report may be induced by the mixture through the same process in vivo as well. The reasons why some cells are affected and other cells are only slightly affected is not at all clear. Possibly there is a difference in the amount of cytochrome *c* and other substances[7] in hepatic cells or other differences based on aging of cells. The fact that the nucleus, the nuclear membrane or the cellular membrane were not affected is an important point. Perhaps, such organelles have some mechanisms of protection against the mixture[9].

Zusammenfassung. Anschwellung und Lysis von Mitochondrien im Reagenzglas (in vivo), durch Zugabe von Glutathiongemisch zur Nährlösung verursacht, wurde elektronenmikroskopisch geprüft. Die Mitochondrien wurden in Mäuseleberzellen nach i.p. Injektion zusammen mit dem Glutathiongemisch untersucht.

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Multinucleated Giant Cells in Organ Cultures of Spleen

Multinucleated giant cells develop in vivo both in physiological and in pathological conditions; similarly, their appearance in vitro has been described in tissue cultures and in cell cultures under different conditions by several authors[1–4].

We have described in a previous communication[5] the appearance of large elements, looking like a sincytium, provided with a great number of nuclei, in organ cultures, of spleen of chicken embryo.

The ultrastructural observation now allowed us to ascertain, firstly, that these nuclei belong to single cytoplasmic areas, and therefore the described elements are multinucleated giant cells.

Materials and methods. Cultivation of 19-day-old chicken embryo spleen fragments, according to the method of Wolff and Haffen[6], was undertaken on glucose agar with addition of a chicken embryo total extract. After 1, 2, 3 and 6 day's incubation these cultures were fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer and postfixed in 1.0% osmium tetroxyde.

Results. Within 48 h incubation and in the following days, large cytoplasmic areas provided with nuclei changing in number from 2–3 to 30–40, develop on the whole surface of the explant facing the culture medium (Figure 1).

Some morphological characters of these cells are alike; other characters differ from cell to cell. In fact, in some cells nuclei are oval, they show little chromatin gathered into a narrow rim adherent to the nuclear envelope, and they are separated from one another by abundant cytoplasm (Figure 2). Free polyribosomes scattered in a low electron density matrix are prominent in every part of the cytoplasm. In other cells, on the contrary, the nuclei show folds and deep indentations, they are more tightly crowded in one part of the cell, and the chromatin is gathered in bulkier peripheral aggregations closed to the nuclear envelope. The plasma matrix appears definitely more dense: ribosomes are singly distributed, free polyribosomes are very rarely observed (Figure 3).

The profiles of these cells are considerably irregular and large cytoplasmic finger-like extensions protrude among the adjacent cells. The plasma membrane shows sometimes a linear profile and sometimes elongated digitations engaging similar structures of contiguous cells in a way reminescent of a fusion of the cells.

The Golgi apparatus is gathered both in the perinuclear zone and in some peripheral areas; granular endoplasmic reticulum is inconspicuous; spherical and rod-shaped mitochondria are extensively spread in large numbers. Large dense bodies of considerable size outlined by a membrane and containing a finely granular material are numerous; besides, many small round vesicles contain a homogeneous material of low density.

Discussion. From this data we consider that the multinucleated giant cells are not a single type of cell with uniform characteristics: the abundance of ribosomes, polyribosomes and mitochondria suggest that these cells display an intense metabolic activity; finally, these elements cannot be considered as cells undergoing regressive changes. At present it seems very difficult to test the factors giving rise to their appearance. However, some points already deserve to be emphasized. Multinucleated cells may form under particular conditions of temperature[7] and medium viscosity: but in the organ culture we have set up, according to the above-mentioned method, both of these factors remain as near as possible to the physiological ones and therefore they cannot be considered as causative factors. Moreover, instead of what happens in the case of the hanging drop cultures, the appearance of these elements is not attributable to the simple contact of cellular elements with a foreign body, as the covership ('Deckglas-Fremdkörperzellen')[8]. It is also important to note that in the cellular cultures in liquid medium[9] giant multinucleated cells make their appearance much later (after 7 days of culture) as compared to their early formation in our cultures. On the other hand, the characteristic distribution of these cells at the explant surface, suggests, in our opinion, two considerations.

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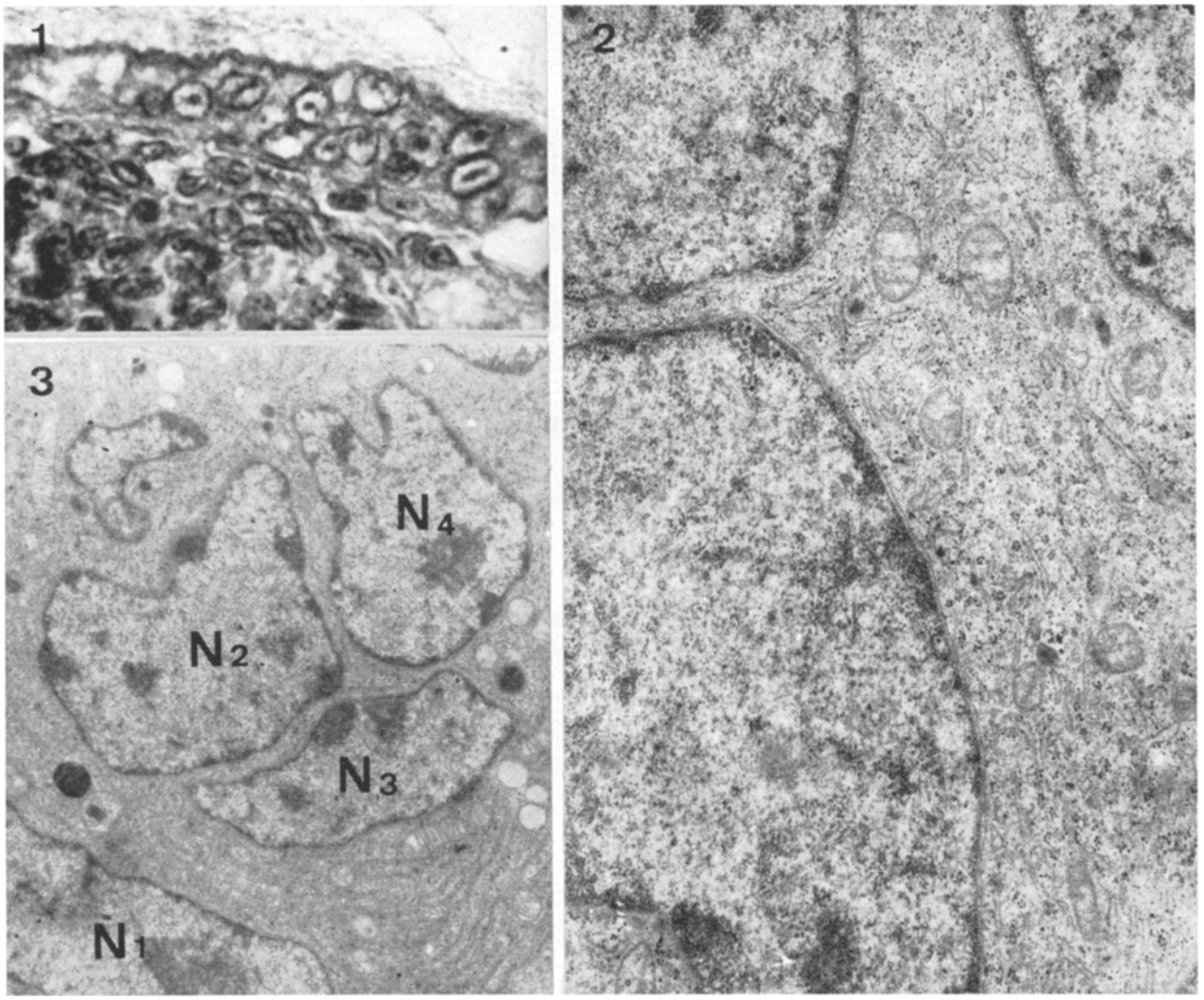

Figs. 1–3. (1) Organ culture of chicken embryo spleen after 48 h incubation. Multinucleated giant cell facing the culture medium. × 780. (2) Detail of a first pattern giant cell showing 3 nuclei shared from one another by abundant cytoplasm provided with a great number or polyribosomes. × 22,400. (3) Four nuclei of a second pattern giant cell; the chromatin characteristic distribution and the irregular profile of the nuclear membrane are evident. × 8500.

An interaction between some substances of the culture medium and lymphoreticular tissue of the explant occurs at their contact face: it is evidenced by some reactive phenomena, such as the appearance of a great number of cells showing phagocytic activity and the differentiation of mastcells (as previously described[10]). Then, we think it possible to presume that the substances of the culture medium, among which are homologous proteins or other substances of high molecular weight may be decisive factors in the process of differentiation of the lymphoreticular tissue.

Besides, we have to emphasize that the explant feeding occurs by adsorption of the material through the medium contact surface, without vascular formation: then it is particularly suggestive that, similarly to what happens for the sincytium-trophoblast in vivo, the multinucleated cells observed develop just in an active exchange zone. Thus, the mitochondria in large numbers and the considerable extension of the plasma membrane of these cells could mean a function of active transport.

Riassunto. In colture organotipiche di milza di embrione di pollo compaiono nella zona di contatto fra le superfici del terreno di coltura e dell'espianto cellule giganti polinucleate. Vengono analizzate le loro caratteristiche ultrastrutturali in base alle quali si evidenziano elementi di almeno 2 tipi. Ipotesi sono avanzate sulle loro possibili attività metaboliche.

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