

The sensitivity of  $G_1$  to protein synthesis inhibition agrees with previous results<sup>8</sup> which showed that the initiation of the S period depends on previous protein synthesis. The lack of sensitivity of the early and middle S period to protein synthesis inhibition was not expected; although completion of DNA replication does not require concurrent protein synthesis, it was logical to assign a role in mitosis to protein(s) synthesized during this period. The present results suggest that proteins synthesized during this period are not strictly required to reach the next division. On the other hand, some protein(s) must be synthesized at the S/ $G_2$  transition in order to trigger the

next prophase, as shown by the great delay recorded after such treatments.

In conclusion,  $G_1$  and S/ $G_2$  boundaries appear as phases highly sensitive to protein synthesis inhibition, while most of S and  $G_2$  do not require protein synthesis for the normal timing in reaching the next prophase. Lastly, this technique is proposed as a simple method to analyze the cycle by specific metabolic inhibitors.

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### The dependence of heart rate and locomotor activity on water temperature in the carp (*Cyprinus carpio* L.)

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**Summary.** Long-term measurements of heart rate and locomotor activity in relatively free moving carps demonstrated dependence on water temperature. Under these conditions, the heart rate has a circadian and circannual rhythm.

As fish are poikilo-thermal animals, their body temperature responds to changes in the temperature of the environment, although little is known of the actual nature of their body temperature maintenance. The environmental temperature and all organic functions of poikilo-thermal animals show positive correlation<sup>2-4</sup>. Rueth<sup>5</sup> found a high positive correlation of heart-rate and body temperature with environmental temperature, in amphibians. Up to now these relations have hardly been investigated in the case of carps. Iriki et al.<sup>6</sup> carried out thermal stimulation of the spinal cord and thus heart-rate in some cyprinid fish. Albrecht<sup>7</sup> was able to show that temperature has an influence on certain physiological changes in inner organs. Little behavioural analysis of temperature-dependence has been carried out, although such analysis could clarify various ecological questions and problems in fishery research<sup>8-10</sup>. Field observations,

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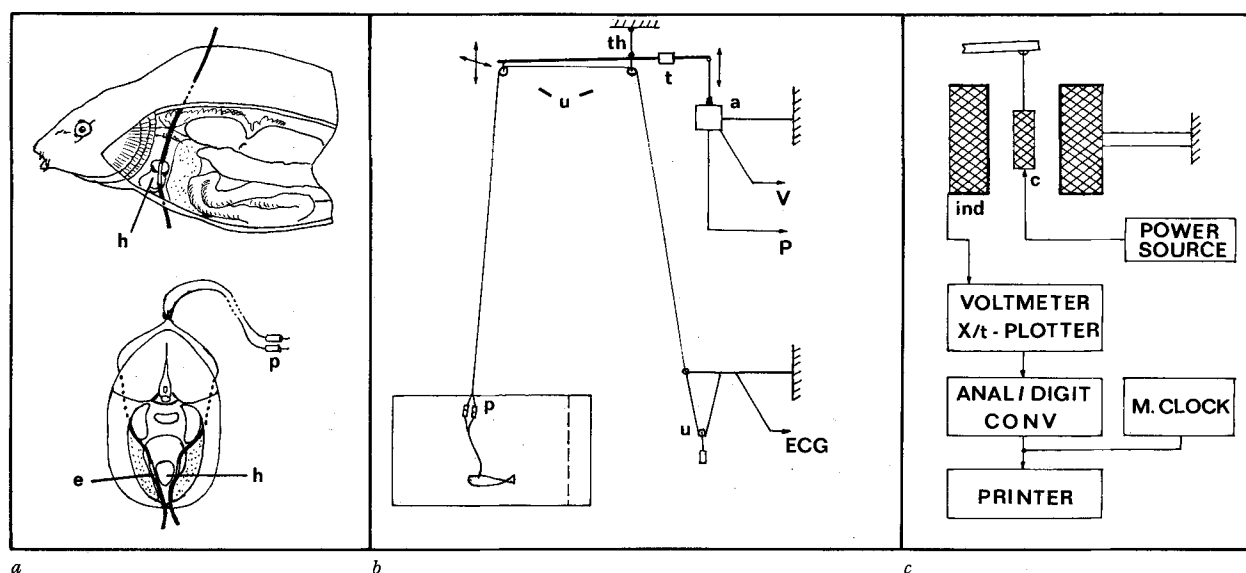


Fig. 1. a Arrangement of the electrodes in the fish: h, heart; e, electrodes. b Recording system: u, turn back rollers; a, activity receiver; th, thread suspension; l, lever; p, plug connection; v, voltmeter; P, power source. c Measuring and processing device for the locomotor activity (schematic); c, current coil; ind, induction coil.

for instance, have confirmed that fish prefer certain temperatures at different times of day<sup>11</sup>. Our previous results<sup>12,13</sup> have shown that heart-rate and locomotor activity show daily and seasonal variations, so that both parameters can be used for comparing investigations of behavioural programs in fish. Here we present the results of long-term measurements of locomotor activity and heart-rate of relatively free-moving fish under different temperature conditions.

**Materials and methods.** 1-year-old carps (12–15 cm length, 50–70 g weight) caught from fish ponds, were used for our experiments. All test animals were maintained in 25-l-aquaria at  $20^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  before and during the tests. The actual water temperature was recorded on a multichannel potentiometric instrument. Gradients in the water temperature did not occur because airing caused a circulation of the water within the aquaria. The oxygen concentration (determined according to Winkler) was practically constant at various temperatures.

For the recording of heart-rate during long-term tests, a stable position of the electrodes is required. Silver wire electrodes were therefore inserted into the fish (figure 1a) and connected to the recording system by plugs weighing 0.2 g. The tractive force of the recording wires (0.1 mm in diameter) was almost independent of the fish's position in the aquarium, due to an improved lever system (figure 1b). A shortening of the wires by twisting was compensated by means of turn-back rollers and adjusted weights; in this way the recording wires remained intact for at least 8 weeks. The ECG-impulses were fed into an ECG-recording device, an electronic impulse counter, and printer. The apparatus is described in more detail in<sup>12</sup>.

The hitherto used method for recording the swimming activity is based upon infrared-light-beams which measure the frequency of passages and which is, under certain conditions, proportional to the amount of activity. A new method has been developed for analogous recording of the

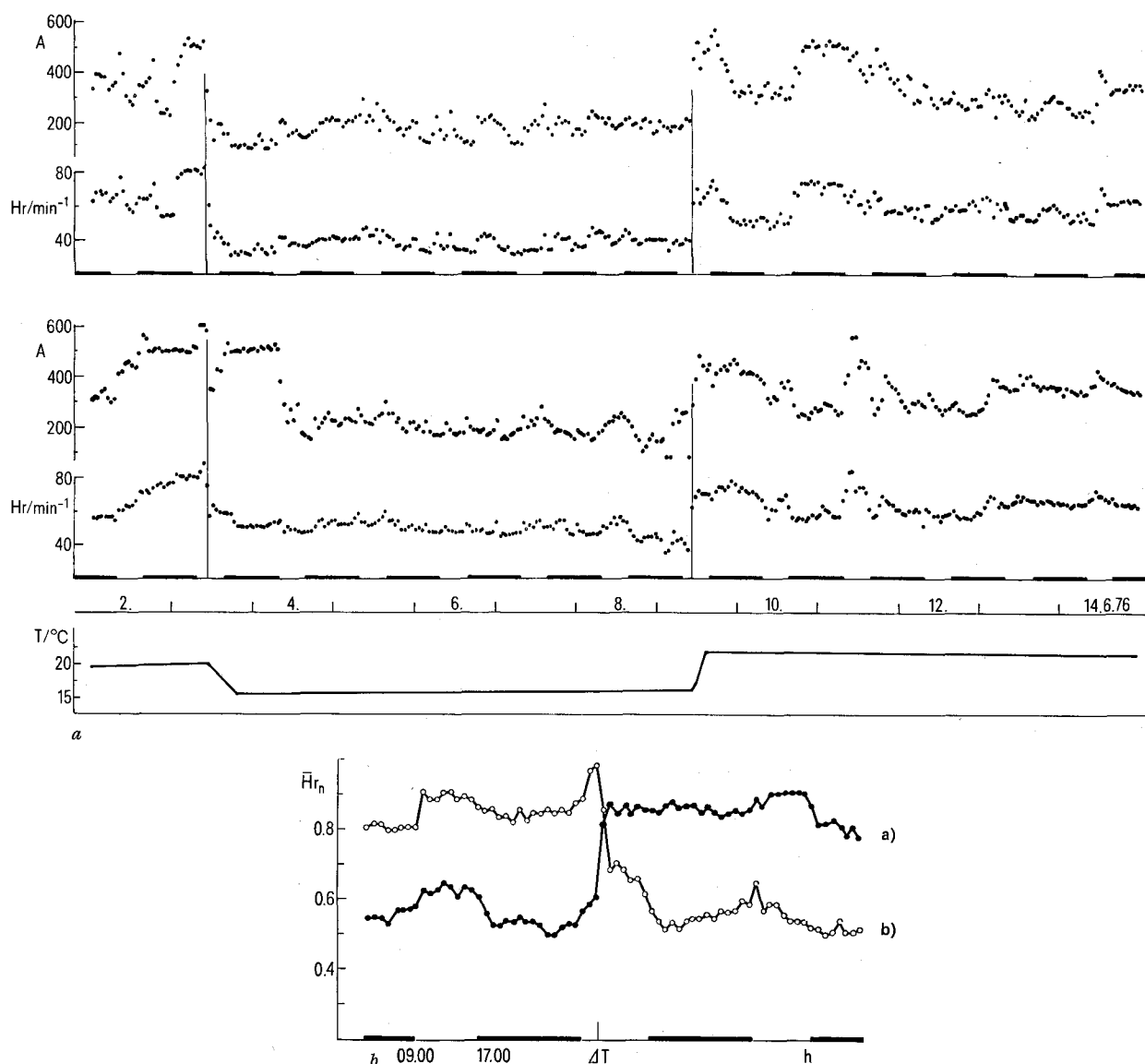


Fig. 2. *a* Correlation of heart-rate (Hr), locomotor activity (A), and water temperature (T) of 2 fish from 2 June to 14 June 1976. Light time 9.00–17.00 h. (Light: dark, 8:16; 55:0 lux.) *b* The dependence of standardized heart-rate on changes of water temperature ( $\Delta T$ ); *a*) from  $16^{\circ}\text{C}$  to  $21^{\circ}\text{C}$  ( $n = 6$  fish), *b*) from  $21^{\circ}\text{C}$  to  $16^{\circ}\text{C}$  ( $n = 6$  fish). Light time 9.00–17.00 h. (Light: dark, 8:16; 55:0 lux.)

locomotor activity which is based on the measurement of induction voltage of a coil (figure 1c). The sensitivity level of the recording device can be varied to such a degree that it is possible to detect fin movements in an otherwise motionless fish.

**Results.** Heart frequency and locomotor activity were mutually correlated to water temperature. By lowering the water temperature from 20°C to 15°C, heart frequency and locomotor activity decreased by about 30% and 50% (figure 2a) respectively. Both parameters reached their initial values after again raising the water temperature. There was a correlation of heart-rate and locomotor activity with  $r = 0.92$  (a) and  $r = 0.72$  (b) and a high significance of  $t_a = 41$  and  $t_b = 18$  for  $p < 0.001$ . Following changes of water temperature of  $+5^\circ\text{C}$  and  $-5^\circ\text{C}$ , respectively, we found a positive correlation of heart-rate and the modification of water

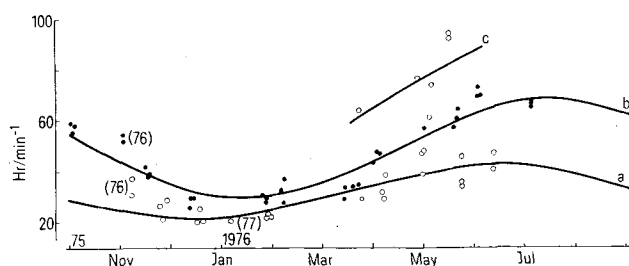


Fig. 3. Seasonal variations of the heart-rate's dependence on water temperature ( $n = 65$  fish). Each value is an arithmetic mean of 5–10 days. Computed curves  $a$   $\circ\circ\circ$  (15–17°C) and  $b$   $\bullet\bullet\bullet$  (20–22°C) fitted by  $f(t) = C_0 + C_{\cos}(\omega t + \alpha)$  with  $C_0 = 31.5$ ;  $C = 10.5$ ;  $\alpha = -195^\circ$  (a) and  $C_0 = 49$ ;  $C = 19$ ;  $\alpha = -225^\circ$  (b).  $c$   $\circ\circ\circ$  (25–27°C).

temperature. With initial temperature of 21°C and 16°C, there was a change of heart-rate of about 40% and vice versa (figure 2b). The SD are  $\bar{\sigma}_n(21-16^\circ\text{C}) = 0.0037$  and  $\bar{\sigma}_n(16-21^\circ\text{C}) = 0.0047$ . All tests show, despite changes of water temperature, the synchronizing effect of the light-dark-cycle.

In recent trials, this positive correlation of heart-rate with changes of water temperature at various time of day can also be found. Seasonal effects do not eliminate this correlation. The results computed and presented to date were obtained from the end of April to June only, in order to exclude seasonal influences. Most daily heart-rates show a maximum in summer and a minimum in winter, and these are distinct seasonal differences in this circadian periodicity<sup>12</sup>. In spite of constant photoperiod (12:12) and water temperature (15°C or 20°C), the heart-rate of *Cyprinus carpio* follows a circannual rhythm. The alteration of heart-rate during the experiments with different temperatures gives a good fit with a cosine function (figure 3). Raising temperature enlarges the amplitude of heart-rate cycle in the course of the year, whereas the maximum at 15–17°C lies in June and at 20–22°C in July. When the carp regularly consumed food during the feeding time, the heart frequency accelerated for about 30–120 min in every case. A circadian rhythm in swimming activity continued in spite of the different temperatures. These results correspond to the circadian locomotor activity recorded simultaneously during these experiments and in previous tests.

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## Transport of the synthetic peptide DSIP through the blood-brain barrier in rabbit<sup>1</sup>

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**Summary.** The synthetic delta sleep inducing peptide (DSIP) passes the blood-brain barrier, since i.v. injection in free moving rabbits (30 nmoles/kg) significantly increases the cortical delta activity and decreases the motor activity during 5 h.

It has been suggested that 'a lipid-insoluble molecule as large as the delta sleep inducing peptide DSIP (a nonapeptide with mol. wt 848.98) does not pass the blood-brain barrier by passive diffusion. A specific transport mechanism would be required and such a system would be unsuited to a substance whose function is to act on brain for long periods.'<sup>4</sup> In order to answer this question, we studied the EEG and behavioral effects of synthetic DSIP i.v. injected into free-moving rabbits. This paper complements the information obtained from intraventricular infusions of synthetic DSIP in the same animal<sup>5,6</sup>. **Methods.** 10 peptide tests (group P) and 9 control tests (group C) were carried out in 7 rabbits under double blind conditions. A dose of 30 nmoles/kg in 0.5 ml Ringer solution, or 0.5 ml Ringer alone as control, was injected within 1 min into the ear vein. Each experiment lasted 7 h (1 h pre-injection period and 6 h post-injection period).

a) **EEG-tests.** The rabbits were submitted to a 60-min pre-adaptation, without recording, on a kinesigraphic table placed in a sound-proof Faraday cage. The following pre-injection period, involving EEG recording, was subdivided into 2 periods of 30 min; the 1st (AP) allowing the

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