MORPHOLOGY AND PATHOMORPHOLOGY

THE EFFECT OF THE MODE OF ADMINISTRATION AND THE DOSE OF A CARCINOGENIC AGENT ON THE DEVELOPMENT OF MORPHOLOGICAL CHANGES IN THE FOCUS OF TUMOR FORMATION

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It has been shown experimentally [1, 2, 4, 6, 11, 12, 16, 17, 19 etc.] that the development of sarcomas induced by carcinogenic substances is preceded by various types of change in the tissues at the site of application of the carcinogen. However, insufficient attention has been paid to the analysis of these reactive phenomena preceding tumor development, and especially of their importance in the outcome of the process.

In our work, which is devoted to the pathogenesis of induced sarcomas, we set out to study the reactive changes arising in consequence of the administration of a carcinogenic agent and also in the later stages of the process of tumor formation, and to determine their importance for the outcome of this process. Naturally, we were particularly interested in the adaptive reactions which could be concerned in delaying this process or bringing it to an end. In order to investigate these reactions more thoroughly and to assess their importance we used various methods of subcutaneous application of the carcinogen (in oil, in animal fat and in the crystalline form), and also various doses of the agent.

As we know from reports in the literature the same carcinogen, depending on the solvent, will produce different amounts of tumor [9, 10, 14, 18, 23 etc.], and also depending on the solvent, the duration of the process of tumor formation (the latent period) is different. The quantity of tumor produced depends also on the doses of the agent.

However, these authors cited are concerned only with the end results of the experiments, and do not scrutinize sufficiently closely the differences between them which are revealed by a comparative study of the development of the morphological changes throughout the whole process – from the moment of administration of the carcinogen to the formation of the tumor.

We consider it useful to make comparisons at the separate stages of the process which were recognized during the investigation and which were characterized by definite reactive manifestations.

EXPERIMENTAL METHOD

A process of tumor formation was induced in rats with 9,10-dimethyl-1,2-benzanthracene. The carcinogenic substance was injected subcutaneously in a crystalline form (1 mg) and dissolved in two solvents – in peach oil (0.3, 0.5, 1 mg) and in rat fat (1 mg). The animals were killed at intervals of 24 to 300 days after injection of the agent. Altogether 254 rats were used in the experiments. Material for histological examination was fixed in 12% neutral formalin and in Zenker formol. Sections were stained with hematoxylin-eosin, picrofuchsin, toluidine blue, Sudan III, iron trioxyhematein by Hansen's method, and silver impregnation by Foot's method.

EXPERIMENTAL RESULTS

Twenty-four hours after injection of the dissolved (in oil or fat) carcinogen into the subcutaneous tissue of the animal an intense inflammatory reaction was observed, with marked edema and large numbers of polymorphonuclear cells surrounding numerous cavities containing the carcinogenic substance dissolved in oil or fat. On the following days (two to five) degeneration and destruction of the polymorphonuclear leucocytes was observed, with an increase in the number of macrophages, histiocytes and fibroblasts; signs of phagocytosis of fat droplets appeared. At the same time in the substance and on the surface of the skin at the site of injection of the agent an accumulation of fatty droplets and of larger masses of fat were observed. This was evidence of secretion of carcinogen onto the surface of the skin through the tissue spaces and the hair sheaths and sebaceous glands. In spite of the considerable quantity of carcinogen, on the 10th to 20th day the number of cells around the cavities showed a marked reduction. However, cellular infiltrations, in which lymphoid cells predominated, appeared at some distance from the cavities containing the agent, in an area bounded by the skin and the underlying skeletal muscles.

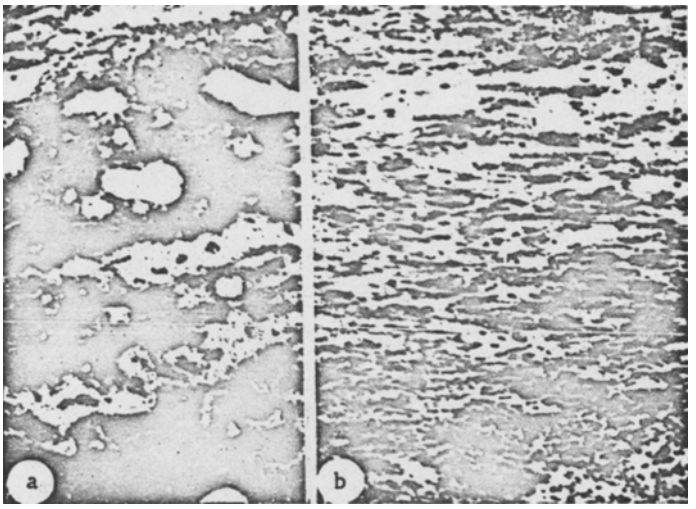

Fig. 1. Hyperplastic processes in the neighborhood of injected carcinogen: a) carcinogen in rat fat (75th day); b) carcinogen in crystalline form (165th day).

As a rule the injection of carcinogenic substance dissolved in rat fat causes a more intense inflammatory reaction than when dissolved in vegetable oil. Around the cavities a more numerous leucocytic infiltration was noted and the phenomena of phagocytosis rapidly reached a high degree of development and remained well defined throughout the subsequent stages of the process. Participation of giant cells in the assimilation of carcinogen in fat was more marked than in assimilation of carcinogen in oil. While the cavities with a fatty solution of carcinogen were quite quickly reduced in size and number, the oily solution of the agent remained unchanged in numerous larger cavities for a long time.

Thus the reactive processes in the first stage (inflammation) lead only to partial removal of the dissolved agent from its main depot. The inflammatory reaction ends with the agent still present in the cavities formed in the connective tissue.

In the second stage (sclerosis), besides the development of connective tissue which as a rule undergoes a period of hyalinosis, a cellular reaction was continued in the form of infiltrations, mainly of lymphoid cells, at some distance from the agent. At the same period (45th to 60th day) proliferation of fibroblasts was observed in the peripheral areas of the focus.

On administration of the agent in rat fat, proliferation of fibroblasts, accumulation of mucopolysaccharides and the appearance of argyrophylic fibers were observed earlier than if injected in an oily solution. However,

the development of sclerotic changes reached a high degree when the carcinogen was given dissolved in oil.

The character of the sclerotic changes in the second stage was reflected by the development and course of the proliferative processes in the third stage of the condition (hyperplasia). For example the solution of the agent in rat fat, causing a less marked sclerotic process, was accompanied by great development of hyperplastic changes, leading in turn to the earlier appearance of a tumor than after administration of a solution of the agent in oil (Figure 1a).

During the formation of a focus of malignant growths (State IV), around the accumulations of proliferating cells with some signs of atypia the appearance of round-cell infiltrations with a predominance of lymphoid cells was observed almost as a rule. On injection of an oily solution of the carcinogen, accumulations of these cells reached a more considerable size than when rat fat was used as a solvent (Figure 2).

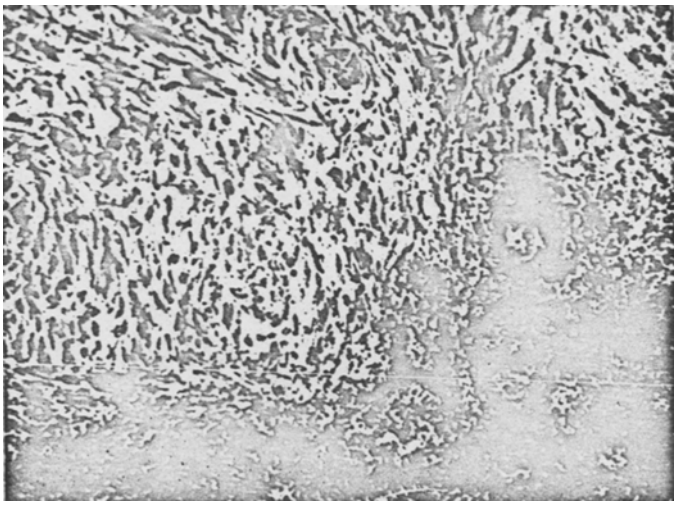

Fig. 2. Carcinogen in oil. 200th day. Focus of incipient malignant change of cells surrounded by a round-cell infiltration with predominance of lymphoid cells.

Injection of the carcinogenic substance in the crystalline form produced a less intense inflammatory reaction than injection of the carcinogen in solution. Around the mass of crystalline substance, surrounded by polymorphonuclear leucocytes, an accumulation of fat was observed from the first few days, both contained in macrophages and as free droplets. This fat was transported from foci of destruction of fatty tissue, as confirmed by the reduction in the number of fat cells in the area of distribution of the carcinogenic substance and by phagocytosis of fat in the foci of destruction. The dry substance was removed from its depot very slowly, following solution. Sometimes the epithelium of the skin took part in this process, by ingrowing in the direction of the deposit of crystals and after surrounding them, took on an eliminatory function, as described by V.G. Garshin. On injection of the carcinogenic substance in a crystalline form sclerotic changes in the neighborhood were particularly well developed. In a number of cases the sclerotic process spread far beyond the limits of the main focus, extending to the adjacent skeletal muscles, and surrounding and isolating groups of muscle fibers (Figure 3). This was confirmed by atrophy, destruction or conversion into myoblastic cells. As a result of changes of this sort rhabdomyosarcomas may arise.

The hyperplastic processes following the use of the agent in the dry form did not reach such proportions as when dissolved carcinogen was used (Figure 1b). This form of application of the agent led to the appearance of a relatively small quantity of tumor and after a longer interval of time than with other forms of administration.

A study of the morphology of the precancerous period after injection of carcinogen dissolved in peach oil in a dose of 0.5 and 0.3 mg showed that whereas the general character of development of the process with smaller

doses was the same, the individual reactive manifestations were more strongly expressed than with a dose of 1 mg. The initial inflammatory reaction pursued a more intensive course, the accompanying phagocytosis was more active, the sclerosis more pronounced and the round-cell infiltration expressed to a greater degree in the period of formation of the focus of malignant growth. Where the lymphoid infiltration was well marked, the formation of the tumor process was sometimes considerably retarded.

Our investigations showed that while the various methods of application of the agent by subcutaneous injection do not change the general character and succession of the changes observed in the precancerous period, they do affect the degree of expression of the reactive manifestations during each individual stage, and thus, in turn, may be reflected in the outcome of the process, i.e., in the development of the tumor itself. The importance of the state in which the agent is administered is shown even in the first, inflammatory stage. On injection of carcinogen in solution, resorption of the agent is observed to a greater degree, especially when dissolved in rat fat. On injection of the agent in powder form this process is drawn out over a long time, which undoubtedly has an effect on the course of subsequent stages of the process.

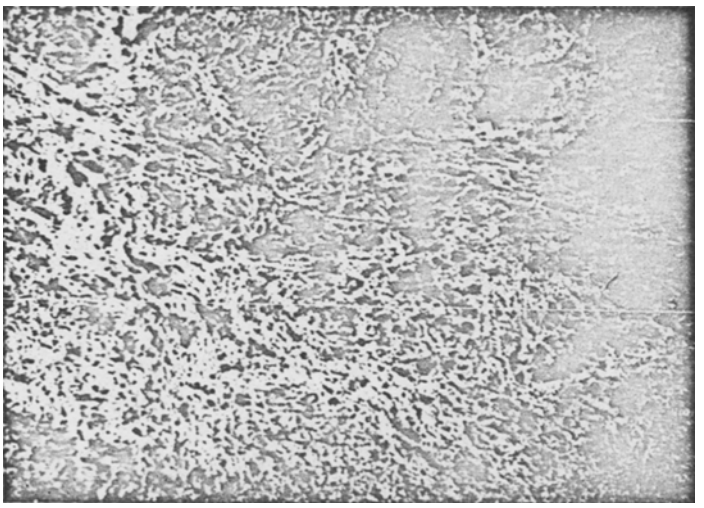

Fig. 3. Crystalline carcinogen. 90th day. Sclerosis, spreading to the adjacent skeletal muscles. Residual altered muscle fibers surrounded by hyaline connective tissue.

The mode of application of the agent affected the character of development of the sclerotic changes in the second stage of the process. These changes were most pronounced and widespread in response to injection of the crystalline carcinogen, and on the other hand, were least pronounced and localized where the agent was dissolved in rat fat. These alterations in the character of the sclerotic changes were to some extent reflected in the next stage — hyperplasia. While with pronounced sclerosis (the crystalline substance) the hyperplastic processes rarely developed to a high degree, with incomplete sclerosis (fatty solutions) these processes developed earlier and reached more considerable proportions. The degree of hyperplasia may in turn be connected with speeding up the malignant change in some cases and in others with the appearance of tumors in small amount and at later periods.

In experiments by Rush [24] in which hydrocarbons were administered in the form of powder, tablets or crystals, tumors also developed less quickly and in a smaller number of animals. Rat fat, used in our experiments as a solvent, produced a more intensive inflammatory reaction. It enabled the removal of a larger quantity of the carcinogen from the focus and, presumably, from the body. Furthermore, the carcinogen which was dissolved in it caused a more pronounced hyperplastic process and led more rapidly to malignant transformation.

The facts which we obtained thus do not confirm the opinion of certain workers of the "anticarcinogenic" action of homologous fat [9, 10, 13, 16, 17], nor the results of those workers who do not find any difference in the

action of the agent when various fats are used as solvents [15, 20]. This may be due to different experimental conditions (carcinogenic substance, dosage, animals).

The greater activity of the carcinogen when dissolved in rat fat in our experiments may be explained by the fact that homologous fat may facilitate the action of the carcinogenic agent on the cells and tissues of the animal, including the nervous system, exposed under the influence of the carcinogen to functional and structural changes [1, 5, 8 etc.].

Besides the above-mentioned adaptive reactions which are regularly observed in the course of experimental tumor formation, we observed throughout the precancerous period and in the beginning of the cancerous period cellular reactions of a protective and adaptative character, but not so constantly found. We have in mind round-cell infiltrations, composed to a large extent of lymphoid cells which are known to be of importance in the immunobiological reactions of the body. In transplantation of tissues infiltrations of this sort are evidence of the heterogeneity of the transplanted tissue. It is possible that in conditions of malignant transformation this reaction indicates the appearance of tissue of a heterogeneous character. It is more pronounced in animals receiving dry carcinogen and solution of the agent in vegetable oil, and less so in animals receiving carcinogen dissolved in rat fat.

In view of the fact that the individual reactive manifestations of a protective and adaptative character were more pronounced with smaller doses of the carcinogen, it may be asserted that carcinogen in small doses and in a less active form (dry substance or oily solution of carcinogen) causes less disturbance of the mechanism of the protective and adaptative reactions than larger doses, or even of the same dose but administered in the form of a solution which facilitates its carcinogenic action (in our experiments – rat fat).

SUMMARY

The effect of subcutaneous application of 9,10-dimethyl-1,2-benzanthracene on the process of tumor formation was studied. Different doses of this preparation were tested, i.e., 1.0 mg in crystalline form, 1.0 mg in rat fat, 1.0, 0.5 and 0.3 mg in peach oil. Various methds of introduction of the stimulant did not affect the regular change of the stages of this process (inflammation, sclerosis, hyperplasia, formation of the focus of malignant growth). However, the method of application and the dose influenced the degree of reactivity during separate stages. The differing character of inflammatory reactions (stage I) influenced the development of sclerotic changes (stage II), which in turn had an effect on the degree of development of hyperplastic changes during the IIIrd stage. In certain cases more rapid carcinogenesis could be connected with pronounced hyperplastic changes, in others – with appearance of a small number of tumors which formed later. The above data allow us to draw a conclusion that the protective-adaptive reactions which are observed in the focus of tumor formation may determine one or another outcome of the whole process.

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