THE EFFECTS OF MELATONIN ON OXYGEN CONSUMPTION RATES IN SMALL TELEOSTS

by

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ABSTRACT

Goldfish, Carassius auratus, exposed to various concentrations of melatonin over a ninety minute time period seem to exhibit a lower rate of oxygen consumption, as compared to controls. The use of a teflon membrane oxygen probe in the isolated controlled environments of the goldfish allowed for the measurement of the change in oxygen content of each fish's environment. From this, the calculation of the rate of oxygen consumption of each fish at specific time intervals was determined. It is proposed that the lower oxygen consumption rates are causally related to the influence of melatonin taken up by the fish directly through their gills.

To Dr. Dale M.J. Mueller, the botany

instructor I never had.

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INTRODUCTION

Numerous studies concerning the effects of the melatonin (N-acetyl-5-methoxytryptamine) on physiological functions have been conducted (for recent review, see Reiter, 1978). These studies have produced a variety of results on how melatonin might be functioning in an organism (Butt, 1976). The investigations have shown that melatonin is a biologically important compound which may play a role in basic physiological functions of vertebrates (Quay, 1974). This suspected hormone is produced by the pineal gland in all vertebrates studies to date. The function of this bulb-shaped gland, located medially to the cerebral hemispheres in the brain of many vertebrates, is not well understood.

Melatonin is biosynthesized from tryptophan via the serotonin pathway, and seems to have different effects on different animals (Altschule, 1975). It has been found to be in higher concentrations at night in diurnally active animals, thus it is possible that it may function to facilitate an overall reduced metabolic rate and rest (Wolstenholme, 1971). In birds and mammals, it primarily has an inhibitory effect on reproduction, showing an antigonadotropic effect (Altschule, 1975; Fenwick 1970a). Investigations on fishes and lampreys have shown melatonin to affect external coloration in

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response to background, producing different degrees of skin blanching at specific melatonin concentration levels (Hafeez, 1970; Ruffin, Reed and Finnin, 1969). This effect has also been shown in studies conducted upon certain amphibians (deVlaming, Sage, and Charlton, 1974). Melatonin has also been shown to affect the seasonality of reproduction and other basic biological rhythms in several species tested (Butt, 1976; Fenwick, 1970b). Although melatonin has been found to reduce some symptions of schizophrenia in psychotic patients, no really definite proof has been demonstrated for its function in humans (Quay, 1974; Altschule, 1975). From studies concerning the effects of this enigmatic molecule, we can suggest that melatonin tends to give a general tonic effect on metabolism in those animals tested. Yet just which basic metabolic, and physiological function or functions are truly influenced by this endocrine-like compound has not been clearly determined. We are suggesting that the basic metabolic parameter of oxygen consumption may be influenced by melatonin. These preliminary experiments are designed to test this possibility.

MATERIALS AND METHODS

A total of 64 adult common goldfish, Carassius auratus, varying in color and size (body wt.:8.69g - 40.75g), and kept in a large aquarium, were used as experimental animals. For acclimation purposes, the fish were randomly chosen 24 hours prior to each testing. They were then placed in individual aquarium environments and fed ad libitum. Melatonin solutions used were freshly mixed each experimental day, dissolving the measured amount of melatonin powder in 0.5 ml of ethanol before diluting. One of the determined concentrations was randomly picked for each test day. On December 12, 1979, Experiment #1 was started by initially aeriating the exactly measured volumes (approximately six-liter) of dechlorinated H_2O of the eight Erylenmeyer flasks to maximum O_2 saturation. Eight previously isolated fish were then individually weighed and transferred (to their individual flasks) for an acclaimation period of 30 minutes. The environment of each flask was then aeriated for 5 more minutes and the temperature of the water in each was recorded. Then by inserting the oxygen probe apparatus and elimenating all air bubbles present, a pre-injection reading of the oxygen content of the water was read from the oxygen meter. At the time of inoculation, using 20cc and 10cc syringes, an exact volume of 1/100 of the flask volume was extracted from the first flask and replaced with the same volume of pre-mixed melatonin solution, yielding a known melatonin

concentration of the environment. The use of a known melatonin concentration directly in the ambient water environment rather than injection by syringe has been demonstrated in previous investigations (Hafeez, 1970). The flask was again stoppered with the probe apparatus and readings were recorded for the initial post-injection 5 minutes. This procedure was repeated for each fish in the other seven respective flasks, with readings also recorded for each 30 and 60 minute time intervals. This overall testing procedure was repeated the next three days, using the same eight fish for the four days of testing and a different melatonin concentration for each testing day . For Experiment #1, melatonin environment concentrations of 0.05 µg/ml, 0.5 µg/ml, 5.0 µg/ml and 0.0 µg/ml (control) were used; the control solution contained the 0.5 ml of ethanol with no melatonin (Hafeez, 1970). Flask volume temperatures for Experiment #1 ranged from 17.0 to 18.5°C and fish body weights ranged from 8.69g to 15.77g. On February 7, 1980, Experiemnt #2 was begun, using the same timed procedure, but was cut short due to concentration mixing problems caused by the amount of alcohol used to dissolve the non-water-soluble melatonin powder into solution. On February 21-23, 1980 Experiment #3 was conducted, but instead using different fish for each experimental run, for each of the three testing days. Readings for each of the eight volumes were also taken at the 90 minute time intervals in the experiment. Similar to other melatonin investigations (Hafeez, 1970), melatonin concentrations of 0.0 µg/ml (control)

5.0 µg/ml and 10.0 µg/ml were used, with one concentration randomly picked for each day of testing. Flask volume temps. for Exp. #3 ranged from 20.0 to 25.0°C and fish body wts 15.04 to 30.50g. On March 20 - 22, 1980, Exp. #4 was conducted, testing Exp. #3 for reproducability. For Exp. #4, flask volume temps were from 20.5 to 21.5°C and fish body wts 19.30 - 40.75g.

RESULTS

Exp. #1:

The oxygen consumption rates determined from Exp. #1 for each solution used at each time interval are presented in Table 1 and Fig. 1. From the analysis of variance (ANOVA), it was found that none of the mean O_2 consumption rates of the four treatment concentrations were significantly different at the three time intervals tested. At the 5 minute interval, mean O_2 consumption rates showed wide variation with standard errors of each overlapping. Although not significantly different from the control solution, all three melatonin concentrations used (.05 µg/ml, .50 µg/ml, 5.0 µg/ml) resulted in fish yielding lower mean oxygen consumption rates at the 30 and 60 minute intervals. From the largest of the three melatonin concentrations used, 5.0 µg/ml, fish exhibited the lowest mean rates of oxygen consumption. From three results, a casual trend was observed: As melatonin concentration was increased there was at least the suggestion of a lower oxygen consumption rate at the 30 and 60 minute intervals. It is from this observed trend that further modification of experimental techniques was indicated, using greater melatonin concentrations and longer time intervals of testing.

Exp. #2:

No results were obtained for this round of testing due to procederal complications while making up the melatonin solution

TABLE 1

Mean Oxygen Consumption Rate Values for Concentrations Used at Each Time Interval in Carassius auratus[a] - Exp. #1

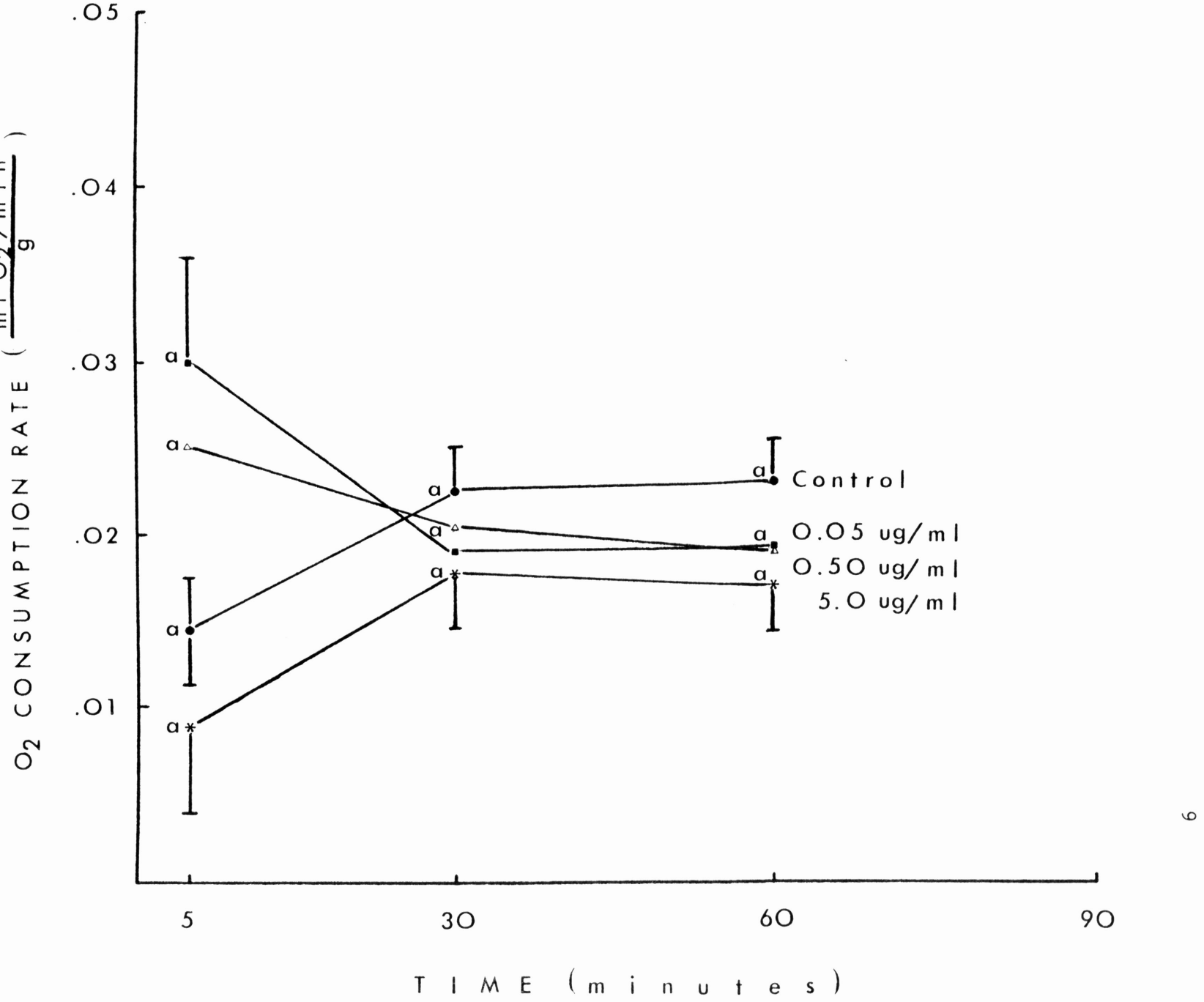
TIME	TREATMENT	MEAN $\frac{\text{ml } O_2/\text{min}}{\text{g}}$	$s/\sqrt{n}$
5 Min.	Control	.0144	.0033
	0.05 ug/ml	.0300[c]	.0059
	0.5 ug/ml	.0252[c]	.0094
	5.0 ug/ml	.0087[c]	.0048
30 Min.	Control	.0225	.0027
	0.05 ug/ml	.0191[c]	.0014
	0.5 ug/ml	.0203[c]	.0028
	5.0 ug/ml	.0178[c]	.0032
60 Min.	Control	.0223	.0026
	0.05 ug/ml	.0188[c]	.0020
	0.5 ug/ml	.0186[c]	.0020
	5.0 ug/ml	.0166[c]	.0026

[a] Mean $\pm$ S.E.M. (n=8 in each group) .

[b] Significantly different than control ($P < .05$, S.N.K.) .

[c] Not significantly different than control .

Figure 1. Exp. #1: The effects of Melatonin Concentrations on Mean Oxygen Consumption Rates of Goldfish, Carassius auratus, at Specific Time Intervals. Vertical lines represent the standard error of the mean. In each group n = 8.


.05
.04
.03
.02
.01
O_2 CONSUMPTION RATE ($\frac{ml\ O_2/min}{g}$)
a
a
a
a
a
a
a
a
a
Control
0.05 ug/ml
0.50 ug/ml
5.0 ug/ml
5
30
60
90
TIME (minutes)

concentrations. Due to the large volume of non-water-soluble melatonin powder required for stronger melatonin solutions, a larger volume of ethanol (5.0 ml instead of 0.5 ml) was required to dissolve the melatonin before dilution. This resulted in having the fish become more affected by the alcohol present than the melatonin. The unusual behavior of the fish was evident in just a few minutes of observation.

Exp. #3:

The mean O_2 consumption rates derived from Exp. #3 for each concentration used at each time interval are presented in Table 2 and Fig. 2. Modification of experimental design included using different fish for each individual test on each of the three consecutive testing days. This eliminated the possible stress factor caused by repeatedly using the same fish. This modification of the protocol also apparently reduced treatment variability. The ANOVA indicated that the mean O_2 consumption rates at all four time intervals were significantly different. Next, the Student-Newman Keuls Test, used to differentiate which means are significantly different, showed that at all four time intervals both melatonin solutions used (5.0 µg/ml and 10.0 µg/ml) gave O_2 consumption rates that were significantly lower than rates of oxygen consumption from fish exposed to the control solution. It is also important to note that at all time intervals except the 5 minute interval, the mean O_2 consumption rates of fish subjected to the 5.0 µg/ml

TABLE 2

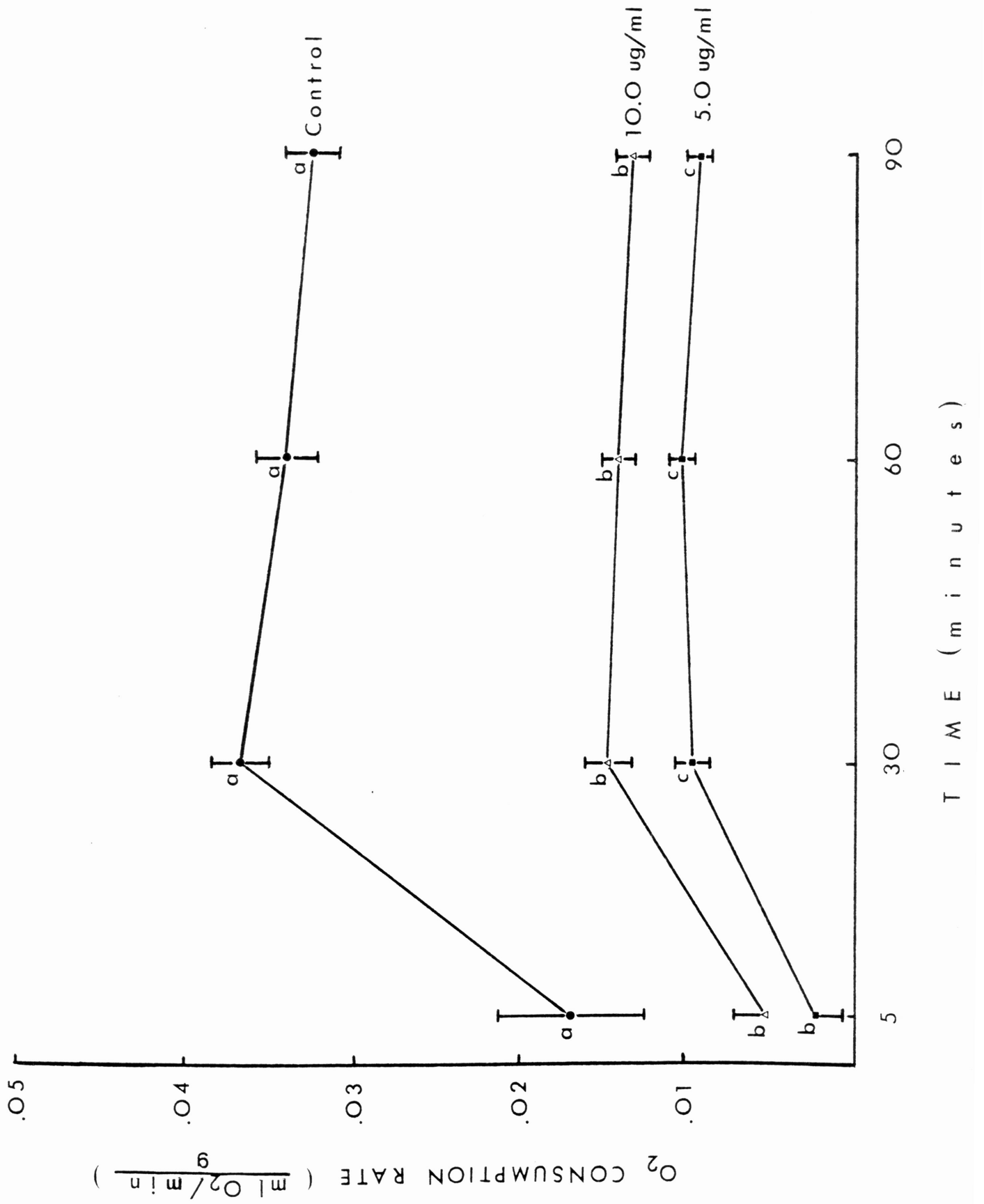
Mean Oxygen Consumption Rate Values for Concentrations Used at Each Time Interval in Carassius auratus[a] - Exp. #3

TIME	TREATMENT	MEAN $\frac{\text{ml } O_2/\text{min}}{g}$	$s/\sqrt{n}$
5 Min.	Control	.0169	.0046
	5.0 ug/ml	.0023[b]	.0016
	10.0 ug/ml	.0054[b]	.0017
30 Min.	Control	.0368	.0017
	5.0 ug/ml	.0096[b]	.0012
	10.0 ug/ml	.0147[b]	.0013
60 Min.	Control	.0340	.0018
	5.0 ug/ml	.0102[b]	.0006
	10.0 ug/ml	.0140[b]	.0009
90 Min.	Control	.0324	.0017
	5.0 ug/ml	.0094[b]	.0006
	10.0 ug/ml	.0133[b]	.0010

[a] Mean $\pm$ S.E.M. (n=8 in each group) .

[b] Significantly different than control ($P < .05$, S.N.K.) .

Figure 2. Exp. #3: The Effects of Melatonin Concentrations on Mean Oxygen Consumption Rates of Goldfish, Carassius auratus, at Specific Time Intervals. Vertical lines represent the standard error of the mean. In each group n = 8.


O2 CONSUMPTION RATE (ml O2/min / g)
.05
.04
.03
.02
.01
5
30
60
90
TIME (minutes)
Control
10.0 ug/ml
5.0 ug/ml
a
a
a
b
b
b
b
c
c
c

solution were significantly lower than mean rates of fish exposed to the more concentrated 10.0 µg/ml melatonin solution.

Exp. #4:

In order to verify the reproducibility of the results obtained in Exp. #3, the same procedures were repeated in Exp. #4. The mean oxygen consumption rates derived from Exp. #4 at each time interval are presented in Table 3 and Fig.3. The ANOVA and SNK tests indicated that at each time interval except the 5 minute mark, both melatonin solutions caused fish to yield oxygen consumption rates significantly lower than rates from fish treated with the control solution. At the 5 minute interval, only fish subjected to the 10.0 µg/ml melatonin solution yielded rates that were significantly lower than controls. It was only at this interval that mean O_2 consumption rates of the two melatonin concentrations were significantly different.

Comparing mean rates of these two melatonin concentrations at the 30, 60, and 90 minute intervals to those of Exp. #3 gave a "reversed relationship". In Exp. #3, fish subjected to the 5.0 µg/ml solution gave mean O_2 consumption rates significantly lower than those exposed to the 10.0 µg/ml melatonin concentration. Yet in Exp. #4, the 5.0 µg/ml concentration yielded rated that were greater (although not significantly) than those exposed to the 10.0 µg/ml melatonin solution at these intervals.

TABLE 3

Mean Oxygen Consumption Rate Values for Concentrations Used at Each Time Interval in *Carassius auratus*[a] - Exp. #4

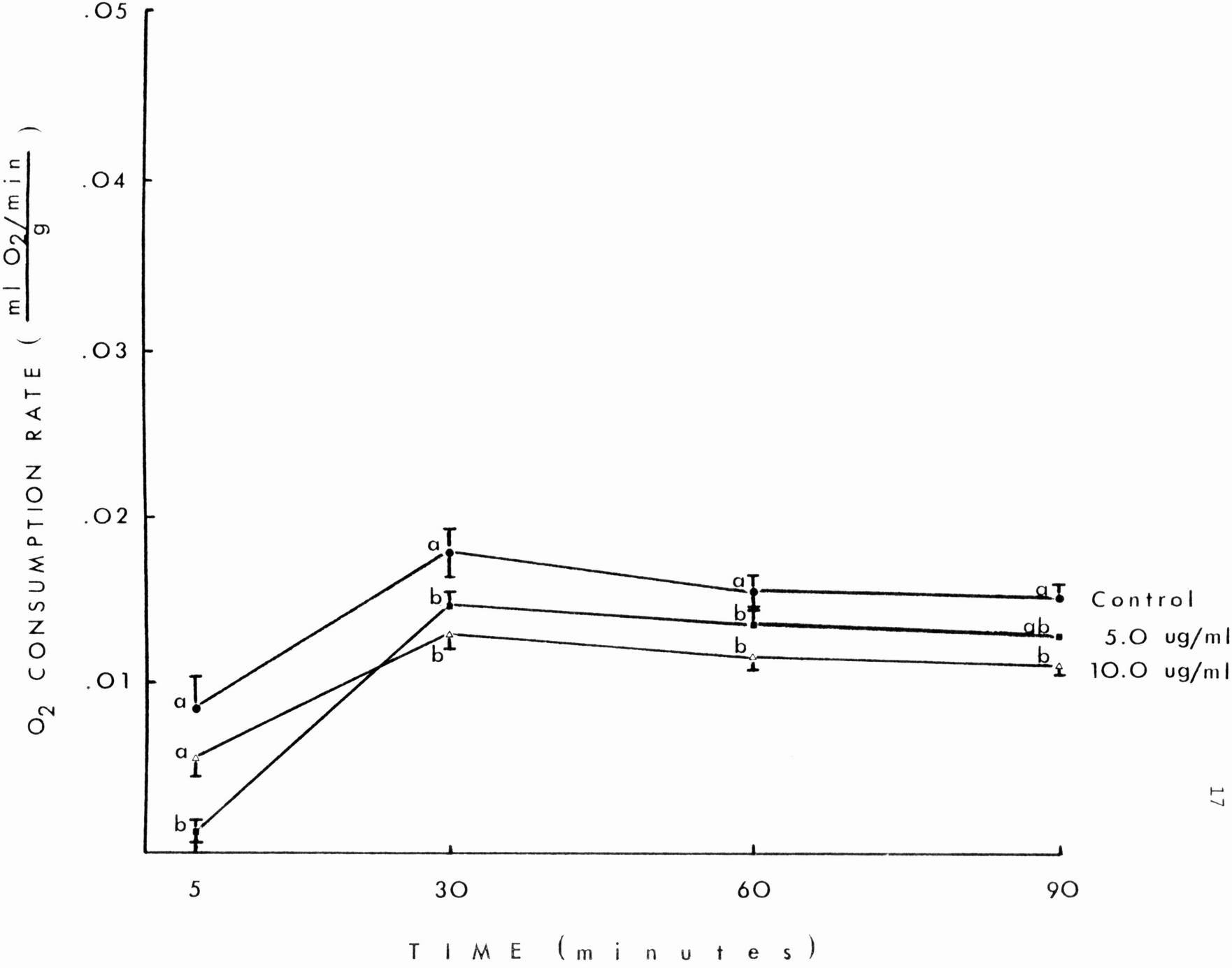
TIME	TREATMENT	MEAN $\frac{ml\ O_2/min}{g}$	$s/\sqrt{n}$
5 Min.	Control	.0084	.0019
	5.0 ug/ml	.0013[b]	.0008
	10.0 ug/ml	.0057[c]	.0014
30 Min.	Control	.0178	.0015
	5.0 ug/ml	.0147[b]	.0006
	10.0 ug/ml	.0129[b]	.0008
60 Min.	Control	.0155	.0010
	5.0 ug/ml	.0136[b]	.0008
	10.0 ug/ml	.0116[b]	.0007
90 Min.	Control	.0151	.0009
	5.0 ug/ml	.0127[b]	.0009
	10.0 ug/ml	.0112[b]	.0015

[a] Mean $\pm$ S.E.M. (n=8 in each group) .

[b] Significantly different than control ($P < .05$, S.N.K.) .

[c] Not significantly different than control .

Figure 3. Exp. #4: The Effects of Melatonin Concentrations on Mean Oxygen Consumption Rates of Goldfish, Carassius auratus, at Specific Time Intervals. Vertical lines represent the Standard error of the mean. In each group n = 8.


.05
.04
.03
.02
.01
O_2 CONSUMPTION RATE ($\frac{ml\ O_2/min}{g}$)
a
b
a
b
a
b
a
ab
b
a
b
b
5
30
60
90
TIME (minutes)
Control
5.0 ug/ml
10.0 ug/ml

DISCUSSION

From this preliminary study, certain relevant conclusions can be derived in accord with the data. First of all, there is the strong indication that Goldfish, Carassius auratus, under the influence of exogenous melatonin exhibit a significantly lower rate of oxygen consumption compared to controls. It was shown that in all cases at the 30, 60, and 90 minute time intervals, the fish subjected to the melatonin concentrations of 5.0 µg/ml and 10.0 µg/ml yielded significantly lower mean oxygen consumption rates compared to mean O_2 consumption rates of controls.

Secondly, it was shown that a consistant response between dosage and oxygen consumption did not occur. In Exp. #3, between the 30 and 90 minute time intervals, mean O_2 consumption rates resulting from use of the 5.0 µg/ml melatonin solution were significantly lower than mean rates derived from the use of the 10.0 µg/ml solution. Yet in Exp. #4, a repeat of the same experimental procedures of Exp. #3, between the same intervals, mean oxygen consumption rates resulting from use of the 5.0 µg/ml solution were greater (although not as statistically proven higher) than those mean rates derived from use of the 10.0 µg/ml solution. Thus a somewhat reversed relationship was observed between the true melatonin concentration solutions used. Explanation of this is difficult, however, it has been shown by Hafeez (1970) that moderate dosages

of melatonin seem to affect certain physiological functions of animals to a greater degree than large dosages of this compound. Further study on dasages is definitely required in order to understand this unique relationsihp.

Thirdly, these preliminary experiments suggest that mean oxygen consumption rates were relatively consistant between 30 and 90 minute intervals. It has been concluded that at the 5 minute interval, the flask environment was not completely stabilized, and equilibrium of the environment with the injected melatonin solution had not been reached. This is shown by the data in Experiments #3 and #4, where 5 minute time intervals' means deviated greatly compared to 30 and 90 minute intervals. Rates between the 30 and 90 minute intervals were relatively consistant in these two experiments.

Taking all of the above into consideration, it is my resulting speculation that natural elevations in nocturnal melatonin may serve to reduce oxygen consumption and metabolism in resting animals. This reduced O_2 consumption could then affect several other parameters which have been shown to have melatonin and pineal gland involvement. For example, poikilothermic animals generally prefer lower temperatures at night even when heat sources are available (Ralph, Firth, Gern, Owens 1979). Thus, reduced O_2 consumption may be the mechanism by which thermal preferences are based. In order to conclusively verify this statement, further experimentation concerning melatonin testing its dosages possibly over longer time intervals, is required.

Determination of exact melatonin levels in the blood via radioimmunoassays is necessary for verification of melatonin uptake by experimental organisms. This was not done because it is beyond the scope of these preliminary experiments. Our data support this view and aid in further investigation of this subject.

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