drug between individuals. However, fish pretreated with thiourea at 16C and acclimated to 29C reduced liver $Qo_2$ to levels similar to those obtained for 29C fish not pretreated with thiourea. P for the difference between these means is greater than 0·1.

The rate of increase of liver $Qo_2$ during thiourea treatment at 16C was investigated in a separate experiment, and the results are shown in figure 4. Thiourea treated fish showed no significant increases in $Qo_2$ after 19 days treatment. After this, there was a progressive increase in $Qo_2$, accompanied by an increase in scatter.

### LIVER WATER CONTENTS

The temperature treatments alone had insignificant effects upon liver water contents. Treatment with thiourea at 16C for thirty three days increased mean water content over controls by 4·2% and for 47 days the mean increase was 3·6%.

### ORGAN WEIGHTS

The considerable variations in liver metabolic activity suggest the possibility of consequent temperature induced changes in organ sizes. Figure 5 shows the effect of transfer of fish from 16C to 29C upon organ weights. In the case of liver and spleen there was a marked reduction in weight, virtually complete after fourteen days at the higher temperature. Heart changes were not as marked, nevertheless the difference between the means for day 0 and day 30 was highly significant (P less than 0·001). Controls left at 16C and measured on day 0 and day 30 showed no significant changes and brain weight was unaltered.

Although actual volumes were not determined, cursory examination showed obvious volume changes, in the case of liver and spleen.

Treatment with thiourea did not abolish these temperature induced reductions in weight and the amounts of reduction were of a similar order to those observed for fish not treated with thiourea. The reductions in weight were highly significant, in all cases, Thiourea treatment alone also induced a highly significant decrease in weight of spleen.

## DISCUSSION

### NORMAL FISH

The modification of liver respiration obtained when normal fish are acclimated to different temperatures is in agreement with previous work, in particular that to Precht *et al* (1955) who report decreased goldfish liver methylene blue decolouration time in Thunberg tubes with higher acclimation temperatures. Freeman (1950) found a drop of almost 40% in $Qo_2$ of goldfish brain after acclimation to 27C, and compared with 12C. Kanungo and Prosser (1960) found a 43% increase in liver respiration of goldfish acclimated to 20C when compared with fish acclimated to 30C. These responses to a changed environment have obvious adaptive advantages, serving to maintain a level of activity closer to an optimum than would otherwise be the case.

The alterations in the weights of the liver, heart and possibly spleen at different temperatures are significant. The lack of any modification of water content indicates that the changes in weight are due to size rather than density changes, for livers at least.

We have not found work reporting results of this nature, and yet such results may be expected, since rate-temperature curves are not identical for different processes. The equilibrium of integrated and catenary processes at 16C cannot hold at 29C. A change in the "optimal" level of activity of an organ, which is related to its integrated and controlling processes, and which may be induced by a temperature change, may result in compensatory modifications in size.

It is of interest to note that acclimation from 16C to 29C involves an absolute reduction of liver $Qo_2$ of more than 75% since both size and $Qo_2$ are about halved. The brain showed no size modification, which is to be expected since there is no proof of any correlation between brain size and activity.

ACCLIMATION IN THIOUREA TREATED FISH

In mammals, thiocarbamides inhibit thyroxine production and liberation from the thyroid, with compensatory hyperactivity of the hypophysis with consequent increased release of thyrotrophin. This results in spurious thyroid development, or goitre, (Astwood 1944). The histological changes associated with thiocarbamide induced goitre in mammals (Williams and Clute 1944, and many others) are almost identical with those described for *T. sparrmanii*, which indicates considerable impairment of normal thyroid activity in the latter.

Acclimatory changes as a result of temperature increase occurred in both normal fish and in goitred fish and the changes were of the same order in both groups. Compensatory reductions in gill ventilation rate and in organ size, still occur. Furthermore, although thiourea itself causes considerable increase in liver $Qo_2$, the reduction during warm acclimation overrides this effect completely.

These experiments show that the thyroid is not of prime importance in controlling temperature acclimation in *T. sparrmanii.*

ACTION OF THIOUREA OF LIVER

On the basis of mammalian studies, the increase in liver $Qo_2$ of 16C fish after thiourea treatment is contrary to expectation. In *T. sparrmanii*, calculations show that this effect is only slightly due to the change in dry to wet liver weight ratio, resulting from the increase in hydration.

This effect must be confined largely to the liver; if a general tissue effect, an increase in total oxygen consumption to this extent would be shown by increases in ventilation rate. Ventilation rates were generally slightly lower after thiourea treatment (figure 2) but not significantly so. Chambers (1953) quotes references reporting reduction in whole fish oxygen consumption with thiourea treatment, and others (e.g .Matthews and Smith 1947) find no alteration. Detoxification occurs mainly in the liver and the result shown in figure 4 may demonstrate the slow induction of a detoxifying system. In rabbits, detoxification of this carbamides takes place mainly in the liver. The complete mechanism is unknown, but the overall reactions are $R\text{-}NH\text{-}CS\text{-}NH_2 \longrightarrow R\text{-}NH\text{-}CN + H_2S$ and then $R\text{-}NH\text{-}CN + H_2O \longrightarrow R\text{-}NH\text{-}CO\text{-}NH_2$ (Williams 1961). In the case of thiourea, this final compound would be urea. It is possible that some steps require adenosine triphosphate (ATP) and the depletion of liver glycogen with thiourea treatment reported by Chambers (1953) supports this possibility. Detoxification of thiourea would thus involve an increase in liver $Qo_2$, and account for the apparently anomalous result obtained in the present investigation.

On this basis, the increase in mortality upon elevation of the temperature is understandable, since with a large proportional reduction in oxidative metabolism at the higher temperature, considerably less ATP would be available and detoxification of thiourea less effective. Fortune's (1955) results, showing that thiourea causes hyperplasia and colloid reduction more readily at high temperatures in *Phoxinus* and *Lebistes* are also explicable in these terms since a less effective detoxification mechanism, proportionately, would result in a higher level of circulating thiourea.


## SUMMARY

1. Persistent temperature change causes significant alterations in the size of various organs of *Tilapia sparrmanii*, and in the respiration rate of excised liver. There is no change in liver water content.
2. Prolonged thiourea treatment induces hyperplastic goitre, similar to mammalian exophthalmic goitre. It also induces an increase in liver respiration, but does not inhibit the adaptive responses of the liver to increased temperature, nor the compensatory changes in ventilation rate.



## ACKNOWLEDGEMENT

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