CHARACTERISTICS OF CARCINOGENESIS IN MUSCLES WITH VARYING METABOLISM

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Numerous investigations have shown that malignant tumors differ from normal tissues in certain facets of their metabolism. This pertains in particular to energy metabolism [8]. The works of Warburg, which established that tumor cells obtain their energy basically through glycolytic splitting of carbohydrates, and the hypotheses formulated in them on the origin of malignant tumors [10-12], have all stimulated intense study of tumor metabolism. The numerous data that have accumulated as a result have occasionally been contradictory. To a large degree, this is explained by the fact that different investigators studied tumors of the most diverse origin. Data on the metabolism of one or another tumor was extended to malignant neoplasms in general. In addition, a large portion of the biochemical, and later, histochemical, investigations were carried out on ascitic forms of tumors, which, although doubtlessly presenting great advantages for study, at the same time are more rapidly, artificially modified by the unicellular organisms which have adapted to new conditions of existence. Therefore, their metabolism differs from the metabolism of solid tumors, which are characterized by organoid structure, a large portion of which carries out manifest glycolysis.

Inasmuch as conversion of the normal metabolism of tissues to the tumor form is accomplished during the pretumor period, the question regularly arises: does the original metabolism of the tissue have any significance for the rate and frequency of formation of tumors? Provided there is a difference in the end result of the process (frequency and rate of formation of a tumor), studying the dynamics of changes at the site of injection of a carcinogen, in tissues that are morphologically uniform but differ in certain aspects of their metabolism, can also help in qualitatively appraising the various changes which potentiate or inhibit the formation of a tumor.

Chickens serve as an ideal subject for the pursuit of this investigation, since they have anatomically separate white and red striated muscles. Besides differences in the concentration of myoglobin and a number of enzymes and chemical substances, these two types of muscles differ specifically in the nature of their carbohydrate–phosphorus metabolism, which is the foundation for the energy metabolism of muscle activity. The white muscles are characterized by the anaerobic route of carbohydrate splitting, while the red muscles actively oxidize carbohydrates [1].

Tumors from the striated muscle tissue of mice and rats were obtained by a number of investigators [2, 6, 7]. The regeneration of muscle tissue has been well studied [4]. The possibility of obtaining malignant tumors in chickens was demonstrated through the injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) into thoracic muscle [5].

EXPERIMENTAL METHOD

In a preliminary experiment, 20 cocks of the Russian White breed, weighing 1000-1200 g, were injected with 20 mg of DMBA, dissolved in 0.5 ml of peach oil. The injection was administered into the left thoracic (white) muscle. An additional 20 cocks were injected in the left femoral (red) muscle with the same preparation and dose. Two other groups of birds, 15 in each, were injected with the carcinogen in the same dose, into the thoracic or femoral muscle. These latter birds were sacrificed at various intervals following the carcinogen injection, beginning with the 3rd day. Pieces of the corresponding muscles were fixed in Susa fluid and imbedded in celloidin-paraffin. Sections were stained with hematoxylin-eosin, according to Van Gieson, and with iron hematoxylin by the method of Heidenhain. To demonstrate fat, sections were prepared on a quck-freeze microtome and stained with Sudan III.

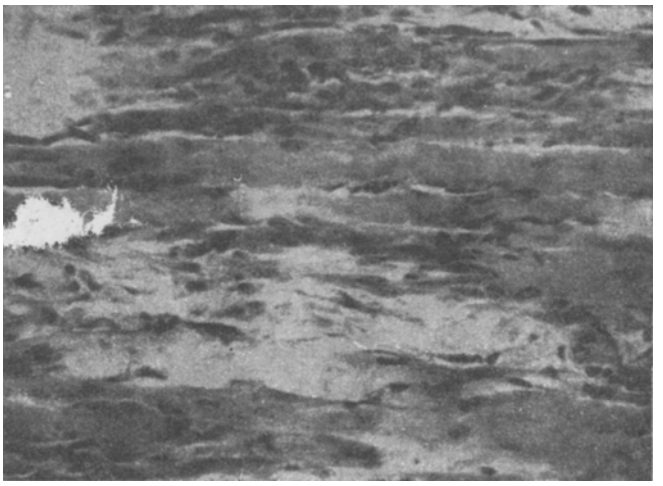

Fig. 1. Dedifferentiation of muscle fibers on the 3rd day following injection of carcinogen into the femoral muscle. Ob. 24 ×, ocul. 6 ×.

EXPERIMENTAL RESULTS

In the preliminary experiment, tumors arose only in the birds in which the carcinogen was injected into the thoracic muscle, occurring from 2-5 months after its injection. The experiment was continued over a period of 10 months.

It must be emphasized that, in addition to differences in the metabolism of the two types of muscles, there are also differences in their functioning, vascularization, and innervation, which doubtlessly must manifest themselves in the process of carcinogens in the given muscle, in relation to the intensity of the cellular and vascular reactions, etc.

Differences in the reactions of the white and red muscles were already manifested at early intervals after injection of the carcinogenic substance (3rd and 7th days). This was true of the cellular reaction, which was more apparent in the femoral muscle. In addition, reactive changes immediately arose in this muscle (Fig. 1). Edema was noted in both muscles at the site of injection of the carcinogen; drops of fat containing the carcinogen were located between the separated muscle fibers. Between the muscle fibers and drops of fat we observed cellular elements, represented by macrophages and fibroblasts (3rd day), to which were added polyblasts, special leukocytes and a small number of plasma cells (7th day). The polyblasts formed small perivascular infiltrates. We noted dilation of the blood vessels, and small hemorrhages. All the changes indicated were intensified by the 7th day.

Subsequently, the carcinogen-containing fat was delimited through the formation of cavities. Directly adjacent to the coalesced fat droplets lay large, elongated cells with small, round nuclei and cytoplasm filled with fine fat droplets. Some distance from these cells there were slender collagen fibers, arranged in a circular fashion. We noted a lymphoid reaction. Initially, the lymphoid cells formed small accumulations, primarily next to blood vessels; subsequently, they appeared next to the cavities. The plasma cell reaction intensified. By the 15th day, the number of cellular elements in the thoracic muscle decreased. As before, there were degenerative changes in the muscle fibers next to the cavities, but in addition, the muscle fibers were fragmented, at the ends of some of the fibers the sacroplasm had become homogeneous, and nuclear multiplication had occurred; we also encountered fibers in which the nuclei were arranged in a chain, along the axis of the fiber. The reactive phenomena of the muscle fibers themselves were more manifest in the femoral muscle.

In the subsequent intervals, the process of delimiting the carcinogen continued. It must be noted that in the thoracic muscle, the cavities were larger, and the formation of collagen fibers more intense, than in the femoral muscle. Collagen collected not only in the form of fibers, but also in clumps of varying dimensions. In the femoral muscle, certain cavities were lined with giant cells. Here it was possible to observe the disappearance of the cavities, which were replaced by large, spherical cells, filled with drops of fat. The cellular reaction remained more manifest in the femoral muscle, where the predominant cellular elements were macrophages and special leukocytes. The lymphoid and plasma cell infiltration intensified, with the formation of massive lymphoid infiltrates. Muscle fibers were detected in the carcinogen depot only in the femoral muscle, where they were represented by symplasts of varying size,

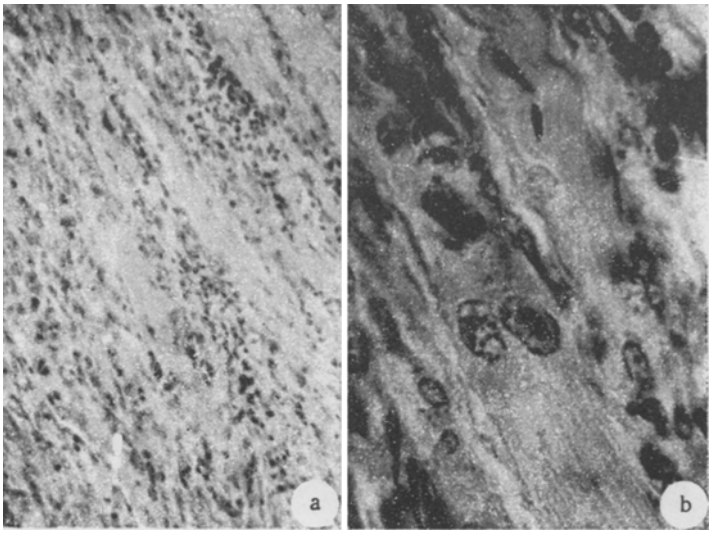

Fig. 2. Malignant changes in the muscle tissue on the 51st day after injection of carcinogen into the left thoracic muscle. a) Formation of tumor cells from the muscle fibers. Ob. 24 ×, ocul. 6 ×; b) atypical nuclei in the muscle fiber, the latter retaining its cross striation. Ob. 100 ×, immer., ocul. 6 ×.

basophilic fragments of muscle fibers, and myoblasts, degenerating remnants of muscle fibers. In the thoracic muscle, muscle tissue was only observed at a certain distance from the cavities, where the muscle fibers, surrounded by connective tissue fibers, were fragmented or degenerated. Here we also observed dedifferentiation of the muscle fibers, and the formation of myoblasts. In both muscles we noted proliferation of the cells in the adventitia of the vessels, and widening of the connective tissue laminae.

Up to approximately the end of the first month after injection of the carcinogen, the changes in both types of muscles essentially differed quantitatively. However, beginning with the 2nd month, the changes in the white and red muscles acquired qualitative differences.

In the femoral muscle, intense resorption of the carcinogen-containing fat continued, the number of cavities decreased, and the cellular reaction diminished. In the carcinogen depot, we observed numerous myosymplast formations, and the number of myoblasts increased, forming "streams" which arranged themselves between the fibers and cellular elements of the connective tissue. Sometimes it was possible to observe small accumulations of myoblasts, with certain atypical features. These accumulations were always located in the center of lymphoid infiltrates. The most characteristic subsequent changes in the femoral muscle were further decreased in the cellular reaction, with restoration of the muscle tissue at later intervals. We noted partial replacement of the defect with scar connective tissue.

In the thoracic muscle, evacuation of the carcinogen was markedly retarded. The fat was located in large cavities, surrounded by a thick connective tissue capsule containing a large number of cellular elements (fibroblasts, histiocytes). The lymphoid and plasma cell reactions intensified. Massive lymphoid infiltrates appeared. The carcinogen depot was delimited from the surrounding muscle tissue. The internal surface of some of the cavities was lined with large, vacuolated cells. Muscle tissue was seen only along the edge of the carcinogen depot, where we noted degenerative changes in certain of the muscle fibers and the appearance of fragmentation against a background of sclerosing of the muscle tissue.

At this time, the number of nuclei in the muscle fibers surrounding the carcinogen depot increased to a considerable extent, and weak basophilia was noted. Isolated muscle fibers, or portions of them, appeared markedly basophilic; intense amitotic division of the nuclei occurred in them. Further observations permitted us to regard this state of the muscle tissue as pretumoral. The earliest malignant changes of this muscle tissue that we observed were on the 51st day. This process, characterized by the absence of transitional forms, occurred simultaneously in a considerable area around the carcinogen depot. Atypical cells detached themselves directly from the muscle fibers, a portion of which

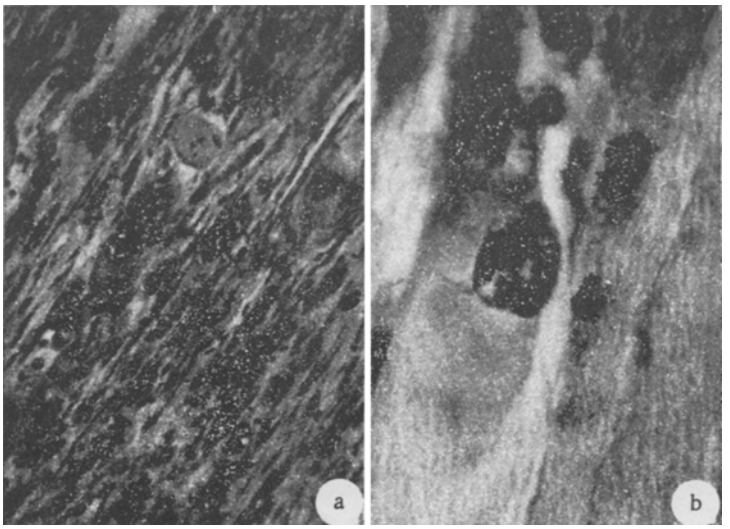

Fig. 3. Rhabdomyosarcoma, formed after injection of carcinogen (DMBA) into the left thoracic muscle. a) Atypical tumor cells. Ob. 24 ×, ocul. 6 ×; b) detachment of an atypical cell from the muscle fiber at the periphery of the tumor; cross striation retained. Ob. 100 × immer., ocul. 6 ×.

retained myofibrils with cross striation (Fig. 2). In these fibers it was possible to observe the formation of large, hyperchromic nuclei, with homogenization of the sarcoplasm in the periphery. The atypical cells, forming symplasts, retained the orientation of the muscle fibers from which they originated.

Tumors appeared in 9 of the 20 roosters that survived 2 months after the carcinogen injection. Eight tumors were classified as rhabdomyosarcomas, and one was a round cell sarcoma. All the rhabdomyosarcomas were formed at the site of injection of the carcinogen, and attained large dimensions, especially in those birds which died as a result of the tumor development. We did not observe metastases in any of these cases.

In the bird with the round cell sarcoma, in addition to a tumor node at the site of injection of the carcinogen, we observed secondary nodes in the thoracic muscle on the contralateral side, and in the muscles of the anterior abdominal wall.

Microscopically, the rhabdomyosarcomas appeared as spindle cell sarcomas, with marked polymorphism. They were characterized by the presence of multinucleated giant cells and ribbon-like formations with a large number of nuclei, arranged in chain form. The connective tissue of the tumors accomplished marked development (Fig. 3, a). The periphery of the tumors was of special interest—there we observed the formation of atypical tumor cells out of muscle fibers, similar to what took place during the genesis of the tumor (Fig. 3, b).

Thus, the tumors arose in tissue which is characterized by intense glycolysis and a low level of oxidative processes. Apparently, reduction of the level of oxidative processes as a whole not only potentiates the development, but also the origin of malignant growth, and may serve as one of the conditions for metastasizing.

Analysis of the dynamics behind the morphological changes in the two types of muscles shows a cellular reaction in the femoral muscle, in the phase of exudation, which is significantly more manifest in intensity and duration. Apparently, this holds great significance in the evacuation of the carcinogen. In the thoracic muscle, in addition to a less manifest cellular reaction, we observed encapsulation of the fat in which the carcinogen was dissolved, which, along with delimiting the material, led to its retention in a depot.

The red muscle fibers were more resistant to the nonspecific injurious action of the carcinogen, which is apparently explained by their intense metabolism. At the same time that the degenerative changes in the white muscle fibers predominated over the reactive changes throughout the entire pretumoral period, in the red muscle the regenerative phenomena arose very early, and were very intense in character. The development of connective

tissue, which is a necessary condition for the normal regeneration of muscle [4], was not abundant in this period. At the same time, the pronounced sclerotic changes in the carcinogen depot and the white muscle fibers surrounding it could substantially hinder, or even distort, the course of regeneration.

Certain investigators have observed the formation of tumor elements from differentiated muscle fibers, in the process of the genesis and growth of rhabdomyosarcomas in mice and rats [2, 3, 7]. Apparently, this conversion of muscle fibers in the process of malignant change is usual for the thoracic muscle of chickens.

In order to increase the depth of analysis of the pretumoral changes, we will carry out cytochemical investigations.

SUMMARY

Two groups of cocks received the same dose of carcinogen (9,10-dimethyl-1,2-benzanthracene) into the thoracic (white) or femoral (red) muscles. Tumors (mostly rhabdomyosarcomas) occurred only in the thoracic muscles in periods of 2 to 5 months after the start of the experiment. Carbohydrate-phosphorus metabolism of this muscle is characterized by the prevalence of anaerobic glycolysis. The dynamics of morphological changes was studied at the site of carcinogen administration in the indicated two types of muscles during the pretumor period.

The muscle tissue reaction and protective-adaptive response to the carcinogen administration were different.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
