without added cAMP. The TCA precipitable ^{32}P was counted on a Packard liquid scintillation spectrometer and the total pmole of phosphate incorporated from the known specific activity of ^{32}P-ATP in the reaction mixture was calculated. Fischer rats carrying sarcoma MCA, R3230 AdCa, mammary carcinoma AAF and DMAAB hepatoma for 27 days (tumor weight was 2–5 g) and control rats were bled then sacrificed by cervical dislocation and their spleens removed into cold HBSS. Spleen cells and spleen lymphocyte suspensions were prepared and homogenized in MES buffer. Aliquots of the $50{,}000 \times g$ supernatants were assayed for the cAMP-dependent protein kinase activity at varying concentrations of cAMP. Statistical significance of the data was determined by Paired Student's test using $n=4$.

Results and discussion. The protein kinase assay was standardized using the enzyme from normal rat spleen cells and spleen lymphocytes. The reaction was linear over the range of 1.25 to 20×10^5 cells, has a K_m for histone phosphorylation of 10 µg/ml and has K_a and V_{max} values for cAMP activation of 3 mM and 17.8 pmoles/min/1×10^6 cells, respectively. The pH optimum is 7.0 and the reaction is linear from 25 to 40 °C.

The results in figure 1 show that there is a loss of cAMP-dependent protein kinase activity in spleens of tumor-bearing animals. The decrease was highest in CMA-bearing (highly immunogenic) and lowest in R3230 AdCa-bearing rats (lack detectable immunogenicity). The results in figure 2 show that blood lymphocytes from tumor-bearing animals displayed a decrease in cAMP-dependent protein kinase activity. The magnitude of the decrease was highest in lymphocytes of CMA-bearing and lowest in lymphocytes of R3230 AdCa-bearing rats. There appears to be a correlation between the loss of cAMP-dependent protein kinase activity and the immunogenicity of the tumor.

Studies on enzyme variations as a consequence of cell-mediated immune response should yield useful information regarding this complex phenomenon. Studies from these laboratories[4,7,8] demonstrated that when incorporated into the culture media of human mammary adenocarcinomas, protease inhibitors accelerated cAMP accumulation and sustained proliferation of human neoplastic cells. The results obtained from the described experiments indicate that cAMP-dependent protein kinase is depressed in whole spleens and lymphocytes of tumor-bearing animals. The magnitude of depression increased with the immunogenicity of the tumor. The results in table 2 indicate a possible relationship between accumulation of cAMP in and immunogenicity of the tumor cells. Although the level of cAMP is lowered in transformed cells[13–15], it is definitely increased in progressively growing tumors. The magnitude of the increase is inversely proportional to the immunogenicity of the tumor.

The results in table 3 summarize the relationship between protein kinase activities of the tumor cells on various substrates in presence and absence of cAMP. All the 4 tumors contained cAMP-dependent protein kinase. The enzyme activities were highest in phosphorylating histone. The magnitude of the activity varied with the immunogenicity of the tumor. Studies of the enzyme specific activities of adenosine 3′,5′-monophosphate-dependent protein kinase in tumor cells and spleen lymphocytes of tumor-bearing animals are in progress.

1 Mailing address: 180 Longwood Drive, Kankakee, Illinois 60901, USA.
2 C.W. Parker, T.J. Sullivan and H.J. Wadner, Adv. Cyclic Nucleotide Res. *4*, 1 (1974).
3 J.W. Hadden, R.G. Coffey and F. Spreafico (eds), Immunopharmacology, p.1. Plenum Medical Book Co., New York 1977.
4 A.A. Hakim, Proc. 3rd Int. Conf. Cyclic Nucleotides, Th.P 254, New Orleans 1977.
5 A.A. Hakim and N.G. Grand, J. pharm. Sci. *65*, 339 (1976).
6 A.A. Hakim, Pharm. Acta Helv. *51*, 98 (1976).
7 A.A. Hakim, Naturwissenschaften *61*, 222 (1974).
8 A.A. Hakim. XXVI Int. Cong. Physiol. Sci., vol. xi, New Delhi 1974.
9 T.M. Simon, A. Lorber and H.T. Homes, J. immun. Meth. *12*, 193 (1976).
10 W. Boyles, Transplantation *6*, 761 (1965).
11 H.G. Nimmo and P. Cohen, Cyclic Nucleotide Res. *8*, 145 (1977).
12 J.D. Corbin, T.R. Soderling and C.R. Park, J. biol. Chem. *218*, 1813 (1973).
13 J. Otten, G.S. Johnson and I. Pasten, Biochem. biophys. Res. Commun. *44*, 1192 (1971).
14 M.L. Heidrich and W.L. Ryan, Cancer Res. *21*, 1313 (1971).
15 J.R. Sheppard, Nature, New Biol. *236*, 14 (1972).

Cell division and cell death during regression of the chick embryo Müllerian ducts

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Summary. In 9-day-old chick embryos, decreased DNA synthesis and enhanced necrosis were observed in a defined area of the right female Müllerian ducts, supporting the idea of the existence of a regression process in this organ. In the male ducts, decreased DNA synthesis and a low level of necrosis were present all over the studied portion of the organ.

The mechanism of the regression of the Müllerian duct (MD) is not well understood and there are opposite views concerning the regression of the right female duct. Groenendijk-Huijbers[3] has called it the 'female-type regression', others[4,5] however, claimed that the organ does not regress but merely stops growing. Necrosis in the right female duct has been previously reported[6]. In the present work DNA synthesis is measured in different areas of the MD to see whether the rate of cell division is affected all over the organ, or if it is localized in preferential zones.

Materials and methods. MD from 9-day-old chick embryos were excised still attached to the mesonephros. In this way it was possible to manipulate the anterior region of the MD without touching it directly. Because of handling difficulties, the posterior part of the organ was not studied. For comparison with the MD of 9-day-old embryos, ducts of 8-day-old and 10-day-old embryos were also analyzed. The genetically sexed[7] 8-day-old embryos were kindly supplied by Dr J.M. Gasc (Institut d'Embryologie du C.N.R.S. et du Collège de France, Nogent-sur-Marne, France). The incorporation of tritiated thymidine (40 µCi ^{3}H-methyl thymidine/ml tyrode solution, pH 7.2, s.a. 25 Ci/mmole, CEA Saclay, France) was carried out for 1 h in an incubator at 38 °C with constant shaking. The MD were fixed in 95%

ethanol and acetic acid (3:1) and embedded in paraffin following the classical method. Slides with histological sections of 6.6 µm were made and after deparaffinization they were treated with cold 5% perchloric acid to eliminate acid-soluble molecules, dipped in Ilford K5 emulsion diluted 1:1 with water and exposed for 2 weeks. The autoradiographs were developed in Kodak microdol X and fixed in 20% sodium thiosulfate. The amount of autoradiographic silver grains was measured with a Leitz microphotometer, the sensitivity being adjusted in the same way for all the measurements. The anterior part of the MD was divided into 6 zones (I–VI), each of them containing 5 sections. A total of 30 equidistant sections with identical areas were analyzed throughout the total length of the duct between the ostium and the posterior part of the mesonephros of 9 male and 10 left and right female ducts. 4 measurements in different places (containing about 5 cells) of each section were carried out. These 4 values were added together to give the labelling rate of the section, expressed in arbitrary units. The background was substracted automatically. Mitoses were scored on Feulgen-stained sections of the 6 zones in 3 ducts of each type. About 260 cells in the epithelium and 2160 in the mesenchyme were counted per zone; the mitotic indices are given as a percentage of total cell counts. Sections containing necrotic clusters were also scored. The labelling rate of the individual nuclei of the zones were established as described previously[6].

Results and discussion. Comparing the different zones of the developing left female MD (figures 1, A, and 2, A), one can observe a significantly higher level of DNA synthesis in the posterior part (especially zone VI of the epithelium) of the duct. The mitotic indices and the DNA labelling rates are significantly lower in the mesenchyme as compared to the epithelium. No necrosis was observed in either case. The level of incorporation of tritiated thymidine into the nuclei of both the epithelium and mesenchyme of the male MD is significantly lower than the incorporation level in the left and right female ducts (figures 1 and 2). This is in agreement with earlier observations showing reduction of DNA content in the regressing male MD[8]. Overall DNA synthesis in the organ was also lower than in the developing left female ducts[6]. No significant difference in mitotic indices was observed between the zones and necrosis is present all over the analyzed portion of the male duct. In the right female duct of 9-day-old embryos, reduced DNA synthesis was found in zone IV, accompanied by the presence of necrotic clusters which sometimes occupied the entire section of the autoradiographs. No necrosis was present in the anterior zones where particularly high DNA synthesis and mitotic indices were observed (figures 1, B, and 2, B). The high DNA synthesis level was the result of heavy labelling of the individual nuclei (table 1). A comparison of DNA synthesis in the MD epithelium and mesenchyme of the 9-day-old embryos with that of younger and older embryos is shown in table 2. Incorporation of tritiated thymidine decreases gradually in MD of 8–10-day-old embryos. This diminution seems to be more rapid in zone IV.

The question concerning the regression or non-regression of the right female ducts have given rise to controversy. Lutz-Ostertag[9] and Groenendijk-Huijbers[3] reported a cranio-caudal (female-type regression). On the other hand, Thiébold[4] and Vergnaud[5] stated that the organ does not really regress but merely stops growing. The observations described here support the idea of the existence of a regression process in the right female duct. In the 10 organs examined, a zone of low DNA synthesis was found frequently accompanied by extensive cell necrosis. The occurrence of necrosis in the right female duct (absent in the left duct) has already been shown in a previous report[6]. It appeared here that, contrary to the male duct, necrosis is not present all over the right female duct. In particular the ostium, which is a part of zone I, was not affected. In this part of the duct, the rate of DNA synthesis was significantly higher than in the corresponding zones of the developing female duct. This unexpected high thymidine incorporation in the anterior part of the right MD compared to the left one is not necessarily in contradiction with the degenerative state of the right duct. The simultaneous presence of particularly high and low levels of DNA synthesis in declining cellular systems has been previously observed. It has been shown that in the regressing male ducts a small amount of nuclei had a particularly high DNA synthesis level, while in a great number of them DNA synthesis was low[6].

Other examples of high incorporation of ^{3}H-thymidine into injured cells have been observed, for example in irradiated chick fibroblasts[10], in irradiated cancer cell cultures[11] and also in irradiated MD[6]. This phenomenon has been explained[10] as an increase in the number of initiation sites for DNA synthesis by irradiation. This could also be the explanation for the increased DNA synthesis in the anterior part of the right female MD. Another explanation of this high DNA synthesis level might be the occurrence of increased permeability of the cell membrane in the first phase of cell degeneration in the right female duct. As a matter of fact, increased cell permeability has been observed in aging human fibroblasts[12]. The high mitotic

Table 1. Mean incorporation of tritiated thymidine into individual nuclei of left and right female Müllerian ducts of 9-day-old embryos expressed in arbitrary units

Zones	Epithelium		Mesenchyme	
	Left ♀ MD	Right ♀ MD	Left ♀ MD	Right ♀ MD
I	68±10*	102±14*	78±17*	122±22*
II	67±11	79±13	83±15*	130±20*
III	73±10	76±12	104±18	114±19
IV	64±7*	50±12*	89±11	81±15
V	78±12	81±13	111±15*	133±23*
VI	85±10	95±14	110±14*	179±30*

Uncertainties are SD of the means calculated for $p = 0.05$. * Differences between left and right female MD are significant.

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Table 2. Comparison of mean incorporation of tritiated thymidine into right female Müllerian ducts of 8-, 9- and 10-day-old embryos. The labelling rate of the sections in the different zones are expressed in arbitrary units

Zones	Epithelium (right ♀ MD)			Mesenchyme (right ♀ MD)		
	8-day	9-day	10-day	8-day	9-day	10-day
I	153±20	134±32*	89±12*	93±25	93±26*	54±22*
II	140±28	120±18*	75±25*	110±20	100±20*	63±28*
III	111±20	104±24*	76±30*	107±31	91±24*	55±21*
IV	98±26*	66±20*	47±15	84±15*	68±12	53±22*
V	124±30	109±24*	76±24*	124±23	105±28*	58±30*
VI	130±18	123±28*	78±40*	122±38	136±44*	73±29*

Uncertainies are calculated for $p = 0.05$. * Differences between the values of the zones are significant.

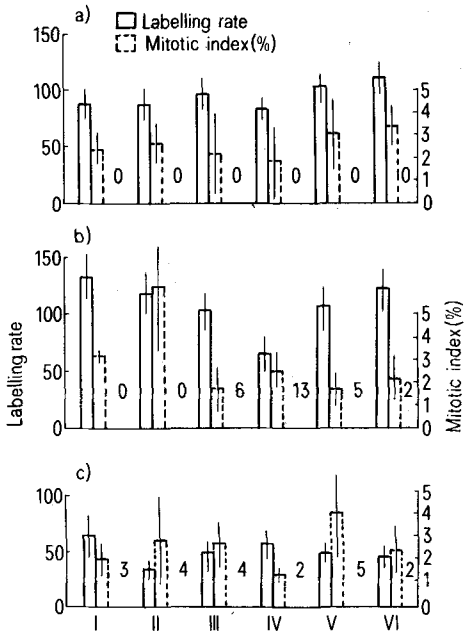

Fig. 1. DNA labelling expressed in arbitrary units, mitotic indices (%) and number of necrotic clusters (arabic numerals) observed per section in 6 (I–VI) zones of the left female (a), right female (b) and male (c) Müllerian ducts' epithelium. The values found for each zone of all ducts were pooled and the differences analyzed with the t-test. The uncertainties are SD of the mean calculated for probability $p = 0.05$. Left: the anterior zones; right: the posterior zones.

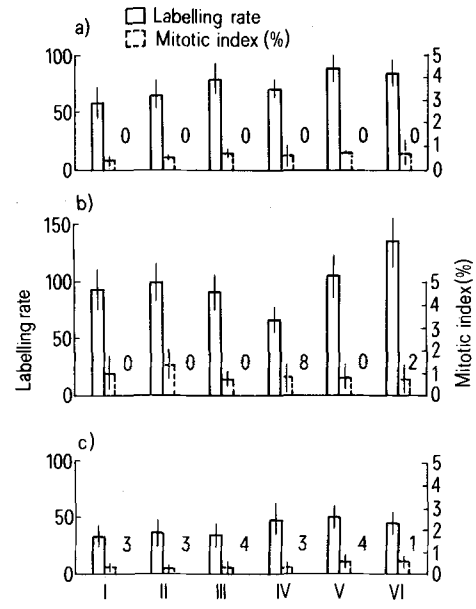

Fig. 2. DNA labelling expressed in arbitrary units, mitotic indices (%) and number of necrotic clusters (arabic numerals) observed per section in 6 (I–VI) zones of the left female (a), right female (b) and male (c) Müllerian ducts' mesenchyme. The values found for each zone of all ducts were pooled and the differences analyzed with the t-test. The uncertainties are SD of the mean calculated for probability $p = 0.05$. Left: the anterior zones; right: the posterior zones.

indices in this zone might be occasioned by mitotic arrest of the cells and mitotic death[13]. Thus, localized cell degeneration (which is not the result of mechanical injury and which is too extensive to be attributed to 'unspecific necrosis') and reduced DNA synthesis might indicate that the right female MD actually degenerates to a certain extent, starting in the more median zones.

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3 M.G. Groenendijk-Huijbers, Anat. Rec. *142*, 9 (1962).
4 J.J. Thiébold, C.r. Soc. Biol. *167*, 338 (1973).
5 O. Vergnaud, R. Maraud and R. Stoll, C.r. Soc. Biol. *167*, 250 (1973).
6 R. Beaupain, Radiat. Res. *75*, 66 (1978).
7 J.M. Gasc, C.r. Acad. Sci. Paris *277*, 1925 (1973).
8 T.H. Hamilton and C.S. Teng, in: Organogenesis, p. 681. Ed. R.L. De Haan and H. Ursprung. Holt, Rinehart and Winston, New York 1965.
9 Y. Lutz-Ostertag, Bull. Biol. *88*, 333 (1954).
10 C.M.A. Kuyper, S. Liébecq-Hutter and S. Chèvremont-Comhaire, Exp. Cell Res. *28*, 459 (1962).
11 R. Beaupain, C.r. Acad. Sci. Paris 274, 588 (1972).
12 L. Berumen and A. Macieira-Coelho, Mech. Age Dev. *6*, 165 (1977).
13 L.E. Hopwood and L.Y. Tolmach, Radiat. Res. *46*, 70 (1971).

Synapse-like profiles in regenerating sensory nerve fibres of Herbst corpuscles

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Summary. During regeneration sequences of Herbst corpuscles, the synapse-like structures are described between pairs of non-myelinated sensory profiles. The probable significance of this unusual for sensory corpuscles finding is discussed.

It is well known that ultrastructural criteria for presumptive chemical synapses have not been elaborated in avian and mammalian mechanoreceptors[1-3]. During the electron microscopical investigation of regenerated Herbst corpuscles[4], it has been noted that one of the most characteristic feature of the new receptors is the presence of numerous non-myelinated nerve fibres in their inner core. The regenerated axons are usually encircled by long cytoplasmic processes of the Schwann receptor cells which subsequently build up the inner core of the receptors. Some of the axonal profiles lie close to each other and between them synaptoid or synapse-like contacts have been observed. The precise description of this unusual finding is an object of the present communication.

Material and methods. The 22 ducks were used as an experimental material. The animals were divided into 2 groups. The suborbital nerve branches of the 1st group were crushed, whereas the same branches of the 2nd group