Abstract of thesis by P.R. Cook: 'Studies on differentiation in vertebrate cells'.

A hen erythrocyte is biosynthetically inert; it can synthesis neither DNA, RNA or protein. Heterokaryons can be constructed between the hen erythrocyte and a mouse fibroblast by the technique of virus-mediated cell fusion. When the nucleus of a hen erythrocyte is introduced into the cytoplasm of a mouse cell in this way, it resumes the synthesis of DNA and RNA. This thesis is concerned with the following question. Can the RNA synthesised within the reactivated erythrocyte nucleus specify the synthesis of hen proteins? Three hen proteins – cell surface antigen, the soluble enzyme inosinic acid pyrophosphorylase and the differentiated product characteristic of the red blood cell, haemoglobin – have been studied.

The first chapter summarises the techniques of cell fusion and the second chapter reviews the observations that have been made on reactivation of the hen erythrocyte nucleus in the mouse cytoplasm. The methods used for the culture and the provision of cells are given in Chapters 3 and 4. Here the properties of the different red blood cells used in subsequent experiments are described.

Chapter 5 deals with the synthesis of hen-specific surface antigens in hen erythrocyte-mouse A_9 cell heterokaryons. Hen-specific cell surface antigens are detected by the method of mixed immune haemadsorption. Surface antigens, which are introduced into the heterokaryon on cell fusion, are eliminated from the surface of the heterokaryons during the first two to three days after cell fusion. This occurs despite the synthesis of DNA and RNA in the hen erythrocyte nucleus. Only later, when the erythrocyte nuclei develop nucleoli do hen specific surface antigens reappear on the surface of the heterokaryons and progressively accumulate. When red blood cells from 12-day-old chick embryos are used in the fusion, the surface antigens reappear on the surface of the heterokaryon sooner; again the surface antigens appear concurrently with the appearance of nucleoli in the chick red cell nuclei. If the red blood cells from 5-day-old chick embryos are fused with the mouse cells, nucleoli appear in the chick red blood cell nuclei on the first day after fusion and chick surface antigens never disappear from the surface of the heterokaryons. In all cases the correlation between the production of the chick surface antigens and the appearance of nucleoli holds.

In Chapter 6 the study is extended to include a soluble enzyme, inosinic acid pyrophosphorylase. The A_9 cell used in these experiments is deficient in inosinic acid pyrophosphorylase. The development of this enzyme in chick-mouse heterokaryons was measured using two techniques. One involved the incorporation of tritiated hypoxanthine into nucleic acid and the other was by direct assay of the enzyme in cell homogenates. Chick and mouse enzymes were distinguished by an electrophoretic method. As was found with the chick surface antigen, chick inosinic acid pyrophosphorylase did not appear in heterokaryons until nucleoli had developed in the chick erythrocyte nuclei. This was again true for heterokaryons constructed from a variety of chick red blood cells of different stages of maturity. From these studies the conclusion was drawn that the genetic information of the chick nucleus was not expressed in these heterokaryons until the chick erythrocyte nuclei had acquired nucleoli.

When haemoglobin was studied, it was found that this protein was not synthesised in adult hen erythrocyte-mouse fibroblast heterokaryons (Chapter 7). This was so despite the appearance of nucleoli in the erythrocyte nuclei in the heterokaryons. Very immature red blood cells from 5-day-old embryos actively incorporate ^{59}Fe into haemoglobin. When these red blood cells were incorporated

into mouse cells, the resulting heterokaryons did synthesise haemoglobin. This appeared to be due to the fact that chick cytoplasm, actively engaged in haemoglobin synthesis, was incorporated into the heterokaryon. However, with time, there was a decay in haemoglobin synthesis. The reasons for the inability of these heterokaryons to synthesise haemoglobin are discussed.

Two types of proliferating hybrid cells were produced from the fusion of human and mouse cells and chick and mouse cells. In Chapter 8, the very interesting properties of these hybrids were discussed.

Finally, in Chapter 9, the conclusions from the work on heterokaryons were discussed against a background of experimental work in other systems, and the role of the nucleolus in the expression of genetic information is considered.