sis to hydroxyproline in the fibrous proteins were carried out by the method of Fischer and Llaurado[6]. These samples, containing hydroxyproline, were adjusted to pH 6-7 and hydroxyproline was estimated by the method of Woessner[7]. The hydroxyproline content thus obtained was multiplied by a factor of 7.46 for collagen estimation and by a factor of 43.4 for elastin determination[8]. These estimated values for collagen and elastin were expressed in terms of percentages of the dry defatted weight of the aorta. The statistical significance of the differences between the 2 groups (C and R) was determined by Student's t-test.

The results are summarized in the table. Percentages of the dry defatted weight represented by these components varied in different areas of the aorta. With respect to sedentary rats, the percentage of collagen was 28.6% in the thoracic aorta and 42.2% in the abdominal aorta, while that of elastin amounted to 54.9% in the thoracic aorta and 38.1% in the abdominal aorta. These control values for collagen and elastin were comparable with those found in the ascending and the abdominal aorta in mongrel dogs, as reported by Fischer and Llaurado[6]. After forced running, the estimated values for collagen were unchanged in the thoracic aorta, while the amount of this fibrous protein in the abdominal aorta decreased by 5.2% of the control ($p < 0.05$). In this case, elastin in the thoracic and abdominal aorta was also reduced by 4-8% ($p < 0.05$).

Reduction of collagen in the abdominal aorta indicates that the distal area of the aorta is more distensible in the trained animals, at a high transmural pressure in the aortic region, because collagen is stiff and resists further stretch. We also noted a decrease in elastin in the thoracic and the abdominal aorta after training. As elastin, unlike collagen, stretches freely to accomodate a given blood pressure, free energy developed in this elastic component in the process of the aortic expansion is probably released during diastole in order to facilitate blood flow against the resistance of circulation. Accordingly, the decrease in the aortic elastin may indicate that the diastolic flow in the trained animals cannot be enhanced during exercise, unless the peripheral vasoconstriction is less pronounced.

In studies on trained rats, Harri[9] found an increased sensitivity of $beta_2$-adrenoceptors and/or decreased sensitivity of alpha-adrenoceptors in the peripheral vessels. This would suggest that in the trained animals, sympathetic activation during exercise augments vasodilation and accelerates the diastolic flow with less free energy expenditure of the aortic elastin. It has been shown for rabbits that the circulation in the capillary beds of skeletal muscle increased as a result of training[10], thereby suggesting an elevated vasodilation of arterioles in the muscle during exercise. Moreover, resting diastolic pressure in healthy men was reduced after training, and was ascribed to a decrease in the peripheral vascular resistance[11].

In our experiments, we detected no increase in the aortic fibrous proteins after exercise, this being in contrast to the overproduction of collagen and/or elastin induced by epinephrine and thyroxine[2], by hypoxia[3] and by hypertension[4,5].

Bassler[12] speculated that marathon runners may have an immunity to fatal atherosclerosis, on the bases of overproduction of collagen and of accumulation of lipid materials. As described above, we used only female rats in the running exercise. Estrogen is a known antiatherosclerotic agent, as explained by Kishi and Numano[13] and suppresses the reduction of cyclic AMP levels in blood vessels and inhibits vascular permeability to protect the walls from lipid intrusion. Thus, estrogen must be considered in evaluating such protective effects of exercise. However, as trained female subjects often have irregular menstruation[14], the question arises as to whether estrogen activity would be stimulated by physical training. When taking into account overproduction of collagen (collagen α B chain)[15] in an early stage of atherosclerotic plaques and considering a reduction of collagen in the distal area of the aorta in the trained rats, daily running for a long period seems to inhibit the proliferation of this stiff component in the arteries. Further studies are warranted on the effect of physical training with regard to metabolism of lipid and connective tissue, including collagen and elastin, in peripheral blood vessels.

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2 Langner, R.O., and Fuller, G.C., Biochem. biphys. Res. Commun. *36* (1969) 559.
3 Crossley, H.L., Johnson, A.R., Mauger, K.K., Wood, N.L., and Fuller, G.C., Life Sci. *11* (1972) 869.
4 Ooshima, A., Fuller, G.C., Cardinale, G.J., Spector, S., and Udenfriend, S., Proc. natl Acad. Sci. *71* (1974) 3019.
5 Wolinsky, H., Circulation Res. *26* (1970) 507.
6 Fischer, G.M., and Llaurado, J.G., Circulation Res. *19* (1966) 394.
7 Woessner, Jr, J.F., Archs Biochem. Biophys. *93* (1961) 440.
8 Neuman, R.E., and Logan, M.A., J. biol. Chem. *186* (1950) 549.
9 Harri, M.N.E., Eur. J. appl. Physiol. *42* (1979) 151.
10 Vannotti, A., and Pfister, H. Arbeitsphysiologie *7* (1933) 127.
11 Hanson, J.S., and Nedde, W.H., Circulation Res. *26-27* (1970) suppl. 1, 1-49.
12 Bassler, T.J., Ann. N.Y. Acad. Sci. *301* (1977) 579.
13 Kishi, Y., and Numano, F., Mech. Aging Dev. *18* (1982) 115.
14 Iwata, M., J. Am. med. Ass. *101* (1933) 723.
15 Ooshima, A., Science *213* (1981) 666.

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Strain differences in responses of the circadian system to light in the Syrian hamster[1]

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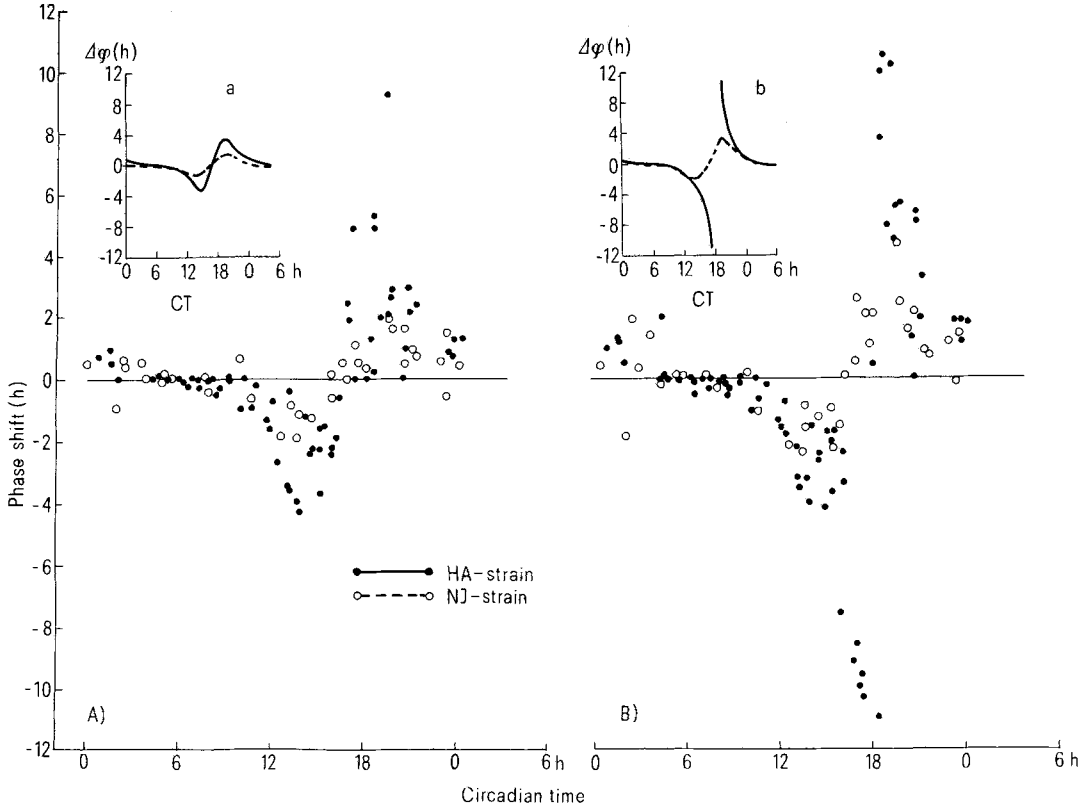
Summary. The circadian systems of 2 strains of the Syrian hamster responded differently to single short light pulses. The differences in the amplitudes of the phase response curves were associated with different ranges of entrainment of the circadian rhythms to periodic light pulses.

The Syrian hamster *(Mesocricetus auratus)* has been one of the most extensively used mammalian species in the research of daily (circadian) and annual periodicities[2-8]. Valuable models have been proposed that describe ex-



Phase responses of activity rhythms of 2 strains of male Syrian hamsters to 1-h light pulses (ca. 150 lx). Background illumination was 0.02–0.05 lx. *A* Immediate responses; *B* steady-state responses. Positive phase shifts ($+\Delta\varphi$) = advances, negative phase shifts ($-\Delta\varphi$) = delays. Points represent single measurements. The phase response curves shown as inserts (a, b) are eye-fitted. The mean freerunning periods (τ) of hamsters of both strains were close to 24 h. Circadian time (CT) 12.00 h = activity onset.

perimental results with regard to entrainment mechanisms[5] and the involvement of the circadian system in the control of the annual reproductive cycle[7,8].

By measuring phase response curves (PRC) of the circadian system of male Syrian hamsters to single light pulses, it was recently found that hamsters bred in our laboratory showed responses that were markedly different from those previously reported[2,4,5,9]. To confirm this result, hamsters of 2 strains were tested under the same experimental conditions: one which originated in the 'Zentralinstitut für Versuchstierzucht' in Hannover, FRG, (HA-strain), the other one, in the 'Lakeview Animal Farm' in Newfield, New Jersey (NJ-strain)[10]. 9 animals of each strain were exposed to constant low light intensity (0.02–0.05 lx). Single 1-h light pulses (ca. 150 lx) were given at different phases of the circadian rhythm of running activity in 2–3-week intervals. Activity was measured by conventional methods and PRC's were determined according to Daan and Pittendrigh[5]. On the left diagram of the figure (A), phase shifts of activity onset measured on the 1st day (period) after the pulse (immediate response) are shown; on the right diagram (B), phase shifts measured between steady-state conditions before and after the pulse (steady-state response) are demonstrated for the same individuals. It is obvious from the data points and from the eye-fitted mean PRC's, shown as inserts in the figure (a, b), that the immediate and steady-state maximal advance and delay shifts of the 2 hamster strains differ significantly: they are more than twice as large in hamsters of the HA-strain as in hamsters of the NJ-strain.

From comparative functional analyses of the effects of short light pulses on the circadian systems of various mammalian species, in particular nocturnal rodents, it was expected that species or individuals showing different phase-dependent responses of their circadian rhythms to light pulses should also have different ranges of entrainment to the respective light stimuli when given periodically as Zeitgebers with different periods (T)[5,11]. Testing the range of entrainment of the rhythms to periodic 1-h light pulses with different T's in hamsters of the HA-strain revealed that the animals can entrain to T's ranging from about 22 to 26 h. This range was more than twice as large as that reported by Elliott[4] using hamsters of the NJ-strain (between 23.25 and 24.75 h). A similar short range of entrainment has been found for 15-min light pulses as Zeitgebers in NJ-hamsters studied by Daan and Pittendrigh[5]. It is worth noting that the 2 observed ranges of entrainment for periodic 1-h light pulses were directly proportional to the mean 'ranges' of the PRC's (maximal advances plus maximal delays) for immediate responses in the 2 strains.

The differences in PRC-amplitude and related functions between the 2 strains of the Syrian hamster have several implications: 1. They provide a tool for testing the entrainment model proposed for nocturnal rodents[5] as well as an experimental basis for further insight into functional mechanisms of circadian systems. 2. They claim extreme caution in interpreting conflicting results obtained by investigators using different strains of hamsters. Further experiments have revealed that the differences in responsiveness to light are not genetically determined but apparently due to specific conditions during breeding and maintenance over many generations (manuscript in preparation).

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2 DeCoursey, P.J., J. cell. comp. Physiol. *63* (1964) 189.
3 Aschoff, J., Figala, J., and Pöppel, E., J. comp. physiol. Psychol. *85* (1973) 20.
4 Elliott, J.A., Thesis, University of Texas, Austin 1974.
5 Daan, S., and Pittendrigh, C.S., (see also: Pittendrigh, C.S., and Daan, S.), J. comp. Physiol. *106* (1976) 223, 253, 267, 291 and 333.
6 Rusak, B., J. comp. Physiol. *118* (1977) 145.
7 Elliott, J.A., Fedn Proc. *35* (1976) 2339.
8 Reiter, R.J., Chronobiologia *1* (1974) 365.
9 Takahashi, J.S., personal communication.
10 The author is very grateful to Dr M. Menaker and J.S. Takahashi (University of Oregon, Eugene) for shipping hamsters of the NJ-strain to our laboratory and for scientific encouragement.
11 Aschoff, J., and Pohl, H., Naturwissenschaften *65* (1978) 80.

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New low-molecular inhibitors of pancreatic elastase with possible in vivo application: Alkylamides of N-acylated tripeptides

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Summary. Out of a series of alkylamides of N-acylated tripeptides, Glt-$(Ala)_2$-Pro-NH-Et and Glt-$(Ala)_3$-NH-Pr were found to be potent inhibitors of porcine and human pancreatic elastase, and because they are free of toxic groups they might be considered for in vivo application.

Hassall et al.[1] introduced alkylamides of N-alkyl- and N-cycloalkanoyl-dipeptides as novel pancreatic elastase inhibitors with potential for action in vivo. Their design does not, however, take into account some new data on elastase-substrate interaction. In previous reports[2-4] we established the prerequisites for a synthetic substrate for pancreatic elastase. This knowledge was used in the synthesis of alkylamides of N-acylated tripeptides. Their inhibitory effect on pancreatic elastase of various origins is reported.

Materials and methods. Inhibitors. The alkylamide residue at the P_1-position (nomenclature of Schechter and Berger[5]) was -ethylamide (NH-Et) or -propylamide (NH-Pr). The acyl residues at the P_5-position included acetyl- (Ac), maleyl- (Mal), succinyl- (Suc) and glutaryl- (Glt). The amino acid sequence at the P_4-P_3-P_2 position was alanine-alanine-alanine (Ala_3) or alanine-alanine-proline (Ala_2-Pro). The synthesis has been described elsewhere[6]. The series of inhibitors is surveyed in the table.

Substrates. Succinyl-tetraalanine-4-nitroanilide (Suc-Ala_4-NAn) and glutaryl-tetraalanine-4-nitroanilide (Glt-Ala_4-NAn), synthesized according to our earlier method[3] were used.

Enzyme. 1. Porcine pancreatic elastase (E 1) Serva (cat. No.20929) was dissolved in 1 mM AcOH. The enzyme concentration was 15.5 nM. 2. Human duodenal contents (pH 7.2-7.5) were aspirated separately from gastric juice after cholecystokinin stimulation. Elastase (E 2) was isolated by chromatography on a DEAE-Sephadex A-50 column[7]. 3. Human pancreatic juice after secretin-cholecystokinin stimulation was obtained by cannulation of the main pancreatic duct (E 3). The lyophilized sample was dissolved in 0.1 M Tris buffer pH 8.2 with Ca^{2+}-ions (5 mM). Zymogens were activated with trypsin (0.3 mM).

Assay of elastase inhibition. The incubation medium consisted of 0.1 ml substrate solution (in dimethylsulphoxide), 1.3 ml 0.1 M Tris buffer pH 8.0, 0.05 ml inhibitor in Tris buffer, and 0.05 ml elastase solution. The enzymic activity was measured at 25 °C by monitoring continuously the split-off 4-nitroaniline (410 nm). K_m-values of Suc-$(Ala)_4$-Nan and Glt-$(Ala)_4$-NAn were estimated at 3 substrate concentrations (0.3125-0.625-1.25 mM) by a Lineweaver-Burk plot. Inhibition constants (K_i) were determined at 2 inhibitor concentrations using a Lineweaver-Burk or Dixon plot (for E 1) and at 1 inhibitor concentration using the intercept with 1/V which was determined by a Lineweaver-Burk plot (for E 2 and E 3).

Results and discussion. K_m-values of Suc-$(Ala)_4$-NAn and Glt-$(Ala)_4$-NAn amounted to 0.356 mM and 0.314 mM respectively. The inhibition constants (K_i) of ethyl- and propylamides of acylated trialanine- and dialanine-proline peptides are summarized in the table. These substances are distinctly more potent inhibitors than the alkylamides of N-alkyl or N-cycloalkanyol-dipeptides introduced by Hassall et al.[1]. In contrast to these authors, we found that the inhibitory effect is further increased, if ethylamide at P_1 is replaced by propylamide. This finding supports our earlier opinion[3], that a substrate with α-aminobutyric acid at the P_1-position may be very suitable for pancreatic elastase. Proline at the P_2-position is superior to alanine both in substrates[4,8] and inhibitors. From the various acyl residues at P_5-position, the presence of an anionic residue of dicarboxylic acid, particularly glutaryl-, appears to be most favorable. These findings hold true for porcine as well as

Inhibition constants (K_i) of ethyl- and propylamides of N-acylated trialanine and dialanine-proline-peptides

Inhibitor	K_i (μM) Suc-$(Ala)_4$-NAn			Glt-$(Ala)_4$-NAn		
	E1	E2	E3	E1	E2	E3
Ac-$(Ala)_3$-NH-Et	19	238	69	21	123	117
Mal-$(Ala)_3$-NH-Et	35	87	69	30	73	117
Suc-$(Ala)_3$-NH-Et	20	76	29	29	89	47
Glt-$(Ala)_3$-NH-Et	8.8	15	7.4	8.4	19	19
Suc-$(Ala)_2$-Pro-NH-Et	6.0	14	23	4.0	19	17
Glt-$(Ala)_2$-Pro-NH-Et	1.6	10	2.9	2.0	14	3.8
Suc-$(Ala)_3$-NH-Pr	6.8	12	13	4.5	21	14
Glt-$(Ala)_3$-NH-Pr	2.5	11	3.8	2.5	7.2	4.5

Source of enzyme: E1, porcine pancreatic elastase; E2, elastase of human duodenal contents after cholecystokinin stimulation; E3, elastase of human pancreatic juice cannulated via the main pancreatic duct (stimulation: secretin-cholecystokinin). Substrates: Suc-$(Ala)_4$-NAn and Glt-$(Ala)_4$-NAn.