CYTOLOGY

Cell Transport and the Bursa of Fabricius

THE use of grafts and parabionts with marked chromosomes in investigations on the mammalian thymus has shown that there is substantial cellular traffic in this organ[1,2]. Using chick parabionts, it should be possible to make an assessment of the role of cellular immigration in the development of the lymphoid organs of this species.

Our own interest centres on a lymphoid organ unique in Aves, the bursa of Fabricius. Birds have the great advantage over mammals that parabiotic experiments are possible at much earlier states of development. On the other hand, techniques for the utilization of avian chromosome markers for cell identification have only recently become available. Indeed, the cytology of birds has suffered neglect pending the development of a convenient technique for the display of avian chromosomes[3,4].

With this method, we have used hetero-specific and sex chromosome markers to identify mitotic cells in the bursa and spleen of parabiont pairs. Two types of parabionts were used: unlike sexed chick twins and parabiosed chicken–turkey embryos. Twin chick embryos were obtained by incubating fertile double yolked eggs. In these, anastomosis of the blood vessels of the chorio-allantoic membranes (CAM) occurs around 7–8 days of incubation. We were able to ascertain this fact by dissection of the membranes and by the recovery of labelled cells from one chick, following injection of labelled blood cells into the other.

Incubation of double yolked eggs was continued for as long as possible. Experience indicated that the majority of twin embryos die on or around day 18, although in some batches of eggs they survived to 20 days and in several cases it has been possible, with help, to hatch one of the members of the pair. For this investigation the data were taken from embryos 17–18 days old.

Chicken–turkey parabionts were made when the chick embryo was 10 days old, as anastomosis of the CAMs was rarely achieved before this age. The age of the turkey embryos was 17 days, to allow for the difference in developmental rate and permit both partners to hatch, if possible. Parabiosis was carried out by removing a small triangle of shell over a well vascularized part of the CAM. A drop of sterile saline was placed on the shell membrane and a fine incision was made in it, care being taken not to damage the CAM. Negative pressure applied with a rubber bulb through a small hole over the air space allowed the CAM to drop. The shell membrane could then be removed from the triangle.

Two eggs prepared in this way were brought together and sealed around the area of the join with a mixture of beeswax and paraffin. After the operation eggs were returned to the incubator and turned regularly in the normal way.

The embryos or hatched chicks were injected with colcemide 3 h before killing and preparations made from the required tissues[3].

Systematic scanning of the preparations was carried out to identify satisfactory metaphase figures. In the twin embryos, the sex chromosome was used as the marker and in the chicken–turkey parabionts the difference in karyotype between the two species was utilized. The principal cytological difference between them is that the turkey lacks a large submetacentric chromosome pair, present in the chicken[5].

The results given in Tables 1 and 2 show that considerable exchange of cells occurred. This reveals a transport of cells into the bursa and spleen at least in natural and artificially produced twins. These birds appear normal, and it is reasonable to assume that cell migration is a regular feature in the development of these tissues. No clue to the nature or origin of the immigrant cells is provided by these experiments.

Table 1. CHICK'S OWN CELLS AND THOSE OF ITS TWIN OF OPPOSITE SEX IN METAPHASE FIGURES OF BURSA AND SPLEEN PREPARATIONS

	Sex of embryo	No. male cells	No. female cells	Immigrant cells (%)
Bursa	Female	6	24	20
	Male	38	9	19
Spleen	Female	7	27	21

Table 2. TURKEY AND CHICKEN CELLS IN METAPHASE FIGURES OF BURSA PREPARATIONS FROM NEWLY HATCHED TURKEY POULTS PARABIOSED WITH CHICK EMBRYOS

	Species of embryo	No. turkey cells	No. chick cells	Immigrant cells (%)
Bursa	Turkey	32	22	40

These results, however, suggest a dual origin for bursal lymphoid cells, which have variously been held to be mesenchymal[6] or epithelial derivatives[7]. It seems possible that they may originate homoplastically from pre-existing elements as well as heteroplastically by vascular invasion of mesenchymal cells.

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MICROBIOLOGY

Spheroplasts from *Candida albicans*

SINCE Eddy and Williamson[1] showed that the gut juice of the snail *Helix pomatia* could be used in isolating protoplasts from certain strains of *Saccharomyces carlsbergensis* and *Sacch. cerevisiae*, several attempts have been made to isolate protoplasts from yeasts or fungi, using various enzyme preparations[2–14]. In general, protoplasts or spheroplasts of the susceptible strains were obtained from young culture and at very dilute cell suspensions. Recently, Duell, Inoue and Utter[15] improved the method of isolation of the spheroplasts of the yeast, *Saccharomyces cerevisiae*, by pretreating the yeast cells with 2-mercaptoethylamine and ethylenediaminetetraacetate, followed by treatment with an enzyme from snail gut juice.

I have applied this method to *Candida albicans*, which is resistant to the enzyme action of snail gut juice when the ordinary technique is used, and have obtained spheroplasts in good yield. Cells of *Candida albicans* were grown in Sabouraud's broth for 6 h with aeration and were then collected, washed with water, and the wet cells were suspended in a solution containing 0·15 M mercaptoethanol (mercaptoethylamine gave similar results) and 0·04 M EDTA (2·5–3 ml./g of cells). After 30 min at 30° C, the cells were spun down, the pellet was resuspended in the same amount of a solution containing 0·72 M sorbitol, 0·02 M citrate-phosphate (*p*H 5·8), and 10 mg of 'Helicase' (snail gut juice preparation, L'Industrie Biologique Française) and 100 units of penicillin per ml. were added to the solution. The cells were mixed thoroughly and incubated at 30° C with occasional stirring. The formation of spheroplasts was followed by observation with a phase contrast microscope and by diluting a sample with water. Under the conditions described, 50–75 per cent of the cells were converted into spheroplasts within 30 min; after 60 min approximately 95 per cent were in spherical forms. Longer incubation made the reaction more complete, but some of the spheroplasts began to burst (Figs.