obtained was corrected according to Bach[8]. From each block, a silver ultrathin section was double stained with uranyl acetate and lead citrate and microphotographed at a primary magnification of 4000. The exact final magnification for each set of electron micrographs was calibrated by means of a carbon grating replica; as an extreme variation of less than 5% of the mean was found, all the calculations were made using only the mean value (×12,000). From each ultrathin section, 5 electron micrographs were recorded; fields were chosen at random and those not containing nuclear profiles of thyroid cells were discarded. A double quadratic lattice test system of 400 points and 225 cm^2 was used to calculate the fractions of the nuclear volume occupied by nucleoli and by nuclear bodies (V_v), the surface-to-volume ratio of nuclei (S/V) and the number of nucleolar and nuclear bodies profiles per unit area of nuclear profiles (Na). In order to determine all these parameters, the techniques described by Weibel and Bolender[9] were used; no corrections were made concerning Holmes effect. The classification of the nuclear bodies was made according to Bouteille et al.[10]. Individual morphometric data were averaged and SD and SE were calculated. In order to compare the results, Student's 2-sided test was used; 2 means were considered significantly different if the probability of error (p) was smaller than 0.05.

Results and discussion. At the optic level, most of the nuclei of the carcinomas displayed a ground-glass appearance and contained prominent nucleoli, while those of goitres had coarse chromatin and rarely contained prominent nucleoli.

The statistical analysis of the results obtained in the morphometric study is summarized in the table and shows several significant differences between the nuclei of the goitre group and those of both types of thyroid carcinomas, as well as a significantly higher surface-to-volume ratio of the sclerosing carcinoma nuclei in comparison with those of common papillary carcinomas. Furthermore, it shows that the increased volumetric density of nucleoli in carcinomas, when compared with goitres, does not depend on an increased number of nucleoli, but on an increase of their individual volumes, which is in keeping with their prominence in light microscopy.

The significant positive correlation ($r = 0.555$; $p < 0.05$) that was found between the surface-to-volume ratio of the nuclei and the volumetric density of their nucleoli, matches Burns et al.[11] statement about the close relationship between the size of the nucleo-cytoplasmic contact and the degree of nucleolo-cytoplasmic interaction, and points to increased nuclear and nucleolar activities in papillary thyroid carcinomas.

Significant positive correlations ($r = 0.701$; $p < 0.005$ and $r = 0.770$; $p < 0.001$) were also found between the volumetric density of nucleoli and the volumetric density of nuclear bodies, as well as between the volumetric density of nucleoli and the number of complex nuclear bodies per unit area of nuclear profiles. Since it is known that the central core of complex nuclear bodies is made of ribonucleoproteins[12], these findings also point to an increased nucleolar activity in papillary thyroid carcinomas, although they do not rule out the hypothetical involvement of a disturbed RNA metabolism in their pathogenesis[7].

8 G. Bach, in: Quantitative Methods in Morphology, p. 23. Ed. E. R. Weibel and H. Elias. Springer-Verlag, Berlin and New York 1967.
9 E. R. Weibel and R. P. Bolender, in: Principles and Techniques of Electron Microscopy, p. 239. Ed. M. A. Hayat. Van Nostrand Reinhold Company, New York 1973.
10 M. Bouteille, S. R. Kalifat and J. Delarue, J. Ultrastruct. Res. *19*, 474 (1967).
11 E. R. Burns, B. L. Soloff, C. Hanna and D. F. Buxton, Cancer Res. *31*, 159 (1971).
12 A. M. Dupuy-Coin, S. R. Kalifat and M. Bouteille, J. Ultrastruct. Res. *38*, 174 (1972).

Invasive properties of the ovarian cortex in birds

M. Callebaut[1]

Laboratorium voor Anatomie en Embryologie, R. U. C. A., Groenenborgerlaan 171, B–2020 Antwerpen (Belgium), 31 May 1977

Summary. In quail/chicken ovarian cortex associations , grown on chicken chorioallantoic membrane (CAM), epithelial and/or germ cells from the quail ovary may invade the cortical rim of the chicken ovary. In certain experimental conditions, ovarian cortical cells may leave a transplanted ovary and grow over or invade the mesenchyme of the surrounding CAM. So a germinal epithelium completely free from other ovarian cell groups can be obtained.

In a recent study[2] we have found experimental evidence that, in the embryonic Japanese quail ovary (transplanted on CAM), surface epithelial cells penetrate into the ovigerous cords and finally give rise to the development of follicle cells. The present investigation demonstrates that the penetration potentialities of this surface epithelium and/or its derivatives are not necessarily limited to its own cortex.

Material and methods. A. Quail-chicken ovarian cortex associations: left ovaries of 9- to 11-day-old chicken or Japanese quail embryos were used for this study. First the quail ovary, with its cortical side directed upwards, is transplanted on the CAM of an 8- to 9-day-old chicken embryo, according to the technique of Harris[3]. 1 day later, a chicken ovary of the same age is placed crosswise over the quail ovary, taking care that part of the ovarian cortex of both ovaries remains in close contact.

B. Quail ovarian cortex in contact with the chorionic epithelium of chicken CAM: ovaries (with their cortical side downwards) from 16-day-old quail embryos or parts from 9-day-old quail ovaries, already grown for more than 1 week on CAM, are grafted on the CAM of 8-day-old chicken embryos. 7–10 days later, grafts from both experimental group A and B were excised and fixed in acetic-alcohol (1:3) for 1 h. After embedding in paraffin, the transplants were sectioned at 7 μm thickness. After deparaffination, the sections were stained with the PAS

1 The author is very grateful to Prof. Dr L. Vakaet, R.U.C.A., Antwerp, for his valuable suggestions and to Mrs S. De Wolf-Van Rompaey, for her excellent technical assistance.
2 M. Callebaut, Experientia *32*, 1337 (1976).
3 J. Harris, Ann. Acad. Sci., New York *76*, 764 (1958).

technique[4]. Nuclear counterstaining was performed with methylgreen (0.2% in water, during 15 min) followed by rapid alcoholic dehydration (15 sec, in each bath). Some sections were also stained by the Feulgen nucleal reaction.

Results and discussion. The PAS-methylgreen staining allows: 1. To distinguish somatic quail nuclei from somatic chicken nuclei. Indeed, the quail nuclei present a typical chromatin distribution (first described by Callebaut[5]). 2. Owing to the feeble concentration of the methylgreen solution, the PAS stainable structures (e.g. the basement membrane) remain clearly visible.

A. Sections through quail-chicken ovarian cortex associations, transplanted on CAM, show that at its rims part of the surface epithelium of the chicken ovary has been ingrown by surface epithelial cells from the quail ovary (figure 1). From there, numerous quail epithelium cells invade the chicken ovigerous cords, However, the quail cells always remain localized within the boundaries of the ovigerous cords (separated from the surrounding chicken ovarian stroma by the continuous PAS positive basement membrane). A migration of chicken somatic cells into the quail germinal epithelium and its derivatives can sometimes be observed, but not with the same accuracy as the reverse migration. Indeed in chicken-quail cell associations, a single cell whose nucleus does not represent the typical quail aspect is not necessarily a chicken cell. Therefore we have only concluded for the migration of chicken cells into the quail ovigerous cords, when at least a group of cells without the typical quail chromatin distribution was distinguishable. Our previous descriptions of the cytological characteristics of oogonia, reticulated nuclei, early and late preleptotene stages after the Feulgen nucleal staining in both the chicken[6,7] and quail ovary[8,9] made it possible to distinguish quail – from chicken female germ cells. So, migration of some germ cells from the ovarian rim of one species into the ovarian rim of the other species could be observed. When during early development the germ cells colonize the gonad and reach the surface epi-

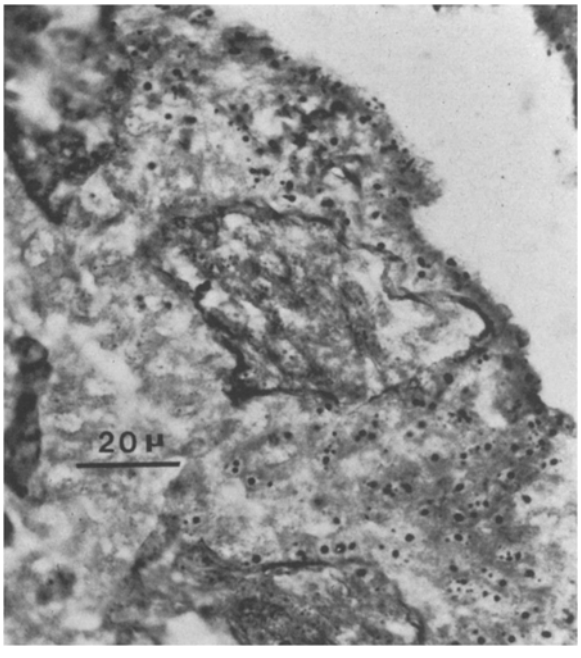

Fig. 1. Section through the rim of a chicken ovarian cortex grown on CAM in the immediate neighbourhood of a quail ovarian cortex. The nuclei of the invading quail epithelial cells present usually 1 or more intensely staining central or subcentral chromatin granules, surrounded by a clear zone. PAS-methylgreen stain.

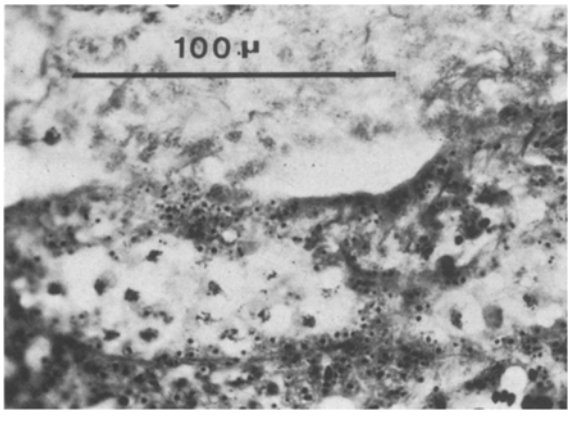

Fig. 2. 2 Contact zones between the central part of a quail cortex (bottom) and the central part of a chicken ovarian cortex (top), grown close together on CAM during 8 days. No intermingling of quail and chicken cells occurs. PAS-methylgreen stain.

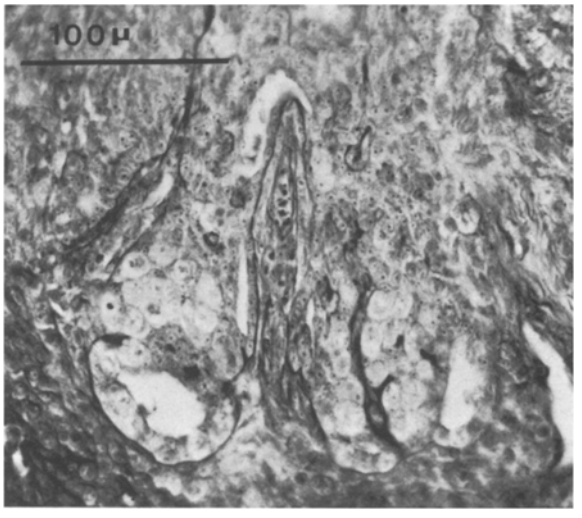

Fig. 3. Invasion of chicken CAM mesenchyme by quail ovarian cortex. Several cortical buds have developed from a common stalk. PAS-methylgreen stain.

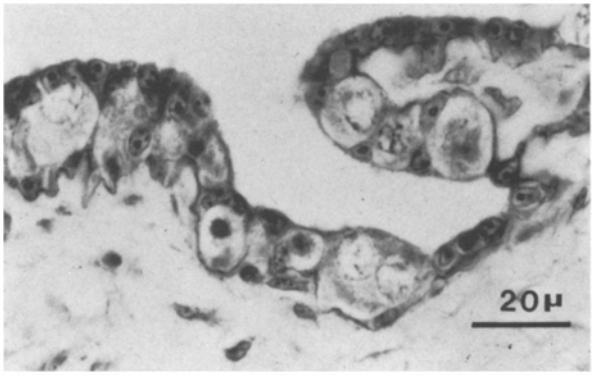

Fig. 4. Section through part of the wall of a paraovarian CAM cavity lined by quail germinal epithelium containing oocytes at beginning diplotene (after 2 successive 10 day sojourns on CAM). Note the absence of quail medullary tissue in the CAM mesenchyme (bottom). PAS-methylgreen stain.

thelium cells by chimiotactic attraction[10], the latter present a syncytial aspect, probably caused by lytic activity of the former[11]. Since the rims of the ovarian cortex in birds are much less advanced in development than the central parts, it may be possible that such phenomena still occur in our experimental conditions. Where the central part of the surface of the quail's ovarian cortex comes in close contact with the corresponding central part of the chicken's ovarian cortex, no invasion of quail cells into the latter (or vice-versa) can be seen (figure 2).

B. Part of the cortex of some transplanted ovaries from 16-day-old quail embryos penetrates into the underlying CAM mesenchyme. The ingrowing quail cell mass may invade even the deeper layers of the CAM mesenchyme, at a considerable distance (300 µm) from its point of origin. Several cortical buds may develop from a common stalk (figure 3). In the central part of the buds, usually a lumen appears. The lumen is lined by a narrow layer of quail oocytes and quail epithelial cells, separated from the CAM mesenchyme (composed of chicken somatic cells) by the PAS-positive basement membrane. Between the chicken somatic cells of the CAM mesenchyme surrounding the buds, some quail cells may be seen. These cells are accompanying ovarian stroma or medullary cells. The buds formed by the ingrowing quail ovarian cortical cells have some resemblance to the acini found in the gonads of gastropods[12].

Developing transplants are usually progressively enclosed in a paraovarian CAM cavity. After a prolonged sojourn (for instance after 2 successive transplantations on CAM), the quail germinal epithelium and oocytes are able to separate themselves completely from the medullary tissue, by invading the epithelial lining of this paraovarian CAM cavity (figure 4). As contrasted with the opinion of other authors[13,14], our results seem to indicate that an isolated germinal epithelium (completely free from adhering ovarian cells) is no longer able to give rise to/or to induce the formation of medullary tissue. Our observations are in agreement with the conclusions of Erickson[15] that the factors that control the differentiation of the female germ cells reside in the cortex ovarii.

4 J. Macmanus, Nature, Lond. *158*, 202 (1946).
5 M. Callebaut, Experientia *24*, 1242 (1968).
6 M. Callebaut, J. Embryol. exp. Med. *18*, 299 (1967).
7 M. Callebaut, Experientia *25*, 978 (1969).
8 M. Callebaut, Experientia *29*, 471 (1973).
9 M. Callebaut. Thesis. Ruca, Antwerpen 1975.
10 D. Simon, Archs Anat. microsc. Morph. exp. *49*, 93 (1960).
11 D. Cuminge and R. Dubois, C. r. Acad. Sci., Paris *268*, 1200 (1969).
12 M. De Jong-Brink, Cell Tissue Res. *171*, 195 (1976).
13 E. Wolff and K. Haffen, Archs Anat. microsc. Morph. exp. *48b*, 331 (1959).
14 D. Scheib and K. Haffen, J. Microsc. Biol. Cell *25*, 237 (1976).
15 G. F. Erickson, Devl Biol. *36*, 113 (1974).

Association of asymmetric unit membrane plaque formation in the urinary bladder of adult humans with therapeutic radiation[1]

J. Alroy, B. U. Pauli, R. S. Weinstein and F. B. Merk

Departments of Pathology, Rush-Presbyterian-St. Luke's Medical Center, 1753 W. Congress Parkway, Chicago (Illinois 60612, USA), and Tufts University School of Medicine, Boston (Massachusetts 02111, USA),
14 January 1977

Summary. Asymmetric unit membrane (AUM) is a component of the luminal membrane of urinary bladder in many species. In normal human adults it is inconspicuous, but it becomes prominent following incidental exposure to therapeutic irradiation.

One component of the luminal membrane of normal bladder urothelium is 'asymmetric unit membrane' (AUM) plaques. These plaques are present in many species[2]. They are found in humans during childhood, but are relatively inconspicuous in adults[3]. The current report describes an increase in AUM-plaques in areas of normal appearing urothelium of adults who have been irradiated for transitional cell carcinoma elsewhere in the urinary bladder.

Materials and methods. Biopsies of histologically normal appearing urothelium were obtained during surgery from 2 categories of patients: a) 7 adult patients without a history of bladder irradiation; 4 who had benign lesions, i.e. urethral obstruction or benign prostatic hyperplasia, and 3 who had transitional cell carcinomas elsewhere in the bladder; and b) 7 adult patients who had histories of irradiation for transitional cell carcinomas. The 7 irradiated patients had received 5000 R to the bladder. One was irradiated a month and a half, four 3 months, one 4 months and one 4 years prior to the biopsy. We also examined histologically normal bladder urothelium obtained at the autopsies of 3 human neonates who were free of genitourinary disease and were never irradiated. The specimens were cut into 1–2 mm³ tissue blocks and fixed in cold 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h. They were post-fixed in 0.1 M cacodylate buffer with 1% osmium tetroxide, dehydrated through graded ethanol solutions and embedded in Epon 812. 50–70 nm thin sections were cut for electron microscopy on an LKB 8801 A ultramicrotome. They were stained with uranyl acetate and lead citrate and photographed with a Philips EM 300 electron microscope. In addition, glutaraldehyde-fixed 1–2 mm³ tissue blocks from 2 irradiated bladders were soaked in 20% glycerol in 0.1 M Millonig's phosphate buffer and then freeze-fractured at –100 °C in a Balzers model BAF 301 M freeze-etch machine, by the method of Moor and Mühlethaler[4].

Results and discussion. The luminal membranes of superficial urothelial cells of newborn humans contain abundant AUM-plaques which are morphologically identical to those described in other species[2]. As is typical of AUM-plaques in general, the human AUM-plaques are 12 nm

1 This work was supported by the Otho S.A. Sprague Memorial Institute.
2 J. A. Firth and R. M. Hicks, J. Anat. *116*, 31 (1973).
3 F. B. Merk, B. U. Pauli, J. B. Jacobs, J. Alroy, G. H. Friedell and R. S. Weinstein, Cancer Res. *37*, 2843 (1977).
4 H. Moor and K. Mülthaler, J. Cell Biol. *53*, 609 (1963).