THE CAPACITY OF NUCLEI FROM NORMAL AND TUMOR CELLS TO STAIN WITH AMMONIACAL SILVER

(UDC 616-006-018-076.1 + 578.65 : 616-006-018)

Ya. G. Érenpreis

Morphology and Physiology Section, Latvian Institute of Experimental and Clinical Medicine, Academy of Medical Sciences, Riga, USSR
(Presented by Academician V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 3, pp. 80-83, March, 1965
Original article submitted September 23, 1963

Black and co-workers discovered that in smears fixed in formalin the chromatin structures of the normal cell nucleus stains with ammoniacal silver. In contrast to this the nucleus of tumor cells are only weakly and diffusely stained, except for the chromosomes of tumor cells which are dividing [2]. The basic conclusions drawn by these investigators was confirmed by Frankfurt [1]. In both papers it was also established that the compounds "responsible" for the nuclear staining of normal cells are histones.

The present study deals with the examination and further study of the differences in capacity for normal and tumor cell nuclei to take up the ammoniacal silver stain.

METHODS

Rats inoculated with sarcoma M-1 (29 animals) and rabbits inoculated with Brown-Pearce carcinoma (15 animals) were the subjects of the study. Impressions of the tumor and of the spleen, liver and mucosa of the small intestine of the same animals were taken on glass slides. The preparations were dried at room temperature and treated according to the technique of Black, as described below.

The impression-preparations are fixed in 10% formalin brought to pH 6.90-6.95 (15 min) with sodium acetate, washed with seven changes of distilled water (two minutes), stained with ammoniacal silver (five sec.), rinsed in five changes of distilled water (two minutes), then 3% neutral formalin with shaking (two minutes) three changes of distilled water (three minutes) dehydrated in alcohols and embedded in balsam. Preparations of the ammoniacal silver: 10% solution of silver nitrate is added in drops with continuous shaking to a concentrated solution of ammonia until the next drop remains as a weakly opalescent insoluble precipitate. Approximately four parts of sodium nitrate solution are added to one part ammonia. The ammoniacal silver is prepared ex tempore; it is pipetted onto the preparation and used one time.

RESULTS

Experiments on rats. Small bowel epithelium. After the reaction has been carried out the chromatin type of staining described by Black is observed (Fig. 1a). The silver granules accumulate mainly along the nuclear membrane and around the nucleolus; single granules or thin chains of them are seen also in the remaining karyoplasm. The cytoplasm remains completely free of silver.

Spleen. The nuclei of different cells of the lymphoid tissue react by staining with variable intensity (Fig. 1b). In the large, round nuclei of the lymphoid type cells the number of silver granules is not dense and they diffusely fill the entire karyoplasm, only the nucleolus remaining free of them. The bean-shaped nuclei of, apparently, the monocytes are stained in almost the same manner. With smaller size nuclei a more dense accumulation of silver granules is observed. Lymphocyte nuclei appear filled with silver granules, whereas polymorphonuclear leucocyte nuclei give a moderately intense reaction. The chromosomes of cells which are in a state of karyokinetic division stain actively with silver. The cytoplasm of all cells remains entirely clear.

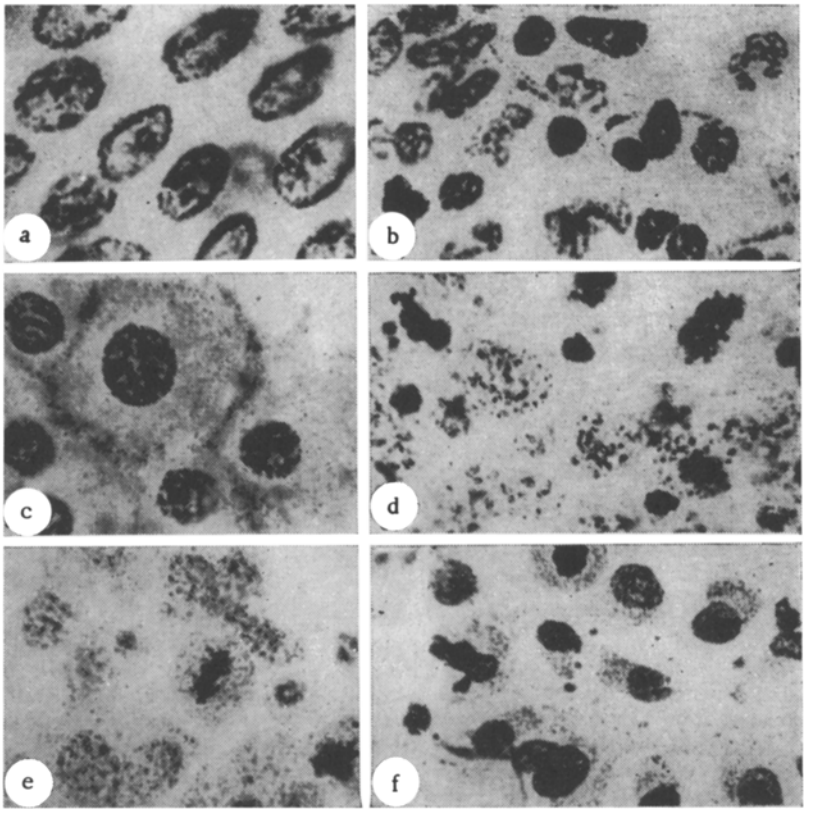

Fig. 1. Staining of cells from different tissues and tumors of rats by ammoniacal silver: a) Small bowel epithelium; b) spleen; c) liver; d,e) cells of sarcoma M-1, which do not give the chromatin type reaction; f) cells of sarcoma M-1, which give the chromatin type reaction. Magnification, 900×.

Liver. In the nuclei of liver cells the silver granules are spread out diffusely or are in chains. Around the nucleolar part of the cell silver is observed to accumulate. A weak, diffuse granularity is also noted in the cytoplasm (Fig. 1b).

Sarcoma M-1. Upon study of tumor imprints from 29 rats, a picture similar to that described by Black when he studied different human and mouse tumors was noted in eight cases (Fig. 1d). Silver granules fill the cytoplasm and the nucleus of the majority of cells almost equally, so that their internal structure is indiscernible. Only in single cells is the topographical relation of the granules to the nuclear structures perceptible (usually as an accumulation of silver around the nucleolus). In no case was silver accumulation observed at the membrane of the tumor cell. The number of granules in one cell varies markedly but is always less (maximum 3-4) than in normal cells. An intense reaction is noted in nuclei of small, wrinkled, evidently dystrophic tumor cells and in the chromosomes of tumor cells in a state of karyokinesis (Fig. 1e). Leucocytes found between the tumor cells react as in the spleen.

In contrast to this description tumor cells from nine rats reacted normally. Not only chromosomes from cells in mitosis but also the nuclei of inter-mitotic cells appeared filled with silver granules markedly localized to the chromatin (Fig. 1f). Their granularity, as a rule, was very fine, often giving a brownish tone instead of the black usually observed. In parts of the cells a faint granularity was also seen in the cytoplasm.

In 12 instances the picture seen was intermediate between the typical states of tumor and normal cells. The nuclei in the majority of tumor cells here contained silver granules with clearly expressed chromatin localization, the density of which was less than in nuclei from normal cells.

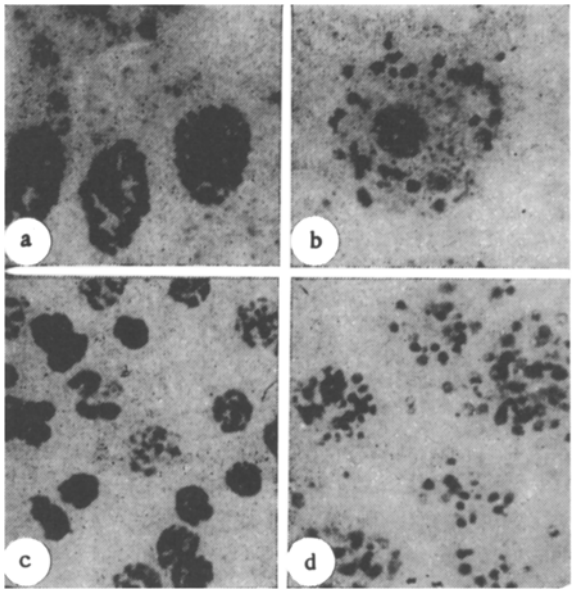

Fig. 2. Staining of cells from different tissues and from tumors of rabbits by ammoniacal silver. a) Small bowel epithelium; b) liver, coarse granularity in cytoplasm is pigment; c) spleen; d) Brown-Pearce carcinoma. Magnification, 900×.

Experiments on rabbits. Small bowel epithelium. After the reaction with ammoniacal silver the same picture was seen as in the rat (Fig. 2a). Silver granules had a chromatin localization, forming more dense accumulations along the nuclear membrane and around the nucleolus.

Spleen. As in the rat, the larger nuclei of lymphoid cells gives a diffuse reaction of moderate intensity. The reaction increases in the smaller nuclei and the nuclei are filled with silver granules (Fig. 2c).

Liver. The nuclei of the hepatic cells of the rabbit react weakly (Fig. 2b). A very fine silver granularity is spread throughout the karyoplasm and only forms denser accumulations around the nucleoli of individual cells.

Brown-Pearce carcinoma. The number of silver granules varies in different tumor cells within rather wide limits. Some of the cells contain only single granules which may be scattered both in the nucleus and in the cytoplasm. In other cells more than 10 granules may be counted, also without any pattern of localization. Finally, in some of the cells a predominantly or exclusively nuclear localization of silver is observed. But in these instances the number of silver granules is considerably smaller than in normal cells, they are distributed in the karyoplasm and the only pattern in their distribution is the accumulation around the nucleolus of single cells (Fig. 2d). Localization of silver along the nuclear membrane of tumor cells is never seen. In none of 15 tumors studied was a picture similar to the reaction of normal cells seen. Chromosomes in karyokinesis react intensely as do the nuclei of dystrophic cells.

Our material confirms the data of Black, that nuclei from normal cells fixed in formalin react with ammoniacal silver to produce a precipitation of metallic granules mainly on the chromatin structures. In studies on the rabbit Brown-Pearce carcinoma we also confirmed the finding that tumor cells, except for those undergoing nuclear division, do not give the chromatin type of reaction. But the reaction of rat sarcoma M-1 cells to ammoniacal silver does not always correspond to this rule. The classic "tumor" type of staining was observed in tumor cells taken from only eight rats, whereas sarcomatous cells from nine rats reacted normally and in 12 instances an intermediate type of reaction was observed.

According to the data of Frankfurt, the reaction with ammoniacal silver unmasks a type of bond between histones and DNA. The argyrophilic groups of the histones of tumor cells are linked with DNA, which also conditions the absence in the tumor cells of the chromatin type of staining characteristic for unchanged cells. The tumor type of reaction may be transferred to normal by treatment of the malignantly degenerated cells with compounds that produce linkage between DNA and the histones and also free argyrophilic groups of the latter [1]. It is possible that the normal reaction we observed in cells of sarcoma M-1 is conditioned by a similar disruption of the DNA-histone bonds which occurs spontaneously or after some type of treatment. The elucidation of this question will require further study.

LITERATURE CITED

1. O. S. Frankfurt, Thesis lecture, Eighth International Cancer Congress [in Russian], (Moscow, 1962), p. 147.
2. M. M. Black, F. D. Speer, and L. C. Lillick, J. nat. Cancer Inst., 1960, Vol. 25, p. 967.