muscle. It may even be that profiles with numerous large granular vesicles, together with small granular vesicles (Figures 2–5), actually represent axons separate from axons containing almost exclusively small granular vesicles (Figure 1). If it is so, the retractor penis muscle of the bull would be innervated by 3 morphologically different types of nerve fibres: 1. axons containing small agranular vesicles, presumably cholinergic; 2. axons containing small granular vesicles, presumably adrenergic; 3. axons containing large and small granular vesicles, whose nature may differ from that of adrenergic axons. It must, however, be pointed out that the relative number of large granular vesicles may vary in different parts of the same axon.

In any case, numerous large granular vesicles characterize most of the axon profiles of the smooth muscle of the retractor penis and the penile artery of the bull, in contrast with those of the vas deferens and the metatarsal artery of the same animal, in which typical adrenergic axon profiles were seen with numerous small, but few large granular vesicles. In view of the pharmacological evidence for the presence of non-adrenergic, non-cholinergic inhibitory nerves in the retractor penis muscle[2], it is of special interest that large opaque vesicles have been considered as a characteristic of the inhibitory non-adrenergic, non-cholinergic, possibly purinergic axons innervating various tissues[15,16]. In those axons, small vesicles are present besides the large ones[16,17]. It is, however, unlikely that adenosine triphosphate serves as an inhibitory transmitter in the retractor penis muscle or the penile artery of the bull[2]. On the other hand, studies with different fixatives are necessary in order to characterize the fine structure of the so-called purinergic nerves.

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Origin of Ovarian Follicle Cells in Birds

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Summary. In the embryonic Japanese quail ovary, transplanted on chicken chorioallantoic membrane (CAM), follicle cells are derived from somatic cells of the ovarian surface epithelium. No evidence was found for a contribution of other cell groups of the quail ovary in the formation of follicle cells. This may be demonstrated on PAS stained sections, by following the transfer of carbon particles, initially applied on the surface epithelium.

It was classically accepted that follicle cells originate from the somatic cells of the 'germinal epithelium'. However, in several groups of vertebrates, the origin of follicle cells is still a matter of dispute[2]. For instance, in mammalia there is much doubt as to the cortical origin of follicle cells[3–6]. In birds, another higher vertebrate class, the formation of secondary sex cords (Pflüger or ovigerous cords) from the surface epithelium can usually be seen distinctly. However later, when the cortex has fully developed, a possible ingrowth of ovarian stroma cells into the ovigerous cords cannot be entirely ruled out after routine histological investigations.

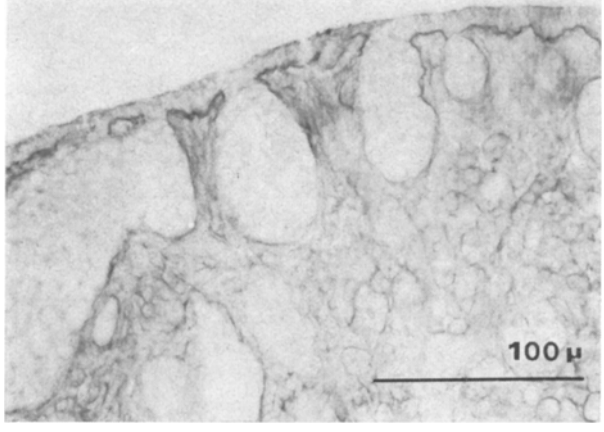

Fig. 1. PAS-stained section of the left ovary from a 15-day-old chicken embryo.

Material and Methods. Ovaries of 8- to 11-day-old chicken or Japanese quail embryos were used in this study. After opening the embryo's abdomen and removing the intraperitoneal organs, a small quantity of ultrasonicated animal charcoal is sprinkled on the surface of the left ovary, while it is still in situ. After excision the left ovary is transplanted on the CAM of a 7- to 9-day-old chicken embryo, according to the technique of Harris[7]. 1 to 9 days later, the graft is excised and fixed in acetic-alcohol (1:3 v) for 1 h. The sex of the host embryo is noted for each transplant. After embedding in paraffin, the transplanted ovaries are serially sectioned at 5 to 7 µm thickness. After deparaffination, the sections are stained with the PAS technique[8]. PAS-stained sections of ovaries from older embryos are used as controls. Some of the sections are counterstained with methyl green or Unna.

Results and discussion. During the formation of the cortex, PAS stained sections of embryonic quail or chicken ovaries clearly show the development of vase-shaped buds connected with the surface epithelium via a

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narrow neck (Figure 1). Both the surface epithelium and the buds are distinctly separated from the underlying or surrounding ovarian stroma by an intensely staining PAS-positive basement membrane. In our opinion, only the PAS stain allows a clear-cut definition of the basement membrane on the whole section. The buds and ovigerous cords appear as clearer zones in which no fibrillar PAS-positive material can be discerned. Counterstaining of the PAS stained sections with methyl green or Unna reveals cytological details in the ovarian cells, but makes the boundaries of the germinal epithelium and its derivatives more vague and hardly distinguishable.

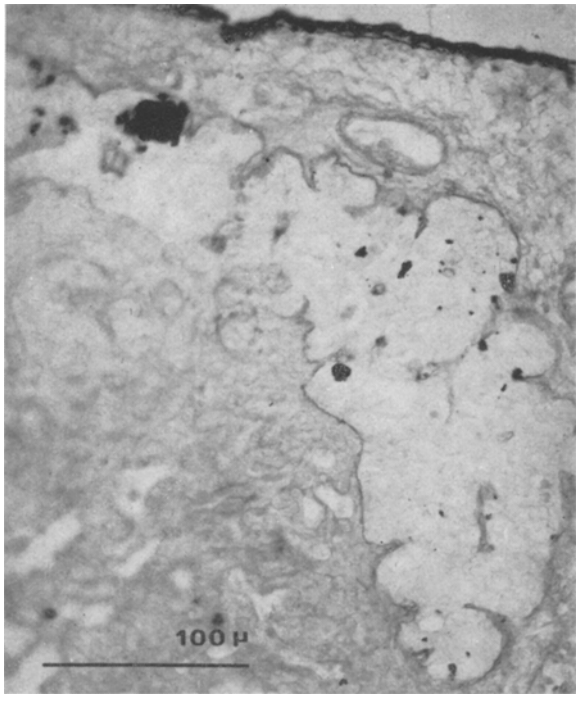

Fig. 2. PAS-stained section of left ovary from a 10-day-old quail embryo, on which surface epithelium carbon particles have been applied, grown on CAM for 9 days. Nearly all the carbon particles are found in the ovigerous cords.

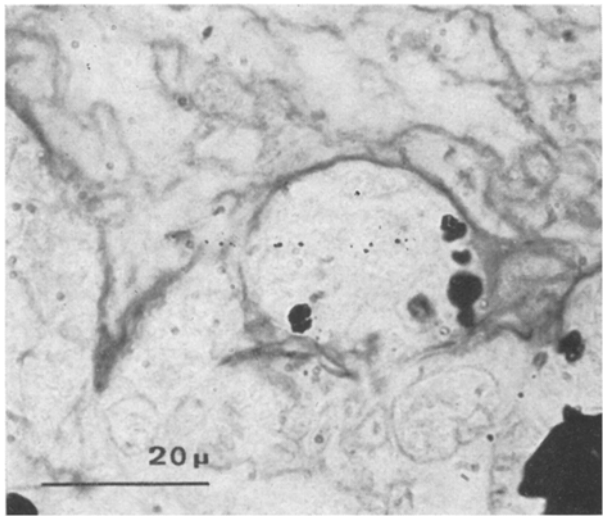

Fig. 3. Developing follicle of the same ovary as in Figure 2. Carbon particles are localized in the follicle cell layer at the inner side of the basement membrane.

The development of the ovigerous cords and cortex of the transplanted ovaries seems to be similar to that of non-transplanted ovaries, found in normal female quail of corresponding age.

Thus the grafted ovaries continue their normal development on the CAM and seem not to be influenced by the hormonal state of the chick embryo on which they have been transplanted[9]. This is in agreement with the observations of BRADLEY[10], WOLFF[11] and GROENENDIJK-HUYBERS[12], that bird gonads, transplanted after their sexual differentiation either on the CAM or in the coelomic cavity of younger embryos, continue to develop according to their genetic sex.

One to 2 days after the transplantation of the ovaries on CAM, the carbon particles are usually no longer found on the surface epithelium, but may be seen in the narrow connections of the cords with this epithelium. After 6 to 9 days of transplantation, almost all the carbon particles are found within the boundaries of the secondary sex cords (Figure 2). Exterior to the basement membrane, which separates the cords from the surrounding ovarian stroma, carbon particles are seldom seen. The incorporated particles are irregular in shape and volume. The cros sectional areas of the larger ones can usually be recognized in successive sections. In the Japanese quail ovaries of 10-day embryos, transplanted for 9 days (corresponding approximately to ovaries from 2-day-old quails), a few developing intrafollicular oocytes can be seen. The follicle develops from a round bud of the sex cord and always remains separated from the ovarian stroma by the basement membrane. In some of these follicles, carbon particles are incorporated between the developing follicle cells, derived from the somatic cells of the secondary sex cord (Figure 3). This indicates that, in the transplanted ovaries, follicle cells develop from somatic cells present in the ovigerous cords, which in turn are derived from the surface epithelium. No evidence was found for a contribution of other cell groups of the quail ovary to the formation of follicle cells. The bulk of the surface epithelium cells of 10-day-old quail embryos is formed by somatic cells. Only a small number of germ cells are still localized in the surface epithelium at this stage. Thus the carbon particles must have been mainly if not exclusively adherent to the somatic cells of the germinal epithelium. Our observations show clearly that the particles migrate from the surface of the ovary, via the connection of the surface epithelium with the ovigerous cords, into the latter. Finally some of the particles are found in some of the developing follicles. This migration is not the result of gravitation, since the particles are found both in the superficial and deeper regions of the cords. Thus the particles seem to be transported into the sex cords by ingrowing epithelial cells. This indicates that growth of the cords is not exclusively the result of localized multiplication of cells within them, but that there is an additional supply of somatic cells originating from the germinal epithelium.

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Characteristic organelles of follicle cells, the so-called 'lining-bodies'[13] were also found in the surface epithelium of the left gonad of chick embryos from the 6th day onwards[14]. These ultrastructural observations are likewise in favour of the classical hypothesis of the origin of avian follicle cells. RAHIL and NARBAITZ[14] found 'boursouflures' (emerging from the somatic cells of the ovarian epithelium[15] together with a network of fine microfilaments, especially concentrated at the apical poles of the surface epithelium cells. According to the latter authors, these organelles perhaps play a rôle in the change of cell shape, and may be related to the formation of sex cords. Our observations seem to afford additional evidence for this supposition.

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Association of Mastopoiesis with Haemopoietic Tissues in the Neonatal Rat

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Summary. Mast cells in the newborn rat occur in haemopoietic foci of liver and spleen, but disappear from those foci as extramedullary haemopoiesis ceases during the initial postnatal month. At the same time, mast cells increasingly populate bone marrow and connective tissues of heart, lung, stomach and portal tract of liver.

In the foetal rat, mast cells appear on the 15th or 16th day of gestation in caudal mesenchyme near the ventral portion of the developing brain[2–7]. They subsequently become widely distributed, being particularly associated in the adult with connective tissues, but are sparse in parenchymal organs such as liver and kidney[8]. On the other hand, there is little information on the distribution and population density of mast cells in the mature foetus, or on the proliferation of these cells in the neonatal rat to achieve their abundance in the adult[9, 10].

To compare the distribution and density of mast cells in the newborn rat and young adult, batches of 4 albino rats (each animal from a different litter) were sacrificed at intervals of $^1/_2$ to 90 days after birth. Pieces of heart, lung, thymus, spleen, liver, stomach, kidney, abdominal skin and tibia from each animal were fixed in Lillie's neutral buffered formalin, and 5 µm paraffin sections were stained for 5 sec with 0.25% eosin Y in 80% ethanol containing 0.25% acetic acid, rinsed briefly in tap water and then stained for 10 min with 0.01 or 0.1% toluidine blue in aqueous 0.3% acetic acid. Mast cells were enumerated in 100 fields per section with a Carl Zeiss N.A. 0.95 × 40 objective and × 8 compensating oculars (field diam = 440 µm), the number of cells being expressed as the corresponding number per mm³ of tissue. No cell corresponding to the basophil leucocyte of non-murine mammals has been identified in the present work, a result consistent with reports of the very low incidence of basophils in the bone marrow of adult rats[11]. In the present investigation, mast cells have been distinguished from basophils by a histochemical technique based on the vigorous hydrolysis of naphthol AS-D chloroacetate obtained with mast cells and the feeble hydrolysis with basophils[12]. Distinction of mast cells from 'mucosal mast cells' has been verified by the inability to demonstrate metachromasia in the latter when fixed in buffered 10% formalin[13].

Enumeration of mast cells in neonatal rats has revealed that the above organs fall into 4 groups (Figure). In the 1st, which comprises skin and thymus (Figure, block 1), the count at birth is relatively high and remains so throughout the initial 90 post-natal days. In skin, mast cells occur mainly in the dermis and panniculus adiposus, while in the thymus 80–90% lie in or near the interlobular connective tissue.

In the 2nd group – viz., liver and spleen (Figure, block 2), and to less extent, thymic parenchyma, the population of mast cells is characterized by an initial high count followed by a rapid decline to virtual absence by the 30th day of life and thereafter. In the liver, mast cells during the initial 14 days almost invariably lie free in the sinusoids of the haemopoietic foci. The hepatic count becomes minimal by the 18th day, but then steadily rises (Figure; see below). In the spleen of neonatal rats, mast cells occur mainly in the red pulp and frequently in the lumen of associated small blood vessels. Beyond the 30th day, a few mast cells appear in the capsule. Of the 10–20% of thymic mast cells not associated with interlobular stroma (see above), rather more occur in medulla than in cortex. But even in the cortex, the mast cells are closely associated with fibres of reticulin. In thymic parenchyma, the number of mast cells becomes maximal by the 6th day and progressively declines thereafter.

In the 3rd group (Figure, block 3), comprising the *connective tissue* of heart, lung, gastric wall and liver, mast cells are practically absent at birth, but rapidly and

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