refractoriness occurs following application of each substance.

1 Present address: Royal Prince Alfred Hospital, Missenden Road, Camperdown (N.S.W. 2050, Australia).
2 This work was carried out while L.P.B. was supported by a Medical Postgraduate Scholarship from the National Health and Medical Research Council of Australia.
3 H.R. Schumacher, Ann. rheum. Dis. *32*, 212 (1973).
4 H.R. Schumacher, Arthritis Rheum. *12*, 387 (1969).
5 L.P. Bignold and A.W.J. Lykke, Experientia *31*, 671 (1975).
6 D.M. Grennan, W. Mitchell, W. Miller and I.J. Zeitlin, Br. J. Pharmac. *60*, 251 (1977).
7 R.S. Cotran, E.R. Suter and G. Majno, Vasc. Dis. *4*, 107 (1967).
8 D.A. Rowley and E.P. Benditt, J. exp. Med. *103*, 399 (1956).
9 I.A. Oyvin, P.Y. Gaponiuk and I.V. Oyvin, Experientia *23*, 925 (1967).
10 A. Baumgarten, G.J.H. Melrose and W.J. Vagg, J. Physiol., Lond. *208*, 669 (1970).
11 M. Greaves and S. Schuster, J. Physiol., Lond. *193*, 255 (1967).

Ultrastructural studies of Morris hepatoma cells reversely transformed by ginsenosides

H. Abe, S. Arichi, T. Hayashi and S. Odashima[1]

Research Institute of Oriental Medicine, Kinki University, Sayama, Osaka (Japan 589) and Department of Pathology, Kanazawa Medical University, Uchinada, Ishikawa (Japan 920-02), 2 April 1979

Summary. Ginsenosides, which were extracted from Panax ginseng, C.A. Meyer, induced well the development of subcellular organelles in cultured Morris hepatoma cells (MH_1C_1).

The basic action of an extract from the root of Panax ginseng, C.A. Meyer, has been reported to be the stimulation of various metabolic reactions of liver cells in vivo[2-4]. Recently, Odashima et al.[5] have shown that ginsenosides induce a reverse transformation of Morris hepatoma cells (MH_1C_1) as follows. The ability of these cells to grow in 0.33% soft agar suspension culture, which is one of the assays for neoplastic transformation of cells, was reduced to one-fourth of that of the control cells. In addition, a remarkable increase of L-^{3}H-ornithine in arginine deficient medium, an increase in the activity of succinate-cytochrome c reductase and a decrease in the activity of 5′-nucleotidase were also observed. These results suggest that ginsenosides might induce the reverse transformation of MH_1C_1 cells. The present paper describes ultrastructural features of these cells to provide further documentation of the structure and function of these cells.

Materials and methods. Extraction and purification of ginsenosides. Ginsenosides were isolated from the roots of Panax ginseng, C.A. Meyer, as described in a previous report[5]. Cells and growth media. Morris hepatoma cells (MH_1C_1) were grown in Leibovitz L-15 medium supplemented with 10% fetal serum, 50 units/ml of penicillin and 50 µg/ml of streptomycin. The effects of ginsenosides on MH_1C_1 cells were examined using a culture medium containing 100 µg/ml of ginsenosides. The details of our procedures for observing the behaviour of ginsenosides have been previously reported[6-8]. Light and electron microscopy. Cultured MH_1C_1 cells were examined under normal and inverted phase contrast microscopes and under the electron microscope according to the following method. Cultured cells were fixed in situ with 2% glutaraldehyde and post-fixed in 1% osmium tetroxide. After dehydration in graded ethanol solutions, the cultures were covered with Epoxy resin. Capsules filled with epon were inverted over the cultures and the epon polymerized. Sections were stained with uranyl acetate and examined at 100 kV with a Hitachi HS-9 electron microscope.

Results and discussion. Morris hepatoma cells (MH_1C_1) have an irregular form and are relatively small in size ($18.95 \pm 0.78 \times 11.33 \pm 0.58$ µm). The cells have a large nucleus with prominent nucleoli, and scanty cytoplasm as shown in figure 1. The Morris hepatoma cells cultured in the medium containing 100 µg/ml of ginsenosides for 24 subcultures (about 170 days) are much larger ($32.68 \pm 0.8 \times 21.53 \pm 0.65$ µm) than the control Morris hepatoma cells and have abundant cytoplasm containing numerous small particles. They form a typical epitherial pattern and the appearance of them is very similar to that of normal cultured liver cells (figure 2)[5,7]. This process was named 'reverse transformation or redifferentiation' of Morris hepatoma cells, since it appeared to reverse the formation of many of the characteristics assumed by cells treated with malignant transforming agents[5].

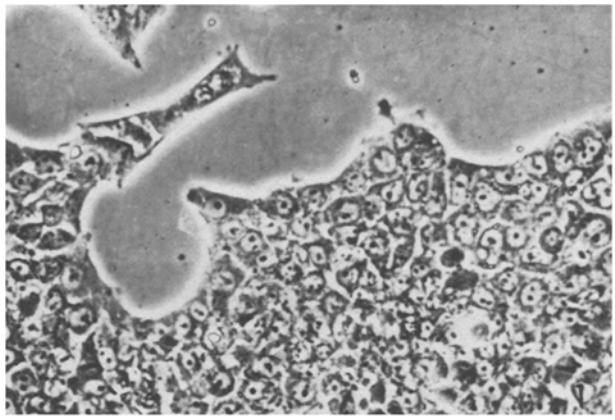
Electron microscopically, Morris hepatoma cells are characterized by small mitochondria and a decreased rough endoplasmic reticulum. Mitochondria are sparse and pleomorphic, averaging 0.2–2.0 µm in diameter as shown in figure 3. Rough endoplasmic reticulum is distributed randomly and has never been seen to form large stacks.

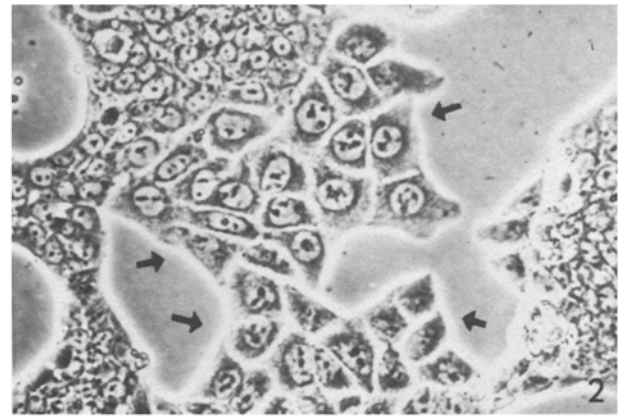
In contrast with this, the subcellular organelles of Morris hepatoma cells cultured in medium containing ginsenosides are well developed and their distribution is also well organized. Mitochondria increase remarkably in number and size (0.5 ± 0.2 µm in diameter and 1.3 ± 0.1 µm in length). Most of these mitochondria with a matrix of normal density are encircled by single cisternae of rough endoplasmic reticulum (figure 4). This observation seems to be closely related to the results, reported in a previous paper[5], that the activities of succinate-cytochrome c reductase and ornithine uptake in arginine deficient medium were significantly increased in reversely transformed Morris hepatoma cells. Golgi complexes are also prominent and consist of enlarged cisternae of smooth membrane (figure 5). Some of these Golgi complexes seemed to be polarized towards the structures resembling bile canaliculi which developed well in comparison with control Morris hepatoma cells.

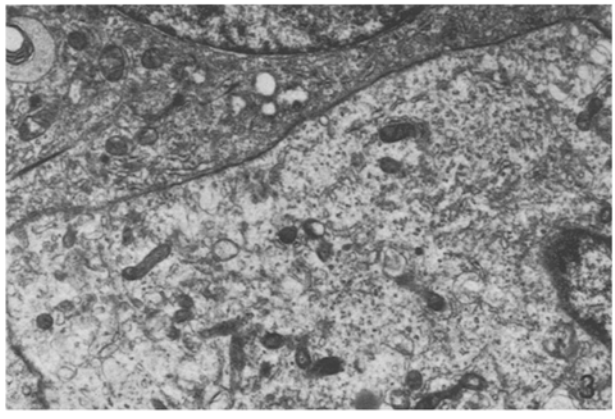
This extensive development and reorganization of subcellular organelles is consistent with our unpublished result[9] that ginsenosides stimulate protein synthesis, particularly production of albumin and α-globulin, in MH_1C_1 cells.

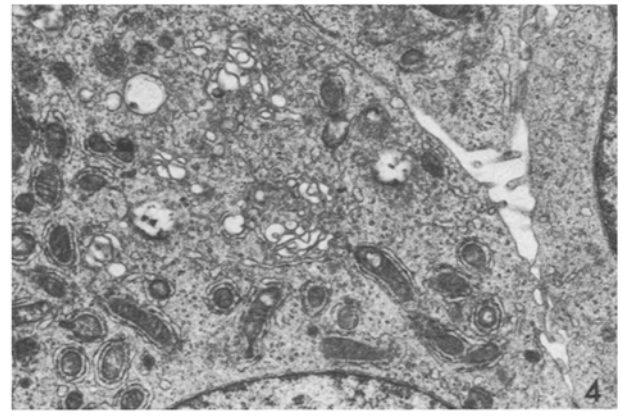
Another structural characteristic of the cells reversely transformed by ginsenosides is that desmosomes are well developed and tonofilaments are seen on the electron dense desmosome plate (figure 6). This result is one of the most important pieces of evidence, since the cells in tumors arising in vivo fail to develope desmosomes[10] and even in normal cultured liver cells the establishment of desmosomes is rarely encountered[11].

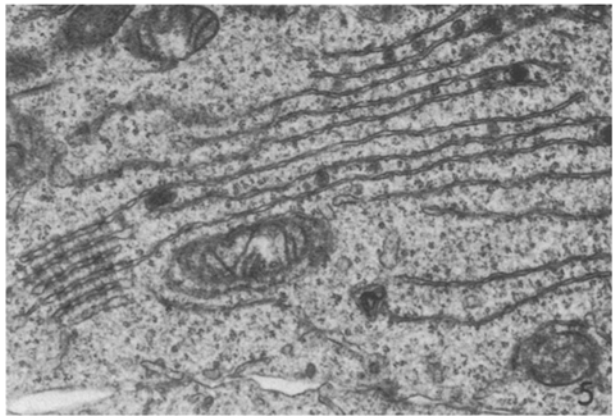
These morphological observations on the reversely transformed cells compare well with those obtained by biochem-











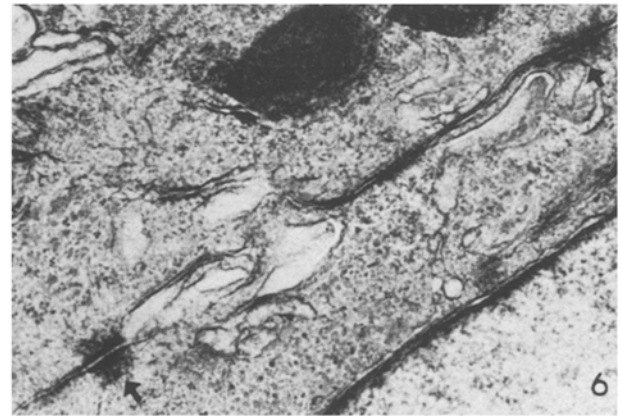


Fig. 1. Phase-contrast micrograph showing control Morris hepatoma cells (MH_1C_1). × 250.

Fig. 2. Phase-contrast micrograph showing hepatoma cells reversely transformed by ginsenosides. Arrows indicate hepatoma cells reversely transformed by ginsenosides. These cells are relatively large, form the typical epitherial pattern and the cytoplasm is granular. × 250.

Fig. 3. Transmission electron micrograph of control MH_1C_1 cells. Small mitochondria are dispersed in the cytoplasm and RER is poorly developed. × 7000.

Fig. 4. Transmission electron micrograph of hepatoma cells reversely transformed by ginsenosides. Note large mitochondria and well developed Golgi complex. Single RER cisternae completely encircle each mitochondrion. × 7000.

Fig. 5. An area of RER in a cell reversely transformed by ginsenosides. Well developed parallel stacks of RER are shown in the cytoplasm of cells grown in medium containing ginsenosides. × 12,000.

Fig. 6. Parts of 2 adjacent reversely transformed cells showing differentiation of plasma membranes resembling desmosomes. × 35,000.

ical studies. But it should be clear from the work reported here that much remains to be learned about the actual process and mechanisms by which ginsenosides induce redifferentiation of Morris hepatoma cells.

1 Acknowledgments. We wish to thank Mr H. Konishi, Mrs H. Makino, Mrs Y. Nakayabu and Miss N. Honjo for their excellent technical assistance.

2 H. Oura, S. Hiai, Y. Okada and T. Yonezawa, J. Biochem. *77*, 1057 (1975).
3 Y. Shibata, T. Nozaki, T. Higashi, S. Sanada and J. Shoji, Chem. pharm. Bull., Tokyo *24*, 2818 (1976).
4 T. Yokozawa, H. Seno and H. Oura, Chem. pharm. Bull., Tokyo *23*, 3095 (1975).
5 S. Odashima, Y. Nakayabu, N. Honjo, H. Abe and S. Arichi, Eur. J. Cancer *15*, 885 (1979).
6 U. Richardson, A.H. Tashjian, Jr and L. Levine, J. Cell Biol. *40*, 236 (1967).
7 S. Odashima, N. Honjo, Y. Nakayabu, H. Abe and S. Arichi, J. Kanazawa Med. Univ. *3*, 91 (1978).
8 S. Odashima, N. Honjo, Y. Nakayabu, H. Abe and S. Arichi, J. Kanazawa Med. Univ. *3*, 95 (1978).
9 S. Odashima, Y. Nakayabu, H. Abe and S. Arichi, in preparation.
10 S.L. Robbins and M. Angell, in: Basic Pathology, p. 88. Sanders, Philadelphia and Toronto 1976.
11 S. Odashima, J.M. Sturgess and A. Rothstein, Cell Tissue Res. *169*, 178 (1976).

The effects of actinomycin D and chloramphenicol on the rat preimplantation embryos

E. Giavini, M. Prati and C. Vismara

University of Milano, Institute of Zoology, Laboratory of Embryology and Experimental Morphology, Via Celoria 10, I-20133 Milano (Italy), 5 February 1979

Summary. Actinomycin D and chloramphenicol, injected in the rat on day 3 or 4 of gestation, induce embryolethality and embryotoxicity. These effects are revealed on day 5 of pregnancy by reduced number of blastocysts and by decrease of mean blastomeres number.

Although it is well known that drugs and other common environmental chemicals pass from the general circulation in the uterine fluid and readily penetrate the embryo in preimplantation stages[1,2], there is little information about the effects of chemicals on the conceptus during the earlier stages of pregnancy.

The flat-mount technique used by Lutwak-Mann[3] in order to study the embryotoxic and dismorphogenetic effects of thalidomide on the rabbit blastocyst is a useful method in this field, but not appliable to rodents. The purpose of the present investigation was to find a ready and efficient routine method for detecting the effects of exogenous agents on the preimplantation development.

In order to evaluate the techniques effectiveness, we tested 2 antibiotics: Actinomycin D (AcD), known to impair the mouse blastocyst development in vitro[3-5], and Chloramphenicol (CAF), teratogen in the rat at very high dosage (more than 1000 mg/kg) but probably not harmful on the preimplantation stages[6].

Materials and methods. Nulliparous Sprague-Dawley rats (Charles River), 200±20 g body weight, were paired overnight with males and the morning on which a sperm-positive vaginal smear was observed, was considered to be day 1 of pregnancy.

The mated females were treated with 300 µg/kg i.p. of AcD on day 3 or 4 of gestation (groups A and B). The females of the groups C and D were treated during the same days with 250 mg/kg i.p. of CAF and the females of group E were treated with 1 ml/kg i.p. of saline on days 3 and 4. Females were killed at 15.00 h on day 5 of pregnancy. The blastocysts were collected in watch glasses by flushing the uterine horns with buffered saline (0.5 ml/horn) and their number was recorded. The blastocysts were afterwards placed in 0.6% sodium citrate for 15 min at room temperature. With the aid of a micropipette, every blastocyst was drawn from this solution, placed in the middle of a slide and fixed with few drops of acetic alcohol (ethyl alcohol + glacial acetic acid 3:1)[7]. The air-dried slides were stained with toluidine blue (2% aqueous solution, for 3 min). This preparation makes it possible to count the scattered blastomeres (figures 1 and 2).

Results and discussion. The results of the investigation are summarized in the table. The CAF administration does not change the average number of collected blastocysts inde-

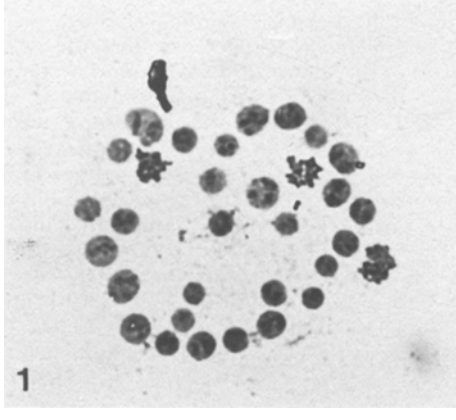


Fig. 1: Blastocyst of control prepared with the air-drying method. 32 blastomeres (86×).

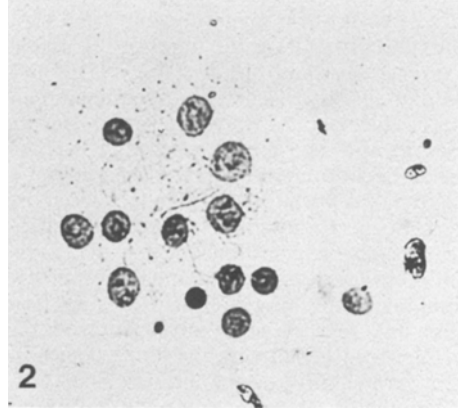


Fig. 2: Blastocyst from female treated with AcD. 13 blastomeres (86×).