boxypeptidase B with isopropylfluorphosphate (Worthington Laboratories, Delaware, USA) was employed.

Result and discussion. The i.v. infusion of 300 units of carboxypeptidase B given to a nephrectomized rat of 250 g produced a complete block of the effect of 1–2 mcg of bradykinin given i.v. This inhibition lasted 60–120 min. The response to bradykinin was restored only very slowly. However, no inhibition whatsoever was observed in the pressor action of incubated plasma or its active fraction (VA). 0.1 ml of the incubated plasma or 2 mg of VA produced before the administration of carboxypeptidase B a pressor effect equivalent to 10 ± 0.12 ng of angiotensin II. An equivalent effect either with VA or incubated plasma was recorded after the enzyme was given, whereas the bradykinin effect was completely blocked (Figure).

These results give support to the concept that the pressor action of incubated plasma is not due to the release of a plasmakinin (bradykinin or kallidin). Either the incubated plasma or its active fraction (albumin) do not provide the substrate (kinogens) or the enzyme (kallikrein) needed to liberate the natural plasmakinins. Since the experiments were performed in nephrectomized rats (16 h) renin can be excluded as an enzyme involved in any step of the pressor mechanism elicited by the incubated plasma.

Résumé. L'action vasopressive chez le rat néphrectomisé, produite par l'injection du plasma humain lors de l'incubation à 38 °C pendant plusieurs heures n'est pas due à la libération de plasmakinines. La carboxypeptidase B in vivo à des doses que produisent l'abolition des effets de la bradykinine et d'autres plasmakinines, ne modifient pas l'action vasopressive soit du plasma incubé soit de sa fraction active.

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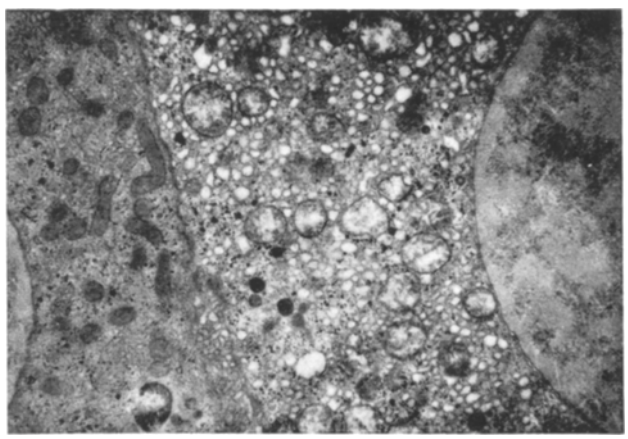
Electron Microscopic Studies on Hepatic Cells in Mice After the Administration of Glutathione Mixtures

The present studies were undertaken to determine the changes in hepatic cells in mice after the injection of oxidized and reduced glutathione mixtures. The dd/YF female 90-day-old mice were injected i.p. with 0.5 ml of physiological solution containing 25 mg of synthetic reduced glutathione which had 99.1 % purity and had no possibility of metals being added in the processes of synthesis (Yamanouchi) or the mixture of 6.15 mg of synthetic reduced glutathione and 61.5 mg of oxidized glutathione (Sigma), and with 0.5 ml of physiological solution. Mice were sacrificed under a non-anaesthetic condition 60 min after the injection and a piece of liver tissue was removed to be fixed in 2% of osmium tetra oxide for 2 h, dehydrated in graded alcohol solutions and embedded in Epon. Hitachi HU-11A, HS-7 and Nihon Denshi JEM-7A electron microscopes were used.

No abnormal picture was found in hepatic cells in mice after the injection with physiological solution, the solution of synthetic reduced glutathione, or oxidized glutathione. In hepatic cells in mice after the administration of glutathione mixture, remarkable swellings of mitochondria were observed, including irregular cristae, many vacuoles and many vesicles which appear to result from changes in the smooth endoplasmic reticulum. The parts of some mitochondria were unclear. Some of the vacuoles appeared to be the result of dissolving lipoid granules. The endoplasmic reticulum, especially rough endoplasmic reticulum, could hardly be found in the altered cells. The nucleus, the nuclear membrane, the intercellular space and the cellular membrane were not affected morphologically. Among altered cells, many unaffected or slightly affected cells were found.

The swelling and lysis of mitochondria from hepatic cells in rats are induced in vitro by the addition of the glutathione mixture to the suspending medium through the process of peroxidation of lipid[1–3]. Such a change is not found in the case of administration of reduced glutathione[4] and is modified with additions of very small amounts of various substances to the suspending medium[2,5–8]. The changes in hepatic cells in the present



Remarkable swelling of mitochondria including irregular cristae, the unclear membranes of mitochondria, many vacuoles and vesicles were found in hepatic cells in mice after the administration of glutathione mixture, but the nuclear membrane and the cellular membrane of the altered cell, the intercellular space and the neighbouring cell are not affected. × 8400.

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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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