to these properties it resembled rat sarcoma virus RASV isolated from inbred animals [7, 8]. Incidentally, the virus was isolated from noninbred rats. We are aware of only one report of isolation of a nononcogenic type C virus from wild rats after treatment of the animals with methylcholanthrene [6].

The results thus demonstrate that the genome of endogenous retrovirus is potentially active in embryonic cells of noninbred rats. As in the majority of inbred rats the virus is repressed, but it can be activated by vaccinia virus and, as a result of recombination, rat sarcoma virus can be formed.

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DETECTION OF DIFFERENTIATION FACTORS IN MALIGNANT HUMAN MELANOMAS

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Melanoma is one of the most malignant human tumors [1]. The low adhesiveness of cells of the primary tumor and invasion of thin-walled blood vessels enable it to metastasize rapidly. The primary tumor of a melanoma is small in volume and is distinguished by the high heterogeneity of its cell composition [1, 3]. Active melanogenesis takes place in the cells of this tumor, and it is probably the incompleteness of this process which determines its high degree of malignancy [1, 2]. Several workers have investigated dependence of melanogenesis on the influence of surrounding tissues [7, 13, 14], but the concrete mechanism of induction of melanogenesis has been identified by the use of the periodic albinism mutation in clawed frogs (Xenopus) genus [8, 9]. These investigations showed that melanogenesis is induced on the basis of the action of a specific melanogenic factor of protein nature, synthesized by certain meseodermal derivatives and, in particular, by the axial complex [9-11].

The aim of this investigation was to detect differentiation factors (DF) in human melanomas, and to compare them with factors acting in nonpigmented tumors, and also with certain factors acting during embryogenesis.

EXPERIMENTAL METHOD

Three sources of DF were used: human melanomas (I and II) cloned in nude mice, human papillomas, and also wild-type (+/+) Xenopus embryos at the stage of neurula and early tail

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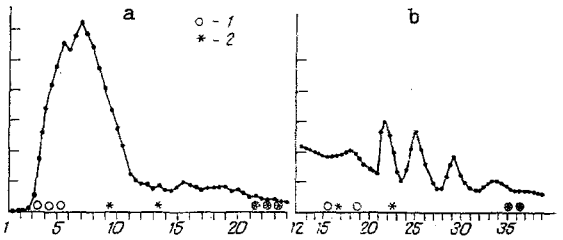

Fig. 1. Elution profiles of proteins of melanoma I extract (a) and extract of Xenopus embryos at the neurula–early tail bud stage (b). Abscissa, nos. of fractions; ordinate, optical density at 280 nm (in relative units). 1) Fraction with marked mesodermalizing activity; 2) fraction with melanogenic activity. a) Column with Biogel P-100 (2.2 × 42 cm), eluent 1M acetic acid, rate of elution 2.2 ml/h, volume of fractions 2.2 ml; b) column with Biogel A-0.5m (1 × 100 cm), eluent 1M acetic acid, rate of elution 2.9 ml/h, volume of fractions 2.8 ml.

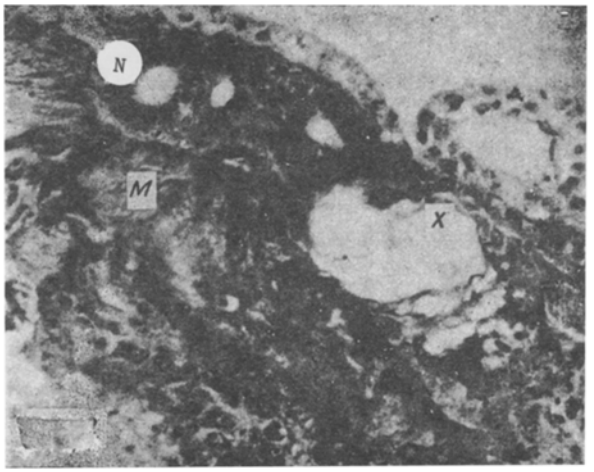

Fig. 2. Structures of axial complex induced by MF. X) Notochord, M) muscles, N) neuroid.

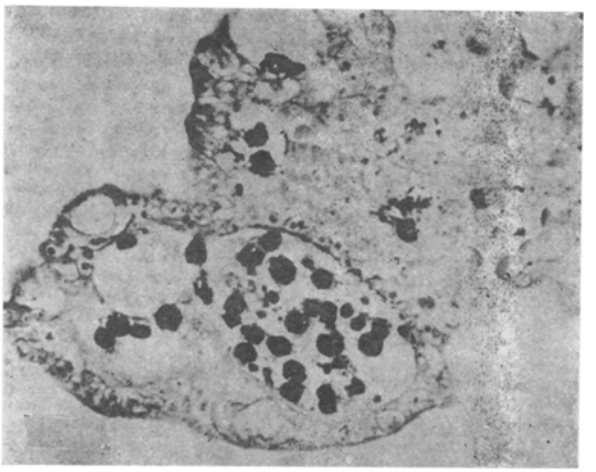

Fig. 3. Melanophores surrounded by atypical epidermis, induced by MgF.

bud. Active synthesis of DF, especially of the melonogenic factor, is observed at these stages in embryonic tissues [4, 9]. The test system consisted of ectoderm of the early gastrula of Xenopus (+/+). EEG cells can differentiate into all cell types of the adult frog under the influence of the corresponding inducing agent; in the absence of such, EEG cells develop into atypical epidermis [4].

In the first experimental series activity of DF from melanoma and papilloma tissues fixed with 70% ethanol was studied. For this purpose pieces of fixed tissue were carefully washed to remove the ethanol and placed in direct contact with EEG. In the control experiments tissue fragments from the swim bladder of bony fishes, one of the strongest sources of mesodermalizing factor [12], were used. In the next series of experiments activity of DF from extracts of the test tissue and fractions obtained from the same extracts by chromatography was determined. To isolate DF from both sources, a technique originally suggested for the isolation of transforming growth factors [15] was used. The method comprises three basic stages: extraction of the tissue homogenate with acid alcohol, precipitation of the extracted proteins with a mixture of ether and alcohol, and gel-chromatography on columns. Aliquots of the fractions obtained were lyophilized, dissolved in Niu–Twitty physiological saline, and tested on EEG by the method in [5]. Before fixation the explants were treated with cAMP (3 μg/ml) to reveal the shape of the melanophores formed. The embryonic cultures were fixed after 5 days, then stained histologically by the standard method. Altogether 1298 explants were used in all the series.

EXPERIMENTAL RESULTS

Melanoma I and II tissues, fixed with ethanol, induced the formation of all cell types which arise in normal development from mesoderm in the Xenopus EEG. The appearance of neural differentiation in the cultures always correlated with formation of the notochord, which in the early stages of its development is a source of neuralizing factor [4]. The character of differentiation of polypotent EEG cells under the influence of melanoma indicates that it contains mesodermalizing factor (MF). Papilloma tissues fixed with ethanol exhibited very low DF activity (Table 1).* In the control, under the influence of the swim bladder, the same spectrum of cell types appeared as under the influence of melanoma tissues.

Extracts from melanoma tissues induced differentiation of the same cell type in EEG as tissues fixed with ethanol. It can accordingly be concluded that the extraction method used is adequate.

Testing fractions obtained by chromatography of proteins of the melanoma extracts on a column with Biogel P-100 revealed several fractions with induction activity (Fig. 1a). Proteins of fractions 3-5 induced the formation of structures of the axial complex in EEG (Fig. 2), including notochord, somites, and also the neural tube. A few melanophores also were observed in these same cultures. Like neural differentiations, they appeared as a result of secondary induction factors arising from the axial mesoderm [9]. Structures of the axial complex were formed in the cultures under the influence of fractions 21-23 also. However, in this case, many well differentiated dendritic melanophores appeared together with them, evidence that these fractions contained not only MF, but also melanogenic factor (MgF). Cultures containing many stellate and dendritic melanophores, in the absence of axial mesoderm (Fig. 3) developed under the influence of fractions 9 and 13. Estimation of the molecular weight of proteins of the active fractions gave the following values: for MF 11-13 kD (fractions 21-23) and over 70 kD (fractions 3-5); for MgF 11-13 kD (fractions 21-23), 24-26 kD (fraction 13), and 40-43 dK (fraction 9).

On primary chromatography of the proteins of the Xenopus embryonic extract on a column with Biogel P-60, maximal mesodermalizing and melanogenic activity was observed in fractions corresponding to the void volume of the column. Subsequent chromatography of the active material on a column with Biogel A-0.5m revealed several fractions containing MF and MgF (Fig. 1b). Structures induced in EEG by the active fractions were analogous to those described above. MF was found in fractions containing proteins with mol. wt. of 12-13 kD (fractions 35-36), about 33 kD (fraction 18), and about 45 kD (fraction 15). The following values of mol. wt. were obtained for MgF: 12-13 kD (fractions 35 and 36), 25-26 kD (fraction 22), and about 40 kD (fraction 16).

It can be concluded from these experiments that human papilloma and melanoma tissues, like amphibian embryonic tissues, contain MF and MgF, although their content is higher in melanoma. These factors may be present in the form of high-molecular-weight aggregates with other proteins, which dissociate in the course of the isolation procedure. Both MF and MgF were shown to be heterogeneous for molecular weight. Values of molecular weight determined for MgF isolated from the different sources coincide. Heterogeneity of DF for molecular weight may perhaps be linked with the ability of the factors to form oligomers. For example, assuming that the low-molecular-weight fractions (12-13 kD) correspond to the monomeric form of MgF, the other two fractions will contain di- and trimers of the factor.

Minimal values of molecular weight for MF isolated from two different sources also coincide. The same value of molecular weight (13 kD) was also obtained for the factor with mesodermalizing activity, also isolated from 11-day chick embryos [6].

DF analogous to the factors acting in amphibian embryogenesis are thus found in tumor tissue, evidence of the high evolutionary conservatism of DF. Comparison of the high activity of melanogenesis-inducing factor, acting during normal development, with that of the factor of the same name acting in malignant melanomas, shows that initiation of melanogenesis itself is not a sufficient explanation of the special malignancy of melanomas.

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*Table 1 omitted in Russian Original – Publisher.

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