Amino Acid Incorporation by Müllerian Ducts Isolated from Chick Embryos[1]

Müllerian ducts of chick embryos are a classical research material in the study of hormone-dependent, developmental tissue degeneration. In normal embryos only the left female duct develops to the adult stage, giving rise to the unpaired oviduct. The development of the right female duct is gradually retarded from the ninth day of incubation, and a slow anterioposterial regression follows. Both male organs degenerate rapidly from the eighth day. These events are triggered by the sexual differentiation of the gonads[2,3].

In ducts destined to undergo hormone-dependent cell degeneration, ribonuclease and other lytic enzymes accumulate in cytoplasmic particles, supposedly lysosomes. Later, these enzymes are found in the soluble fraction of homogenized ducts[3-5]. This has been interpreted to indicate that in degenerating cells they are released into the cytoplasm ('suicide' function of the lysosomes). The initial increase in activity of the lytic enzymes suggests that the induction of cell degeneration is mediated through alterations in the pattern of protein synthesis. In analogy with current thinking, a control mechanism operating at the gene level has accordingly been postulated[3]. This has made the 'suicide' concept fall somewhat into the background, and it has been suggested that the increased lysosomal fragility is a consequence rather than a cause of cell degeneration[5,6].

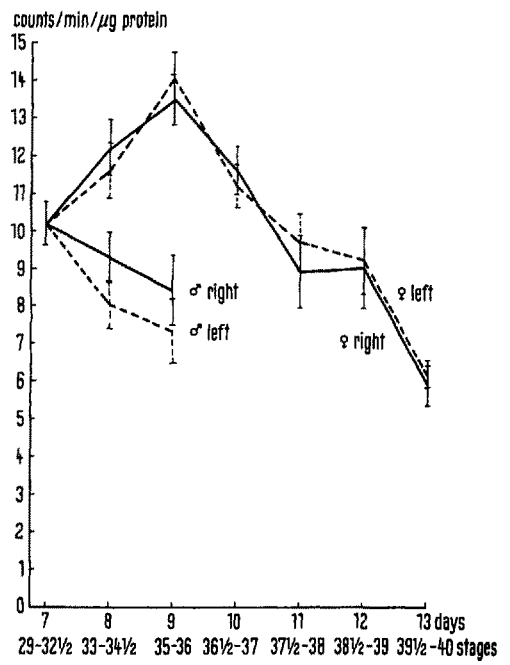
Both hormone-induced repressions (whether at the transcriptional or translational level) and increased ribonuclease activities in the cytoplasm might be expected to decrease the proportion of active polysomes, and cut down the rate of protein synthesis in the cells. We were interested to learn whether the involution of the Müllerian ducts was preceded by a reduced capacity of the isolated organs to incorporate labelled amino acids into protein.

Experimental. Müllerian ducts were carefully excised under microscope from White Leghorn chick embryos, and were incubated for 30 min at 35 °C under carbogen gas in a mixture of 0.45 ml KREBS-HENSELEIT[7] medium, 0.05 ml 0.25 *M* glucose and 0.125 µmoles ^{14}C-L-leucine (2 mC/mmole). After repeated extractions at 90 °C with 5% trichloroacetic acid containing unlabelled leucine, the ducts were treated with organic solvents and finally dried in ether[8]. The radioactivity was determined in a liquid scintillation counter (Packard Tricarb) with a solution of 0.5% 2,5-diphenyloxazole and 0.015% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene in toluene as scintillator. Control experiments with ducts, recounted after solubilization in formic acid and evaporation in the counting vials, indicated that no self-absorption occurred. After washings with ether, the protein content of the ducts was determined by the LOWRY[9] method. The increase in the specific activity of the proteins was approximately linear for at least 1 h. When $5 \cdot 10^{-4}$ *M* puromycin or 10^{-4} *M* 2,4-dinitrophenol was added to the incubation medium 15 min before the labelled amino acid, the incorporation was inhibited 99% and 93% respectively. This served as a control of the specificity of the technique.

Results and discussion. As is shown in the Figure, the specific incorporation activities of the preparations of male and female ducts diverged progressively from the seventh day. While the activity of the female ducts continued to increase, that of the male ducts gradually declined. The % of activity loss was, however, fairly moderate. Even on the ninth day, when the size of the male ducts was noticeably smaller, and the DNA and RNA contents per mg wet weight had decreased by nearly 50%[3], the average incorporation activity in our material was reduced by only 23%. Some of this decrease in specific activity may furthermore be accounted for by a higher proportion of inert, intercellular protein in the preparations. No reliable measurements could be made on the tenth day due to the minute size of the duct remnants.

The incorporation activity of the preparations of female ducts was greatest on the ninth day. The subsequent decrease may possibly reflect a gradual maturation of the embryonic tissues. Of particular interest was the fact that the right ducts, although their growth had ceased, showed specific incorporation values not significantly different from those of the actively growing left ducts.

Our experiments with both male and female ducts indicate that the hormone-dependent cell degeneration was not preceded by any strikingly reduced capacity of protein synthesis in the preparations. In fact the actively



Incorporation of ^{14}C-L-leucine into protein by Müllerian ducts isolated from male and female chick embryos. Continuous lines represent right ducts, broken lines left ducts. Each point represents the mean value of 9–26 independent measurements. The vertical bars indicate the standard errors of the means. On day 7 no difference was observed between male and female ducts, and the indicated value represents the mean of all values. The staging was made according to HAMBURGER and HAMILTON[13].

[1] Supported by a grant from the Swedish Cancer Society.

[2] E. WOLFF, Experientia *9*, 121 (1953).
[3] T. H. HAMILTON, XIIIth Int. Orn. Congr. 1004 (1963).
[4] J. BRACHET, M. DECROLY-BRIERS and J. HOYEZ, Bull. Soc. Chim. biol. *40*, 2039 (1958).
[5] D. SCHIEB-PFLEGER and R. WATTIAUX, Devl Biol. *5*, 205 (1962).
[6] J. W. SAUNDERS, Science *154*, 604 (1966).
[7] H. A. KREBS and K. HENSELEIT, Hoppe-Seyler's Z. physiol. Chem. *210*, 33 (1932).
[8] R. J. MANS and G. D. NOVELLI, Archs Biochem. Biophys. *94*, 48 (1961).
[9] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

growing 13-day-old left female ducts showed a lower average specific incorporation activity than the partially involuted 9-day-old male ducts. Although the sizes of the intracellular amino acid pools have not been determined in the various kinds of preparations due to the minute size of the organs, it seems safe to conclude that proteins were synthesized at a remarkably high rate even by regressing ducts. Thus, the negative protein balance of the regressing organs is not readily explicable on the basis of a general loss in the proportion of active polysomes, but seems mainly due to an increased rate of protein degradation. The experiments suggest that the breakdown of the cell contents primarily takes place in an organized manner, e.g. by 'focal degradation'[10-12], since vital cell functions seem to go on without much interference until fairly late in the degeneration process.

Zusammenfassung. Es wird gezeigt, dass die Müllerschen Gänge weiblicher Hühnchenembryonen die gleiche Fähigkeit zum Einbau von Aminosäuren besitzen, trotzdem der rechte Gang sich allmählich zurückbildet. Auch erwies sich die Differenz zwischen männlichen und weiblichen Embryonen im Hinblick auf den Aminosäureneinbau als nicht besonders auffällig.

A. SJÖQVIST and T. HULTIN

Department of Cell Physiology, Wenner-Gren Institute, Stockholm Va (Sweden), 31st January 1967.

[10] H. SWIFT and Z. HRUBAN, Fedn Proc. Fedn Am. Socs exp. Biol. *23*, 1026 (1964).
[11] A. B. NOVIKOFF, E. ESSNER and N. QUINTANA, Fedn Proc. Fedn Am. Socs exp. Biol. *23*, 1010 (1964).
[12] D. SCHEIB, C. r. hebd. Séanc. Acad. Sci., Paris *260*, 1252; *261*, 5212 (1965).
[13] V. HAMBURGER and H. L. HAMILTON, J. Morph. *88*, 49 (1951).

Pressor and Oxitocic-Like Effects of Angiotensin Affected by Aldosterone Pretreatment in Guinea-Pigs

It has been proved that there are many connections between the production and biological activity of angiotensin and the secretion of aldosterone. LARAGH et al.[1], CARPENTER et al.[2] and KAPLAN et al.[3] observed that angiotensin is able to stimulate aldosterone secretion respectively in man, in intact animals and also in vitro. Later DAVIS et al.[4] reported that angiotensin is a true aldosterone-stimulating-hormone (ASH). Observations were also made on the reduced vascular reactivity towards angiotensin in adrenalectomized dogs[5] and cats[6] and on increased vascular reactivity towards the same peptide in dogs pretreated with aldosterone[7,8]. Moreover, reductions of the NaCl content of the suspension media of isolated guinea-pig ileum preparations caused conspicuous losses of the spasmogenic activity of angiotensin[9-11].

No report was found in the literature about the in vivo effects of angiotensin on extravascular smooth muscles in aldosterone pretreated animals.

Materials and methods. Guinea-pigs weighing 400–650 g were anaesthetized with ethyl urethane (1.0–1.5 g/kg i.p.). Uterine activity in situ was studied following ROTHLIN's procedure[12]. The 2 uterine horns were suspended to a strain-gauge microdynamometer (Ditta Ugo Basile, Via Campiglio 9, Milano, Italia) and their movements recorded by means of a d'Arsonval galvanometer writing on a smoked paper kymograph. Blood pressure was recorded from the common carotid artery using a mercury manometer.

The animals were set into 2 groups (7 non-pregnant guinea-pigs in each one) and treated as follows: control animals received daily saline (1 ml s.c.) for 5 days and were then prepared for the experiment; treated animals were administered daily for 5 days with aldosterone (Aldosten® CIBA) at the dose of 200 µg/kg/day in 1 ml of saline s.c. and then prepared for the experiment.

Synthetic angiotensin (α-L-Asp[1]-Val[5]Hypertensin II – Ipertensina® CIBA) was administered i.v. through a cannula in the external jugular vein. The injections of the peptide followed the same regimen both in control and in treated animals: 0.5, 1.0, 2.0, 10.0, 20.0 µg/kg i.v., according to preliminary experiments and to the data reported by FREGNAN and GLASSER[13].

Results. The averages of the results obtained in control and in aldosterone pretreated animals are given graphically in the diagram. Both in control and in treated animals, angiotensin showed a pressor activity at all the doses employed.

At the doses of 0.5, 1.0 and 10.0 µg/kg i.v. angiotensin has shown higher pressor activity in pretreated than in control animals. From the graph it can be seen that there is a clear difference in the 2 groups of guinea-pigs: in control animals the pressor effects are always increased by increasing the doses, while in pretreated animals the

[1] J. H. LARAGH, M. ANGERS, W. G. KELLY and S. LIEBERMAN, J. Am. med. Ass. *174*, 234 (1960).
[2] C. CARPENTER, J. O. DAVIS and C. R. AYERS, J. clin. Invest. *40*, 2026 (1961).
[3] N. M. KAPLAN and F. C. BARTLER, J. clin. Invest. *41*, 715 (1962).
[4] J. O. DAVIS, C. J. CARPENTER and C. R. AYERS, Circulation Res. *11*, 171 (1962).
[5] G. C. SALMOIRAGHI and J. W. MCCUBBIN, Circulation Res. *2*, 280 (1954).
[6] F. CHIESA, C. BERETTA and R. OBEROSLER, Nuova Vet. *10*, 14 (1964).
[7] R. BERETTA, F. FANTINI, P. LANUCARA and U. MARINI, Atti Acad. med. lomb. *19*, 192 (1964).
[8] R. BERETTA, F. FANTINI, P. LANUCARA and U. MARINI, Atti Acad. med. lomb. *19*, 208 (1964).
[9] C. BERETTA, F. CHIESA and G. AGUGGINI, Nevrasse *14*, 1 (1964).
[10] C. BERETTA, Rc. Ist. lomb. Sci. Lett. *99*, 79 (1965).
[11] J. R. BLAIR-WEST and J. S. MCKENZIE, Experientia *22*, 291 (1966).
[12] E. ROTHLIN, Schweiz. med. Wschr. *33*, 971 (1938).
[13] G. B. FREGNAN and A. H. GLASSER, J. Pharm. Pharmac. *16*, 744 (1964).