OBSERVATIONS ON THE DESOXYRIBONUCLEIC ACID COMPONENT OF PIGMENTED SKIN TUMORS*

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The fundamental role played by the nucleic acids in the economy of the cell has become increasingly apparent in recent years, despite the fact that their precise mode of action has not been satisfactorily explained. Although many investigators from widely separated fields of endeavor have contributed to the general fund of knowledge regarding the nucleic acids, relatively little work has been done on these compounds as they pertain to pigmented skin tumors. In succeeding paragraphs will be presented first an introduction to the subject of the nucleic acids, followed by an account of observations on the desoxyribonucleic acid component of pigmented skin tumors.

The Nucleic Acids

The two general types of nucleic acids are known as ribonucleic acid (RNA) and desoxyribonucleic acid (DNA). Current evidence suggests that these compounds should be regarded as classes of substances rather than as specific chemical entities (1). Partial hydrolysis of the nucleic acids yields nucleosides and nucleotides while complete hydrolysis yields pyrimidine and purine bases, a sugar component and phosphoric acid (2). RNA occurs chiefly in the cytoplasm of the cell and to a lesser extent in the nucleolus and the chromosomes (3). DNA is confined to the nucleus of the cell. The amounts of RNA fluctuate greatly with altered cell states, being particularly high when the cells are active in biosynthesis (3). The mean amount of DNA, on the other hand, although varying quite widely from species to species, is apparently constant for nuclei of the different somatic tissues of a given species (2). DNA appears, in fact, to be the least variable of all cell constituents (2). This concept of a fixed amount of DNA per nucleus apparently holds for bacterial as well as for animal cells (2). Of the many roles assigned to the nucleic acids by various investigators, those dealing with protein synthesis, cell division and multiplication, the maintenance and transmission of genetic specificity and the transformation of one type of bacterial cell into another with different morphologic characteristics appear to be among the most important and best documented. Several qualitative and quantitative methods for the demonstration of nucleic acids in tissue have been devised during recent years (2).

MATERIALS AND METHODS

At the outset it was decided to confine this investigation to a study of the DNA component of the various tumors. Seborrheic keratosis, intradermal nevus,

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blue nevus, junction nevus, lentigo, pigmented basal cell carcinoma and malignant melanoma were the pigmented skin tumors selected for this investigation. Several individual specimens of each were examined, and subsequent descriptions and comments are based, in each instance, on the study of several lesions. Each lesion was surgically excised, fixed in 10% formalin, sectioned and stained with hematoxylin and eosin in the usual fashion (although formalin is not the ideal fixative for the demonstration of DNA by the technic described below (10), it proved itself quite serviceable in this study). When the clinical diagnosis had been verified by microscopic examination, further sections of each specimen were cut at five microns and all were treated simultaneously by the Feulgen technic (4), which stains DNA a purple or violaceus color. The principle of the Feulgen technic is that hydrolysis with N hydrochloric acid splits off purines from nucleic acid, exposing, in the case of desoxy sugars, an aldehyde group which combines with reduced fuchsin to give a purple color (5). The depth of color appears to be directly related to the amount of DNA (8, 9).

OBSERVATIONS

Seborrheic keratosis—Microscopic examination of H. and E. stained sections showed the typical "stuck-on" character of the lesions, and the usual hyperkeratosis, acanthosis (resulting from the proliferation of basal type cells), papillomatosis, clods and clumps of melanin and cystic inclusions of horny material. In Feulgen stained sections, nuclei of the densely packed basal type cells, which comprised a large proportion of each lesion, were of a moderately deep purple color. Those at the periphery of the lesions were more strongly Feulgen positive than those toward the central portion of the lesions. The cystic inclusions of horny material were Feulgen negative.

Intradermal nevus—Microscopic examination of H. and E. stained sections showed masses, clumps, nests, columns and cords of typical nevus cells located in the dermis. Multinucleated nevus cells were occasionally encountered. Melanin was present in many of the lesions. In Feulgen stained sections the nuclei of all nevus cells were purple in hue but those at the periphery and at the base of the lesions were of a deeper purple color. Multinucleated and melanin-containing nevus cells did not appear to differ from their neighbors in DNA content, as judged by color intensity.

Blue nevus—Microscopic examination of H. and E. stained sections showed typical masses of spindle-shaped melanocytes, and an abundance of melanin, in the dermis. In Feulgen stained sections, the nuclei of these melanocytes were of a notably light purple hue.

Junction nevus—Microscopic examination of H. and E. stained sections showed nevus cells at the epidermal-dermal junction, with many clear cells, and varying amounts of melanin. In Feulgen stained sections the nuclei of cells at the epidermal-dermal junction, especially those of clear cells, were of a much deeper purple color than other cells of the epidermis (Fig. 1). The presence or absence of melanin in cells at the epidermal-dermal junction did not appear to alter their staining properties with the Feulgen technic.

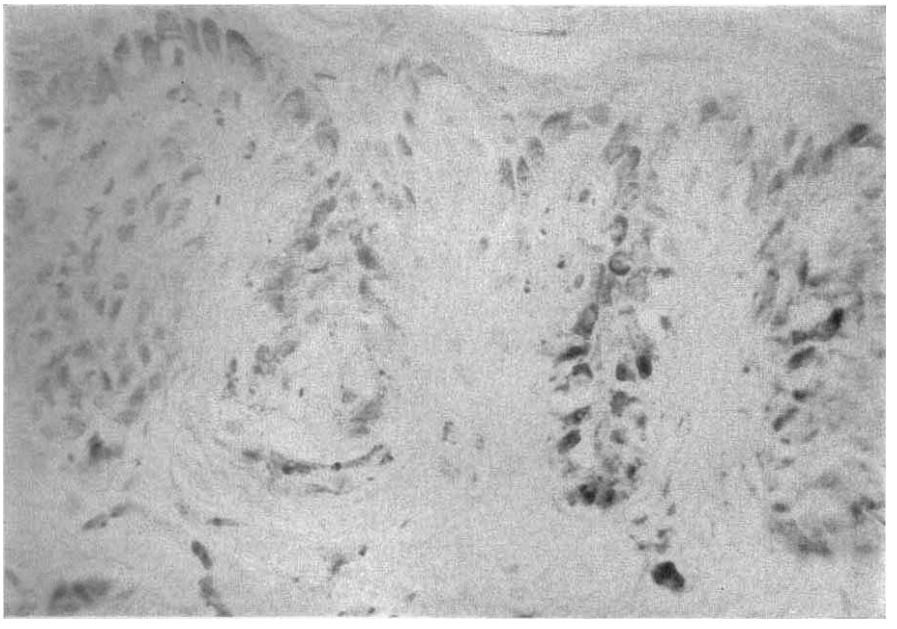

FIG. 1. Junction nevus, Feulgen stain. Note the darkly stained nuclei of the clear cells. These nuclei were deep purple in color. ×580.

Lentigo—Microscopic examination of H. and E. stained sections showed elongated, club-shaped rete pegs, from some of which projected eccentric thumb-like buds, hyperpigmentation, and a notable increase in clear cells. In Feulgen stained sections the microscopic findings resembled those of junction nevus rather closely. Nuclei of clear cells were of a deep purple hue, far overshadowing other epidermal cells in depth of color. Melanin-containing cells did not differ from their neighbors in color intensity.

Pigmented basal cell carcinoma—Microscopic examination of H. & E. stained sections showed the usual masses of dark staining basal type cells and varying amounts of melanin. In Feulgen stained sections there was a notable difference in staining properties in different parts of the individual tumors (Fig. 2). Some of the neoplastic masses were deep purple, while others, even in close proximity, were of a light purple hue. In general, however, the nucleus of each tumor cell in each individual mass of cells stained almost exactly like its neighbors. The presence or absence of melanin in the tumor masses apparently did not alter the staining properties with the Feulgen technic.

Malignant melanoma—Microscopic examination of H. and E. stained sections showed considerable variation in the findings. In most of the sections there was junctional activity and invasion of the dermis by large, atypical cells. The amount of melanin varied from lesion to lesion, as did also the inflammatory infiltrate. Mitotic figures were present in most of the lesions and in some the epidermis was invaded by the neoplasm. In Feulgen stained sections the nuclei

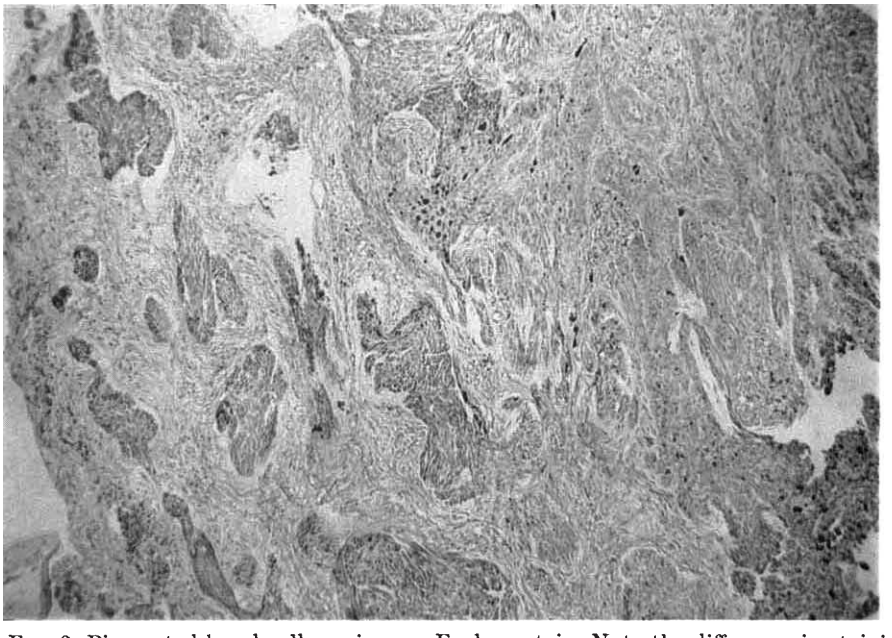

FIG. 2. Pigmented basal cell carcinoma, Feulgen stain. Note the difference in staining properties of the neoplastic masses in different parts of the lesion. The dark staining masses were deep purple in color. ×115.

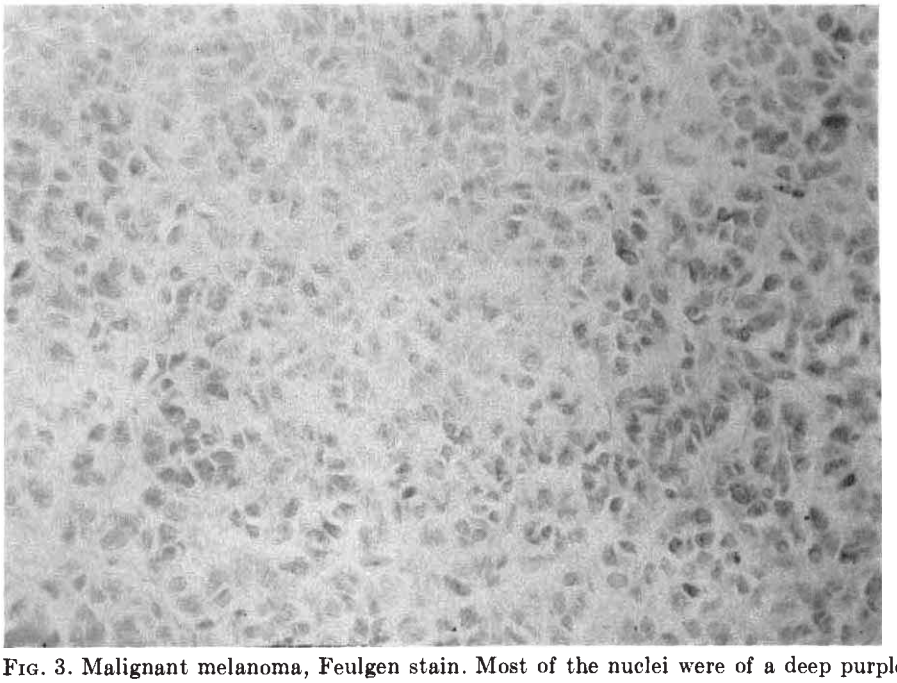

FIG. 3. Malignant melanoma, Feulgen stain. Most of the nuclei were of a deep purple color. ×580.

of neoplastic cells were deep purple in color (Fig. 3). It was notable that neoplastic cells at the periphery of the lesions, especially where the tumor was actively invading the dermis, were more strongly Feulgen positive than neoplastic cells in other portions of the lesions.

COMMENT

It has been shown that the nucleic acid concentration of tumor tissue varies over such a wide range that generalizations cannot be made (2) other than to say that highly cellular neoplasms are rich in nucleic acids while those which contain much fibrous tissue are low in nucleic acids (2). There is ample evidence (6) to indicate, however, that many tumors show a disturbance in either or both the RNA and DNA, and that in most instances the DNA is increased (6) as compared with homologous tissue. In the light of this knowledge, several of the data elicited in the course of the investigation warrant further comment.

(a) The Feulgen reaction showed that the nuclei of all the pigmented skin tumors under investigation contained DNA. The two malignant neoplasms of the group, basal cell carcinoma and malignant melanoma, appeared to be richest of the pigmented skin tumors in DNA from an overall standpoint. This latter observation was not unexpected in the light of what has already been said about the nucleic acid content of tumors.

(b) The nuclei of the cells of blue nevus were only weakly Feulgen positive, their pale purple color being especially notable when compared with Feulgen stained nuclei of the other pigmented skin tumors. This observation may reflect the sluggish demeanor of blue nevus, as compared with the aggressive behavior of such pigmented skin tumors as basal cell carcinoma and malignant melanoma.

(c) Melanocytes of the various pigmented skin tumors (exclusive of the clear cells of lentigo and junction nevus, which are considered by some authors to play a part in pigment formation) did not differ from their neighbors in DNA content, as judged by color intensity of the nuclei in Feulgen stained sections.

(d) The nuclei of the clear cells of both lentigo and junction nevus were far more strongly Feulgen positive than other cells of the epidermis. This observation may lend support to the hypothesis that clear cells give rise to nevus cells.

(e) Of the four pigmented skin tumors which were comprised of many densely packed cells (seborrheic keratosis, intradermal nevus, malignant melanoma and pigmented basal cell carcinoma) the nuclei of the first three were more strongly Feulgen positive at the periphery, where growth and multiplication was doubtless taking place (7), than in the central areas. In pigmented basal cell carcinoma, on the contrary, there was notable variation in color intensity from one tumor cell mass to another (although the nucleus of each cell in each individual mass stained almost exactly like its neighbors), which suggests that growth and multiplication in pigmented basal cell carcinoma takes place in various foci throughout the tumor, rather than at the periphery of the lesion as apparently happens with seborrheic keratosis, intradermal nevus and malignant melanoma.

SUMMARY

Pigmented skin tumors, including seborrheic keratosis, intradermal nevus, blue nevus, junction nevus, lentigo, pigmented basal cell carcinoma and malignant melanoma were examined for desoxyribonucleic acid (DNA) by means of the Feulgen reaction. The results showed that the nuclei of all the pigmented skin tumors under investigation contained DNA, but that the two malignant neoplasms of the group, basal cell carcinoma and malignant melanoma, appeared to be richest in DNA from an overall standpoint. The nuclei of the cells of blue nevus were only weakly Feulgen positive when compared with Feulgen stained nuclei of the other pigmented skin tumors. Melanocytes of the various pigmented skin tumors (exclusive of the clear cells of lentigo and junction nevus, which are considered by some authors to play a part in pigment formation) did not appear to differ from their neighbors in DNA content. The nuclei of the clear cells of both lentigo and junction nevus were far more strongly Feulgen positive than other cells of the same epidermis.

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