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Effect of Handling and Methylpentynol Anaesthesia on Serum Glucose Levels in Goldfish, Carassius auratus Linnaeus

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ABSTRACT

In comparison to "pre-stress" levels, goldfish anesthetized with methylpentynol and "handled" showed a significant increase in serum glucose level one day after handling, and apparent continued effects for the next three days. Fish handled but not anesthetized, and control fish which were neither anesthetized nor handled did not exhibit similar changes.

INTRODUCTION

Fish show a hyperglycemic response to stress stimuli. Studies by Chavin (1964) and Chavin and Young (1970) have reported that merely transferring goldfish between aquaria is sufficient stimulus to evoke a hyperglycemia lasting for several days (4 days in 1964 study; 2 days in 1970 study). A means for reducing such a marked change in the internal environment is desirable in order to maintain experimental fish closer to normal physiological levels. Methylpentynol has been shown to partially inhibit the rise of blood sugar which normally follows stress in rats (Watson and Steinberg, 1958).

This study was undertaken to examine the effects of methylpentynol on the stress reaction of goldfish to capture and handling, as indicated by changes in serum glucose levels.

METHODS AND MATERIALS

On arrival from Ozark Fisheries, Stoutland, Missouri, goldfish (comets; mean standard length 11.1 cm, range 9.3-14.5; mean weight 46.4 g, range 25.0-93.9) were sorted into 18 water-filled 75.5 liter plastic buckets, five fish per bucket. The fish were maintained for approximately 4 months at 14 C ($\pm$2 C), with a 12:12 photoperiod. They were fed chopped frozen shrimp daily until two

days before being fin clipped, at which time feeding was terminated until completion of the experiment. Marking the fish allowed comparison of stressed levels to original levels of specific fish. After being marked, the fish were returned to fresh water to which 5 ml of terramycin had been added. Initial blood samples (approx. 1 ml) were taken one week after fin clipping, by cardiac puncture with a #22-gauge needle and heparinized tuberculin syringe. This technique produced 7% mortality. Serum glucose was determined for three surviving individuals per bucket with a Beckman Glucose Analyser ($\pm$ 1 mg/100 ml tolerance), using glucose oxidase.

Seven days following determination of initial levels the 18 containers were divided into three groups of six. We anesthetized the first group of fish (group I) by placing sufficient 98.5% methylpentynol in the water to achieve a final concentration of 4 ml/liter. Fish were left in the solution until they lost equilibrium and swimming activity had ceased, approximately 20-30 minutes. The fish would, however, still respond to deep pressure, corresponding to stage III and between planes 1 and 2 of anesthesia classification of fishes (Klontz, 1965). In the "stressing" procedure the anesthetized fish were netted, held out of water for 20 seconds to standardize transfer times, and returned to fresh water to which 8 liters of non-treated water from their original bucket had been added.

The group II received treatment identical to group I (netting and handling) but were not anesthetized. Group III was used as a control. Control fish were not removed from buckets. Their water was siphoned off until 8 liters remained, then fresh water was added. Thus, for environmental constancy, all buckets had fresh water and 8 liters of original water.

Post-stress sampling of the three groups began 14 hours after stressing and continued daily for the succeeding five days. At each of these times, blood was taken from three fish per group, providing for two serum glucose readings (initial and post-stress) per fish. The time of day and sampling technique were the same for the post-stress sample as for the initial sample. The post-stress sampling produced less than 2% mortality. Both paired and unpaired T tests were used for statistical analysis of data.

RESULTS AND DISCUSSION

Initial serum glucose levels for the 54 fish ranged from 20.3 to 50 mg/100 ml with a mean of 29.7 mg/100 ml (SD $\pm$ 10.5). This

compares favorably (N.S. difference, $P > .2$, unpaired T test) with the results of Chavin and Young (1970) who obtained a mean of 28.5 mg/100 ml (SD $\pm$ 9.6) for 300 goldfish.

The use of methylpentynol resulted in considerable changes (Table 1) in serum glucose levels of anesthetized fish. The post-stress serum glucose levels of anesthetized fish deviated markedly

TABLE 1: MEAN SERUM GLUCOSE (mg/100 ml) FOR THREE GROUPS OF GOLDFISH BEFORE AND FOR SIX DAYS AFTER STRESSING

	Methylpentynol and Handled	Handled Only	Control
Initial Level (IL)	24.0 (4.6) a	20.7 (1.5)	23.3 (2.2)
Day 1	47.3 (18.5) b	26.0 (8.5)	26.0 (15.7)
IL	35.0 (5.3)	26.0 (17.4)	20.3 (1.2)
Day 2	24.3 (2.9) c	31.3 (12.3)	18.7 (5.5)
IL	26.0 (6.2)	37.7 (22.7)	32.3 (7.8)
Day 3	16.7 (2.9) d	32.0 (17.4)	29.3 (8.0)
IL	22.0 (7.5)	29.3 (4.0)	28.0 (5.6)
Day 4	38.0 (11) e	32.7 (20.3)	29.3 (1.5)
IL	33.3 (10.4)	27.3 (2.3)	34.0 (5.6)
Day 5	30.3 (13.8)	33.3 (15.0)	28.00 (11.1)
IL	24.3 (4.2)	41.0 (10.0)	50.0 (20.4)
Day 6	18.3 (4.2)	33.7 (4.0)	44.0 (17.5)

a - one standard deviation from the mean
b - significantly different ($P < .05$, paired T test) from initial level
c,d,e - different from initial level ($P < .1$, paired T; $P < .05$, unpaired T)

from their initial levels for days 1 through 4 (Table 1). Small sample size is probably responsible for the Day 2-Day 4 figures being below the level of statistical significance. The graphic presentation (Fig. 1) depicts a damped oscillation returning to the norm. This type of

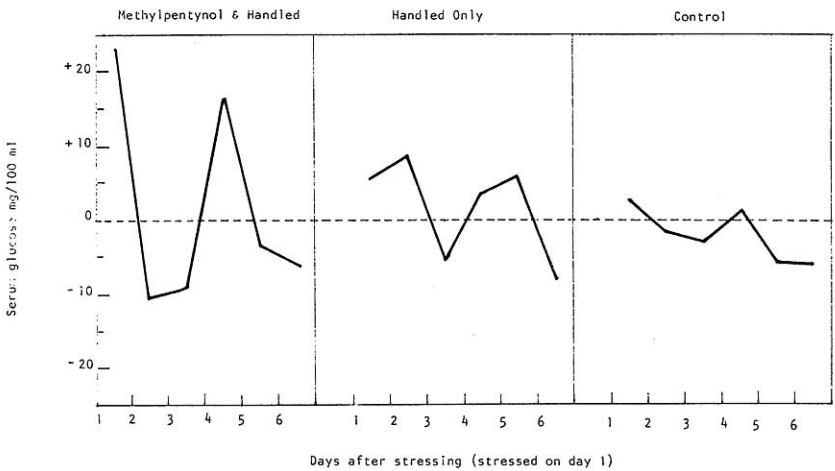

FIGURE 1. A comparison of serum glucose level from anesthetized and handled, and control groups before and after stress. The center line depicts means of three fish per group before stress application. The graph line connects serum glucose levels of the same three fish per group after stressing. Sampling on Day 1 occurred 14 hours after stressing.

oscillation may indicate a typical feedback situation in which physiological processes repeatedly overcorrect in an effort to return some parameter to the norm. Such an overcorrection or "hunting" occurs in man when serum glucose equilibrium is severely disturbed (Tepperman, 1968). The large initial increase in serum glucose may result from a number of factors. Several anesthetics such as ether (Hedner and Rerup, 1962) and MS-222 (Crowley and Berinati, 1972) are known to increase serum glucose levels. Methylpentynol may similarly cause a pharmacological increase in glucose titers apart from external stress stimuli. In contrast, behavioral changes; e.g., pre-anesthesia thrashing and lunging in goldfish (this study) and other species (Howland and Schoettger, 1969) indicate methylpentynol may in itself be a stress stimulus. Finally, there may be an additive or synergistic effect of methylpentynol and stress; i.e., either stress of capture and being

held out of water, or the stress of anesthesia induction. Whatever the cause, results indicate that methylpentynol is not a suitable anesthetic or tranquilizer for use in reducing a hyperglycemic reaction in stressed fish. It should not be used by investigators attempting to maintain fish at "normal physiological levels".

We were unable to duplicate Chavin's (1964) and Chavin and Young's (1970) results of significant increases in serum glucose for a period of days after merely transferring fish between identical aquaria. Neither the group which was handled but received no anesthetic (group II) nor control fish (group III) show significant differences between any individual day's serum glucose level and its "norm". These conflicting results are, however, no doubt reconcilable considering the numerous factors which affect serum glucose in fish (Chavin, 1964; Chavin and Young, 1970), a probable answer lying in differing physiological states of the experimental animals, e.g. at differing stages of the life cycle.

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