Table 2. DA levels in control and PCS rats (7 days of postoperatory) and after levodopa + LPDI administration

DA levels (ng/mg)	Dopaminergic system		Noradrenergic system	
	Olfactory	Striatum	Amygdala	Hypothalamus
Control rats	2.68 ± 0.12 (18)	4.18 ± 0.50 (16)	0.76 ± 0.08 (17)	0.59 ± 0.04 (17)
Control rats + levodopa + LPDI	7.05 ± 0.39 (9)**	5.90 ± 0.11 (10)**	3.40 ± 0.12 (10)**	4.70 ± 0.14 (10)**
PCS	2.03 ± 0.18 (17)*	3.22 ± 0.48 (18)*	0.98 ± 0.20 (17)	0.90 ± 0.09 (16)*
PCS + levodopa + LPDI	10.09 ± 0.76 (10)**	9.12 ± 0.65 (10)**	7.38 ± 0.32 (9)**	6.79 ± 0.42 (10)**

Number of experiments in parenthesis. Statistically significant values compared to control group: * $p < 0.01$; ** $p < 0.001$.

DA levels diminished in dopaminergic areas after PCS ($p < 0.01$) (table 2). Levodopa + LPDI administration produced a significant increase in DA levels in control and PCS groups ($p < 0.001$) but regional difference patterns were not apparent (table 2). The increase was higher in PCS rats. Statistical comparison of results was made by Student's t-test.

CA levels in the CNS vary early after PCS in rats, which means that hepatic alterations, such as a diminished portal blood flow, induce changes with regard to its metabolism. These changes are reflected in a depletion of CA levels in the olfactory tubercle and striatum nucleus after 7 days of PCS but are reversible by administrating levodopa associated with LPDI.

Zieve et al.[17] showed an improvement in rats with ammonia coma after levodopa, which could be accounted for by the peripheral effect of DA on renal function. However the DA and NE increases in dopaminergic and noradrenergic areas after administration of levodopa plus LPDI in rats after 7 days of PCS may indicate a central action.

The mechanisms of levodopa action in the CNS in this experimental model may be the inhibition of serotonin synthesis[18], the increase of oxygen cerebral consumption[11] and the increase of NE synthesis. All of these mechanisms are secondary to the increase of DA synthesis.

On the basis of these experimental results it is possible to believe that the improvement obtained after levodopa + LPDI administration in the same patients in an earlier state of PSE may be explained by an increase of the CA levels in the CNS.

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3 S. Sherlock, W.H.J. Summerskill, L.P. White and E.A. Phear, Lancet *2*, 453 (1954).
4 L. Zieve and D.M. Nicoloff, A. Rev. Med. *26*, 143 (1975).
5 H.O. Conn and M.M. Lieberthal, in: The hepatic coma syndromes and lactulose, p.46. Williams and Wilkins Co., 1979.
6 A.M. Hoyumpa, P.V. Desmond, G.R. Avant, R.K. Roberts and S. Schenker, Gastroenterology *76*, 184 (1979).
7 J.E. Fischer and R.J. Baldessarini, Lancet *1*, 75 (1971).
8 J.E. Fischer, in: Artificial liver support, p.31. Pitman, London 1975.
9 J.D. Parkes, P. Sharpstone and R. Williams, Lancet *2*, 1341 (1970).
10 M. Stefanini and E.N. Hetherington, J. Am. med. Ass. *220*, 1247 (1972).
11 M. Lunzer, I.M. James, J. Weinman and S. Sherlock, Gut *15*, 555 (1974).
12 H. Michel, M. Solere, P. Granier, G. Cauvet, J.P. Bali, F. Pons and H. Bellet-Hermann, Gastroenterology *79*, 207 (1980).
13 S.M. Lee and B. Fisher, Surgery *50*, 668 (1961).
14 J. Arias, F. Andres-Trelles and A. Alsasua, Archs Pharmac. Tox. *3*, 205 (1977).
15 D.M. Jacobowitz and J.S. Richardson, Pharmac. Biochem. Behav. *8*, 515 (1978).
16 J. Glowinsky and L.L. Iversen, J. Neurochem. *13*, 655 (1966).
17 L. Zieve, W.M. Doizaki and R.F. Derr, Gut *20*, 28 (1979).
18 M. Goldstein and R. Frenkel, Nature New Biol. *233*, 179 (1971).

The relation between cell proliferation and adenylate cyclase activity in grafts of 3-methylcholanthrene induced mouse uterine cervical tumors

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Summary. In transplanted uterine cervical tumors, induced by 3-methylcholanthrene, a significant negative correlation was obtained between adenylate cyclase activity and cell proliferation.

The role of cyclic adenosine 3′-5′-monophosphate (cAMP) in the control of growth in normal and malignant cells is still a matter of controversy[2-6]. Some of the discrepancies in opinion could be explained by different test cells being used; as a rule, chemical carcinogens or promoters increase the basal level of cAMP while tumorigenic viruses tend to decrease it. The cAMP level is crucial; abnormally high concentrations may arrest cell growth while moderate levels facilitate the growth of some cells. An active adenylate cyclase has been described in solid tumors[7,8] but great variations have been reported in hormone sensitivity, fluoride stimulation and basal activity[9-11].

In an earlier study[12] a trend could be traced for a correlation between adenylate cyclase activity and cell prolifera-

tion. As the sample size used was rather small we found it important to look for a possible significant correlation in a larger sample of tumors.

6-9-week-old female NMRI mice were used. Uterine cervical tumors were induced with 3-methylcholanthrene (Sigma) as described earlier[13]. After reaching palpable size, 2–4 small pieces of the tumors were grafted s.c. on the abdominal wall of newborn female mice, within 24 h after birth. About 2 weeks later growing tumor grafts were regularly found. Parts of these tumors were used for different purposes; for histological study, for studies on ^{3}H-thymidine incorporation or for retransplantation. The remaining part of the tumor was frozen and stored in liquid nitrogen for later homogenization. Some tumors have been transplanted for 30 generations. The 24 tumors used in this study were all derived from 4 primary tumors, with different generations being represented.

9 small pieces of each tumor graft were incubated in Leighton tubes (3 pieces in each tube), containing 2 ml medium supplemented with 5 µCi/ml of ^{3}H-methyl thymidine (^{3}H-TdR). The medium, incubation conditions, DNA determination and scintillation counting was as described earlier[12]. The mean value of the triplicates for ^{3}H-TdR incorporation per DNA unit (cpm/µg DNA) was used as a measure of cell proliferation.

The frozen tumor material was homogenized[12] and assayed for adenylate cyclase, using the method of Salomon et al.[14], with the modifications earlier described[12]. Linearity of enzyme activity with time (at least 10 min) and protein concentration (50–300 µg per assay) was checked in 10 homogenates. Adenylate cyclase activity was measured in triplicates, incubation time 10 min, protein concentration 200–250 µg. Results were expressed as pmoles cAMP produced per min and this value was related to mg DNA or protein in the homogenate[12].

In the figure (A and B) is illustrated the regression of basal adenylate cyclase activity, (pmoles newly synthesized cAMP per mg DNA or protein) on proliferative activity (incorporation of ^{3}H-TdR per µg DNA) in the tumor grafts. After transformation of the X-values to lnX, a significant straight line regression was obtained for adenylate cyclase activity on proliferative activity (per mg DNA: $y = 6234.2 - 853.3$ lnX; per mg protein: $y = 1241.7 - 176.6$ lnX). A variance analysis for the regressions resulted in the following F-values: that for adenylate cyclase per mg DNA = 9.48 at 1 and 22 d.f. ($0.01 > p > 0.001$) and that for adenylate cyclase per mg protein = 11.86 at 1 and 22 d.f. ($0.01 > p > 0.001$). Significant correlation coefficients were obtained irrespective of enzyme activity being related to DNA or protein content; in the former case $r = -0.55$ ($t = 3.08$ for 22 d.f., $0.01 > p > 0.001$), in the latter case $r = -0.59$ ($t = 11.86$ for 22 d.f., $p < 0.001$).

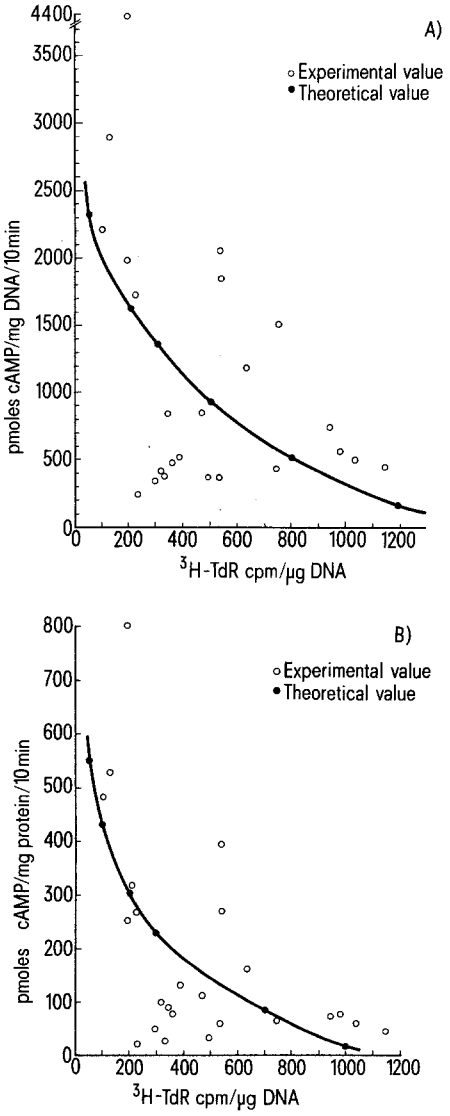
All the tumors used for this report were of the same well-differentiated type, without any obvious relation between histology and enzyme activity.

A significant association thus exists between basal adenylate cyclase activity and cell proliferation in our tumor grafts, the enzyme activity declining with increasing proliferative activity. Actively growing DMBA-mammary tumors have lower adenylate cyclase activity compared to spontaneously regressing or stable tumors[15]. Ovariectomy-induced regression of DMBA tumors is associated with increased cAMP content, and increased activities of adenylate cyclase and cAMP-phosphodiesterase activities[16,17]. Treatment with cAMP or agents elevating the intracellular level of cAMP often causes growth inhibition and death of tumor cells, both in vitro and in vivo[6,16]. Fast growing Morris hepatomas have a decreased basal activity of adenylate cyclase compared with normal liver tissue[7].

Because the neoplastic state may involve mutational changes at both major steps in the control of the cAMP level, the adenylate cyclase and cAMP-phosphodiesterase enzymes, no conclusions should be drawn from the cAMP level to the enzyme affected or from enzyme activity to the cAMP level. A malignant transformation may also interfere with cAMP-dependent protein kinase activity[18].

In this study, only basal adenylate cyclase activity was measured. In our earlier study[12], using the same type of tumor material, the activity was stimulated by NaF in homogenates. Moreover, the enzyme activity was increased after treating the host animals with progesterone[12]. These results indicate that the adenylate cyclase enzyme is sensitive to both NaF and hormones.

A comparison between adenylate cyclase activity in cervical tumor tissue and normal tissue is hardly possible. The normal uterine cervix is dominated by the stromal compartment while just the opposite occurs in tumors. Enzyme studies on normal cervical tissue will not be representative for the cell type from which the tumors originate.



The regression of basal adenylate cyclase activity (ordinate) on cell proliferation (abscissa). Non-transformed X-values. The adenylate cyclase activity was related to the DNA (A) or protein (B) content of the homogenate.

1 Reprint requests to J.-G.F., Department of Anatomy, Biskopsgatan 7, S-22362 Lund (Sweden).
2 A.L. Boynton, J.F. Whitfield, R.J. Isaacs and R.G. Tremblay, Life Sci. *22*, 703 (1978).
3 F.J. Chlapowski, L.A. Kelly and R.W. Butcher, Adv. Cyclic Nucleot. Res. *6*, 245 (1975).
4 L.I. Rebhun, Int. Rev. Cytol. *49*, 1 (1977).
5 W.L. Ryan and M.L. Heidrick, Adv. Cyclic Nucleot. Res. 4, 81 (1974).
6 M.J. Tisdale, Cancer Treatm. Rev. *6*, 1 (1979).
7 W.E. Criss, P. Muganda, A. Sahai and H.P. Morris, Adv. exp. Med. Biol. *92*, 489 (1977).
8 F.R. de Rubertis, R. Chayoth and J.B. Field, J. clin. Invest. *57*, 641 (1976).
9 R.A. Hickie, Adv. exp. Med. Biol. *92*, 451 (1977).
10 N.H. Hunt, J.R. Shortland, V.P. Michelangeli, J.C. Hammonds, D. Atkins and T.J. Martin. Cancer Res. *38*, 23 (1978).
11 R.M. Niles and J.S. Makaraski, J. Cell Physiol. *96*, 355 (1978).
12 S. Kvinnsland, P.E. Lønning and J.-G. Forsberg, Acta path. microbiol. scand. *88A*, 111 (1980).
13 J.-G. Forsberg and L. Stray Breistein, J. natl Cancer Inst. *49*, 155 (1972).
14 J. Salomon, C. Londos and M. Rodbell, Analyt. Biochem. *58*, 541 (1974).
15 C. Ip and T.L. Dao, Cancer Lett. *8*, 227 (1980).
16 S.J. Bodwin and Y.S. Cho-Chung, Cancer Lett. *3*, 289 (1977).
17 R.J. Matusik and R. Hilf, J. natl Cancer Inst. *56*, 659 (1976).
18 R.E. Scott and T. P. Dousa, Cancer Res. *40*, 2860 (1980).

Morphological alterations of basal cells of vaginal epithelium in neonatally oestrogenized mice[1]

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Summary. Neonatal oestrogen treatment results in the development of vaginal cancers in the mouse. A morphological sign which probably indicated early invasion of altered vaginal cells into the stroma through gaps in the basal lamina was first seen at 10 months of age in neonatally oestrogenized C57Black mice. Prior to this, a decrease in the numbers of cellular attachment organelles such as half desmosomes and desmosomes was observed by 3 months.

Neonatal treatment of mice with sex steroids causes a hormone-independent, irreversible proliferation of cornified vaginal epithelial cells, which is finally converted to neoplastic growth at an advanced age[2-4]. It is also known in the human that vaginal cancers appear in the daughters of mothers given diethylstilbestrol at a critical stage during pregnancy[5]. Since it has been reported that absence and/or disruption of the basal lamina may occur at an earlier stage of local invasion of cancer cells[6], we have attempted to investigate the morphological evidence suggesting early signs of the invasive growth of the mouse vaginal cells possibly converted to neoplastic cells by neonatal exposure to oestrogen.

Materials and methods. Mice used in this experiment were C57Black/Tw strain. This strain originated from C57Black/6 transferred to the Zoological Institute, Faculty of Science, University of Tokyo in 1963 from the National Institute of Genetics, Mishima, Japan, and was maintained by the strict brother × sister mating. 70 female mice were injected s.c. with 20 µg of 17β-oestradiol in 0.02 ml of sesame oil for the first 5 days after birth. 81 controls were given the vehicle only. Mice of both groups were sacrificed at intervals from 3–24 months of age. Histological and electron microscopic studies were performed on the vaginae of oestrogenized and control mice. Paraffin sections of vaginae were stained with haematoxylin and eosin or the alcian blue-PAS technique[7]. For electron microscopy, thin sections of vaginae, fixed in glutaraldehyde-paraformaldehyde, post-fixed in osmium tetroxide, and embedded in epoxy resin, were stained with uranyl acetate and lead citrate. Numbers of half desmosomes, desmosomes and mitochondria per vaginal basal cell cross section were counted on electron microscope photographs.

Results and discussion. In control mice, the basement membrane of the vaginal epithelium stained red with the alcian blue-PAS technique, indicating the presence of glycoproteins (fig. 1a). At 3–10 months of age, vaginal basal cells rested on a basal lamina with a distinct and continuous contour. At 12 months and thereafter, duplication and folding of the basal lamina were observed in some areas; and at 18 months, it was not infrequently found to be discontinuous. In oestrogenized mice, the basement membrane stained red with the alcian blue-PAS technique; however, the intensity of staining was usually weak and its contour was obscure (fig. 1b). Further disruption of the basal lamina was often observed at 3 months, and frequent-

Numbers of half desmosomes, desmosomes and mitochondria per basal cell cross section*

Groups	Age at autopsy (months)	Half desmosomes	Desmosomes	Mitochondria
Oestrogenized	3	$14.2 \pm 0.5^{abc**}$	7.9 ± 0.4^{g}	11.0 ± 0.5^{k}
	12	11.0 ± 0.7^{ad}	7.2 ± 0.5^{h}	11.4 ± 0.7^{l}
	18	11.2 ± 0.5^{b}	7.2 ± 0.3	11.7 ± 0.8
Control	3	21.9 ± 0.9^{ce}	10.4 ± 0.4^{gi}	16.8 ± 0.6^{km}
	12	19.7 ± 0.7^{df}	10.8 ± 0.4^{hj}	14.7 ± 0.6^{ln}
	18	12.1 ± 0.6^{ef}	5.8 ± 0.5^{ij}	10.6 ± 0.7^{mn}

* Numbers of half desmosomes, desmosomes and mitochondria per vaginal basal cell cross section were calculated for 30 cells from each mouse. All groups consisted of 5 mice and data obtained were assessed by Student's t-test. ** Mean values which have the same superscripts are significantly different each other at 0.01 level.