AN ELECTRON-MICROSCOPE STUDY OF THE 
IN VITRO TRANSFORMATION OF HUMAN 
LEUCOCYTES 

I. TRANSFORMATION OF LYMPHOCYTES TO 
BLASTOID CELLS IN THE PRESENCE OF 
PHYTOHAEMAGGLUTININ 

J. A. CHAPMAN, M. W. ELVES* AND J. GOUGH† 
Rheumatism Research Centre, University of Manchester, and 
Department of Clinical Haematology, The Royal Infirmary, Manchester 

SUMMARY 

Electron-microscope studies of cultured small lymphocytes from human peripheral blood 
transforming into larger blastoid cells in the presence of phytohaemagglutinin (PHA) show that 
the transformed cell possesses the preliminary stages of development of a protein-synthesizing 
system. The transformed blastoid cell has abundant ribosomes, although, in contrast with 
in vivo protein-secreting cells, many of these occur as single particles with only a small pro-
portion linked in polysomal clusters. Endoplasmic reticulum membranes occur to a very limited 
extent and with a marked paucity of attached ribosomal particles; the few attached particles 
are usually located in groups. Some endoplasmic reticulum membranes revealed degenerative 
changes in otherwise normal cells. A moderately well-developed Golgi apparatus was a charac-
teristic feature of the cells. Apart from the relatively low proportion of polysomes, in vitro 
PHA-transformed blastoid cells are identical in fine structure to in vivo blast cells (otherwise 
known as immunoblasts, haemocytes, etc.) occurring in the immune response. It is sug-
gested that messenger-RNA production in PHA-stimulated transformed cells may be reduced 
and that this could explain the limited number of polysomes and the restricted development of 
the endoplasmic reticulum. 

INTRODUCTION 

There is now ample evidence that the small lymphocyte of peripheral blood can 
transform in vitro into at least two other cell types. One type of transformation occurs 
in the presence of phytohaemagglutinin (PHA) (MacKinney, Stohlman & Brecher, 
1962; Carstairs, 1962; Elves & Wilkinson, 1963; Cooper, Barkhan & Hale, 1963), as 
a result of antigenic stimulation in a secondary immune response (Pearmain, Lycette 
& Fitzgerald, 1963; Elves, Roath & Israels, 1963; Schrek, 1963) or when lymphocytes 
from two individuals are mixed (Bain, Vas & Lowenstein, 1964; Elves & Israels, 
1965). This transformation leads to the development and proliferation of large 
blastoid (blast-like) cells similar to cells found in vivo which have been variously 

* Present address: The Charles Salt Research Centre, Robert Jones and Agnes Hunt Ortho-
apaedic Hospital, Oswestry, Salop. 
† Present address: Department of Pathology, University of Manchester.
described by others as transitional cells (Fagraeus, 1948), large lymphocytes (Trowell, 
1958; Nossal & Mäkelä, 1962), activated reticular cells (Marshall & White, 1950), 
large pyroninophilic cells (Gowans, McGregor, Cowen & Ford, 1962), haemocyto-
blasts (Fagraeus, 1960; André-Schwartz, 1964; Wellensiek & Coons, 1964), immuno-
blasts (Dameshek, 1963; Movat & Fernando, 1965) and blasts (Nossal & Mitchell, 
1963; de Petris & Karlsbad, 1965). In this paper the large cells which develop in 
cultured peripheral blood in the presence of PHA will be referred to simply as 
PHA-transformed cells' or, more generally, as 'blastoid cells'.

The other type of in vitro transformation occurs in the absence of stimulating agents 
(such as PHA) and results in the development of macrophages. A cytochemical study 
of this transformation has recently been made by Gough & Elves (1966, 1967).

The purpose of this paper is to describe the salient ultrastructural features of blastoid 
cells proliferating in cultures of human peripheral blood in the presence of PHA. 
A separate paper (Chapman, Gough & Elves, 1967) is devoted to a similar study of 
macrophages developing in cultures in the absence of PHA.

MATERIALS AND METHODS

Leucocytes were obtained from the peripheral blood of normal healthy human 
subjects by dextran sedimentation, using 2 ml of 6% dextran in isotonic saline ('Dex-
traven' from Benger Laboratories, Holmes Chapel, Cheshire, England) per 20 ml 
of heparinized blood. For the study of the blastoid transformation, 0.1 ml of Difco 
phytohaemagglutinin P (PHA) was added to the separated leucocytes, which were then 
divided into 2–3 ml aliquots; these were made up to 10 ml each by the addition of 
TC 199 (Glaxo). The cultures were placed in universal containers and to some of them 
500 µg of hydrocortisone were added not more than 10 min after the addition of PHA. 
Cultures were sacrificed after 10–30 min, 24, 48 and 72 h by centrifugation at 800–
1000 rev/min for 10 min. After withdrawal of the supernatant medium the cells were 
wash with isotonic saline, collected again by centrifugation and fixed for electron 
microscopy.

Fixation was in 1% ice-cold osmium tetroxide in veronal-acetate buffer at pH 7.2–
7.4, made isotonic with sodium chloride, and the duration of fixation was 15–20 min. 
Two methods were used for the treatment of cells. In the first method the cells were 
fixed in suspension and pre-embedded in agar on a microscope slide as previously 
described (Elves, Gough, Chapman & Israels, 1964); in the second method a centri-
fuged button of cells was fixed for 5 min, cut into small fragments with a cataract 
knife and then fixed for a further 15 min. Both methods gave satisfactory results but 
the second method was more convenient and yielded a greater concentration of cells 
in the sections.

Specimens were dehydrated in increasing concentrations of ethanol and embedded 
in Araldite. Thin sections cut with a Huxley ultramicrotome were mounted on carbon-
filmed grids and examined at instrumental magnifications ranging from × 5000 to 
× 80000 in a Siemens Elmiskop I electron microscope operating at 60 or 80 kV. Most 
sections were stained with aqueous lead hydroxide, aqueous lead citrate, or methanolic
Transformation of lymphocytes to blastoids

uranyl acetate (Stempak & Ward, 1964). Sometimes a combination of lead and uranyl staining was used.

RESULTS

Initial cultures

In cultures sacrificed at the start of the culture period (10–30 min) neutrophils and small lymphocytes were predominant; occasional monocytes and eosinophils were also seen. The ultrastructural features of these cell types were as described by others (Grey & Biesele, 1955; Low & Freeman, 1958; Paegle, 1961; Bessis & Thiéry, 1961; Bernhard & Leplus, 1964) and will not be described again here.

PHA cultures

Those ultrastructural features of PHA-transformed lymphocytes that have already been considered by others (Tanaka, Epstein, Brecher & Stohlman, 1963; Inman & Cooper, 1963; Johnson & Roberts, 1964) will be described only briefly.

In 24-h PHA cultures, neutrophils showed signs of degeneration, evidenced by pyknosis and vacuolation. The predominant lymphocytes were, in many instances, increased in size and the enlarged cytoplasm in such cells contained many ribosomal particles, identifiable by their size, electron-optical staining properties and distribution. Other cytoplasmic features included mitochondria, centrioles and occasional compound vacuoles (Low & Freeman, 1958). In addition there were small numbers of vesicles, 500–1000 Å across, occurring as membrane-bounded structures with electron-transparent contents, and distributed in isolated form, in chains of vesicles or as short cisternae. Although, in 24-h cultures, these vesicles were only rarely associated with ribosomal particles, observations on later cultures suggest that they may be regarded as developing vesicles of a limited endoplasmic reticulum.

By 48 h these larger lymphoid cells (which may now be described as blastoid cells) were present in greater numbers and in a greater size range, many of them with diameters up to 15 μ. Cytoplasmic organelles were similar to those described for the 24-h cells but ribosomal particles and endoplasmic reticulum vesicles were more abundant. Some of the vesicles of the endoplasmic reticulum appeared to contain material of increased electron opacity and a poorly developed Golgi apparatus was sometimes present. In addition, large electron-opaque granules were commonly observed; these granules, up to 1 μ in diameter and membrane-bounded, occurred in rounded form or as irregular stellate structures. In a few instances the membrane surrounding these granules was seen to be continuous with the membrane of an endoplasmic reticulum or Golgi vesicle (Elves et al. 1964). Similar granules observed by others have been described as lipid droplets (Johnson & Roberts, 1964). Some of the smaller granules are probably lysosomes (Parker, Wakasa & Lukes, 1965). Evidence of mitotic division was occasionally noted and in a few cells the nuclear membrane seemed to be shedding vesicles into the cytoplasm. In these 48-h cultures typical small lymphocytes were still present although in reduced numbers.
In 72-h PHA cultures the blastoid cells, now very much larger than the few remaining small lymphocytes, were the predominant cell type in almost all specimens. Nucleus and cytoplasm were both increased in volume, compared with earlier cultures, although the increase in volume of cytoplasm was significantly greater than that of the nucleus. Nuclei possessed finely-divided chromatin, in marked contrast to the clumped pattern present in the nuclei of small lymphocytes; one or two well-developed nucleoli were usually visible in a sectioned nucleus. In suitably-oriented sections the nucleus displayed a deep indentation, frequently branched. Fig. 2 shows a typical blastoid cell in which many of these characteristics are apparent. At this magnification the most notable feature of the cytoplasm of these cells is the paucity of the more highly organized cytoplasmic organelles. Mitochondria were always present but were mostly small and round in section, with elongated forms occurring infrequently; in most cells the mitochondria possessed numerous well-defined cristae, although evidence of mitochondrial swelling and disruption was commonly noted. In many cells round or stellate electron-opaque granules were prominent; several of these granules occur in the cell of Fig. 9; some stellate granules are present in Fig. 8 but granules are absent from Fig. 2. No 'crystalline structures' showing regular periodicity, as described by Inman & Cooper (1963), were observed. Compound vacuoles and multivesicular bodies (Low & Freeman, 1958) were noted in both blastoid cells and small lymphocytes.

At higher magnification it is immediately apparent that the bulk of the cytoplasm of the larger blastoid cells (such as that shown in Fig. 2) is occupied by ribosomal particles. A rough calculation shows that a typical cell may contain from $10^5$ to $10^6$ ribosomes, comparable with the number found in haemoglobin-synthesizing rabbit reticulocytes (Mathias, Williamson, Huxley & Page, 1964). The ribosomes, illustrated here in Figs. 4–7, occurred singly as isolated particles or in clusters containing up to 11 ribosomal particles per cluster; in many of these the ribosomes were arranged in rosettes having the typical appearance of polysomes (Slayter, Warner, Rich & Hall, 1963; Mathias et al. 1964). In a few instances the ribosomes of a polysomal cluster could be seen to be connected by a thin strand, not more than about 20 Å in diameter and usually located on the inner side of the rosette (Figs. 4, 6).

In an attempt to obtain a rough quantitative estimate of the fraction of the total ribosomal population occurring as polysomes, a histogram was plotted to show the distribution of ribosomal particles in clusters of various sizes. In Fig. 1 this distribution in PHA-transformed blastoid cells in vitro is compared with the corresponding ribosomal distribution observed by dePetrir, Karlsbad, Pernis & Turk (1966) in immunoblasts in vivo. Although only a small number of cells was used to obtain the distribution it was clear from visual inspection of many other cells that the results were typical. The two distributions show that a far higher proportion of ribosomes occurs as single particles in PHA-cultured cells, compared with in vivo immunoblasts; the proportion is also very much greater than that found in haemoglobin-synthesizing rabbit reticulocytes (Rifkind, Danon & Marks, 1964). To a rough approximation, when certain correcting factors are taken into account (see legend to Fig. 1), it would seem that little more than half the total ribosomal population of PHA-transformed cells are grouped together into polysomal aggregates, compared with not less than 90% in...
Transformation of lymphocytes to blastoids

immunoblasts. Nor is it certain that all the multiple clusters of ribosomes are, in fact, polysomes for many of the clusters consisted only of irregular aggregates of particles, unrecognizable as typical polysomal rosettes (see Fig. 4).

Although endoplasmic reticulum could be detected in most 72-h blastoid cells, it was severely limited in extent and usually restricted to isolated, elongated cisternae (Figs. 2, 3) or strings of smaller vesicles. It was rare to find more than 4 or 5 easily recognizable elongated profiles in a single cell in section, and the field of view of Fig. 3 contains an unusually high density of profiles. A conspicuous and puzzling feature of most of these endoplasmic reticulum profiles was the paucity of attached ribosomes. Compared with the dense packing of ribosomes on the endoplasmic reticulum membranes in normal protein-synthesizing and -secreting cells, the membranes of the blastoid cell endoplasmic reticulum were relatively devoid of particles, despite the occurrence of ribosomes in large numbers in the surrounding cytoplasm.
Some attachment of particles to membranes did occur, usually with several ribosomes in close proximity (Figs. 4, 5, 7), suggesting an attachment of polysomes to the membrane. No trace of the thin connecting strand was visible in such situations, implying that the strand, if present, was not far removed from the membrane. Ribosomal particles, singly and in groups, were occasionally observed attached to the outer surface of the perinuclear membrane.

Most 72-h blastoid cells had one or more regions containing a moderately well-developed Golgi apparatus; this was frequently located in proximity to the nuclear cleft and consisted of numbers of flattened sacs and rounded vesicles, all with smooth-surfaced membranes (Figs. 2, 8).

A peculiar, but not uncommon, characteristic of blastoid cells was the presence of single elongated membranes adjacent on one side only to an electron-transparent zone; several of these structures are apparent in Fig. 2. Although probably degenerative or artifactual in origin, they are not immediately interpretable as embedding or sectioning artifacts, as the relative orientation of the membrane component and the clear zone was independent of the cutting direction; ribosomes or polysomes were sometimes attached to one side of a membrane (away from the clear zone), suggesting that the membrane was derived from a degenerating endoplasmic reticulum sac. In some cells these peculiar structures took the form of complex whorls.

Hydrocortisone was added to PHA cultures in an endeavour to bring about a stabilization of intracytoplasmic membranes but few significant effects resulted. Electron-opaque granules tended to be fewer and it seemed that polysomal rosettes were slightly more abundant in hydrocortisone-treated cultures; the histogram of Fig. 1 was obtained from cell cultures to which hydrocortisone had been added. Hydrocortisone is known to depress the incorporation of labelled amino acids and to reduce the numbers of polysomes in thymus cells in rats pre-exposed to the steroid 6–12 h before sacrifice (Gabourel & Fox, 1965), but the effect does not occur if hydrocortisone is added directly to the incubation vessel during the amino acid incorporation period (Gabourel & Comstock, 1964).

The plasma membrane of blastoid cells showed occasional pseudopodial extensions and a tendency for adjacent cells to be in close apposition. No well-developed filopodia were seen and there was no evidence of phagocytic activity; pinocytotic vesicles were rare.

A few older PHA cultures were examined. After 5–6 days, extensive necrosis had occurred and the few remaining non-degenerate blastoid cells did not differ significantly in appearance from 72-h blasts.

DISCUSSION

It is now generally accepted that the small lymphocyte is the precursor of the blastoid type of cell in PHA-cultured human blood (Carstairs, 1962; MacKinney et al. 1962; Elves & Wilkinson, 1963; Schrek, 1963). The salient ultrastructural features of the PHA-transformed blastoid cell as described by others (Tanaka et al. 1963; Inman & Cooper, 1963; Johnson & Roberts, 1964) are in general agreement with the observations made in the present investigation.
Transformation of lymphocytes to blastoids

The main object of this paper is to derive, from a study of blastoid cells at higher electron-optical magnifications, further information about the significance of this in vitro transformation. It is evident from our observations that the PHA-transformed cell is one showing the preliminary stages of development of a protein-synthesizing system. The concentration of ribosomes in a typical PHA-transformed cell is of the same order of magnitude as that encountered in rabbit reticulocytes, known to be synthesizing haemoglobin in large amount (Mathias et al. 1964). It has been shown, however, that, in contrast to reticulocytes and immunoblasts, little more than about half the total population of ribosomes in PHA-transformed cells is present as polysomes ('polyribosomes'). In such polysomal clusters a thin connecting strand is sometimes resolvable and it seems reasonable to postulate that this strand is messenger RNA (Penman, Scherrer, Becker & Darnell, 1963; Gierer, 1963; Slayter et al. 1963; Mathias et al. 1964).

The restricted development of the endoplasmic reticulum is a characteristic feature of PHA-transformed cells. Although protein can be synthesized in the absence of intracytoplasmic membranes (as in reticulocytes), a well-developed endoplasmic reticulum does appear to be a functional requirement for the synthesis of proteins intended for subsequent secretion, as in pancreatic exocrine cells (Caro & Palade, 1964), fibroblasts (Chapman, 1961, 1962; Ross & Benditt, 1961) and plasma cells (Braunsteiner & Pakesh, 1955; Thiéry, 1955, 1957, 1958; Bernhard & Granboulan, 1960; Movat & Fernando, 1962). The limited numbers of isolated cisternae occurring in PHA-transformed cells are recognizable as endoplasmic reticulum sacs by the occasional attachment of ribosomal particles, but extensive parallel arrays of elongated profiles are never found. The attachment of ribosomal particles to the few existing membranes is sporadic and some membranes are almost entirely devoid of particles. Moreover attached particles tend to occur in groups, indicating the attachment of polysomes rather than individual ribosomes; this could mean that messenger RNA is necessary before attachment can occur.

These results are consistent with light-microscope studies in which PHA-transformed cells have been shown to synthesize RNA (McIntyre & Ebaugh, 1962; Epstein & Stohlman, 1964; Hayhoe & Quaglino, 1965; Darzynkiewicz, Krassowski & Skopinska, 1965; Rubin & Cooper, 1965; Gough & Elves, 1966) and protein (Sell, Rowe & Gell, 1965).

In a recent electron-microscopic study of lymphoid tissue during antibody formation Movat & Fernando (1965) have described the fine-structural changes occurring during maturation of plasma cells following primary and secondary antigenic stimulation and have shown that the large pyroninophilic blast cells (designated by these authors as 'immunoblasts') become plasmablasts by the development of a polysome-studded endoplasmic reticulum; plasmablasts, in turn, develop into plasma cells. It is significant that the 'immunoblast' of Movat & Fernando (1965) bears a striking morphological resemblance to the in vitro PHA-transformed cell of the present investigation (figs. 21 and 30 of Movat & Fernando's paper should be compared with Fig. 2 of the present work). Like the PHA-transformed cell the immunoblast possesses abundant ribosomal material, with variable but restricted amounts of endoplasmic
reticulum, moderate numbers of mitochondria and a complex nucleolus. Moreover these investigators present ultrastructural evidence indicating that the immunoblast develops from the small lymphocyte, both in the diffuse lymphoid tissue of lymph nodes and periarteriolar lymphoid tissue of the spleen and also in the germinal centres. The only significant ultrastructural difference between the in vivo immunoblast of Movat & Fernando and the in vitro blastoid cell of the present study is that the ribosomes of the former occur almost entirely in polysomal clusters; these migrate to the endoplasmic reticulum membranes as the immunoblast develops into a plasmablast. Similarly, abundant polysomes and few single ribosomes are seen in lymph-node cells involved in antibody synthesis (de Petris & Karlsbad, 1965), and during the development of contact hypersensitivity (de Petris et al. 1966). In other respects these cells are similar to PHA-transformed blastoid cells. These experiments strongly support the view that in vivo blast cells are derived from lymphocytes, and that a proportion of them can develop into plasmablasts, and then into plasma cells, in the immune response. In this sense the blast cell can be regarded as transitional in character. It is therefore pertinent to enquire why the PHA-transformed cell develops in vitro only as far as the blast ('blastoid') stage.

The morphological evidence alone is insufficient to provide a clear-cut answer to this question. It is possible that a simple nutritional deficiency in the culture medium could result, for example, in defective or inadequate membrane production. The apparently degenerate endoplasmic reticulum membranes noted in many blastoid cells lend some support to this possibility, although other membrane systems, such as that of the Golgi apparatus, show little evidence of degeneration or retarded development.

An alternative explanation is that the limiting factor is the supply of messenger RNA to the ribosomes. An essential feature of the transformation, both in vitro and in vivo, is the increase in ribosomal content. The present investigation has shown, however, that cultured cells contain a significantly smaller proportion of ribosomes arranged in polysomal clusters, compared with the high proportion of polysomes present in blast cells in vivo. The observed ribosomal distribution in vitro is entirely consistent with a substantially reduced production of messenger RNA. That some of the RNA which is produced in PHA-stimulated lymphocytes is messenger RNA is indicated by the pulse-labelling studies of Rubin & Cooper (1965) and Darzynkiewicz et al. (1965), and this is in accord with the observation that some polysomes do occur. Moreover, it has already been noted that ribosomes tend to occur in groups on endoplasmic reticulum membranes, suggesting an attachment of polysomes rather than individual ribosomes to membranes. If development of the endoplasmic reticulum membranes were dependent on the attachment of polysomes, then the limited production of messenger RNA could also explain the restricted growth of the endoplasmic reticulum and the failure of the cultured cells to develop further in the direction of mature protein-synthesizing cells.

The authors are grateful to Professor Kellgren and Dr Israëls for their interest and encouragement and to Mrs H. C. Jeffery and Mr S. Grundy for technical assistance. This investigation has been rendered possible by the financial assistance of the Nuffield Foundation.
REFERENCES


Transformation of lymphocytes to blastoids


(Received 9 September 1966—Revised 10 February 1967)
Figs. 2–9 are electron micrographs of blastoid cells in cultures of human peripheral blood, grown for 72 h in the presence of phytohaemagglutinin (PHA). With the exception of Fig. 2, all micrographs are of cells from cultures to which hydrocortisone had been added.

Fig. 2. Typical PHA-transformed blastoid cell, about 13 x 9 μ in section. The large nucleus (n) is characterized by finely-divided chromatin, two prominent nucleoli and a deep, branched invagination of the nuclear membrane. The cytoplasm contains few organelles apart from numbers of mitochondria (m), a Golgi zone (g) and infrequent endoplasmic reticulum cisternae (er); some membranes, presumed to be derived from the endoplasmic reticulum, show a clear zone on one side (arrows). The bulk of the cytoplasm is occupied by ribosomes. x 16000.
Fig. 3. Endoplasmic reticulum cisternae (er) in the cytoplasm of a blastoid cell. This field of view shows an unusually high density of profiles. \( \times 30000 \).

Fig. 4. Ribosomes as single particles (r) and linked in polysomes (p) in a blastoid cell. In some polysomes the ribosomes can be seen to be connected by a thin strand (mr), presumably messenger RNA. Other groups of ribosomes consist merely of irregular aggregates of particles (a), unrecognizable as polysomes; these were, however, counted as polysomal clusters in the ribosomal distribution of Fig. 1. Some attachment of ribosomes to membranes occurs (arrows), usually with several ribosomes in close proximity. \( \times 100000 \).
Fig. 5. Single ribosomes (r), polysomes (p), irregular aggregates of ribosomes (a) and an endoplasmic reticulum membrane (er) with some attached ribosomes. ×160,000.

Fig. 6. A field of view showing a high density of polysomes. In some instances the thin connecting strand (mr) is detectable. It is possible that this section is grazing the surface of an endoplasmic reticulum cisterna; there is evidence of a membrane system in the lower right-hand corner. ×160,000.
Fig. 7. Attachment of ribosomal particles in groups (arrows) to an endoplasmic reticulum membrane (er). Also shown is the cytoplasmic membrane (cm) and the nuclear membrane (nm). × 160000.
J. A. CHAPMAN, M. W. ELVES AND J. GOUGH (1)
Fig. 8. Golgi apparatus (g), stellate granules (sg), mitochondria (m) and centrioles (c) in a blastoid cell. × 25000.

Fig. 9. Round, electron-opaque granules (rg) and irregular stellate granules (sg) in a blastoid cell. Also shown are a number of incomplete endoplasmic reticulum membranes associated with a clear zone on one side (arrows). × 25000.