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HISTOCHEMICAL EXAMINATION OF CERVICAL CARCINOMA DURING RADIOTHERAPY*

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All branches of medical research, and so also oncology, have profited by the rapid progress of the biochemical methods of examination. Metabolic processes occurring in tumour cells have recently received considerable prominence in oncological studies. Histochemistry allows insight into biochemical reactions in the living organism, into enzymatic processes and metabolic changes.

A number of papers deals with degeneration in the cell nucleus and cytoplasm caused by X-rays and radium. Literature contains also reports on metabolic processes which, occurring in the neoplastic tissue of the cervix uteri, can be demonstrated by histochemical methods.

The present paper deals with histochemical changes induced in cervical carcinoma by exposure to X-rays and the effect of radium.

Material and method

The cancer of the uterine cervix of 23 patients who were receiving radiotherapy was studied by biopsy [1] before the beginning of treatment; [2] after the administration of 2000 to 3000 r; [3] after the administration of 1800 to 2000 mg/hr of radium irradiation and a further dose of 4000 r; [4] after an additional dose of 6000 r; and [5] finally, after a further dose of 8000 r and 1800 to 2000 mg/hr of radium emanation. In some of the patients local lesions improved during the treatment to an extent that made it impossible to collect sufficient biopsy material by means of Volkmann's spoon. Excision of tissue by other means might have been harmful and was not attempted. We obtained material utilizable for histological examinations five times from 11, four times from 7, and three times from 6 patients.

Various staining methods were employed, and the manner of fixation was always adapted to the stain chosen in the given case. The stains and staining methods were haematoxylin-eosin; van Gieson's stain; methylgreen-pyronin; Feulgen's method; periodic-acid Schiff reaction; Hale's and Ritter—Oleson's method, Gomori's alkaline phosphatase method. With a view to obtaining fully reliable results we performed specific digestion tests by means of diastase and ribonuclease. (Diastase ex mould L., *Light & Co. Ltd*; ribonuclease from the *Reanal* Works.).

* Dedicated to Prof. B. HORN on the occasion of his 60th anniversary.

Results

Degenerative changes in the nucleus and cytoplasm, due to irradiation, are beyond the scope of this study. We, too, observed pycnosis, karyorrhexis, karyolysis, vacuolation and hydropic degeneration as described by RUZICSKA and BERTA [15]. We shall restrict ourselves to the registration of histochemical observations.

1. *Alkaline phosphatase reaction*

The intensity of the reaction varied with the type of tumour prior to treatment. Cells of adenocarcinoma (especially their nuclei) displayed the most vigorous activity (Fig. 1). The reaction elicited from the stroma was moderate. Activity did not essentially decrease at the beginning of radiotherapy; its decrease became marked after the administration of 4000 r and 1800 to 2000 mg/hr of radium emanation. The reaction was mostly negative after a dose of 6000 r, and invariably negative after 8000 r and a new 1800 to 2000 mg/hr dose of radium irradiation.

Phosphatase activity persisted in the old portions of the stroma, and became less vigorous only after the full series of X-ray irradiation and the termination of the two Ra-treatments. Phosphatase activity increased in the fresh granulation tissue that was growing partly under the effect of irradiation and partly for the purpose of delimiting and carrying off the deteriorated tumour fragments. A high degree of activity was displayed by the endothelial cells of the lymphatics and capillaries, the fibroblasts and the plasmacytes.

2. *Glycogen and mucopolysaccharides*

Cells of collum uteri cancer contain a considerable amount of glycogen (Fig. 2). It should be noted that a determination of the total amount and the distribution of glycogen was easiest when the material had been fixed immediately after excision in Shabad's fluid [17]. This agent contains the ions of heavy-metal salts (Zn, Cu) for the inhibition of bacterial digestion, and phloridzin for that of enzymatic processes. It has been demonstrated that in necropsy or biopsy material free and bound glycogen is almost completely decomposed within 6 to 8 minutes by enzymatic and bacterial digestion. Oxidation with a 0.23 per cent solution of potassium periodate should, according to SHABAD, render only the glycogen specifically stainable, but in our experiments this treatment affected also neutral polysaccharides, mucoproteins and glycoproteins in a similar manner. Therefore, we applied also staining according to Hale and Ritter — Oleson for the separation of neutral and acid mucopolysaccharides. Apart from much glycogen, peripheral tumour cells contain a moderate amount of acid mucopolysaccharides as well; they stain blue with Hale's dye even after digestion by diastase (Fig. 3). A consider-

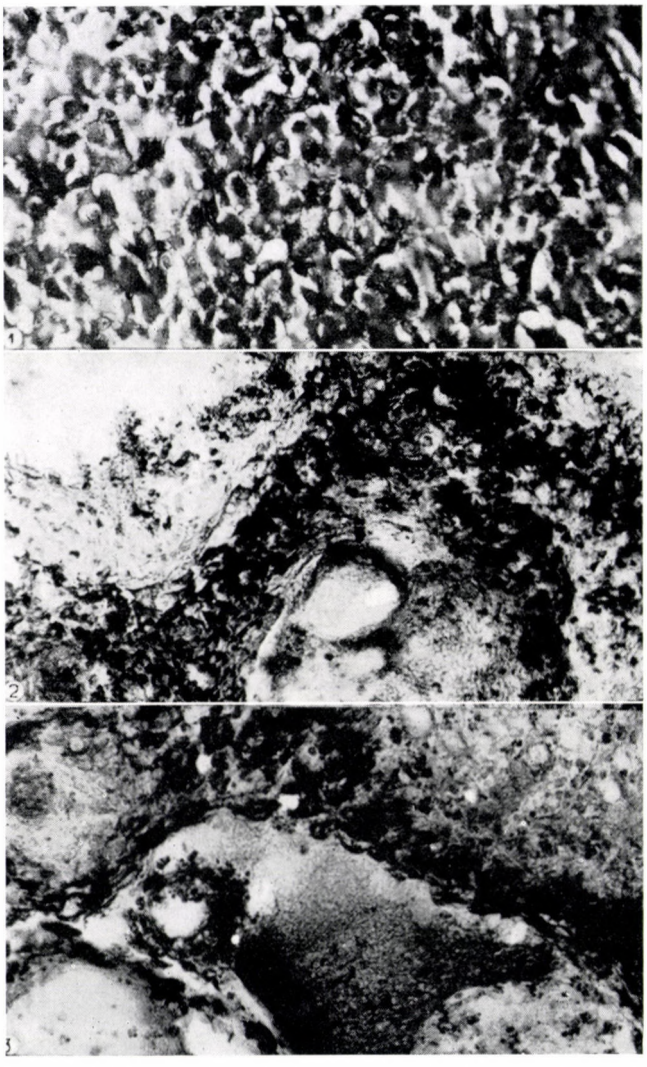

Fig. 1. Intensive phosphatase activity in tumour cells before the beginning of radiotherapy. Gomori's alkaline phosphatase reaction, ×200
Fig. 2. Much glycogen in tumour cells. PAS; ×80
Fig. 3. Blue-staining acid mucopolysaccharides in peripheral tumour cells. Hale's stain; ×80

able quantity of acid mucopolysaccharides was observed in the surrounding richly vascularized stroma and in the intercellular spaces (Fig. 4). The central necrobiotic and necrotic portions of the tumour contained little glycogen. There was, on the other hand, much neutral mucopolysaccharide in them which — after digestion by diastase — stained red with periodic acid (Schiff) and by the method of Ritter and Oleson (Fig. 5). The cytoplasmic particles

of the tumour cells, disintegrated by radiation, continued to give a positive PAS reaction. Part of them proved to be glycogen, while the rest was found to be composed of neutral mucopolysaccharides (Fig. 6). The granulation tissue between the disintegrating parts of the tumour and its surroundings, contained dilated lymphatics and veins. Their walls appeared to be saturated with and thickened by a homogeneous substance which stained red with eosin. This substance consisted partly of glycogen which gave a positive PAS reaction only before digestion with diastase, and partly of neutral mucopolysaccharides which stained red with PAS and Ritter—Oleson even after digestion with diastase. PAS positivity in the cytoplasm of the vacuolated and hydropic tumour cells was considerably diminished, and the vacuoles themselves gave no PAS reaction.

3. *Ribonucleic acid*

The cytoplasm and the nucleolus of unimpaired tumour cells contained much RNA, while vacuolated and hydropic cells displayed no pyroninophilia. We encounted, on the other hand, giant tumour cells, the cytoplasm of which contained many pyroninophilic granules. Though disintegrating under the effect of irradiation, the cytoplasm was pyroninophilic in all its scattered fragments. Part of the homogeneous substance in the walls and lumina of the dilated lymphatics and veins around the disintegrating tumour showed likewise pyroninophilia and lost this property only after digestion with ribonuclease (Fig. 7). There were many pyroninophilic granules in the granulation tissue around the decomposing tumour fragments, as also in the cytoplasm of the mostly perivascular tumour cells.

4. *Desoxyribonucleic acid*

The amount of DNA contained in the nucleus of tumour cells increased considerably under the effect of irradiation (Fig. 8). There was a conspicuous difference in Feulgen's reaction between tumour cells and the surrounding stroma cells. The DNA content of the tumour cells did not significantly decrease under the effect of irradiation, and even the disintegrating and pycnotic fragments were Feulgen positive, while the homogeneous matter in the lymphatics was Feulgen-negative.

Discussion

Our finding concerning alkaline phosphatase agreed well with HOLLÓ and ZLATOROV's [4] results obtained in animal experiments. It was likewise in adenocarcinoma cells that STOLL et al. [17] found the most intensive phosphatase reaction. ORTIS and LLUSIA [11] observed increased phosphatase

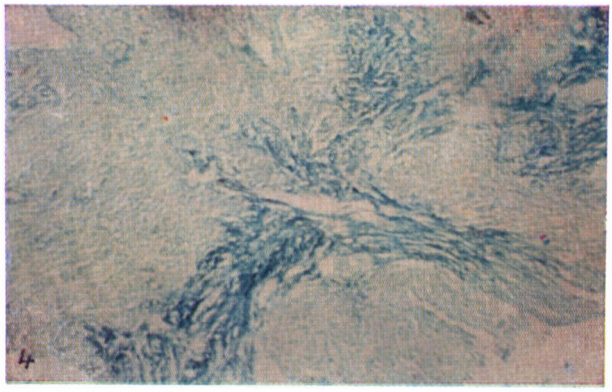

Fig. 4. Large amount of blue-staining acid mucopolysaccharide in richly vascularized stroma around tumour. Ritter—Oleson's stain; ×80

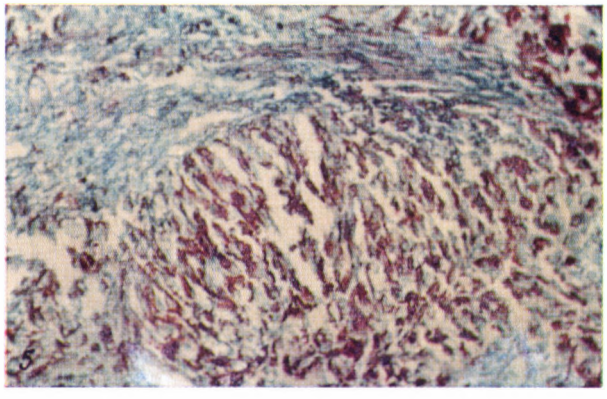

Fig. 5. Large amount of red-staining neutral mucopolysaccharide after digestion with diastase. Ritter—Oleson's stain; ×200

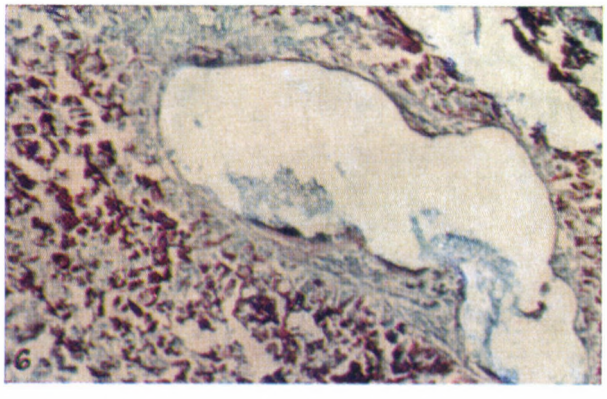

Fig. 6. Red-staining neutral intramural and intravascular mucopolysaccharide after digestion with diastase. Ritter—Oleson's stain; ×200

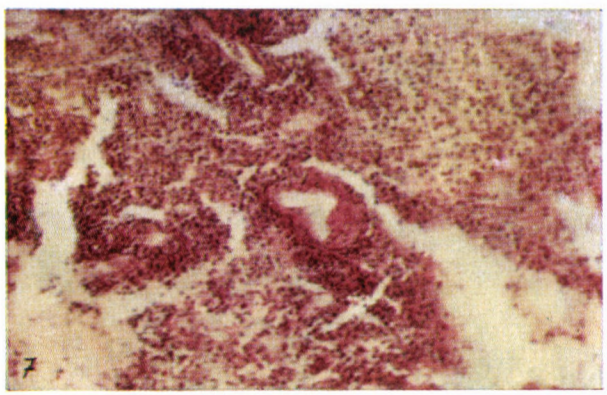

Fig. 7. Subendothelial RNA, staining red before digestion with ribonuclease. Methylgreen—pyronine; ×120

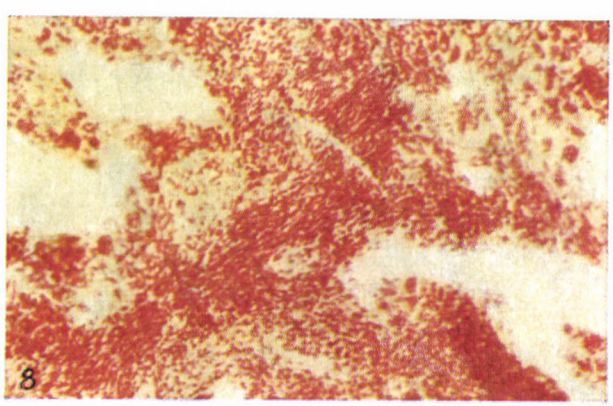

Fig. 8. Large amount of DNA in tumour cell. Feulgen's reaction; ×200

activity in connection with metaplasia and basal cell hyperactivity. RÉV et al. [13] found, in contradiction to our observations, that exposure to gamma rays did not diminish the alkaline phosphatase reaction of the cancerous squamous epithelium of the uterine portion. Their findings concerning the increased activity of the surrounding granulation tissue are in agreement with our results. In our opinion the effect of irradiation manifests itself with a paralysis of the enzymatic metabolism of the tumour cells, which becomes more and more pronounced with the progress of treatment, while a selectivity of the radiation effect may explain the phenomena observed in the stroma and the granulation tissue around the tumour. To wit, carcinoma cells respond to therapeutic doses with necrobiosis, while healthy tissues suffer no damage. SIRACKÝ [16] did not observe any change in the alkaline phosphatase reaction or in the PAS reaction that would have been ascribable to radiotherapy. Changes in connection with DNA or RNA were in many cases indications of radiosensitivity.

This paper is not the first report on the presence of glycogen, glycoproteins and mucopolysaccharides in neoplastic growths. It was as far back as 1903 that E. KROMPECHER [5] discovered a mucoid (termed by him pseudomucin-6) in basal-cell epitheliomas. AVERBAKH demonstrated its presence in neoplasms of the mammary gland. I. KROMPECHER and BERENCSI [7] showed that the PAS-positive substance they had found in mouse cancer, consisted of glycogen, acid and neutral mucopolysaccharides. PAPADIA [12] demonstrated by the PAS reaction the presence of mucin in 75 per cent of epithelial cancers of the uterine cervix. GROSS and DANZINGER [1] found little glycogen in the cells of epithelial cancers both before and after irradiation. HELLWEG [3] observed much mucus in cancers of the uterine portion. All these findings, including our own results, are in sharp contradiction to the observations of LAJOS and PÁLI [8] and MCMANUS [9], according to whom carbohydrates bound to protein are absent in cervical cancers. NOVIKOFF et al. [10] showed that anaerobic glycolysis in neoplastic growth occurs according to Embden—Mayerhof's scheme so that there is no qualitative difference between normal and cancer cells in respect of lactic acid production. There is no reason to suppose that the uterine cervix which abounds in mucoproteins, or neoplastic growths arising in it, should be subject to different laws of metabolism. KROMPECHER and BERENCSI [7] state that acid mucopolysaccharides are produced in tumour cells with good blood supply, and neutral ones in hypoxic cells.

It is commonly known that the level of mucopolysaccharides is considerably elevated in the blood (reaching 1.5 to 2.5 times its original value), when tumours disintegrate and metastases are formed. It was observed that neutral mucopolysaccharides, synthesized under anaerobic conditions, gained access from the tissue interstices via the lymphatics to the blood and this mechanism

was thought to favour the spread of tumours, i.e. the formation of metastases. Particles of the irradiated and disintegrated cytoplasm remained PAS-positive and pyroninophilic, and passed into the lymphatics in our experiments. Viable corpuscles (glycogen, mucopolysaccharides, RNA) transported into the lymph vessels and lymph nodes, may offer favourable conditions for the survival of disjunct but viable cancer cells. Of course, most of these cells are so severely damaged by irradiation that they are unable to survive or propagate even under favourable conditions.

The above described histochemical observations should be regarded as a warning that interventions which may become necessary in the course of radiation treatment have to be made with extreme precaution and always with regard to the oncological aspects. It should be borne in mind that detached tumour cells which have remained viable in spite of irradiation or have suffered but little damage because of insufficient irradiation may, in consequence of non-ablastic surgical interventions, form metastases after having passed into the lymph circulation and found there favourable conditions of survival.

Summary

Biopsy material from the cancer of the uterine cervix of 23 patients has been studied histochemically. It was the alkaline phosphatase activity of the tumour cells which seemed to be most sensitive to X-ray and Ra irradiation; it decreased rapidly in the course of radiotherapy, to disappear completely. On the other hand, enzymatic activity in the surrounding granulation tissue was found to increase under the influence of irradiation. Glycogen, glycoprotein, RNA and DNA were markedly resistant to X-rays and Ra. Even scattered particles of the disintegrated tumour cells remained PAS-positive, pyroninophilic and Feulgen positive. Lymph vessels and plasma cells ensure the transport of the proteins and carbohydrates originating from decomposing tissues.

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HISTOCHEMISCHE UNTERSUCHUNG DES GEBÄRMUTTERHALSKREBSES WÄHREND STRAHLENBEHANDLUNG

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Am Excisionsmaterial von 23 an Gebärmutterhalskrebs leidenden Kranken wurden während der Strahlenbehandlung histochemische Untersuchungen durchgeführt. Die Untersuchungen ergaben, daß die alkalische Phosphatasenaktivität der Geschwulstzellen am empfindlichsten auf die Röntgen- bzw. Radiumtherapie reagiert. Sie vermindert sich rasch im Laufe der einzelnen Behandlungen, um später ganz negativ zu werden. Demgegenüber steigt die Enzymaktivität des Granulationsgewebes, das sich in der Umgebung der Geschwulst bildet. Glykogen und Glykoprotein, Ribonukleinsäure und Desoxyribonukleinsäure zeigen eine starke Resistenz gegen Bestrahlung. Selbst die Teilchen der zerfallenden Geschwulstzellen bleiben PAS-positiv, pyronophil und behalten ihre Feulgenreaktion. Im Abtransport der zerfallenden Gewebseiweiße haben die Lymphgefäße und die Plasmazellen eine entscheidende Bedeutung.

ГИСТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ РАКА ШЕЙКИ МАТКИ ВО ВРЕМЯ ЛУЧЕВОЙ ТЕРАПИИ

Ф. ТОТ, Ш. ЧЕМЕР и Г. СЛЕПКА

Авторы проводили гистохимическое исследование материала пробного иссечения опухолей, полученном у 23 больных раком шейки матки, во время лучевого лечения. Согласно наблюдениям активность щелочной фосфатазы опухолевых клеток чувствительнее всего реагирует на рентгено- и радиотерапию: она в ходе отдельных облучений постепенно снижается, а затем становится совершенно отрицательной. В противоположность этому повышается ферментативная активность грануляционной ткани, образующейся в окрестности опухоли. Гликоген и гликопротеин, рибонуклеиновая кислота и дезоксирибонуклеиновая кислота показывают сильное сопротивление к лучам. Даже распадающиеся частицы опухолевых клеток сохраняют свою ПАСК-положительность, пиронофилию и реакцию Фельгена. В транспорте распадающихся тканевых белков и углегидратов решающую роль играют лимфатические сосуды и плазматические клетки.

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