18–19-day-old age. The similar but stronger development is to be seen in figure 1, *B*, where the maximum responses to NA of 1–2- and 8–9-day-old rats were only 20–30% from those of 18–19-days-old and the adult rats ($p < 0.001$). Responses to an alpha-adrenergic drug, PHE (figure 1, *C*), show that the sensitivity of alpha-receptors developed to the adult stage later than those of beta-receptors, the 18–19-day-old hearts still being subsensitive to PHE but not to ISO and NA.

The dose-response curves to cholinergic drugs, ACh and CCh, indicated (figure 2) that the cholinergic chronotropic sensitivity did not change significantly after birth. The responses at the different concentrations did not differ significantly between the age groups.

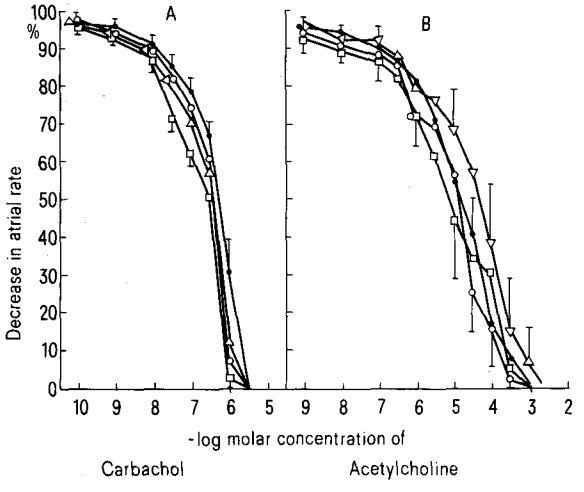

Fig. 2. Log concentration-response curves for the negative chronotropic responses to carbachol (*A*) and acetylcholine (*B*) in the isolated atria from 1–2-day-old –●–, 8–9-day-old –○–, 18–19-day-old –△– and the adult rats –□–. The results are expressed as the percentage decrease from the basic contraction frequencies. The curves are the means ± SE, n=6–8 rats.

Discussion. The present results indicated that the atrial chronotropic sensitivity to adrenergic drugs was lower in the newborn than in the adult rats. The postnatal development of adrenergic sensitivity in the atria observed in this study is well correlated to the development of the adrenergic innervation in the heart[1–3], and the development of neural uptake of H^3-NA[4–6]. Adrenergic innervation[1–3], NA uptake[4,5] and NA concentration of heart[5] increase rapidly during the 3 weeks after birth. Also the chronotropic sensitivity to ISO and NA were already developed to the adult level within 19 days, but the sensitivity to the alpha-receptor agonist, PHE, developed later. In the rat portal vein, it has been shown[10] that the sensitivity to exogenous NA increases during the first 3 weeks. The resting heart rate increases from 300 to 500 beats/min during 20 days in vivo[7] but the base rate of isolated atria did not change with age, as observed in this study and earlier by Adolph[11].

The atrial sensitivity of rats to ACh and CCh did not change further after birth, thus indicating that the cholinergic system in the rat heart is well developed at birth. Hall[8] has estimated that the embryonic heart of the 10.5-day-old rat failed to respond to ACh but hearts of 11.5–14.5-day-old rats responded by temporary diastolic arrest. Ljung and State[10] have found in the rat portal vein that the sensitivity to exogenous ACh does not increase during the postnatal development.

This study shows that there are age-related functional differences in the chronotropic responses of atria to adrenergic agonists but not to cholinergic agonists. The beta-receptor system of the rat atria appear to develop faster than the alpha-receptor system.

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Changes of hormonal status in young mice by restricted caloric diet. Relation to lifespan extension. Preliminary results[1]

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Summary. The maintainance of mice on a reduced caloric diet for 6 weeks starting from weaning time produces persistent changes in their hormonal status as reflected by differences in blood levels of gonadal and adrenal steroids. The changes might express a permanently different hypothalamic regulation. This might account for a prolongation of their life span.

Since the discovery by McCay[3–6], it has been known for many years that a diet restricted in calories prolongs significantly the life span of mice and rats. How this operationally 'simple' procedure can effect such a remarkable prolongation of the life span is still a matter of speculation. A very important aspect which emerged from previous work was that the earlier in life the diet was instituted, the longer the animals lived.

Dilman[7] proposed in 1971 that one of the causes of senescence, and of some of its most compelling pathological manifestations (atherosclerosis, senile diabetes, increased incidence of tumors, a.o.), is an age-associated progressive elevation of the hypothalamic threshold to feed-back suppression. This might involve a compensatory increase of production and release of certain protein hormones (growth hormone, prolactin for example) which promote most of the peripheral metabolic alterations which are typical of ageing. These progressive degenerative changes of the hypothalamus were considered by him to be the cause of a kind of 'physiological' ageing; many factors of hereditary or environmental nature might powerfully contribute to the delay or acceleration of this syndrome[7].

Our work on the role of the thymus in the programming and organization of neuroendocrine functions in early ontogeny[8,9] and the notions that different kinds of manipulations can alter or affect permanently hypothalamic functions, suggested a possible mechanism by which a restricted caloric diet might prolong life. This might be by delaying and influencing the definitive organization of the hypothalamus for adult endocrine functions. The idea

Persistent changes in the hormonal status of specific pathogen-free female MAG mice attributable to a restricted caloric diet for a period of 6 weeks after weaning

Age (days)	Treatment	Average b. wt (g)	Thyroxine (nMol/l)	Progesterone (ng/100 ml)	17-β-estradiol (nMol/l)	Insulin (pg/ml)	Corticosterone (μg/100 ml)
63	Diet*	15.4	84	346	0.26	< 200	n.d.
	Controls	29.1	128	486	0.32	< 200	16.9
78	Diet	27.4	78	507	0.36	244	15.1
	Controls	27.3	72	433	0.29	860	13.6
108	Diet	28.3	129	507	0.37	< 200	15.3
	Controls	30.0	95	417	0.26	200	26.1
138	Diet	30.8	88	750	0.42	372	12.0
	Controls	32.8	117	427	0.27	< 200	16.0
168	Diet	31.0	119	446	0.26	< 200	8.5
	Controls	32.0	98	582	0.33	264	16.0

*The restricted caloric diet was started at 21 and interrupted at 63 days of age. After that time, the mice were fed at libitum. Each value corresponds to 1 or more determinations of 1 pool of sera from 10 mice. n.d., non determined.

was that an alteration of the normal hypothalamic programming before it reaches its final adult organization (prepubertal age) would maintain this organ in a more 'juvenile' condition, thus retarding the degenerative changes and the consequent disruption of the normal hormonal feed-back homeostatic control, as suggested by Dilman[7]. Such a sequence would explain why life is prolonged significantly when the diet is applied in mice immediately after weaning. The crucial aspect of this postulated causal relationship was whether maintainance on a low caloric diet only over a limited time after weaning the mice, that is at a prepubertal age when the definitive programming for adult neuroendocrine functions (e.g. sexual functions) were still incomplete, would permanently alter the hormonal status.

Materials and methods. To verify this hypothesis and the link to Dilman's theory of ageing[7], groups of mice (outbred hybrid MAG strain, females, raised and maintained in specific pathogen-free conditions) were isolated in single cages at 21 days of age. They were given daily 3 g of the common mice pellets, which corresponded to 50% of the daily ad libitum food input for this strain[10]. Water was given ad libitum with addition of vitamins (Multibionta, Merck AG; 0.4 ml/200 ml water). Control groups were given food ad libitum. The restricted caloric diet was maintained for 6 weeks. At the end of this period (9 weeks of age), the mice were given free access to food. At the end of the restricted diet period, and then 2 weeks, 1, 2 and 3 months after ending the diet (11, 16, 20 and 24 weeks of age), groups of 10 mice and an equal number of controls were sacrificed by exsanguination under strictly standardized conditions between 10.00 and 12.00 h, sera were pooled, divided into aliquots and frozen. Determinations of thyroxine, progesterone, 17-β-estradiol, corticosterone and insulin were made. For the interpretation of the results, it must be pointed out that the internal variability of the assays (differences between double determination of a coded sample) or the variability of values between pools of sera from groups of mice of the same age, sex and strain did not exceed 5–10% when they were bled under our standardized conditions. Therefore, constant differences over 20% were considered as significant, although they were more pronounced in most cases.

Results and discussion. The table shows the results. At the end of the 6 weeks, the mice kept on a low caloric diet displayed half the weight of the mice fed ad libitum, but their body weight had reached that of the control group 2 weeks after ad libitum feeding. Still at 3 months after the end of the 6-week restricted diet, the mice showed very significant differences in levels of several hormones when compared to the mice fed permanently ad libitum. The clear tendency of the mice of the low caloric diet groups was to produce and/or release less corticosterone. On the other hand, levels of progesterone and 17-β-estradiol were higher in the low caloric group at 78, 108 and 138 days of age, while a reversal of this situation was observed at 168 days of age. Thus maintainance on a restricted caloric diet for a period of 6 weeks after weaning induced persistent changes in the hormonal status of the mice, as reflected by the levels of hormones in peripheral blood. These changes concerned mainly adrenal and sexual functions.

These findings, although preliminary, give support to the hypothesis of Dilman[7]. They indicate that a diet restricted in calories at a stage when the hypothalamus is not yet fully organized for mature sexual functions, affects persistently its performance to feed-back suppression. It is clear that the complex metabolic processes consequent to high caloric input during growth and in which many 'anabolic' and 'catabolic' hormones are required, makes for a different regulation of the neuroendocrine system,

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depending on the amount of food intake. This influences in a crucial way the still undifferentiated hypothalamic centers and stabilizes the threshold to peripheral feedback stimuli at a different level. Accordingly, at a prepubertal age (between 10 and 16 years of age) a diet relatively poor in calories but otherwise balanced in all its components might be *one* determinant factor in programming the species-specific life span of humans and prolong it to its maximal expectancy. This kind of intervention will not prevent ageing, but it might well postpone the onset of some of its most typical degenerative phenomena (artheriosclerosis, hypertension, increased genesis of tumors, a.o.) which most probably relate to an imbalanced hormonal status.

Potentials in mammalian skeletal muscle from collagenase-treated tissue

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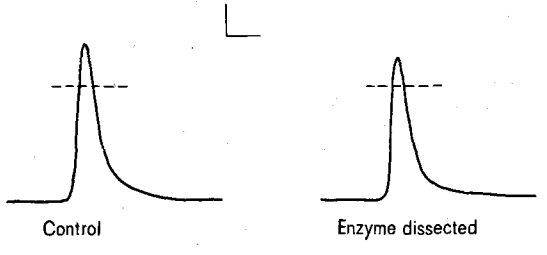
Summary. Transmembrane potentials were recorded from skeletal muscle fibres dissected with the aid of collagenase perfusion. Collagenase treatment had little or no effect on the action or resting potentials.

Because dissection of single skeletal muscle fibres or small bundles of fibres often is difficult due to tough connective tissue, we have looked for a technique that would reduce this component without cellular injury. Collagenases have been shown to digest primarily intercellular proteins and have allowed the isolation of intact smooth muscle[1] and cardiac cells[2]. We have used collagenases to prepare skeletal muscle for homogenization[3], thus we decided to explore the possibilities of enzyme-assisted dissection of mammalian skeletal muscle fibres and bundles. Preliminary experiments demonstrated that simple submersion of the tissue in an enzyme-containing solution was inadequate for disruption. Therefore, a hind-limb perfusion technique similar to the liver perfusion protocol employed in the isolation of liver cells[4] was adopted. The integrity of preparations subjected to this technique was assessed by examining the transmembrane action potential.

Electrical potentials from rat semi-tendinosus muscle

	Normal*	Enzyme-treated*	p
Resting potential	-68.9 ± 1.0 mv (15)	-66.6 ± 1.0 mv (23)	0.09
Overshoot	$+20.4 \pm 2.4$ mv (15)	$+17.6 \pm 1.2$ mv (16)	0.33
Action potential	89.4 ± 2.5 mv (15)	84.1 ± 1.9 mv (16)	0.10

* Indicates that 3 animals were used and numbers in parentheses indicate the number of penetrations recorded. Electrical potentials are the means $\pm$ SE of all the penetrations recorded.



Tracings of action potential recorded from control and enzyme dissected muscle. The dashed line in each represents the isopotential line. Vertical bar is 20 mV. Horizontal bar is 2 msec.

Materials and methods. Sprague-Dawley rats 200–300 g were anaesthetized with ether and a small cannula was introduced into the right femoral artery. After the cannula was tied in place, room temperature (22 °C $\pm$ 2 °C), calcium-free Hank's solution was perfused through the limb by means of a peristaltic pump until the veins were cleared of blood. Then, the femoral vein was severed and the perfusate allowed to escape for a 5-min-period. Subsequently, collagenase (Type I, Sigma Co.) in complete Hank's solution (containing calcium) that had been filtered through Gelman 0.45 µm filtres was perfused, at room temperature, for 5 min. Thereafter, normal Hank's solution without enzyme was perfused for 10 min to remove the collagenase and digestion products. Dissection and removal of small bundles of fibres from the semitendinosus muscle was performed in Hank's solution. Control preparations were dissected from nonperfused, ether anaesthetized animals and placed directly into complete, room temperature, Hank's solution.

For electrical recording, control and test muscle bundle preparations were transferred to a horizontal muscle chamber containing oxygenated Hank's solution. Transmembrane potentials were measured with 3 M KCl filled glass microelectrodes having 5 to 20 MΩ resistance and connected through calomel half cells to a Transidyne MPA-6 preamplifier with stray capacitance neutralization. Stimulation was accomplished with suprathreshold square wave pulses of 0.2 to 0.4 msec duration applied through platinum electrodes. A floating microelectrode arrangement was utilized to minimize mechanical artifacts. Signals were displayed on a Tektronic Type 503 oscilloscope equipped for Polaroid photography.

Results. When perfused with enzyme-containing solution, hind-limb muscles gradually swelled to nearly twice their initial diameter. Under direct microscopic view, it was apparent that muscle fibre bundles were well dispersed and that they responded to electrical stimulation with vigorous contractions. Subsequently, minimal mechanical dissection was necessary to obtain small bundles of muscle fibres that were less readily obtained from un-

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