Effects of acute cold exposure and cold acclimatization on plasma glucagon, FFA and glucose concentrations

		Body weight (g) Initial	At experiment	Glucagon (pg/ml)	FFA (μEq/l)	Glucose (mg/dl)
I	Warm-acclimatized controls (10)	165 ± 1.9	233 ± 8.9	195 ± 12.6	484 ± 52.2	141.3 ± 2.29
	Cold-acclimatized rats (5 °C, 2 weeks) (8)	157 ± 5.2	169 ± 6.4[c]	328 ± 20.5[c]	911 ± 97.7[c]	153.3 ± 4.49[a]
	Acute cold exposure (6 min) (6)	—	207 ± 8.9	176 ± 19.3	516 ± 66.8	145.6 ± 7.12
	Acute cold exposure (60 min) (8)	—	215 ± 11.5	149 ± 15.4[b]	540 ± 69.5	134.2 ± 3.52
II	Warm-acclimatized controls (10)	232 ± 7.9	289 ± 5.2	153 ± 11.7	500 ± 34.3	128.4 ± 1.77
	Cold-acclimatized rats (5 °C, 2 weeks) (10)	240 ± 7.9	249 ± 13.1[b]	305 ± 18.3[c]	929 ± 75.9[c]	161.7 ± 4.13[c]
III	Warm-acclimatized controls (10)	165 ± 2.3	299 ± 3.8	203 ± 10.5	410 ± 33.4	122.0 ± 2.08
	Cold-acclimatized rats (5 °C, 4 weeks) (5)	165 ± 4.1	217 ± 18.0[c]	181 ± 15.5	443 ± 17.5	155.6 ± 4.16[c]

Mean ± SEM. Number in parenthesis indicates the number of animals. P vs warm-acclimatized control rats: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. Values with no marks indicate no significant difference vs warm-acclimatized controls.

The results were summarized in the Table. The increment of body weight was significantly smaller in rats exposed to cold for 2 or 4 weeks, possibly due to higher metabolic rate in the cold. Plasma glucagon concentration was markedly elevated in cold-acclimatized rats for 2 weeks. This finding was confirmed by 2 different series of experiments with younger and older rats as seen in the Table (I and II). However, these elevated glucagon levels were returned to the control values after cold acclimatization for 4 weeks. Such changes observed in plasma glucagon were also the case for plasma FFA; it was increased in cold-acclimatized rats for 2 weeks, while it was not different in those for 4 weeks from that in warm-acclimatized controls. Plasma glucose level remained elevated in cold-acclimatized rats for 2 to 4 weeks. Acute cold exposure did not appreciably affect the plasma concentrations of these three parameters. Further, there were highly significant correlations between plasma glucagon and plasma FFA, or glucose concentrations as a whole in warm-acclimatized and 2 weeks cold-acclimatized rats (Figures 1 and 2).

The present results would appear to indicate that plasma glucagon plays a role in the development of cold acclimatization through its lipolytic and glycogenolytic actions. It is of interest to notice that the blood corticosterone response to histamine stress increased rapidly with the time of cold exposure, reaching a maximum after 14 days in the cold, and then declined, reaching control levels after 28 days in the cold[11]. Consequently, the changes in the plasma glucagon in the cold are comparable to those in the adrenal function in the cold. However, the normalization of plasma glucagon level under continuing cold exposure would not eliminate the possibility of a role of this hormone in cold acclimatization, since cold acclimatization might induce an enhancement of lipolytic action of glucagon as described above[5].

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Heart Rate and Locomotor Activity in Fish: Correlation and Circadian and Circannual Differences in *Cyprinus carpio* L.

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Summary. Long-term measurements of locomotor activity and heart rate in relatively free moving carps demonstrated a correlation between the two parameters examined. Under laboratory conditions, both parameters exhibit a circadian and a circannual rhythm.

Recording of heart rate in fish is an usual method for investigating the effect of exogeneous acoustical, chemical or optical stimuli[1-3]. There is also some information on the correlation of heart rate and respiratory activity under constant and varied environmental conditions[4,5].

Hitherto, we know little about the relation of heart rate and locomotor activity in poicilothermes[4,6-9], and most investigations are based on short term measurements. Therefore we have performed continuous long term measurements of heart rhythm and locomotor activity with carp (*Cyprinus carpio* L.) under laboratory conditions.

Materials and methods. 2 short silver wires were inserted near the heart of anaesthetized carps aged one summer.

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These electrodes were connected to flexible insulated thin copper wires. The ends were dorsally and ventrally fixated to the fish body. The 2 dorsal wires were suspended vertically over the midpoint of the test aquarium (25 l). In our first experiments we used wires with a length of 50 cm in a loose suspension. In that way the fish could move freely, but sometimes were restricted by twisted wires. Therefore in recent investigations a lever system with wires 1 m long and 0.1 mm in diameter is used (Figure 1).

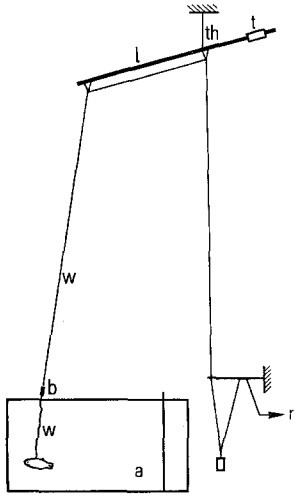

Fig. 1. Lever system used for registration of the electrocardiogram a, aquarium; b, banana plugs; l, lever; r, recording device; t, tare weight; th, thread suspension.

Here the tractive power of the wires for the fish is nearly independent of its position in the aquarium. Now the freedom of movement is better.

The ECG-impulses were fed into an ECG-recording device (3-NEK-116), which was connected to a threshold value regulator, an electronic impulse counter (VA-G-120), and a printer (VA-G-24 A), which printed the number of heart beats every 10 min. The locomotor activity was recorded by an IR-light beam, the number of interruptions of the beam being printed in intervals of 1 h. The tests were carried out in a soundproof Faraday chamber. The animals were exposed to an artifical lightening regime of 8 h light and 16 h darkness (kLD 8:16; 55:0 lux). The temperature of the ventilated test aquaria was held constant at 20 $\pm$ 1 °C.

Directly following the operation the fish were placed in the test chamber. Within 4 days the heart rate reached its normal level and the fish began eating again. Then the heart rate and swimming activity of each animal were continuously recorded for at least 6 days. The fish were disturbed for feeding and technical control only.

For the analysis of the heart rate rhythms, the differences between the hourly means (Hr) and the mean of the 8-hour-light interval ($\overline{Hr}^L$) were related to the value of the hour with the highest value in each 24-hour-period (Hr_{max}). This was necessary to eliminate individual and seasonal variations and to obtain clearer results. For the same reason, the amount of locomotor activity per hour (A) was related to the value of the hour with the greatest degree of locomotion (A_{max}). The results are given on percentage of the daily amount of locomotion.

Results. Figure 2a presents the standardized heart rate and locomotor activity rate of 4 different animals, examined during the months October 1973, January,

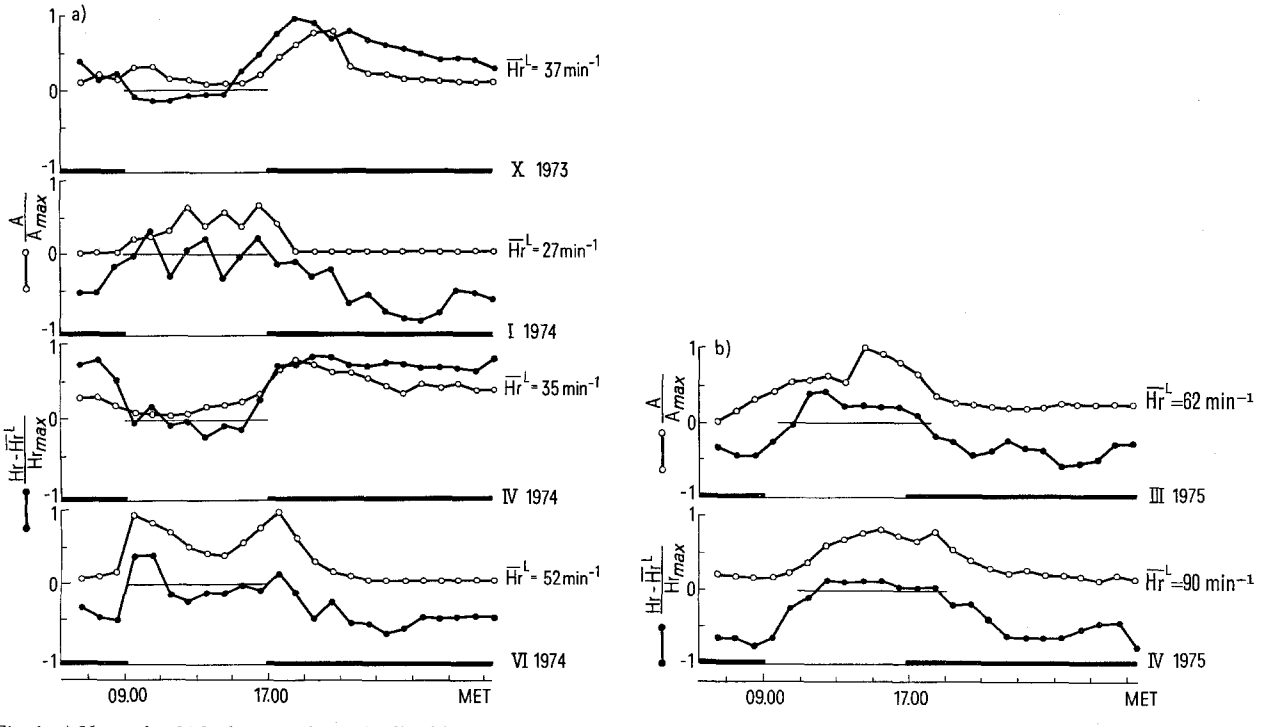

Fig. 2. a) Means for 24-h changes of standardized heart rate (●——●) and swimming activity (○——○) of 4 carps investigated at different seasons. I, 4–8 January 1974 (5 days); IV, 2–6 April 1974 (5 days); VI, 12–19 June 1974 (8 days); X, 24–29 October 1973 (6 days). Note also the seasonal variation of mean heart rate in the light phase (Hr^L) lasting from 09.00 to 17.00 MET.

b) Means for 24-h changes of standardized heart rate (●——●) and swimming activity (○——○) of 6 carps. III, mean for 3 single carps, 6–13 March 1975 (8 days); VI, mean for 3 single carps, 22–27 June 1975 (6 days). These fish were from another source and had a higher heart rate generally.

April, and June 1974. Each point of this figure represents at least 30 single data for heart rate and at least 5 single data for activity. Correlation of heart rate and activity rate. A clear connection between the two parameters can be seen (Figure 2a). High locomotor activity is mostly accompanied by a corresponding increase in heart rate. The coefficients of rank correlation (Spearman) are 0.46 for X 1973, 0,83 for I 1974, and 0.77 for IV and VI 1974. These data are significant (t-test, Student) with $\alpha < 0.1\%$. More recent simultaneous experiments with 3 single individuals gave the same results (Figure 2b). The correlation coefficients are 0.71 for III, and 0.83 for VI 1975 ($\alpha < 0.1\%$). We expect that the degree of correlation is a function of the sensitivity of the activity measuring system[10].

Circadian and circannual differences. All tests clearly show the synchronizing effect of the light-dark-cycle. The most pronounced changes of both parameters are to be seen after light-on and light-off.

There are clear seasonal differences in this circadian periodicity (Figures 2, a and b). Twice a year, in winter (I 1974, III 1975) and in summer (VI 1974 and 1975), the mean values of both swimming activity and heart rate are higher during light-time than during darkness. In spring (IV 1974) and autumn (X 1974) this relation is reversed. Thus a seasonal fluctuation of the daily mean heart rate under constant laboratory conditions can be seen. The daily mean heart rate shows a maximum in summer and a minimum in winter.

A multiple phase shift of locomotor activity is reported for some fish species (*Cottus poecilopus*[11], *Salmo trutta*[12]). Our results give a hint that this phenomenon could occur also in carp.

Regular daily changes of heart rate are known from experiments with *Lampetra fluviatilis*[4] and *Salmo gairdneri*[13]. CLARIDGE et al.[4] found a connection between heart rate and locomotion in relatively restrained *Lampetra*, but pointed out that the connection between breathing rate and locomotor activity is a closer one. Short term measurements in *Salmo gairdneri*[7,8] demonstrated that fish, swimming at varied velocities, show large changes of cardiac output. This results in a slight increase of heart rate and a large increase of stroke volume.

Our results, which demonstrate a close relation between locomotor activity and heart rate, complete these findings. So the heart rate of fish seems to be a more favourable and more sensitive parameter in recording the individual state than some visible behavioural patterns.

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Increased Erythrocyte Permeability to Li and Na in the Spontaneously Hypertensive Rat

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Summary. Red blood cells incubated in a physiological medium in which Li replaces Na (LiPSS) gain Li in exchange for Na and K. The rate of Li uptake is modestly but significantly increased in the spontaneously hypertensive rat (SHR) at 37 °C and at 22 °C. The slow rate of Na gain and K loss during cooling at 2 °C was about doubled in unmodified whole blood samples from the SHR.

Recent observations on ion distribution in experimental hypertension have provided new support for our general view that cell Na regulation plays a central role both in acute vasoconstriction and in the structural redesign of blood vessels characteristic of sustained hypertension[2,3]. We thus consider the bi-directional passive permeability of the vascular smooth muscle cell membrane, together with the transport protein available for actively sustaining the transmembrane Na gradient to be a totality ('net Na pumping activity'). Our present working hypothesis is that any derangement in this totality favouring accumulation of cell Na will, if sustained, be interpreted by the cell as an increase in its work load and a stimulus to increase its work capacity. It will respond by manufacturing new structural and transport protein which will in effect tend to mask the original defect.

It might be expected that in some forms of hypertension the original defect would be sufficiently generalized to affect all or most cell membranes and so be particularly apparent in the erythrocyte, which lacks the ability to synthesize new protein. An increase in red blood cell Na in hypertension in man has recently been reported[4] and BEN-ISHAY et al.[5] have noted enhanced Na efflux in erythrocytes from rats with one form of genetic hypertension. We have approached the problem by looking at the passive permeability of the membrane, using simple indicators which could then be applied clinically if warranted. Analytic rather than tracer methods were chosen for simplicity and movements of Li were compared with Na since passive processes should affect both ions and the larger hydrated size of Li would amplify marginal changes[6].

Materials and methods. Male rats with spontaneous hypertension (SHR-Carworth) and their matching controls (CFN) were used throughout. In each experiment, the animals were lightly anaesthetized with ether, blood

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