ORGANOTYPE CULTURES OF CARCINOMA OF THE PORTIO CERVICIS

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SUMMARY

Fragments of carcinoma of the portio cervicis were cultured by the method of Wolff & Haffen.

The explants retained their organic structure, their proliferating capacity and the cytological characters of the original tissue for the entire period of culture.

These results thus provide an *in vitro* pattern which seems extremely promising for the experimental study of the different aspects of this particular tumour.

Methods of culture *in vitro*, nowadays widely used in the biological and clinical field, can quite profitably be applied in studying the problems of experimental oncology.

In particular, with regard to cultures of neoplasia in the portio cervicis, numerous studies exist, starting with the experiments by Ueno ([21]) to whom credit must be given for having first cultivated cells derived from a squamous carcinoma of the cervix.

Subsequently, Gey *et al.* ([3, 4, 5]) isolated cell strains from the cervix, of which one, under the name of Hela, has enabled a number of interesting investigations to be done on the metabolism of the tumour cell.

Many authors ([2, 6, 7, 8, 11, 12, 13, 14]) have demonstrated that carcinoma of the cervix, contrary to normal epithelium, shows a marked proliferative activity *in vitro*.

A careful analysis of the modes of growth and of the cytological, karyological and ultrastructural characteristics of normal epithelium and of carcinoma *in vitro* was made by Richart (1964) ([16]), Richart & Wilbkans (1966) ([18]), Richart *et al.* (1967) ([17]) Auersperg & Worth (1966) ([1]) and Shingleton & Wilbkans (1974) ([19]). The different proliferative behaviour of the epithelium in the normal and the neoplastic portio was also used as a screening test for diagnostic purposes by Simeckova *et al.* (1962) ([20]).

The above-mentioned research was accomplished entirely by using histiotype methods. So far as we know, there have been no reports in the literature on organ cultures, though these have been applied with success to the experimental study of carcinoma of the endometrium ([9, 10 15]).

This fact is mainly due to the considerable difficulty of culturing cornifying epithelium.

In the present investigations we set ourselves the task of demonstrating a

technique which would enable organ cultures of carcinoma of the portio cervicis to be maintained *in vitro*.

MATERIAL AND METHODS

Seven cases of carcinoma of the portio cervicis were taken into consideration, in patients aged between 35 and 71 years. After hysterectomy, fragments of tumour tissue were obtained aseptically, and these were later cultured on a solid medium.

The basic technique used was the method perfected by Wolff & Haffen [22] which has already given satisfactory results in the experimental study of neoplasm [23, 24, 25, 26, 27, 28, 29, 30] and which we have ourselves already used for culturing adenocarcinoma of the body of the uterus.

The solid culture medium [22] was composed of:

— 50 % agar in 1 % solution in Gey's liquid;
— 25 % embryonal chick extract, incubated for 8 - 9 days;
— 25 % horse serum.

Fragments of vitelline membrane of non-incubated hen eggs were spread over this medium. Over the vitelline membrane were placed fragments of mesonephros of chick embryos incubated for 8 - 9 days. These in their turn were covered by the membrane, folded upon itself; and on the upper surface of this fold were finally scattered small fragments of tumour tissue [31, 32, 33]. The use of this device made it possible to avoid invasion of the mesonephric tissue by the tumour [27] and thus the pure tumour tissue could easily be recovered at the time of fixation. The interpositionof the vitelline membrane does not interfere with the passage of trophic substances processed by the mesonephros. These substances are essential for the maintenance *in vitro* of the neoplastic tissue, from the time when the culture medium, which alone will allow the explants of mesonephros to survive and develop, is insufficient to ensure the development of the tumour interposition of the vitelline membrane does not cells [24, 32]. In some experiments a flat mosaic was used, without interposition of the vitelline membrane between the mesonephros and the tumour tissue.

After a period of incubation at 38 °C for about a week, the explants were fixed in Bouin's liquid, embedded with paraffin, and the sections, between 5 and 7 microns in diameter, were stained with haematoxylin-eosin.

In each case, of course, fragments of tumour tissue were fixed at the time when the samples were obtained, and were used as controls.

RESULTS

The carcinoma maintained its own organic structure, which seemed to be well preserved as regards both its epithelial and connective tissue components, throughout the period of culture.

The epithelium retained the morphological characteristics and differentiating capacity of the original tumour.

In cornifying epidermoid carcinomas (fig. 1), plugs of epithelium can be observed after more than a week of culturing: these go deeply into the stroma, and are made up of cells which even when atypical, as shown by their anisokaryosis and nuclear hyperchromia, reproduce the stratification of normal epithelium with the presence of extensive areas of keratosis, often accompanied by typical horny globules.

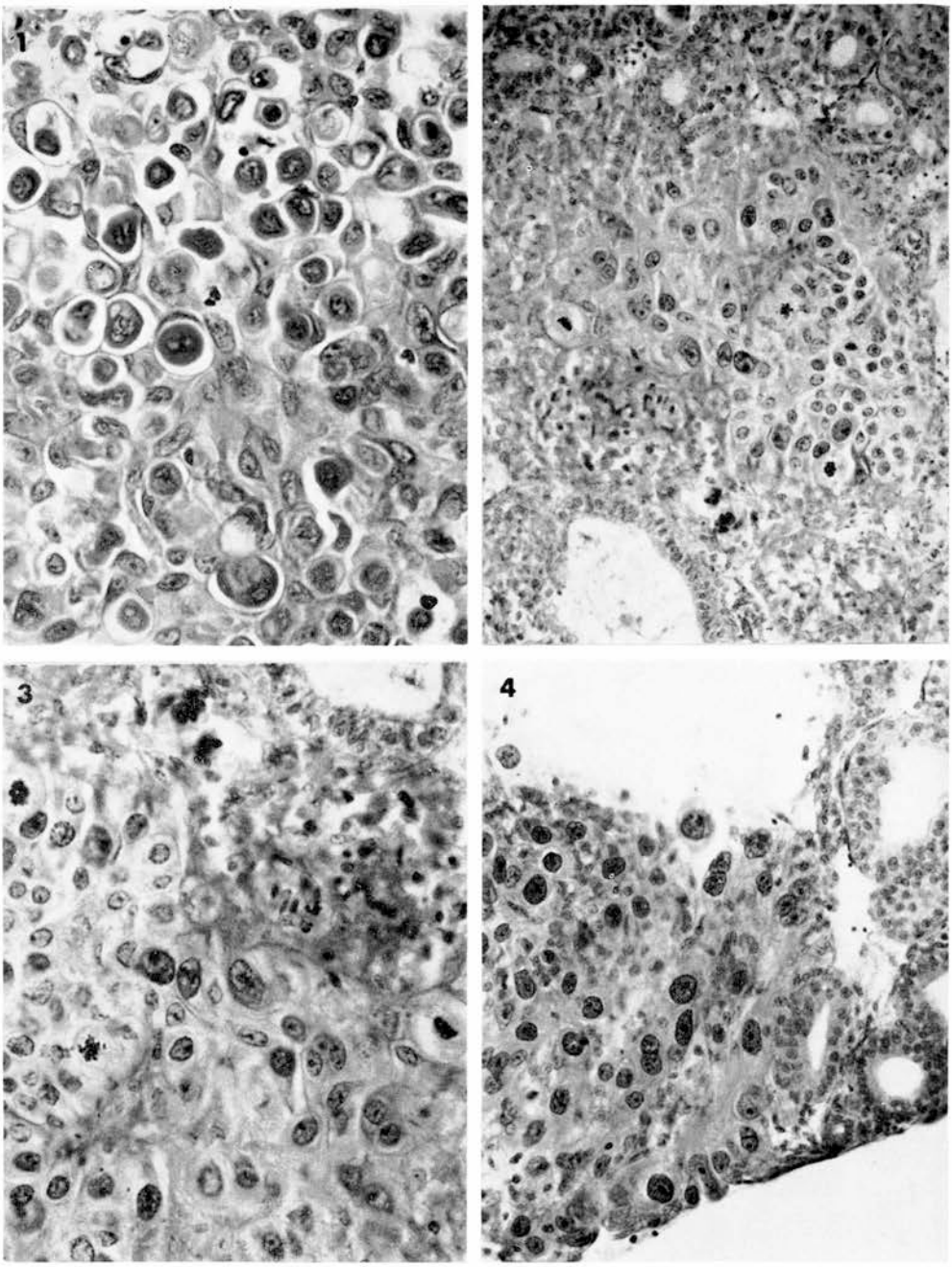
It was also noted that the height of the cells from the basal stratum is exstremely variable, and that the cells of the spinous stratum may show marked anisocytosis.

The connective tissue stroma frequently appeared infiltrated by lymphocytes, and sometimes by polynuclear cells (fig. 2).

In less differentiated carcinomas there is no development in the direction of keratinization and the epithelium is composed essentially of spinous cells (figs. 3, 4) in which anisokaryosis and cellular gigantism are marked; phenomena of karyorrhexis are frequent (fig. 4).

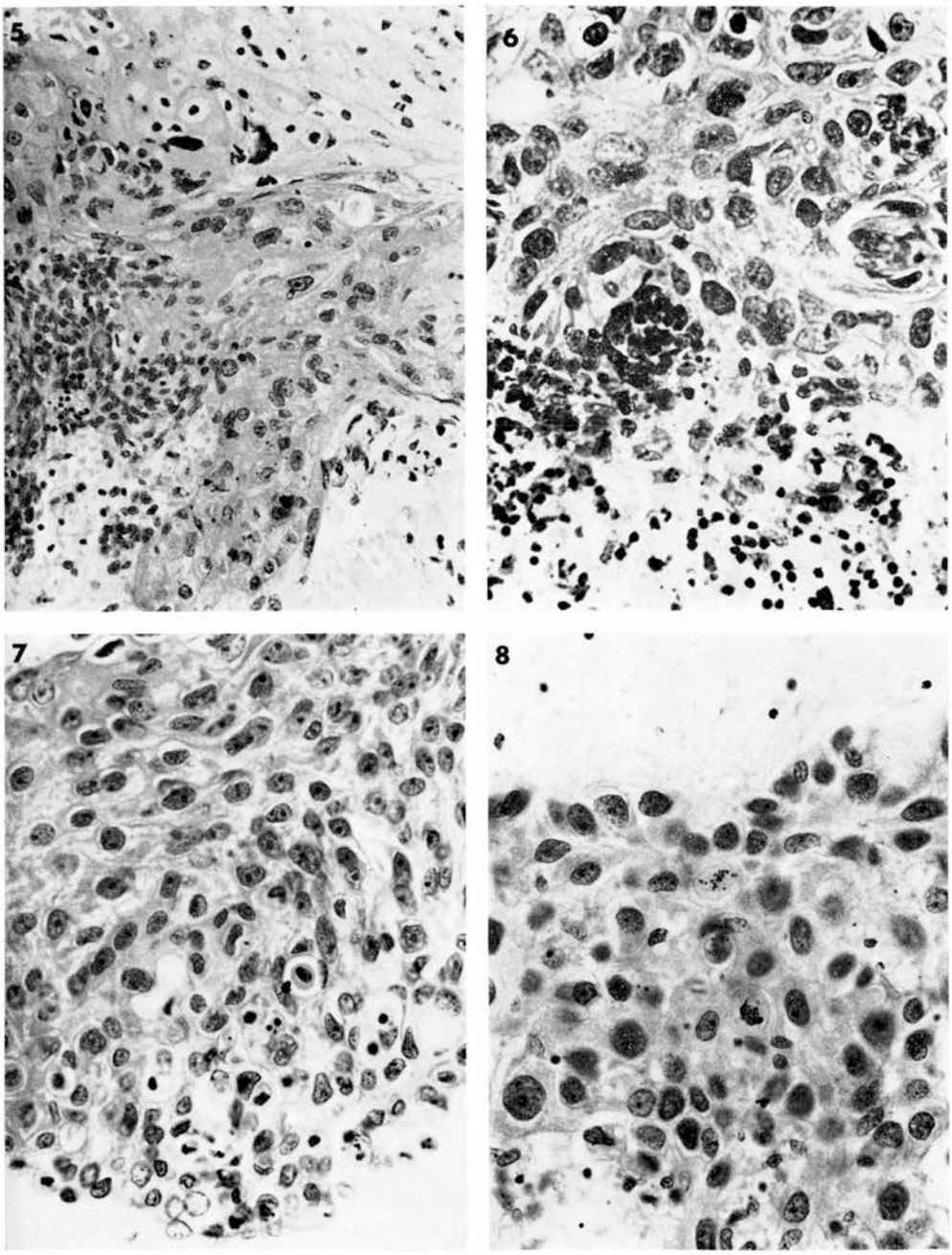
In some cases, phenomena of « cell cannibalism » were observed in the cell population, with phagocytosis of cells keratinized by other less differentiated epithelial cells. But it should be noted that the latter subsequently die and degenerate more rapidly than the phagocytized cells, which, almost mummified, can still be demonstrated at the centre of optically empty areas (fig. 5).

The proliferative activity of the neoplastic cells continues *in vitro* for the entire period of culture, as shown by the



Figs. 1, 2. — Explant of cornifying carcinoma in a woman aged 44. Note a plug of epithelium which has penetrated deeply into the stroma, anisokaryosis, and stromal infiltration by mononuclears.

Figs. 3, 4, 5. — Explant of slightly differentiated epidermoid carcinoma in a woman aged 71. The epithelium is formed of spinous cells; there are no horny laminae. Marked cellular atypia; phenomena indicated karyorrhexis, presence of mitosis (fig. 4) and cell cannibalism (fig. 5).



Figs. 6, 7, 8. — Same case as in previous figures. In these explants the association of tumour with mesonephros has been achieved by means of the flat mosaic technique. The tumour tissue is actively proliferating, as shown by the numerous mitoses, and it is getting nourishment from the mesonephric tissue, in which areas of necrosis are evident.

numerous mitoses present in the sections of the explants.

Finally as regards the relations between the mesonephric tissue of the chick embryo and the neoplastic tissue, since the tumour is nourished at the expense of the mesonephros, wide areas of cell necrosis are found adjacent to normal mesonephric tubules (figs. 6, 7, 8).

CONCLUSION

The present research has made it possible to perfect a system *in vitro* in which explants of a carcinoma of the portio cervicis maintain their organic unity, their proliferative capacity and the cytological characters of the original tissue.

This system is experimentally valid as a model which can be utilized for the study *in vitro* of normal and neoplastic cervical epithelium (understood as an index of biological potential), of dysplastic epithelium and of the carcinoma *in situ*; for the histochemical study of the enzymes and of cell kinetics in cervical lesions; for the study of the part played by viral agents in cervical carcinogenesis, as well as the effect on the tumour cell of drugs of oncological interest, such as chemotherapeutic and cytotoxic substances and hormonal preparations.

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