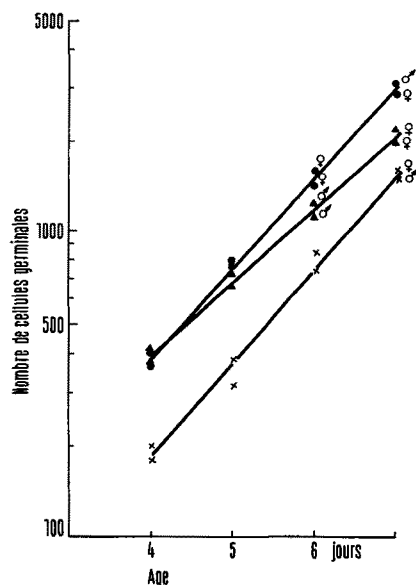


des temps demeurant linéaire, on peut comparer la variation dans le temps de la multiplication de ces cellules chez les 3 espèces (Figure).

1. Comme chez le Poulet et le Dindon, on se trouve chez la Caille dans une phase d'accroissement exponentiel de la population des cellules germinales: les points de référence sont pratiquement alignés. Ce type de progression paraît être assez général<sup>3</sup> et caractéristique de la période considérée puisque les travaux de CHRÉTIEN<sup>4</sup> révèlent une croissance exponentielle de la population des gonocytes de Lapin entre 9 et 14 jours post coïtum, c'est-à-dire également durant la période précédant la différenciation sexuelle.

2. Le nombre de gonocytes primaires est à peu près le même (voisin de 400) chez la Caille et le Poulet de 4 jours, alors qu'il est inférieur de moitié chez le Dindon. Ce fait ne peut apparemment pas s'expliquer par le développement embryonnaire plus lent du Dindon



Evolution des populations de cellules germinales, entre 4 et 7 jours d'incubation, chez l'embryon de Poulet (▲), de Dindon (×) et de Caille (●). Le sexe est indiqué lorsqu'il a été reconnu avec certitude.

(28 jours d'incubation au lieu de 21 chez le Poulet) puisque la Caille qui a un développement encore plus court (16 jours) possède à 4 jours le même nombre de gonocytes que le Poulet. On pourrait essayer de mettre en parallèle les populations de cellules germinales et les niveaux de fécondité (beaucoup plus faible chez la Dinde que chez les deux autres espèces) mais cela nécessiterait des résultats plus nombreux et, en outre, les observations de HARDISTY<sup>5</sup> ne semblent pas conclure à l'existence d'une relation de ce type.

3. Les taux de croissance des populations de gonocytes sont presque les mêmes chez la Caille et le Dindon (les deux droites traduisant leur variation sont à peu près parallèles) alors qu'il est nettement plus faible chez le Poulet (pente inférieure de 8% environ). Alors que le mode exponentiel de croissance paraît être général pour la période considérée, il est possible que le taux d'accroissement soit caractéristique de l'espèce. Dans ce cas, les différences de taux constatées rendraient compte, de façon satisfaisante, des distances immunologiques découvertes par MAINARDI<sup>6</sup> et qui font ressortir un lien phylogénétique plus étroit entre Caille et Dindon (distance immunologique: 11) qu'entre Caille et Poulet (d.i.: 32) ou Dindon et Poulet (d.i.: 22).

**Summary.** Counts were made of the numbers of germ cells in quail embryos aged 4-7 days' incubation. The rate of growth in the population of gonocytes was compared to these of chick and turkey embryos during the same period. The increase is exponential in the three species and the rates are more similar between quail and turkey than between each of them and the chick.

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<sup>3</sup> De façon très générale, cette phase de multiplication exponentielle constitue en fait la période la plus active de la vie d'une population cellulaire.

<sup>4</sup> F. C. CHRÉTIEN, J. Embryol. exp. Morph. 16, 591 (1966).

<sup>5</sup> M. W. HARDISTY, Biol. Rev. 42, 265 (1967).

<sup>6</sup> D. MAINARDI, Nature, Lond. 184, 913 (1959).

## Organotypical Culture of Irradiated and Non-Irradiated Chick Mesonephros

Organotypic cultures are suitable for the study of irradiation effects on cells and tissues. We used the chick mesonephros in our experiments because this organ is fully differentiated and functional. In this sense, it is comparable to adult organs. As an embryonic tissue, it is easy to culture. The differential effect of ionizing irradiation on epithelial and fibrous tissue occupied our special attention.

**Methods.** The 9-day-old chick mesonephros (Stage 34-35 of HAMBURGER and HAMILTON<sup>1</sup>) has been used throughout the present experiments. One of the 2 organs is kept in Tyrode's solution after dissection, while the other is irradiated. The irradiation technique used is mentioned in a previous paper<sup>2</sup>; single doses of 500 rad, 1000 rad, and 2000 rad are delivered.

After irradiation, both control and irradiated organs are cut into pieces, and transferred for incubation to a

semi-solid nutrient medium<sup>3</sup> in culture dishes as described by GAILLARD<sup>4</sup>. The mesonephros is explanted in vitro following WOLFF's technique<sup>5</sup>. The cultures are fixed in Zenker after 6 h, 48 h, 72 h and 7 days of incubation at 37.5°C, embedded in paraffin, sectioned at 8 µm and stained with haematoxylin and eosine, and Azan or Bielschowsky's<sup>6</sup>.

<sup>1</sup> V. HAMBURGER and H. HAMILTON, J. Morph. 88, 49 (1951).

<sup>2</sup> L. DE RIDDER, M. MAREEL and M. VAN VAERENBERGH, *Strahlentherapie*, Sonderband, Teil B (1967), p. 393.

<sup>3</sup> L. VAKAET and L. PINTOLON, C. r. Soc. biol., Paris 153, 147 (1959).

<sup>4</sup> P. J. GAILLARD, in *Methods in Medical Research* (Ed. M. B. Visscher; Year book publishers, Chicago 1951), p. 241.

<sup>5</sup> E. WOLFF, Tex. Rep. Biol. Med. 10, 463 (1960).

<sup>6</sup> B. ROMEIS, *Mikroskopische Technik*, Verbesserte Auflage (R. Oldenbourg, München 1948).

**Results.** After 6 h of incubation, the histological aspect of irradiated and control cultures is the same. Many traumatized tubules are visible. The tubular cells have swollen nuclei and nucleoli, and the cytoplasm is enlarged and eosinophilic. Many intraluminal inclusions, consisting of cell debris, are present. Several glomeruli are traumatized; most are swollen. The interstitial tissue consists of groups of polymorphous cells; a faint, often disrupted argentophilic layer around the individual tubules is seen. Necrotic cells are numerous.

After 48 h of incubation the overall diameter of the tubular lumen increased in the control cultures, especially at the periphery. The tubular epithelium is flattened. The distal tubules are normal. The glomeruli are still swollen. The clumps of polymorphous cells of the interstitial tissue show flattened cells at their periphery. These fibroblast-like cells link up tubules and glomeruli, with the vitelline membrane. Their number also increases around the individual tubules as well as the periphery of the culture, where some isolated cells are found.

In the 500 rad irradiated cultures, cytoplasmic eosinophilic staining is pronounced and fibroblast-like cells are less abundant at the periphery of the cultures; no isolated cells are found.

In the 1000 rad irradiated cultures, cytoplasmic eosinophilic staining and necrotizing cells are present in all tubules and glomeruli. In the interstitial tissue, nuclear polymorphism is visible and flattened cells are

rare. The argentophilic fibers at the tubular periphery are swollen and disrupted; they encircle the culture only partially.

In the cultures irradiated with 2000 rad, proximal and distal tubules as well as the glomeruli are eosinophilic. Necrotizing cells are numerous. The tubules show a considerable variation in diameter and usually contain cell debris. The number of cells per tubule is decreased. The brush border is indistinct, thus making discrimination between proximal and distal tubules difficult. In the interstitial compartment, cell clumps and flattened cells are rare. Argentophilic swollen fibers can be discerned at the tubular periphery and near the vitelline membrane.

After 72 h of incubation the intra-luminal inclusions, consisting of cell debris, are rare in the controls; instead, we find an amorphous meshwork. Most proximal tubules have a clear brushborder. Traumatized tubules are no longer visible. Necrotic cells are scarce in the interstitial tissue. The number of argentophilic fibers has increased.

The 500 rad cultures only differ from the controls by the lack of isolated, fibroblast-like cells.

In the 1000 and 2000 rad cultures the tubular arrangement is disturbed. Many nuclei are pycnotic. The glomeruli of these cultures show hypoplasia of the stroma. Cell clumps in the interstitial compartment have completely disappeared and amorphous substance is filling the gaps between tubules and glomeruli.

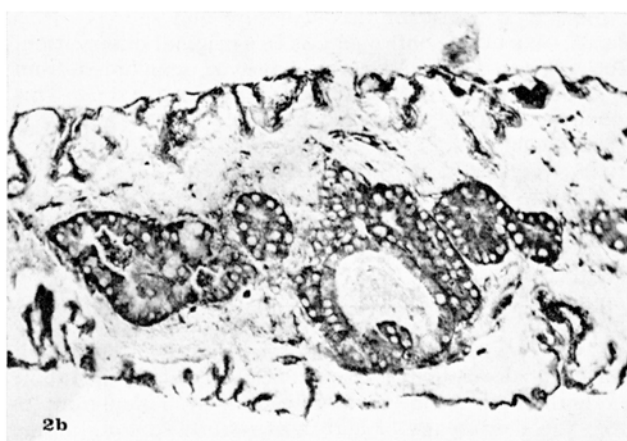
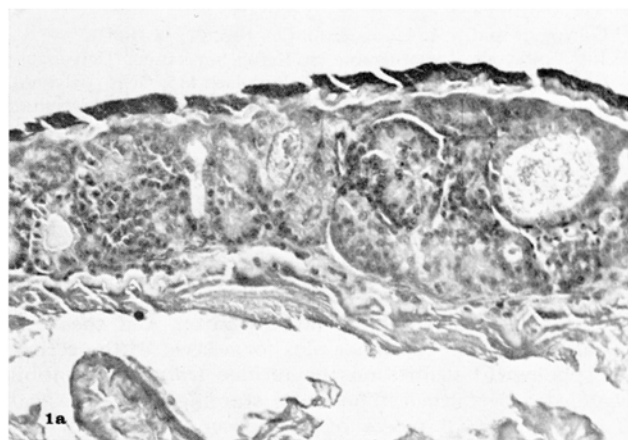


Fig. 1. a) Mesonephros explants of control culture 7 days of incubation. b) Mesonephros explants of 2000 rad irradiated culture 7 days of incubation. Hematoxylin-Eosin stain Fixation in Zenkers' solution.  $\times 500$ .

Fig. 2. a) Mesonephros explants of control culture 7 days of incubation. b) Mesonephros explants of 2000 rad irradiated culture 7 days of incubation. Bielschowsky's stain. Fixation in Zenker's solution.  $\times 500$ .

After 7 days of incubation the control cultures show involution phenomena in both proximal and distal tubules: presence of vacuolization in an eosinophilic cytoplasm, polymorphism of the nuclei, and reappearance of cell debris in the lumen. In the glomeruli, the cell number decreases and Bowman's capsule is thickened.

In the cultures irradiated with 1000 and 2000 rad a wide spread disintegration of tubules and Bowman's capsules is found. The paucity of fibroblast-like cells is remarkable. The swollen and disrupted remnants of argentophilic fibers are still to be seen.

**Discussion.** After 6 h of incubation, both irradiated and control cultures show reversible and irreversible cell damage. In irradiated cultures, recuperation is retarded or completely absent, a phenomenon also mentioned by LASNITZKI<sup>7</sup>. We infer from this that irradiation injury is superimposed on injury caused by cultural procedures, increasing the number of irreversibly damaged cellular components.

The interstitial cell compartment changes considerably during culture. These changes are characterized by the appearance of connective tissue around the organized structures and at the periphery of the culture. Most of these interstitial cells originate from traumatized kidney tubules<sup>8</sup>. These cells flatten and migrate through the whole culture and form argentophilic fibers. Interstitial cells also undergo irradiation damage, as is shown by their swelling. The decreased number of flattened cells cannot be explained by inhibition of multiplication, since in these cells mitotic activity is low. This phenomenon can only be explained by inhibition of cell migration, as also stated by GÄRTNER<sup>9</sup> and GOLDFEDER<sup>10</sup> in fibroblast cultures. The absence of isolated cells at the periphery of the culture supports this theory.

It may be concluded from our experiments that in the chick mesonephros organized structures are less radio-

sensitive than the interstitial cell compartment. This phenomenon is also described by NORRIS and HOOD<sup>11</sup> in cultures of human foetal kidney. The irradiation-induced changes described in our experiments are dose-dependent, as is shown by comparison of the various doses administered.

**Zusammenfassung.** Es wurden Hühnchen-Embryonen mit Einzeldosen von 500, 1000 und 2000 rad in vitro bestrahlt und die Kulturen nachher inkubiert. Nur bei den nicht bestrahlten Kulturen kam es zur Proliferation des interstitziellen Gewebes und zur Vermehrung der argentophilen Fasern. Schädigungen an Tubuli und Glomeruli bei den bestrahlten Kulturen erwiesen sich als strahlendosisabhängig.

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16 October 1970.*

<sup>7</sup> I. LASNITZKI, Br. J. Radiol. 15, 61 (1943).

<sup>8</sup> O. A. TROWELL, Expl Cell Res. 16, 118 (1959).

<sup>9</sup> H. GÄRTNER, Strahlentherapie 103, 620 (1957).

<sup>10</sup> A. GOLDFEDER, Radiology 31, 73 (1938).

<sup>11</sup> G. NORRIS and S. L. HOOD, Expl Cell Res. 27, 48 (1962).

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## CAJAL Cells of the Rabbit Cerebral Cortex

'Special cells' or horizontal cells located in the first layer (molecular or layer I) in the cortex cerebri of small mammals, were first described by CAJAL<sup>1</sup> as cells possessing no axons, and were later classified as neurons by the same investigator<sup>2</sup>. RETZIUS<sup>3</sup> described the same type cell in the brain of the human fetus and coined the name 'CAJAL cells' for this structure and shortly afterwards, VERATTI<sup>4</sup> confirmed CAJAL's original observation. Both RETZIUS and VERATTI, however, concluded from their work that the CAJAL cells contained axons. This conclusion was also reached by CAJAL<sup>5</sup> from his study on the cortex cerebri of the new-born human. LORENTE DE NÓ<sup>6</sup> discussed the possibility that the CAJAL cells of the mouse cerebral cortex could be of the short axon type. In other animal species, the short axon cells located in the molecular layer, have been described as modified CAJAL cells. Since these publications, CAJAL cells are thought to be neurons with axon, though the literature supporting this view is rather inconclusive.

The present study was conducted in order to establish the morphological features of the CAJAL cells of the rabbit cerebral cortex during early development. Tissue samples from the sensory motor cortex of rabbits ranging in age from 6 to 24 days were prepared histologically by the Golgi method. In all brains studied, tissue sample sectioning was performed both perpendicularly and tangentially to the cortical surface. Suspecting a similarity between

CAJAL cells of the rabbit cerebral cortex and the large amacrine cells of the inner plexiform layer of the retina, morphological studies on the retinae from adult rabbit were also performed. The Golgi staining procedure was applied to small pieces of this tissue which was subsequently sectioned perpendicularly to the main surface of the retina. In order to obtain a more complete histological view of the whole retina, it was also subjected to the staining technique of GROS as modified by GALLEGÓ<sup>7</sup>.

In our preparation of the rabbit cortex cerebri, it was possible to demonstrate the occurrence of CAJAL cells and to study their body shape and prolongation in 2 angular sections. These cells, whose soma was located in the middle and lower third portion of molecular layer,

<sup>1</sup> S. RAMON Y CAJAL, Gac. méd. catal. 15, dec. (1890).

<sup>2</sup> S. RAMON Y CAJAL, in *Textura del Sistema Nervioso del Hombre y de los Vertebrados* (Moya, Madrid 1899), vol. I, p. 43.

<sup>3</sup> G. RETZIUS, Biol. Untersuch. 13, 1 (1893).

<sup>4</sup> E. VERATTI, Anat. Anz. 13, 377 (1897).

<sup>5</sup> S. RAMON Y CAJAL, in *Histologie du Système Nerveux de l'Homme et des Vertébrés* (Maloine, Paris 1911), vol. II, p. 526.

<sup>6</sup> R. LORENTE DE NÓ, Trab. Lab. Invest. biol., Univ. Madr. 20, 41 (1922).

<sup>7</sup> A. GALLEGÓ, An. Inst. Farmac. esp. 2, 171 (1953).