Gynaecologic Department of the University in Pécs.
(Director : Prof. L. Lajos)

NEW DATA IN THE BIOCHEMISTRY OF NEOPLASM OF THE FEMALE GENITALS

(HISTOCHEMICAL EXAMINATIONS)

László Lajos and *Kálmán Páli*

(Received, November 16, 1951.)

The statement that the biological properties of tumour cells differ widely from those of normal cells is not new. The biochemical aspects of this difference have been in the focus of interest for quite a while. Numerous reports were concerned with the morphology of tumour cells, their nucleus, nucleolus, the mitochondria, the cytoplasm, the nucleus-plasma ratio, without finding a feature characterizing neoplastic cells. Recently, *Zilber* [1] et al. succeded in demonstrating specific antigens in malignant tumours that could not be transferred by their filtrate. They isolated the nucleoproteid fraction of the tumour by sensitizing guinea-pigs with it.

It seems beyond any doubt that the protein synthesis of the organism undergoes far-reaching alterations in cases of malignant tumours.

Recently, attention has been called increasingly to the intracellular enzymatic processes and the biochemical changes related to them. Their importance in carcinogenesis was especially stressed by the Soviet scientists *Roskin* [2] and his associates.

In this Institute large-scale experiments have been carried out for several years with the *McManus* [3]—*Hotchkiss* [4] histochemical method for demonstrating protein-bound carbohydrates in the tissues. It has been shown that mucopolysaccharids, mucoproteids and glucoproteids had a considerable role in various metabolic processes. It was partly for this reason, partly because the disturbances of protein synthesis in tumorous affections seemed proved, further because this method was applied to the intraepithelial neoplasms of the portio by *McManus* & *Lyman Findley* [5] that we have studied the behaviour of neoplastic tissues with regard to protein-bound carbohydrates.

Our observations with this histochemical method in the preblastomatosis of the portio has already been reported [6, 7, 8, 9]. The method seemed suitable for the diagnosis of the precancerosis of the portio. It became evident that parallel with the progression of the process the areas which do not stain become gradually larger.

First of all, it should be mentioned that the epidermis does not stain with periodic acid. In the female genitals conditions are different. The labia maiora

behave as the skin. Contrary to this, the inner surface of the labia minora, the epithelium of the vagina and the portio, when prepared with periodic acid, react to Schiff's aldehyde reagent with a very intensive violet colour in the stratum polygonale, while the basal layer always remains colourless (Fig. 1).

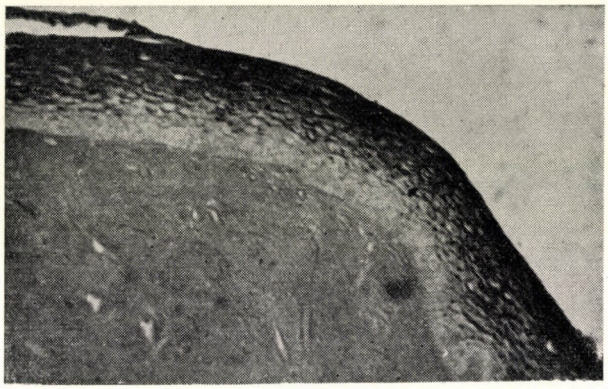

Fig. 1
Normal vaginal and portio epithelium stained by the *McManus—Hotchkiss* method (Schiff's periodic stain). The basal layer and papillae are colourless while the cells of the str. spinosum are vivid violet (100 x)

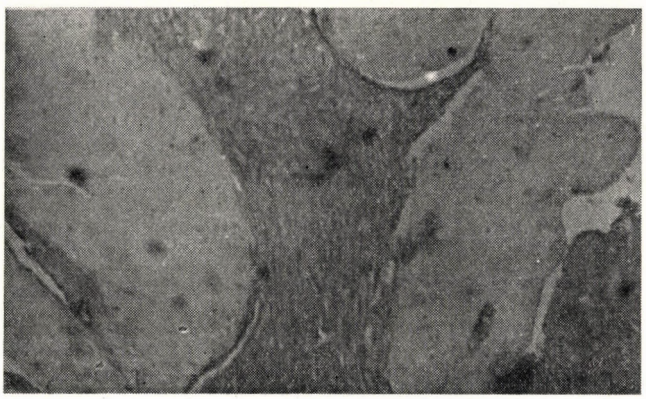

Fig. 2
Squamous cell cancer in the portio. The broad palisades of connective tissue between the cancerous patches stain well. The cancerous cells remain colourless all over the whole picture

Similarly, the papillae projecting from the connective tissue into the stratified epithelium, and the epithelial border surrounding them, i. e. the germinative layer, do not react either. The cylindrical epithelium of the cervical canal, as well as the epithelium of the cervical glands, stain intensively. The endometrium and its glands yield a positive reaction only in the phase of transformation, as a manifestation of progesterone activity. These facts have been stated in an earlier paper [10].

The mucous membrane of the Fallopian tube usually remains unstained, apart from some occasional poor staining of the apical border of the epithelium. Stress must be laid upon the fact that the nuclei do not stain by this method. In positive cases the cytoplasm becomes coloured.

In the following we report our observations made with the *McManus—Hotchkiss* test in cases of cancerous growth of the female genitals. Simultaneously with histochemical reactions, the haematoxylin-eosin stain was also applied in each case. In cases of cancer starting from the epithelium of the vulva, the vagina and the portio, the tumour cell groups proved stain-resisting (Fig. 2).

Regarding the carcinoma in situ of the portio, *Mc Manus & Lyman Findley* claim that the lack of staining is due to the absence of glycogen, positive staining being brought about by the glycogen content

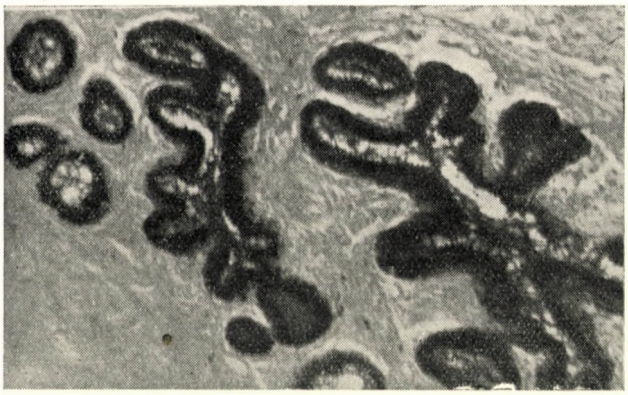

Fig. 3
The epithelium of cervical glands exhibits an intensive reaction under normal conditions (100 x)

of the plasma. Our experiments are not likely to corroborate this contention, because control glycogen staining with Best's carmine performed in series showed that following formalin fixation only traces of glycogen were found in the epithelium of the portio. On the other hand, the intensive hue of the stratum polygonale, noted in positive cases after fixation in aquaeous formalin did not correspond with the poor colouring in the Best-carmine preparations serving as controls, made with the same fixation in aquaeous formalin, wherefore the positive reaction cannot be attributed solely to the glycogen content.

Morphologic observations have led us to accept the view that, in cancers of the skin, vagina, or portio, carcinoma formation starts from the basal layer of the epithelium which, as stated above, remains colourless in normal cases. This may suggest the hypothesis that the negative response of neoplastic parenchyma observed by the above method is due to the fact that it originates in cells wich are devoid of protein-bound carbohydrates under physiological conditions as well. This hypothesis has, however, been refuted by the fact that the

tumours originating in the cervical epithelium or cervical glands (Fig. 3) which are known to stain vividly under physiologic conditions, remain similarly unstained. It should be stressed again that the intensive stain noted in cervical glands under physiological conditions is not

Fig. 4
Adenocarcinoma of the cervix. The glandular epithelium is not stained. In the lumen a secretion containing mucoid substances is visible (100 x)

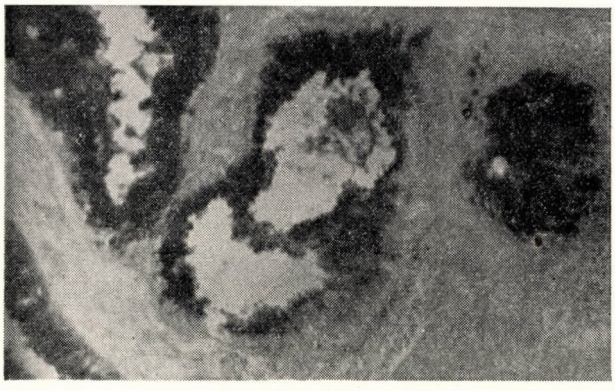

Fig. 5/a
Partial failure of staining in the epithelium of cervical glands (100 x)

induced by glycogen, these tissues failing to show positive glycogen reactions even after fixation in absolute alcohol. On the other hand, the lumina of neoplastic glands frequently contain large masses of mucoid substances. In these instances, the histologic aspect suggests that the epithelial cells have expelled the protein-bound carbohydrates in the course of their malignant transformation (Fig. 4). We observed single colourless areas in several cases within the dark staining cervico-glandular epithelium (Fig. 5a). The identification of these areas on the control haematoxylin-eosin sections revealed morphological signs of a moderate cell accumulation and some unrest (Fig. 5b).

In some parts of the heteroplastic cervical glands the failure to stain was even more striking (Fig. 6a, 6b) although many other heteroplastic glands displayed an intensive colouring. This different attitude of heteroplasias should, from the angle of malignancy, be explained by further experiments.

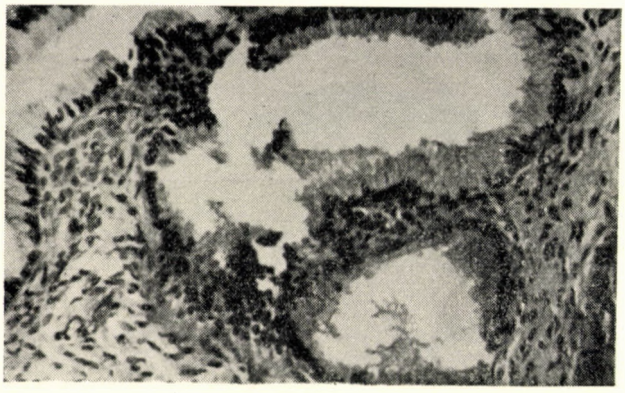

Fig. 5/b
The same with haem.-eosin stain (200 x)

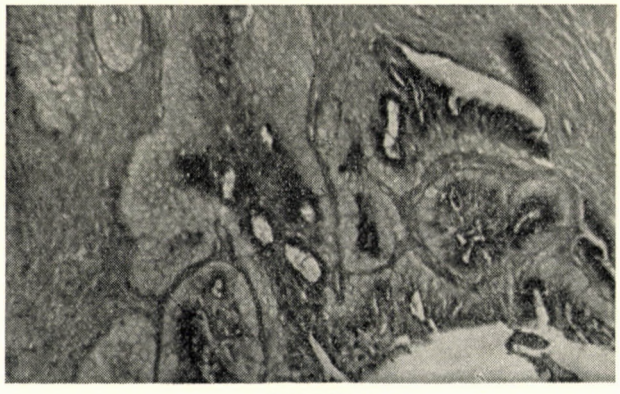

Fig. 6/a
Heteroplasia in the cervical glands showing a negative reaction (100 x)

We shall, at present, confine ourselves to the statement that in the presence of mucoproteids the morphological signs of unrest are absent. The accumulation of cells in the cervical glands, as well as the non-coloured heteroplastic areas, *may eventually be manifestations of the precancerous state.* In order to get a clear picture of these questions, further careful investigations on ample material would be necessary. Obviously, the above observations are in accordance with those made on the preblastomatous portio, which proved that larger non-stained areas correspond to a more advanced state of malignancy.

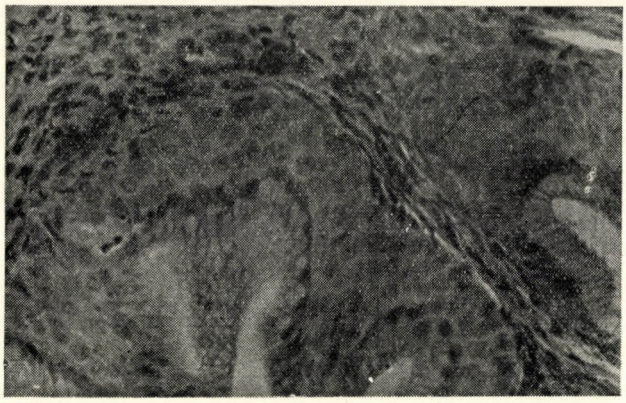

Fig. 6/b
The same with haem-eosin stain (200 x)

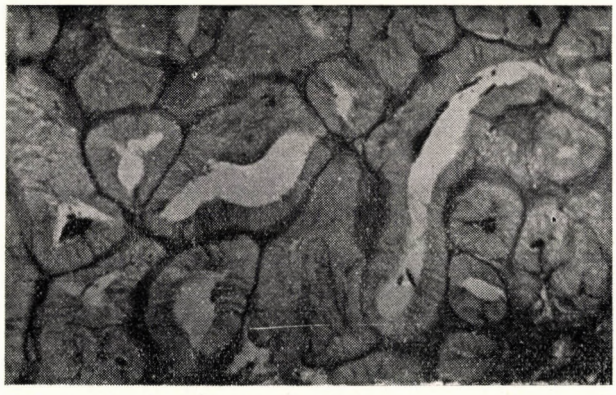

Fig. 7
Adenocarcinoma of the uterine body. The tumour cells remain refractory to staining. Basal membranes take on the dye (100 x)

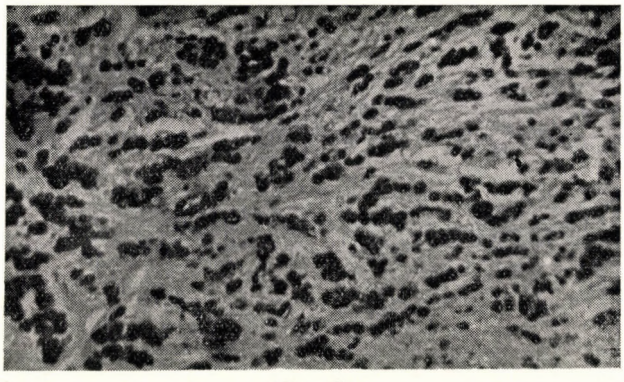

Fig. 8/a
Positive reaction in the mucin-secreting cells of a Krukenberg tumour

These interesting phenomena advocate the opinion that in neoplastic disease, the primary moment is a biochemical change in the cell. This is later followed by morphological changes.

The absence of mucoproteids is demonstrated by the method also in cancers of the uterus and of the tubes (Fig. 7), further in the malignant tumours of the ovary (cancers, granulosa-cell tumors, dysgerminomas). To date, the only exception was the mucus producing Krukenberg-tumour (Fig. 8a & 8b).

Our investigations have thus shown that protein-bound carbohydrates are absent in malignant tumours, as the histochemical procedure failed to

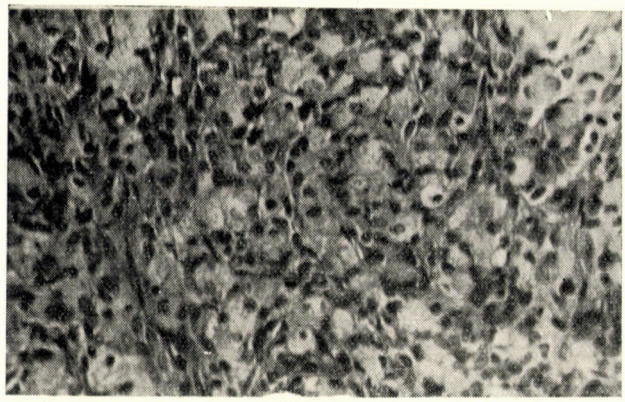

Fig. 8/b.
Krukenberg tumour stained with haem.-eosin. Note the large tumour cells (signet-ring) (200 x)

stain the tumour cells. On the other hand, the cells of benign epithelial tumours react to the stain like the original tissue e. g. cervical polypus stain vividly while adenomas of the endometrium remain colourless after the menopause, like the atrophied glands of the uterine mucosa.

Summary

1. Malignant tumours of the female genitals regularly react to the *McManus—Hotchkiss* histochemical method by failing to take on the dye, quite independently of the fact whether the original tissue responded positively to this method or remained unstained. Krukenberg tumour forms an exception.

2. Consequently, protein-bound carbohydrates are absent in the cells of malignant tumours. According to our investigations, this failure to stain is not due to absence of glycogen.

3. It may be assumed that the *McManus—Hotchkiss* method will also reveal the precancerous conditions of the cervical canal.

4. It seems that the biochemical alteration of the cell is the primary change in the genesis of carcinoma. Morphological transformation of the cell follows later.

5. Benign tumours are stained in the same way as the tissues in which they arise.

6. The examinations have revealed a biological property of the cancerous tissue and may form the basis of further chemical investigations.

REFERENCES

1. *Zilber : (1951.)* A rosszindulatú daganatok specifikus komponense, Onkologia 1. sz. (Original : Успехи Современной Биологии 188. 1950. — Russ.)

2. *Roskin & Struve : (1951.)* After I. J. Elpiner. A rákdiagnosztika biológiai és fizikokémiai módszerei. Onkologia, 1, 44, (Hung.)

3. *McManus : 1946.* Histological demonstration of mucin after periodic acid. Nature 158, 202,

4. *Hotchkiss : (1948.)* Arch. Biochem. 16, 131,

5. *McManus & Lyman Findley : (1949.)* Histochemical Studies on Glycogen in Carcinoma in Situ of the Cervix Uteri. Surg. & Gyn. & Obst. 89, 616,

6. *Lajos—Páli : (1951.)* Uj megfigyelések genitális rákok histochemiai vizsgálata kapcsán. Orv. Hetilap, 6, 179, (Hung.)

7. *Lajos—Páli : (1951.)* Histochemical observations on genital cancer. Nature 167, 721,

8. *Lajos—Páli : (1951.)* Új eljárás a portiorákok korai kórismézésére. Orv. Hetilap 31, 1005, (Hung.)

9. *Lajos—Páli : (1951.)* A new method for the recognition of precancer and of early cancer of the portio vaginalis. The *Journ.* of Obst. & Gyn. of the Brit. Empire. 58, 780,

10. *Páli—Lajos—Bacsó : (1952.)* Az endometriumok histokémiai vizsgálata. Magy. Nőorvosok Lapja 2, 33, (Hung.)

НОВЫЕ ДАННЫЕ К БИОХИМИИ ЗЛОКАЧЕСТВЕННЫХ ОПУХОЛЕЙ ЖЕНСКИХ ПОЛОВЫХ ОРГАНОВ

Л. Лайош, К. Пали

1. При применении гистохимического метода Мек-Мануса-Готшкисс-а (McManus-Hotchkiss) злокачественные опухоли женских половых органов реагируют своеобразно. Они не принимают краски, независимо от того реагировала-ли первоначальная ткань положительно или же оставалась неокрашенной. На опухоли Крукемберга этот закон не относится.

2. Таким образом оказывается, что в клетках злокачественных опухолей нет углеводов, связанных к белкам. По результатам наших исследований эту отрицательную реакцию нельзя объяснить отсутствием гликогена.

3. Можно предполагать, что методом Мек-Мануса-Готшкисс-а можно обнаружить предраковые состояния шеи матки.

4. Кажется, что при возникновении раковой опухоли биохимические изменения клеток являются первичными, и за ними следуют морфологические изменения.

5. Доброкачественные опухоли красятся, также как и ткани в которых они возникли.

6. Настоящие исследования осветили новое биологическое свойство раковой ткани и служат исходной точкой для дальнейших химических исследований.