Ultrastructure of Human Leucocytes synthesizing DNA

The application of autoradiographic techniques in the localization of radioactively labelled compounds in cells by electron microscopy has been developed by a number of workers[1]. The direct coating of ultra-thin sections with photographic emulsion has the disadvantage that long exposure times are required to obtain positive autoradiographs. Furthermore, it is difficult to locate labelled cells in the electron microscope by this method when their frequency is low. In the experiments recorded here the characteristics of the ultrastructure of cells in DNA synthesis were examined by using a 'thick–thin' technique[2]. Two examples of proliferation in atypical lymphocytes were investigated by this method: normal human lymphocytes cultured with phytohæmagglutinin[3–5], and freshly isolated leucocytes from patients suffering from infectious mononucleosis (glandular fever). The cells were incubated with thymidine (^{3}HT) (10 mc./ml., specific activity 1·9 c./mmole) for 30 min and then fixed with osmic acid and embedded in methacrylate. A thick section (about 0·25μ) was cut from a suitable part of the block and mounted on a glass slide. The contiguous ultra-thin section was then cut and mounted on an electron microscope grid. The methacrylate was removed from the thick section with chloroform and the section coated with Ilford *L4* emulsion and stored at 4° C. Three weeks later it was developed in Kodak *D19B* and stained with toluidine blue. A map of the whole section was made from a mosaic of light photomicrographs and the cells with positive autoradiographs noted. This map was used to locate the position of the DNA-synthesizing cells in the ultra-thin section, which were then examined and photographed in the electron microscope.

In general, cells in DNA synthesis (*S* cells) tended to be larger than other transformed cells and their ultrastructure differed in several respects. The nuclear double membrane showed a far greater degree of irregularity in the cells synthesizing DNA. The separation of the two components was variable and resulted in a beaded appearance. In some cases the outer membrane showed evaginations, and in others it was fragmented. It seems likely that this represents a very early stage in the breakdown of the nuclear membrane which is known to precede mitosis. Exceptionally large-diameter nuclear pores were also present in these cells. The chromatin of these nuclei was loosely packed, especially in the central zone of the nucleus; nucleoli were frequently seen and often had a 'bobbined' appearance. The resting transformed cells had more densely packed chromatin and the nuclear membrane was more regular but contained some smaller pores. Nucleoli were less frequently seen in these cells. A few cells with negative autoradiographs were seen to have loose chromatin and highly irregular cell membranes which were breaking down; these were considered to be nuclei in early prophase.

The cytoplasm of the *S* cells had three major features which distinguished it from the organization of the resting transformed cells. The most noticeable was the far higher concentration of free ribosomes and larger number of dense bodies, possibly containing glycogen, scattered in the cytoplasm of the DNA-synthesizing cells. Endoplasmic reticulum was poorly developed in the transformed lymphocytes, but these cells had a more pronounced quantity of smooth endoplasmic reticulum than the resting transformed cells. Among the DNA-synthesizing cells there was considerable evidence of elaboration of mitochondria, especially in the Golgi zone. The other features of the transformed cells—micropinocytosis, the multivesicular bodies, lipid-containing bodies and Gall bodies—were present in the cytoplasm with equal frequency in resting and in DNA-synthesizing cells.

A general ultrastructural description of the characteristic cells of infectious mononucleosis has been given[6,7]. This work suggests that the differences between DNA-synthesizing and resting cells in this cell line were very similar to those described here for phytohæmagglutinin-transformed cells. The chromatin density and nuclear membrane differences were present, but nucleoli were infrequently seen in both resting and DNA-synthesizing cells. The greatly increased cytoplasmic free ribosome concentration in the *S* cells was equally marked, as was the increase in elements of the smooth endoplasmic reticulum. The other cytoplasmic differences, though present, were less marked than in the phytohæmagglutinin-transformed cells.

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Allotypes of Hen Serum Proteins

The allotypic differences in antigenic specificity of rabbit serum proteins were discovered by Oudin[1] in 1956. Oudin's results were confirmed by Dray and Young[2]. Serum groups in rabbits were also described by Dubiski, Dudziak and Skałba[3]. Immunological differences in serum proteins have also been demonstrated in the guinea-pig[4] and the mouse[5].

This paper presents the results of investigations which showed the allotypic specificity of hen serum proteins. The experiments were carried out on four breeds of hen: Rhode Island Red, Sussex, White Leghorn and Greenleg. The isoprecipitins were obtained by immunizing the hens with bacteria mixed with corresponding immune antibacterial hen serum. For the production of antibacterial sera hens were immunized with heat-killed bacterial suspensions of *Proteus vulgaris* (*OXl9*). 0·5 ml. of antibacterial serum was mixed with 0·05 ml. of *Proteus* suspension containing approximately 10^9 cells. The mixture was incubated for 60 min at 37° C and re-suspended in 1 ml. of saline. The inoculum thus prepared was injected intravenously. The injections were made three times a week. During the whole period of immunization (7–10 months) each hen was inoculated with one antibacterial serum only. The birds were bled eight days after the final injection.

The immune sera were tested in 1·5 per cent agar gel by the method of Ouchterlony. The concentration of sodium chloride was 8 per cent in agar gel.

Table 1. Distribution of the Allotypes GA1, GA2 and GA3 in Different Hen Races

Breed	Number of hens	Allotypes GA1	GA2	GA3
Rhode Island Red	112	112	40	41
		1·000	0·357	0·366
Sussex	113	78	65	68
		0·690	0·572	0·602
White Leghorn	120	74	68	48
		0·617	0·567	0·400
Greenleg	117	111	60	37
		0·949	0·513	0·316

Three precipitating antisera were obtained. Three distinct antibodies were identified and the corresponding allotypes of serum proteins. In accordance with the agreed notation for allotypy[6], the allotypic specificities of hen serum proteins were marked GA1, GA2 and GA3.

It is theoretically possible to foresee eight different combinations of the three allotypes, if they were independent.