sarcomere lengths are identical, adjacent sarcomeres still could behave differently in response to single electrical shock. This could be attributed to a difference in the visco-elastic elements.

For the sake of convenience and for ease of analysis, muscles are studied either under isotonic condition or isometric condition. Under isotonic condition, velocity is usually expressed in units of L_o/sec. This at best merely represents average velocity of sarcomere shortening in units of sarcomere length/sec[17]. CLOSE[18] has estimated the speed of shortening per sarcomere by dividing the speed of shortening of the whole muscle by the average number of sarcomeres per muscle fibre within a muscle. His estimations, however, assumed that, a) all the muscle fibres were orientated in the same direction, b) all the muscle fibres were of uniform length, c) each muscle fibre had a uniform number of sarcomeres[19], d) every sarcomere length was uniform, e) the force-generator in each sarcomere was the same and f) the visco-elastic property of each sarcomere was the same.

Likewise in isometric measurements, even with single muscle fibres, GORDON et al.[20] in their studies on the length-tension curves must also assume the validity of c), d), e) and f). Our study indicates that at least in the striated muscle fibres in the horseshoe crab, b), c), d), e) and f) are by no means sound and valid assumptions all the time.

Zusammenfassung. An Beinmuskulatur des Pfeilschwanzkrebses *Tachypleus gigas* wurden während isometrischen Einzelzuckungen die Distanzen zwischen benachbarten Z-Scheiben und A-Banden unter Verwendung einer Televisionsanordnung ausgemessen. In einer ruhenden Einzelfaser erwiesen sich die Längen der einzelnen Sarkomeren als nicht einheitlich. Während einer isometrischen Einzelzuckung verkürzten sich gewisse Sarkomere, während andere in die Länge gezogen wurden. Bei gleicher Ruhelänge von benachbarten Sarkomeren war die Geschwindigkeit der Verkürzung in der Regel ungleich. Dies wird auf Unterschiede im visco-elastischen Verhalten zurückgeführt. Die Resultate zeigen deutlich, dass selbst aus dem Verhalten von Einzelfasern nicht ohne weiteres auf das Verhalten von einzelnen Sarkomeren geschlossen werden darf.

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Effects of Temperature on Osmotic Responses and on Transmembrane Efflux of Urea and Sodium in Vascular Smooth Muscle Cells

In previous studies of osmotic responses in smooth muscle of rat portal vein, we have described an intimate relation between the changes in cell volume and the spontaneous electrical and mechanical activity[1-3]. Anisosmolar solutions containing urea caused characteristic transient changes in activity. A close correlation was demonstrated between the time course of these reponses and the rate of penetration of the molecule through the cell membranes as studied directly by ^{14}C-urea[2]. The present report is concerned with the effects of temperature on such contractile responses and on membrane permeability.

Figure 1 illustrates increases of contractile activity observed in rat portal vein on return to standard solution

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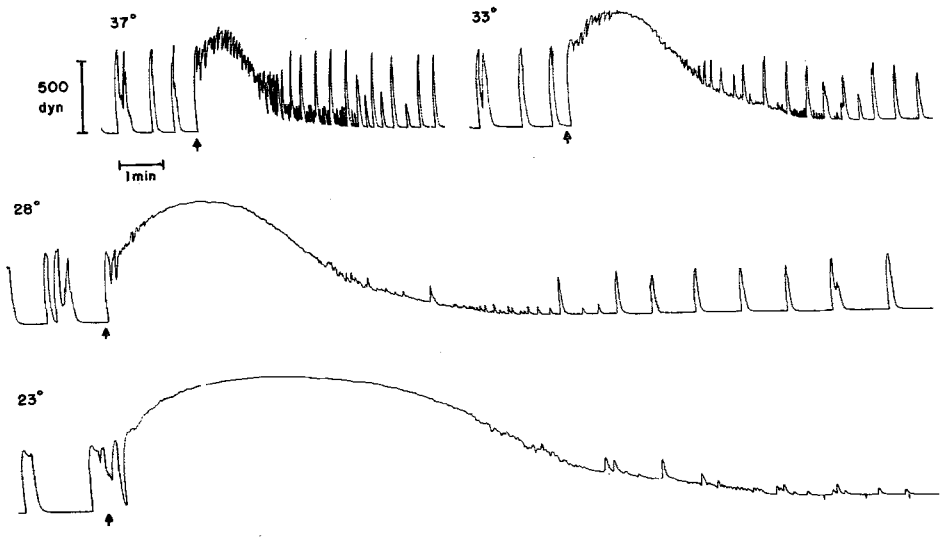

Fig. 1. Time course of the transient increase of contractile activity observed in a portal vein preparation on return to standard Krebs solution (arrows), after previous equilibration in a hyperosmotic solution with 100 m*M* urea. The duration of the excitatory response is markedly increased with decreasing temperature.

after previous equilibration in a hyperosmolar medium containing 100 mM urea (for methodological details see[1]). As discussed previously[1–3], the mechanical response is related to the swelling of the cells and to the subsequent return to normal cell volume during the outward diffusion of urea. The prolongation of the excitatory response with decreasing temperature in Figure 1 therefore suggests that cooling greatly reduced the rate of efflux of urea. A rough estimate of the temperature coefficient from the time course of the tetanic force indicated a Q_{10} around 3.0. This value seemed very high for the efflux of a small non-electrolyte, expected to pass the cell membranes by simple diffusion. We therefore decided to examine this process more directly by studying the wash-out of ^{14}C-urea. In such experiments, the results would not be affected by changes in contractile activity or by net transmembrane fluxes of water, as might possibly occur in the above mechanical experiments. We have included in this study also wash-out of ^{24}Na, which depends for its membrane passage on a number of processes including active transport[4].

Portal-mesenteric veins, prepared and pre-incubated as described previously[5,6], were transferred to flasks wiht standard Krebs solution containing either ^{14}C-urea or ^{24}Na. The uptake period in radioactive solution was 105 min at 37 °C plus 15 min at the temperature of the subsequent wash-out. The efflux of ^{14}C-urea at 15, 25 and 37 °C and of ^{24}Na at 15 °C was studied by transferring the muscles through a series of test tubes containing non-radioactive standard solution. In view of its compartmental complexity, the wash-out of ^{24}Na at 37 °C was too fast to be studied satisfactorily with this method. In this case the muscles were placed instead in a bath which was continuously perfused with non-radioactive solution. The effluent was collected in test tubes, automatically changed by a fraction collector. The radioactivity of the wash-out media and of the muscles at the end of the procedure was determined as described previously[5,6].

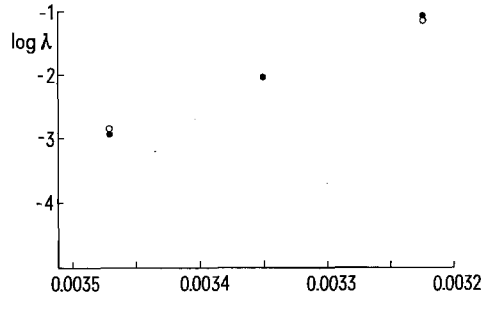

Fig. 2. The natural logarithm of the transmembrane efflux rate constant. λ, for urea (filled circles) and for sodium (open circles) plotted against the reciprocal of the absolute temperature. For details see text.

Results from experiments where the washout of ^{14}C-urea and ^{24}Na was studied at different temperatures

		n	λ min^{-1}	Y_o ml/100 g wet wt.
^{14}C urea	15 °C	7	0.053 ± 0.002	20.5 ± 0.7
	25 °C	4	0.126 ± 0.005	16.2 ± 2.3
	37 °C	7	0.411 ± 0.020	23.0 ± 2.8
^{24}Na	15 °C	8	0.054 ± 0.001	3.51 ± 0.24
	37 °C	10	0.398 ± 0.019	3.84 ± 0.32

λ represents the efflux rate constant of the intracellular component and Y_o its intercept with the ordinate (correction has been made for the influence of the series-coupled extracellular phase).

The elimination curves showed the same general features as the ones obtained earlier[5,6], and they were analyzed in the same way. The results are summarized in the Table. Here λ denotes the rate constant of the intracellular component of the wash out curves. The intersection of this component with the ordinate, Y_o, is given in units of 'space' after correction for the series-coupled extracellular phase[7]. A plot of ln λ versus the inverse value of the absolute temperature (1/T) according to the Arrhenius equation[8] is shown in Figure 2. The values for urea can be fitted by a straight line and the values for Na fall close to this line. The slope of the line corresponds to a Q_{10} of 2.4 (activation energy ~ 16,000 cal/mole).

The results support our previous views on the correlation between the rate of membrane penetration of non-electrolytes and the changes in electrical and mechanical activity of the smooth muscle[2,9]. In view of the electrical forces acting on the sodium ions and the contribution of metabolic processes to their membrane transport, a Q_{10} of 2.4 for ^{24}Na efflux seems quite resonable. A still somewhat higher Q_{10} for ^{24}Na efflux was calculated for guinea-pig taenia coli by GOODFORD[8]. The high temperature sensitivity of the washout of urea was a surprizing finding, indicating that the efflux of this water-soluble, small non-electrolyte cannot be described as simple, free diffusion. The 'porous aqueous pathways' through which urea might pass by simple diffusion seem to occupy a very small fraction of the membrane area in smooth muscle[9], so that, despite its small oil/water partition coefficient, most of the urea will pass through the lipid membrane. This latter transport may show a high temperature coefficient due to chemical interaction between the urea molecules and the cell membrane or due to thermic changes in the viscosity or the molecular configuration of the membrane lipids. The present results seem to imply that the temperature coefficient is an unreliable indicator of the type of mechanism involved in cellular transport of smooth muscle.

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Zusammenfassung. Die Harnstoffpermeabilität der glatten Muskelzellen der Portalvene von Ratten wurde im Temperaturbereich zwischen 15 und 37 °C untersucht. Sowohl die ^{14}C-Harnstoff- wie die $^{24}Na^{+}$-Elimination zeigten einen Q_{10} von etwa 2,4. Aktive und passive Transportprozesse in der glatten Gefässmuskulatur können wahrscheinlich nicht ausschliesslich durch die Bestimmung des Temperaturkoeffizienten unterschieden werden.

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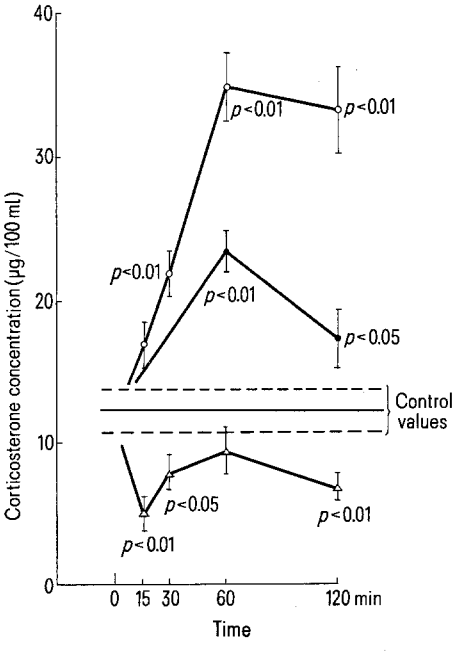
Effect of Stress and Diazepam Treatment During Infancy on the Corticosterone Regulation and Androgenic Activity in Adult Male Rats

Handling, stress or disturbance of the mother-infant relationship during infancy can modify considerably further development of many nervous and somatic functions[1–3]. Increased activity of the pituitary-adrenal system at time of stimulus action may be suspected to be involved in some of these phenomena. One of the approaches to an investigation of this problem seems to be a pharmacological alteration of the adrenal cortex regulating system responsivenes towards stress stimulation during infancy. In this study, diazepam treatment[4] was employed for that purpose, while the simple i.p. saline injection procedure served for the preweaning stress stimulation[5–7]. The experiment was completed by an investigation of ACTH-treated animals[8,9] which were expected to reveal the effects of maximal stimulation of the adrenal cortex.



Serum corticosterone levels in the 2-day-old rats. Ordinate: Corticosterone concentration in µl/100 ml of sera. Abscissa: Time after the injections in minutes. ●, i.p. injection of physiological saline; △, i.p. injection of diazepam; ○, i.p. injection of ACTH. Horizontal line: untreated animals. (Mean ± S.E.). *P*, statistically significant difference in comparison to control values (Student's *t*-test).

Material and methods. The Wistar strain rats were used in these experiments. The methods of corticosterone and serum electrolytes estimations, in vitro adrenal gland incubation and the animal maintenance schedule were described earlier[10,11].

In the first short-time experiment, the corticosterone levels were investigated in serum of 2-day-old animals sampled within 2 h after i.p. administration of 0.1 ml of saline, or single dose of 10 mg of diazepam (Faustan Germed) i.p. or single dose of 1 IU of ACTH (Cortrosyn Organon) i.p. per 100 g body weight. Pooled samples from 2–3 animals were used.

The delayed after-effects were estimated in adult animals which had been treated i.p. from 2nd to 12th day of age once daily by either 0.1 ml of saline, diazepam (total dose 1.3 mg/rat per 10 days), or by ACTH (total dose 1.6 IU/rat per 10 days). There was 7–8 animals per group and they were investigated at age of 200 days. The result of behavioral investigation performed on these animals at age of 90 and 130 days will be published separately[12–14].

Results and discussion. The short-term experiment showed that the exogenous ACTH, as well as the stress due to the i.p. injection procedure, increase the serum corticosterone levels in the 2-day-old pups. In the diaze-

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