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 °C and − 5 °C, respectively, we found a positive correlation of heart-rate and the modification of water 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 °C) = 0.0037 and $\bar{\sigma}_n$ (16–21 °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 eleminate 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[12]. 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.

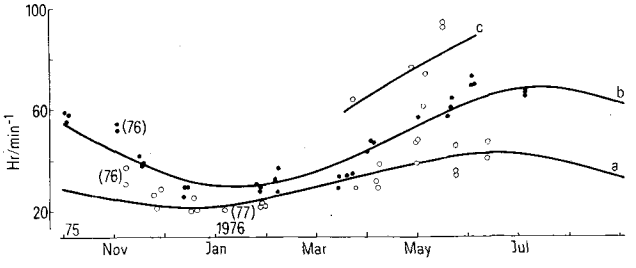

Fig. 3. Seasonal variations of the heart-rate's dependence on water temperature (n = 65 fish). Each value is a arithmetic mean of 5–10 days. Computed curves *a* ○○○ (15–17 °C) and *b*●●● (20–22 °C) fitted by $f(t) = C_o + C_{cos}(\omega t + \alpha)$ with $C_o = 31{,}5$; $C = 10{,}5$; $\alpha = -195°$ (*a*) and $C_o = 49$; $C = 19$; $\alpha = -225°$ (*b*). *c* ○○○ (25–27 °C).

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Transport of the synthetic peptide DSIP through the blood-brain barrier in rabbit[1]

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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.[4]' 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[5,6].

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

1 Acknowledgment. This work was supported by grants from the Swiss National Science Foundation (Nos 3.871–72, 3.525.75, 3.443.074, 3.780.076), Fonds für Lehre und Forschung der Universität Basel, Merck Sharp and Dohme (USA), Ciba-Stiftung Basel.
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rabbit to readapt after connecting the leads with the chronic implanted electrodes, the 2nd used for reference (RP). The EEGs of the frontal and limbic cortex were recorded by means of chronically implanted electrodes and an electro-encephalograph of Schwarzer, as described previously[7,8]. The delta rhythms (2–3 Hz) were continuously quantified by an automatic analog wave analyzer (Faraday Electronic Instr. Ltd.), applying the conventionally amplified signals simultaneously to a set of tuned frequency selected circuits. Their outputs were integrated over epochs of 10 sec and averaged every 50 sec; the values obtained were summed every 5th min ($6 \times 50 = 300$ sec = 5 min). The total recording time was divided into 7 periods of 1 h; for each of them, the mean of 12×5 min delta activity was calculated. As reference level, we chose the 5-min delta value of the 10 peptide and 9 control tests during the 30 min preceding the injection. As shown in the figure, A, this value integrated by the wave analyzer was taken as base-line (97 mm = 100%). After the injection, the 5-min mean delta activity of each h was referred both in the peptide (P) and control (C) groups to the pre-injection reference value and expressed in percent. Finally, the difference between the hourly mean delta values of the P and C groups was indicated in % on each h column, together with the significance (t-test).

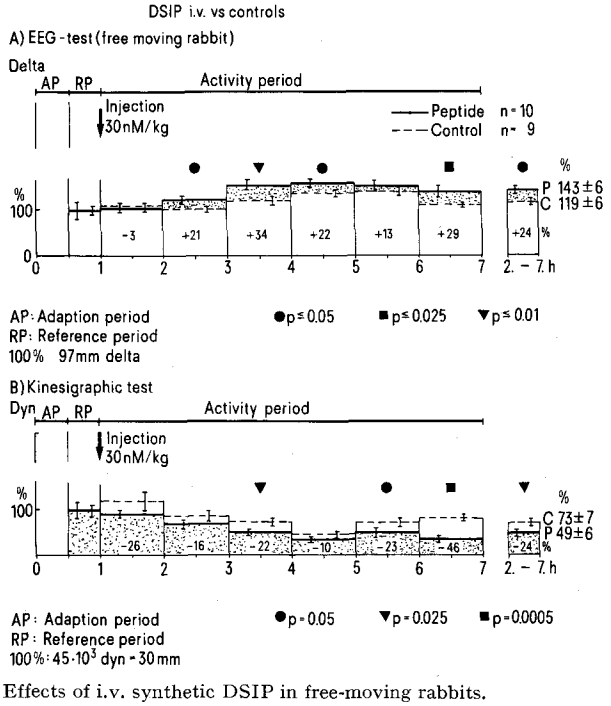
b) *Kinesigraphic behavioral tests.* They were carried out simultaneously with the EEG-tests. The rabbit, supplied with food and water, was placed on the movable detecting plate of a kinesigraph (Electronic activity monitor) previously described[7]. It could move freely, eat and drink ad libitum during the experiment. An electromagnetic system transformed the movements into voltage changes, which were electrographically recorded. As in the EEG tests, the deflexions of the kinesigraph were measured in mm and summed every 5 min. The resulting values were expressed in % of the base-line (30 mm = 100%) and plotted on the ordinate. For a calibration impulse of 15 mm and an amplification of 12%, every mm value corresponded to 1517 dynes. For each of the 6 h activity period, the mean of 12×5 min motor activity was calculated in mm and transformed in dyn. %.

Results. a) *EEG-activity.* The diagrams of the figure show that the delta activity started to increase significantly 1 h after injection of DSIP compared to controls (difference between P and C = + 21%; $p \leqslant 0.05$). The further difference between groups culminated during the 3rd h after injection (+ 34%; $p \leqslant 0.01$). This increased delta activity remained high during the following 4th h, as regards the delta percentage referred to the initial level 100. The P level slightly decreased 5–6 h after injection, but the difference between P and C still remained significant after 6 h: + 29% ($p \leqslant 0.025$). The average delta activity between 2 and 7 h was 143 ± 6% in the P group against 119 ± 6% in the C group. The difference (+ 24%; $p \leqslant 0.05$) expressed the total delta increase.

The proportion of waking and sleep episodes, including the chief sleep states, was determined by scoring the 4th h sample after injection of DSIP. The comparison of the values in the P group and control group confirmed that DSIP chiefly enhances in rabbit the percentage of delta sleep, as well as the total sleep (38% delta + 7% spindles +6% REM = 51%) and reciprocally decreases the waking episodes (41%). In the control group, a reversed proportion was obtained: 40% total sleep; 59% waking state. The difference between sleep and waking episodes reached 18% in this scoring sample.

b) *Motor activity.* The activity quantified with the kinesigraph was diagrammatically represented as was the EEG delta activity (figure, B). The reference level, obtained from the half-hour before injection, was 100% = 30 mm = 45.10^3 dyn. During the activity period, the kinesigraphic curves decreased in both P and C groups, referred to the initial level. However, between the end of the 1st h and $4^1/_2$ h after injection, the decrease was always stronger in the P group, with lowest level in the 4th h. The significance of the difference between P and C groups was $p \leqslant 0.0025$ in the 3rd, and $p \leqslant 0.0005$ in the 6th h. The average motor activity decrease over the 2–7 h activity period, referred to the initial level, amounted to 49 ± 6% in the P group, against 73 ± 7% in the C group. The difference reached – 24% ($p \leqslant 0.025$).

Discussion. I.v. synthetic DSIP enhances in rabbits the delta + spindle EEG-activity (+ 24%) without reducing REM episodes. It concurrently decreases the motor activity (–24%). These EEG and behavioral slow-wave sleep effects start, in the i.v. test, $^1/_2$–1 h after injection and remain significant over 5 h. These observations complement the data from the intraventricular infusion tests restricted to $1^1/_2$ h[5,6] and suggest that DSIP, in spite of its mol. wt (~ 849), may pass the blood-brain barrier. This invalidates Pappenheimer's assumption[4] that DSIP cannot pass the barrier either by passive diffusion, because of its large molecule, or even by a specific blood-brain carrier system. Other sleep-promoting factors exhibit comparable effects when administered intraventricularly or i.p.[4,9,10]; their i.v. effects, however, have not yet been reported.



Effects of i.v. synthetic DSIP in free-moving rabbits.
A EEG test. The injection of 30 nmoles/kg in 0.5 ml induces a progressive EEG delta increase starting 1 h, reaching a maximum 3 h (+34%; $p \leqslant 0.01$) and still detectable 6 h after injection. Total delta increase +24%.
B Kinesigraphic test. The same injection is followed by a progressive decrease of motor activity, with a minimum 4 h and a still detectable effect ($p \leqslant 0.005$) 6 h after injection. Total decrease –24%.

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