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.

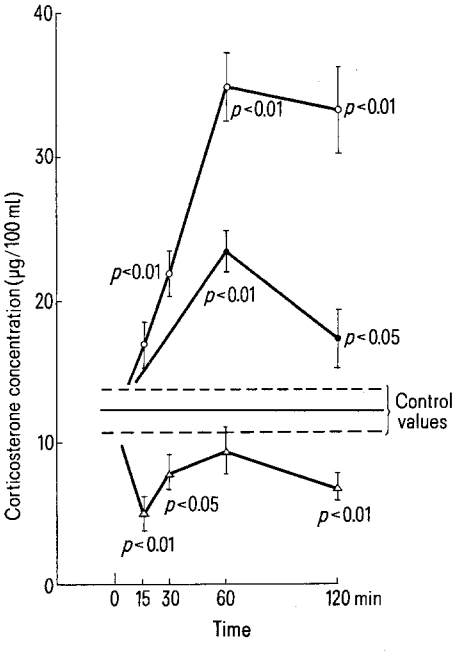
O. JONSSON, B. JOHANSSON, G. WENNERGREN and L. STAGE[10]

Department of Physiology, University of Göteborg, S–40030 Göteborg (Sweden); and Department of Physiology, University of Lund, S–22362 Lund (Sweden), 7 August 1974.

[10] Acknowledgment. This study was supported by grants from the Medical Faculty, University of Göteborg and from the Swedish Medical Research Council No. 14x-28.

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-

[1] R. ADER and L. J. GROTA, in *Drug Effects on Neuroendocrine Regulation* (Eds. E. ZIMMERMANN, W. H. GISPEN, B. H. MARKS and D. DE WIED; Elsevier, Amsterdam, London, New York 1973), p. 395.
[2] O. WEININGER, Science *119*, 285 (1954).
[3] M. KRAUS, in *The Postnatal Development of Phenotype* (Eds. S. KAZDA and V. H. DENENBERG, Academia, Praha 1970), p. 151.
[4] R. KRULÍK and M. ČERNÝ, Activitas nerv. sup. *14*, 31 (1972).
[5] A. M. BARRETT and M. A. STOCKHAM, J. Endocr. *26*, 97 (1963).
[6] J. R. HODGES and S. MITCHLEY, J. Endocr. *47*, 253 (1970).
[7] V. H. DENENBERG and M. X. ZARROW, in *The Postnatal Development of Phenotype* (Eds. S. KAZDA and V. H. DENENBERG, Academia, Praha 1970), p. 123.
[8] M. X. ZARROW, J. E. PHILPOTT and V. H. DENENBERG, in *The Postnatal Development of Phenotype* (Eds. S. KAZDA and V. H. DENENBERG, Academia, Praha 1970), p. 137.
[9] K. MILKOVIČ and S. MILKOVIČ, in *Neuroendocrinology* (Eds. L. MARTINI and W. F. GANONG, Academic Press, New York and London 1966), p. 371.
[10] R. ERDÖSOVÁ and M. KRAUS, Hormones *2*, 216 (1971).
[11] H. DLOUHÁ, R. ERDÖSOVÁ, M. KRAUS and J. ŠKOPKOVÁ, Biologia Neonat. *22*, 38 (1973).
[12] J. LÁT and B. JAKOUBEK, Activitas nerv. sup., in press.
[13] S. FRAŇKOVÁ and B. JAKOUBEK, Activitas nerv. sup., in press.
[14] B. JAKOUBEK, A. DĚDIČOVÁ, R. ERDÖSOVÁ, M. KRAUS and J. LÁT, Physiol. bohemoslov., in press.

pam-treated pups, the difference between values found at 15th and 60th min ($p < 0.01$) indicates that the stressfull effect of the injection procedure was not completely abolished but, in general, the corticosterone levels were suppressed to such an extent that they did not increase above the control values (Figure).

In the 200-day-old males which had, from the 2nd to 12th day of life, been exposed to stress stimulation or treated by exogenous ACTH, most of the observed changes which are listed in the Table may be explained in terms of an increased androgen activity which could result either from increase in androgen secretion or from an increased responsivenes of the target tissues to their influence.

It is known that the increased androgenic stimulation can increase the weight of seminal vesicles[15], kidney weight[16], serum potassium without change in serum sodium level[17], and decrease the corticoid desintegration rate[18], while its effects on adrenal gland weight seems to be an irregular one[19]. As seen in the Table, most of the changes observed in adult animals which had been exposed during the preweaning period to stress stimulation or to the ACTH treatment, fits the hypothesis of increased androgenic activity, the decreased catabolism of corticoids being inferred from increased serum corticosterone levels associated with unchanged or even decreased corticosterone production. Obviously, an involvement of other hormonal systems activity cannot be ruled out, since, for instance, the growth hormone can increase the sensitivity of tissues to the androgen influences and vice versa[20–23]. Similarly, the weight of thymus can be influenced both by corticosteroids and by androgens[24].

In contrast, in the animals in which the pituitary-adrenal response to stressfull stimulation during the early infancy had been suppressed by diazepam, apart from the relatively small change in body weight, no other significant differences were found in adult animals in comparison to the untreated control animals (Table).

It can be concluded that the stimulation of the adrenal cortex activity during the preweaning period can be followed by various changes in features of adult animals. The results indicate further that the ACTH treatment or stress stimulation during infancy, that does not result in an immediate gross damage to the young organism, tends to favour such a somatic after-effects which are likely to be induced by higher androgenic activity, or conversely, the lack of extrastimulation or suppression of the response to undamaging stress by tranquilizer during the early stages of postnatal development is followed subsequently by indices of lower androgenic activity.

Delayed effects of preweaning i.p. injections of saline, diazepam and ACTH estimated in 200-day-old male rats

Controls	Treatment		
	Diazepam	Physiological saline	ACTH
Body weight (g)			
375.6 ± 8.5[a]	436.8 ± 14.1 ($p < 0.01$)	563.4 ± 21.3 ($p < 0.01$)	531.3 ± 33.7 ($p < 0.01$)
Testes (mg/100 g B.W.)			
667.9 ± 30.7	591.0 ± 33.2 n.s.	583.6 ± 24.4 n.s.	679.9 ± 34.6 n.s.
Seminal vesicles (mg/100 g B.W.)			
76.6 ± 12.8	85.0 ± 15.1 n.s.	213.4 ± 37.4 ($p < 0.01$)	203.3 ± 21.4 ($p < 0.01$)
Kidneys (mg/100 g B.W.)			
504.8 ± 31.7	528.1 ± 13.5 n.s.	548.6 ± 31.2 n.s.	612.6 ± 14.1 ($p < 0.01$)
Thymus (mg/100 g B.W.)			
161.4 ± 9.4	173.0 ± 9.5 n.s.	139.4 ± 9.0 n.s.	136.5 ± 4.9 ($p < 0.05$)
Serum corticosterone (µg/100 ml)			
5.8 ± 0.4	8.7 ± 1.1 n.s.	12.6 ± 3.0 ($p < 0.01$)	10.6 ± 1.9 ($p < 0.05$)
Corticosterone production (µg/1000 g B.W./h)			
1.40 ± 0.09	1.30 ± 0.11 n.s.	1.17 ± 0.12 n.s.	0.95 ± 0.09 ($p < 0.01$)
Adrenal gland (mg/100g B.W.)			
9.93 ± 0.29	8.47 ± 0.35 n.s.	7.63 ± 0.32 ($p < 0.01$)	9.54 ± 0.31 n.s.
Serum potassium (mEq/l)			
6.13 ± 0.09	6.46 ± 0.24 n.s.	6.64 ± 0.18 ($p < 0.05$)	6.54 ± 0.12 ($p < 0.05$)
Serum sodium (mEq/l)			
139.6 ± 1.96	139.4 ± 1.24 n.s.	143.4 ± 1.62 n.s.	141.0 ± 0.71 n.s.

Numbers in parentheses: statistical significance in comparison to controls (ANOVAR). [a] Mean ± S.E.

Zusammenfassung. Stresseinwirkung in früher postnataler Ontogenese führt bei erwachsenen Ratten zu Veränderungen, die auf eine erhöhte Androgenaktivität hinweisen. Die Mehrheit dieser Veränderungen kann mittels Diazepam gehemmt werden.

R. Erdösová, B. Jakoubek and M. Kraus

Czechoslovak Academy of Sciences, Institute of Physiology, Budějovická, 14220 Praha 4 (Czechoslovakia), 29 July 1974.

[15] R. I. Dorfman, in *Methods in Hormone Research* (Ed. R. I. Dorfman, Academic Press, New York and London 1962), vol. 2, p. 275.
[16] C. D. Kochakian, Endocrinology *70*, 99 (1962).
[17] A. Molteni, H. D. Colby, F. R. Skelton and A. C. Brownie, Proc. Soc. exp. Biol. Med. *141*, 936 (1972).
[18] F. E. Yates, A. L. Herbst and J. Urquhart, Endocrinology *63*, 887 (1958).
[19] S. N. Roy, J. N. Karkum and S. K. Roy, Indian J. med. Res. *47*, 25 (1959).
[20] A. V. Bocabella, Endocrinology *72*, 787 (1963).
[21] A. J. Lostroh, Endocrinology *70*, 747 (1962).
[22] R. O. Scow and S. N. Hagan, Endocrinology *77*, 852 (1965).
[23] J. J. van der Werff ten Bosch, in *The Pituitary Gland* (Eds. G. W. Harris and B. T. Donovan, Butterworths, London 1966), vol. 2, p. 324.
[24] H. E. Voss and G. Oertel, *Handbuch der experimentellen Pharmakologie* (Springer Verlag, Berlin, Heidelberg, New York 1973), vol. 35/1, p. 280.