The in vitro behaviour of leucocytes has been studied since the early days of tissue culture \(^5\)\(^8\), and the giant cells which appear in these cultures have been investigated in some detail \(^5\)\(^8\). The purpose of this note is to confirm and extend the observations on the formation of the multi-nucleate giant cells through the fusion of monocyctic cells \(^4\), as well as the observation that this process can be accelerated in an acid medium \(^8\).

### Methods

Chicken blood was harvested using approximately 100 units of heparin (obtained from Novo terapeutisk Laboratorium, Kopenhagen) per ml of blood, as anticoagulant. This blood was centrifuged 15 minutes at 2,500 rpm and one half of the supernatant plasma was removed. The blood was resuspended in the remaining plasma and centrifuged in dumbbell-shaped centrifuge tubes (95 mm long, outer diameter of the wide portion = 25 mm, outer diameter of the thin neck = 10 mm) for 5 minutes at 2,500 rpm. The leucocytes could be easily harvested in the narrow neck of the centrifuge tube, relatively free from erythrocytes. The cells were distributed in petri plates, approximately 1 \(\times\) 10\(^6\) cells per 100 mm in diameter, and grown in a nutrient medium consisting of 75% Earle's saline, 20% chicken serum, and 5% embryo extract. After 20 hours growth the leucocytes were spread out on the glass and were then thoroughly washed to remove any erythrocytes and further incubated in fresh nutrient medium. The medium was changed every second day in prolonged experiments.

All microscopic work was done using the Leitz phase contrast system (Heine condensor).

### Experimental Results

#### 1. Growth of leucocyte tissue cultures

The 24 hour old tissue cultures presented an extremely heterogeneous picture; many islands of small cells with rather large nuclei (perhaps thrombocytes) interspersed with the larger macrophage-like cells. After 48 to 72 hours the small cells have completely disappeared and the culture has a rather uniform appearance. When the cultures are very dense, the cells have the appearance of an epitheloid sheet; but when the individual cells have sufficient space, they spread out and often have fibroblast-like...
like extensions. Up to the fifth or sixth day of cultivation the cells may multiply, but later the culture becomes stationary. No successful subcultures of these cells have been made. This type of stationary tissue culture of monocytic blood cells will be called a macrophage culture.

2. Nutrient medium

Many types of media were tested, but the Earle’s saline with 20% chicken serum and 5% embryo extract proved most satisfactory. The most specific requirement seems to be the chicken serum since media containing horse serum or calf serum, in concentrations from 10 to 50% were unable to support growth of the cells. A medium containing Earle’s saline with 1% lactalbumin hydrolysate and 0.5% yeast extract (LaYe) was also tested in combination with from 10 to 40% chicken serum. This medium also proved unsatisfactory although chicken embryo cells grow very well in LaYe with 10% calf serum. The macrophage cells, on the other hand, become highly vacuolated and degenerate in this medium.

3. Production of giant cells

At least two conditions seem necessary for the production of monolayers of giant cells. The first is a suitably high concentration of the macrophage culture. The second is a low pH. Estimates of the pH made by color comparison of standard solutions colored with phenol red, showed that a pH between 5.5 and 6.0 was necessary to induce the transformation. The effect is not due to variations in the $\rho_{\text{CO}_2}$, as demonstrated with cultures held at the same $\rho_{\text{CO}_2}$ but at different pH values by varying the concentration of the bicarbonate.

The macrophages were often observed in the process of “autophagocytosis” which seems to be the main method by which the multinucleate giant cells are produced in this system. Fig. 1 * and 2 show typical examples of autophagocytosis. In fig. 1, a giant cell containing at least 16 nuclei is shown engulfing several smaller macrophages simultaneously. It should be noted that the smaller cells are actually in the same plane as the giant cell. Fig. 2 shows a very large cell engulfing two smaller cells. Note that all three cells are already connected by a network of micro-pseudopodia. Such connections have also been observed in macrophage cultures prior to the formation of the giant cells. Fig. 3 shows a typical giant cell. This cell has some 18 or 20 nuclei, non-uniform in size. Cells containing up to 120 nuclei and 20 – 30 $\mu$ in diameter have been observed. The central portion of these cells, containing the nuclei, is thick whereas the periphery is rather thin. Fig. 4 shows part of a monolayer of giant cells, produced under acid pH conditions.

In order to see if the multinucleate cells arose exclusively by autophagocytosis, the cultures were treated with colchicine in the period during which the giant cells are being formed, in this particular experiment after 4 days incubation. Colchicine was used in concentrations ranging between 0.4 $\gamma$/ml, and 0.8 $\gamma$/ml, and after 18 – 20 hours incubation the cells were stained with Wright-Giemsa stain. No mitoses were observed in the multinucleate cells formed in this time. Furthermore only a few of the mononuclear macrophage cells were observed in mitosis. Some of the giant cells had highly fragmented nuclei and nuclei with very bizarre shapes. It is interesting that this effect of colchicine was also more pronounced at low pH.

Discussion

Giant cells can arise in tissue culture in several ways. Besides the autophagocytosis typical for the phagocytic macrophage system, giant cells can also be produced by damaging the mitotic mechanism in a rapidly dividing cell system. An example of this is the giants produced by the x-irradiation of HeLa cells?. These cells often have a single large nucleus or may have several nuclei plus some nuclear fragments. Giant cells have also been observed in cultures of Earle’s L-cells, where they probably arise due to mitoses without cell division. Giant L-cells with single giant-nuclei, as well as with 4 or 6 smaller nuclei have been observed in our laboratory.

Multinucleate giant cells provide an interesting system for studying the multiplication of animal viruses in tissue culture2. Such cells with their large surface areas, some 10 times larger than the area of the normal macrophage cell, may also be more sensitive to viruses. Some observations suggest this to be true, at least for the giant cells derived from the HeLa system.

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* Fig. 1 — 4 s. Tafel S. 206 d.
Fig. 1. Giant cell engulfing several macrophages. Phase contrast.

Fig. 2. Giant cell (upper right) engulfing two other giant cells (lower left). Phase contrast.

Fig. 3. Typical isolated giant cell. Phase contrast.

Fig. 4. Monolayer of giant cells produced at low pH. Phase contrast.