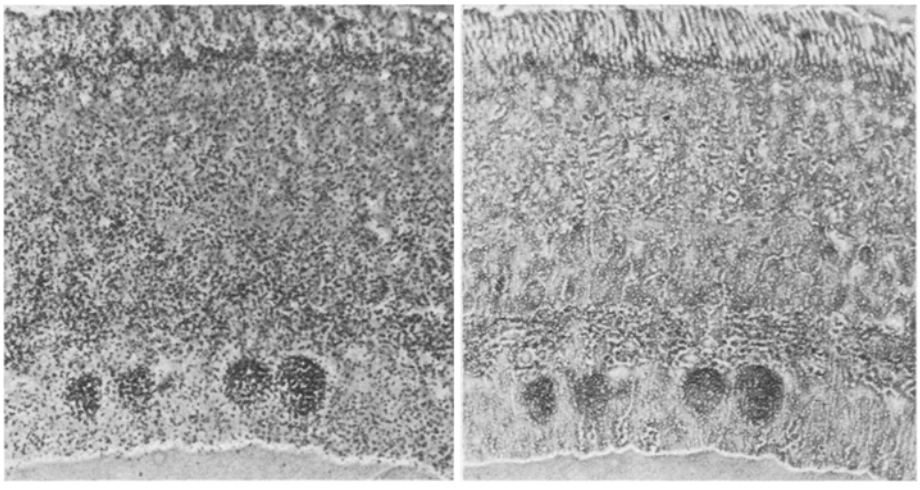

Autoradiograph of rabbit retina, 4 h after the intravitreal injection of 25 μCi ^{3}H-serine (generally labelled). There is radioactivity in 4 ganglion cells, in a layer near the external limiting membrane and for the rest rather diffusely throughout the retina. Left, focus on the grains; right, focus on the section. Phase contrast micrograph. ×450.

artificial laboratory illumination. Other rabbit retinas were incubated (15 min) as previously described[4] with the tritiated serine, 1 μCi/ml before being processed. Exposure times of the autoradiographs were 1 to 3 months.

No convincing selective labelling of retinal amacrine neurons was seen. The radioactivity had apparently accumulated (Figure) to a large extent in glial cells (Müller cells) and in the ganglion cells. Particularly after 24 h there was also a strongly radioactive zone in the photoreceptors. The pattern is very similar to what is seen with a number of other amino acids not suspected of being neurotransmitters or neurotransmitter precursors[6]. By waiting for 24 h after the intravitreal injection, it was hoped to reveal accumulation into the neuronal pool with slow turnover, but no such effect was evident.

Thus, it is not possible to tag the presumed glycinergic neurons with the precursor serine. This is in contrast with what is seen in, e.g., catecholaminergic neurons where dopa is readily converted to dopamine or noradrenaline. The reason for the failure with serine is not apparent, but one possibility is that the enzyme converting serine to glycine is being kept inactive in the cells under the conditions of the experiment resulting in no or little de novo synthesis of transmitter glycine. By comparison the rate limiting enzymes in the catecholamine synthesis are similarly subject to regulation in the neurons[9].

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Penetration of Melanocytes from Embryonic Japanese Quail Peritoneum into Associated Embryonic Avian Gonads, Grown on Chicken Chorioallantoic Membrane

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Summary. After association on chorioallantoic membrane (CAM) of an embryonic bird testis with pigmented peritoneum from a Japanese quail embryo, numerous melanocytes penetrate in its interstitial tissue. If, instead of a testis, an ovary is transplanted under similar conditions, then the melanocytes may be found in the medulla or between the secondary sex cords at the rim of the ovary.

After a period of elective chimiotactic attraction for germ cells[2], the surface epithelial cells of the gonad penetrate together with these germ cells into the underlying stroma and form sex cords. In the present work, we have studied the behaviour of extragonadal pigmented coelomic epithelium (from quail embryos) when placed in contact with avian gonads, just after their sexual differentation.

Material and methods. The left gonad from 9- to 11-day-old Japanese quail embryos, or from 8-day-old chick embryos, was placed on the CAM of 7- to 9-day-old chick embryos according to the technique of Harris[3]. A sheet of pigmented parietal peritoneum from the infero-lateral part of the abdomen (either from female or male 10-day-old quail embryos) was placed over these gonads. Then, 6 to 9 days later, the transplants were excised and fixed in acetic-alcohol (1:3$_v$). After dehydration and embedding in paraffin, the transplants were sectioned at 7 μm thickness. For each transplant the sex of the host on which it developed was noted. The sections were stained with toluidine blue, PAS or Feulgen.

Results and discussion. In the 10-day-old Japanese quail embryo (wild type) pigmented coelomic cells can usually be seen under the stereomicroscope (X120). Their number increases in older embryos and after hatching. They are most numerous on the inferior and posterior walls of the peritoneal cavity.

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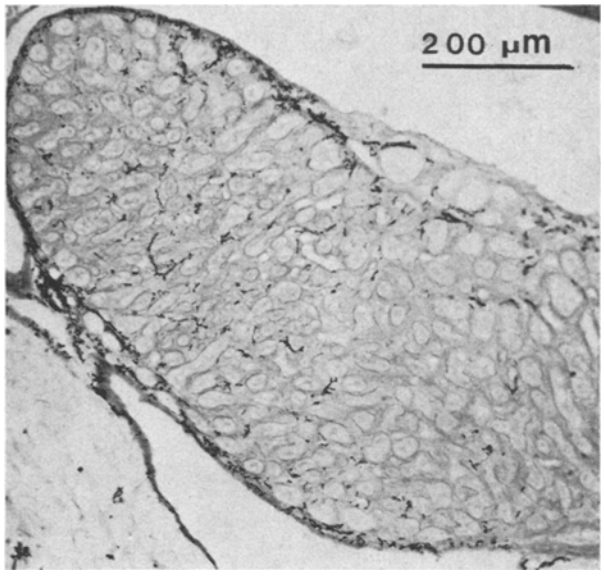

Fig. 1. Section through the left testis (from a 10-day-old Japanese quail embryo), grown on CAM for 8 days, in the presence of pigmented parietal peritoneum from a female quail embryo of the same age. The melanocytes have invaded the testicular interstitial tissue. PAS stain.

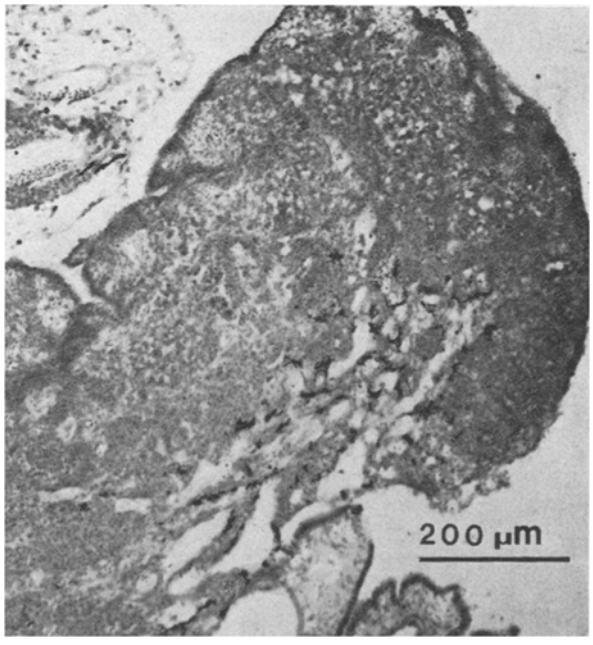

Fig. 2. Section through the left ovary (from a 10-day-old Japanese quail embryo), grown on CAM for 8 days, in the presence of pigmented parietal peritoneum from the same embryo. The melanocytes are chiefly localized in the neighbourhood of the distended medullary sex cords. Toluidine blue stain.

In some places their peri-arterial localization is obvious. Their cell body and its ramifications contain numerous round or ovoid black granules. Under natural conditions, these melanocytes are never found in the surface epithelium of the gonads. In some quails, however, they are present in the peritoneum surrounding the gonadal hilus or even in the hilus. Melanocytes have been observed in the testes of some species of birds[4,5]. In our experimental conditions, the pigmented peritoneal layer usually transforms into a pigmented vesicle which surrounds the associated gonad. In the large meshes of this vesicle, the melanocytes have an irregular distribution. Their melanin granules can easily be seen in unstained paraffin sections and remain visible after Feulgen or PAS staining. When associated with an embryonic avian testis, on CAM, they invade part or the whole surface of this organ, and from there seem to penetrate into the testicular interstitial tissue (Figure 1). They may be seen even in the most central part of the testis. They never penetrate into the seminiferous cords. Some of the melanocytes are in close association with the wall of the blood vessels of the interstitial tissue. Female as well as male melanocytes penetrate into an avian testis grafted on the CAM of female or male hosts. The observed penetration mechanism seems to be elective for interstitial tissue, since, in experiments where in addition to a left gonad also the corresponding adrenal gland or a piece of metanephros was transplanted, no migration of melanocytes into these organs could be observed. Under natural conditions also, we have not found melanocytes in the latter organs, despite the fact that they are partially covered by pigmented parietal peritoneum.

When an ovary of a 9- to 10-day-old Japanese quail embryo (i.e. 3 days after sexual differentiation) is transplanted in association with pigmented parietal peritoneum of the same embryo, then numerous melanocytes may be found in the deeper part of the ovarian medulla (chiefly around the distended medullary cords, derived from the primary sex cords), but never in the cortical region (Figure 2).

When an ovary of an 8-day-old chick embryo (i.e. just after differentiation) is transplanted together with pigmented extragonadal coelomic epithelium of a female quail embryo, melanocytes may be found in the deeper part of the medulla but also between the secondary sex cords at the rim of the ovary (Figure 3).

This seems to indicate that in parts of the ovarian cortex with a less advanced development and with still potentialities of the indifferent gonad, the melanocytes may penetrate also. We do not know if the invasion of the ovarian cortical rim by melanocytes occurs via the medulla or directly through the surface epithelium. Experiments with younger ovaries may probably afford more details of the penetration mechanism. If sections from transplanted chicken gonads, in which quail melanocytes have penetrated, are stained with toluidine blue or by the Feulgen nucleal reaction, no other cells with the typical chromatin distribution of quail nuclei (first described by CALLEBAUT[6]) can be found within the gonad. This would seem to indicate that melanocytes are the only quail cells capable of penetrating the gonad.

GROPP and OHNO[7] assumed that, in mammalia, the interstitial cells of the testis and the follicle cells of the ovary are homologous. In contrast, our results seem to indicate that in birds there is no homology between these structures. Indeed, in our experimental conditions, melanocytes are never found in the tubuli of the testis nor in the germinal epithelium and secondary sex cords (containing the precursors of the follicle cells[8]) of the

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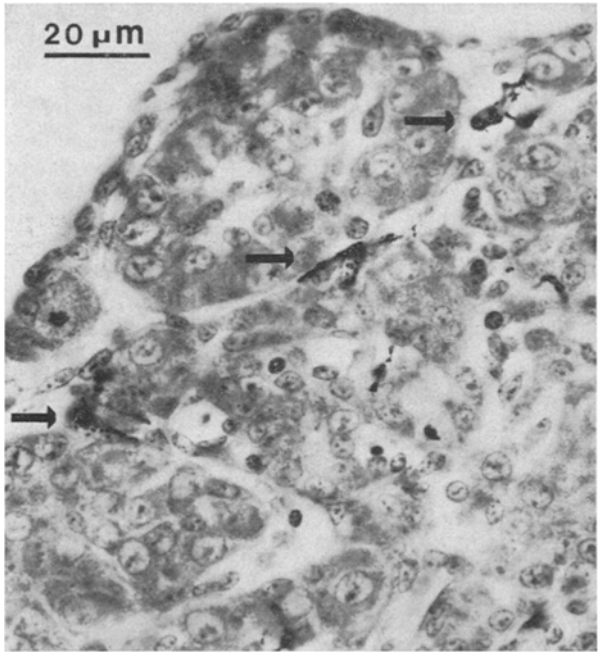

Fig. 3. Section through the rim of the left ovary (from an 8-day-old chicken embryo), grown on CAM for 8 days, in the presence of pigmented peritoneum (from a 10-day-old female quail embryo). A few melanocytes (arrows) have penetrated between the secondary sex cords. Toluidine blue stain.

ovary. We do not know why, during normal development, no melanocytes can be found in the surface epithelium of the Japanese quail gonad, while in the parietal peritoneum, close to the gonadal hilus, numerous pigment cells are present. Our results seem to indicate that the spatial distribution of the melanocytes in the immediate neighbourhood of the gonad determines whether or not penetration will take place.

Avian melanocytes are derived from neural crest material[9]. That melanocytes can settle down in sites of the testis where normally interstitial cells are found, suggests that the latter also may be of neural crest origin. This study demonstrates how quail melanocytes (localized in an easily manipulable sheet of peritoneum) may be used as cellular markers in homospecific as well as in heterospecific tissue associations.

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Persistent and Atypical Lobules in the Human Breast may be Precancerous[1]

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Summary. Persistent human mammary lobules (PL) remaining after the menopause, and certain atypical lobules (ALA) are morphologically similar to the common preneoplastic hyperplastic alveolar nodule (HAN) of mice of strains having a high incidence of mammary carcinoma. This and other evidence suggest that like the HAN of mice human PL and ALA are precancerous.

It is generally agreed that the hyperplastic alveolar nodule (HAN) is the common kind of preneoplastic lesion of the mammary gland of mice having a high incidence of mammary adenocarcinoma[2,3]. HAN are lobuloalveolar in morphology and are more frequently observed in those strains of mice which have a high incidence of mammary adenocarcinoma than in strains which have a low incidence. HAN increase in number with age, and are most frequent in old retired breeders. HAN can be shown by direct experimental means to be preneoplastic in that cancer arizes more frequently in transplanted cell populations derived from HAN than in populations derived from normal mammary cells. Our search for preneoplastic lesions in the human breast provides quantitative morphological evidence that certain kinds of human lobules resembling HAN are precancerous. This evidence is based on quantitative studies of mammary lesions in whole human breasts utilizing a subgross sampling technique with histological confirmation[4].

The purpose of this report is to demonstrate our findings of striking morphological similarities between the common lobular HAN of mice and two kinds of human lobular lesions which we believe to be precancerous to infiltrating duct carcinoma in the human. We have designated these two kinds of human lobules as 1. persistent lobules (PL) and 2. atypical lobules, type A (ALA), and will define these further in the text.

Mammary glands were obtained from 24 female mice as follows: C3H (retired breeders), 2 mice; BALB/c (retired breeders), 10 mice; CF2 (retired breeders), 3 mice; GR (old virgins), 9 mice. Whole glands were fixed overnight in 10% buffered formalin, stained with iron hematoxylin at pH 1.3–1.5, dehydrated, and stored in methylsalicylate[5].

210 whole human mammary glands were fixed for 4 weeks in 10% buffered formalin, embedded in 10%

[1] This work was supported by a contract from the National Institutes of Health. PHS NOI-CB-43908, and funds from the Cancer Research Coordinating Committee, University of California.
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