structure of adenocarcinoma and carcinoma with a low degree of differentiation (Fig. 2e, f). Throughout the period of passage the tumor has preserved in principle a stereotyped structure and cell composition and has corresponded to the picture of a carcinoma with low degree of differentiation. Features of glandular structure (adenocarcinoma), observed in the original material, could not be detected in any of the generations studied (Fig. 2d). Strain RShM, serially transplanted into animals at intervals of 14-15 days, preserved the structure of a nonkeratinizing squamous-cell carcinoma, corresponding to the original tumor from which the HeLa cell line, which was the source of the strain, was obtained (Fig. 2h).

All 14 tumors strains described in this communication, which were transplantable into nude mice and rats, consisted mainly of human cells, as shown by LDH electrophoresis, revealing five peaks.

Strains transplantable into nude mice and rats and obtained from the same human tumor were histologically identical with one another. However, stromal cells of strains transplanted into mice and rats differed, for they consisted of cells of the tumor-bearing host. With the models obtained it is possible to study human tumors under different experimental conditions and they enable transplantable tumors, especially from rats, in which they attain a large size, to be used for biotechnological purposes.

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EFFECT OF ANTICARCINOGENS ON THE TRANSPLACENTAL CARCINOGENIC EFFECT OF N-NITROSO-N-ETHYLUREA

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UDC 615.277.4.015.23:615.277.3].033.01.85.036.8:616-006.6-053.1-092.9

KEY WORDS: transplacental carcinogenesis; N-nitroso-N-ethylurea; anticarcinogens.

The study of the effect of various modifying factors on carcinogenesis is one approach to the elucidation of the pathogenetic mechanicsms of development of tumors and the devising of measures of their prevention. We know that tumors in children, and sometimes even in adults, may be due to the action of carcinogens on the mother during pregnancy [1]. Consequently, experimental data on the effect of anticarcinogens on the realization of transplacental carcinogenesis must be taken into account when measures are devised for preventing prenatally induced neoplasms in man.

In this investigation the action of seven different anticarcinogeneic agents was studied on the development of tumors of the nervous system and kidneys induced transplancentally in rats by N-nitroso-N-ethylurea (NEU). The choice of substances was dictated by the need for the agents used to have an anticarcinogenic action when given to the progeny only in the postnatal period, which is a model of the most suitable approaches for inhibiting prenatally induced neoplasms in man. For this purpose the known inhibitors of carcinogenesis — selenium [8] and vitamin A [11] — were used. Low-molecular-weight polypeptide factors obtained from tissue extracts of the bovine thymus, pineal gland, and bone marrow, acting on the immune and

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(Presented by Academician of the Acadmey of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 73-76, July, 1985. Original article submitted October 12, 1984.

homeostatic systems [5, 7], the antidiabetic agent phenformin [4], and the biostimulator succinic acid [3], whose anticarcinogenic activity has been less well studied, also were used.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats bred at the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. On the 21st day of pregnancy, the mother rats were given a single intravenous injection of NEU in a dose of 75 mg/kg. Offspring of both sexes were divided at birth into nine groups (Table 1). In groups 1 (control 1) and 7 (control 2) the animals received no treatment of any kind in the postnatal period. In group 2 the young rats received sodium selenite with the drinking water in a dose of 4 mg/liter, and in group 3 the rats received retinol acetate with the food in a dose of 300,000 IU/kg food daily, 5 days a week. Both substances were given throughout postnatal life. In groups 4, 5, and 6 the animals received low-molecular-weight thymus (thymalin), pineal (epithalamin), and bone marrow (hemalin) factors, starting with 2.5 months after birth and thereafter for the rest of life in courses lasting 5 days a week, every 3 weeks, in a dose of 0.5 mg per rat daily, subcutaneously. The polypeptide factors of thymus, pineal, and bone marrow were obtained by extraction from the correponsing bovine tissue [5-7]. In group 8 the animals received the antidiabetic biguanide phenformin (from Dibotin, England) per os, starting with the first month after birth and throughout the rest of life five times a week, in a daily dose of 5 mg of the drug per rat in 1 ml of tap water. Rats in group 9 received succinic acid with their drinking water in a dose of 2 g/liter throughout postnatal life. Experiments on animals in the last three groups were carried out 18 months after those in previous groups. Consequently, the first six groups had their own control, and the next three groups had theirs. The offspring remained under observation until death. All rats were autopsied and organs with tumors or changes suggestive of tumor growth were fixed in Kaiserling's fluid and investigated histologically in the usual way. The numerical data obtained in the experiments were subjected to statistical analysis by Student's test and the chi-square test. For statistical analysis of the frequencies of tumors in separate locations, the method in [9] was used.

EXPERIMENTAL RESULTS

As a result of the transplacental action of NEU virtually all the young rats in group 1 (control 1) developed tumors (Table 1). The neoplasms were most commonly found in the brain and spinal cord, and rather less frequently in the peripheral nervous system and kidneys. The index of multiplicity, calculated for all neoplasms, was 2.7 ± 0.1 per rat and the total average latent period of tumor development was 294 ± 5 days. Very similar results were obtained in group 7 (control 2).

Of seven anticarcinogenic agents studied only three (thymalin, epithalamin, and phenformin) inhibited the transplacental carcinogenic effect of NEU (Table 1). Compared with the rats of group 1 (control), in rats exposed to the transplacental action of NEU followed by the postnatal action of thymalin the index of multiplicity for all tumors was lowered to 2.2 ± 0.1 ($P < 0.01$), the frequency of the neoplasms was reduced by half for the spinal cord ($P < 0.001$) and by 12% for the kidneys ($P < 0.05$), and the total average latent period of tumor development was lengthened by 2 months ($P < 0.001$). Compared with control 1 epithalamin reduced the total frequency of the tumors by 10% ($P < 0.05$), the index of multiplicity for all neoplasms to 1.9 ± 0.1 ($P < 0.001$), and the frequency of tumors of the spinal cord by half ($P < 0.001$), of the peripheral nervous system by 15% ($P < 0.05$), and of the kidneys by 67% ($P < 0.01$); it also lengthened the total average latent period of tumor development by 2 months ($P < 0.001$). Meanwhile neither thymalin or epithalamin had any effect on the number of the most commonly found brain neoplasms or on the multiplicity of tumors in individual locations. In the group of animals receiving NEU transplacentally and phenformin postnatally, compared with control 2 the total frequency of tumors was reduced by 17% ($P < 0.01$), and the frequency of neoplasms of the brain by 16% ($P < 0.05$) and of the spinal cord by 17% ($P < 0.05$), and the index of multiplicity for all neoplasms was reduced to 1.9 ± 0.1 ($P < 0.001$). Phenformin also reduced the multiplicity of brain tumors statistically significantly. Thymalin is known to have an immunostimulant action on the T system of immunity, and it can also bring about normalization of the metabolic disturbances developing in the body during carcinogenesis [6]. Epithalamin is a regulator of the neuroendocrine system, it restores hormonal-metabolic disturbances developing during carcinogenesis, and it stimulates reactions of cellular immunity [7]. Phenformin can normalize hormonal-metabolic disturbances developing during carcinogenesis, and above all, changes in lipid and carbohydrate metabolism, and can abolish the phenomena of metabolic immunodepression [4]. It was evidently on account of these mechanisms

TABLE 1. Effect of Anticarcinogenic Factors on Transplacental Carcinogenic Effect of NEU

Group of animals	Experimental conditions	Number of young rats		Number of young rats with tumors in different situations					Number of tumors		
		total (a)	with all tumors	brain	spinal cord	peripheral nervous system	kidneys	elsewhere	total	index of multiplicity (b)	average latent period, days
1-	NEU (control 1)	89	87 (97,8%)	54 (60,7%)	55 (61,8%)	37 (41,6%)	33 (37,1%)	7 (7,9%)	235	2,7±0,1	294±5
2-	NEU + selenium	55	54 (98,7%)	33 (60,0%)	32 (58,2%)	21 (38,2%)	20 (36,4%)	5 (9,1%)	147	2,7±0,1	310±9
3-	NEU + retinal acetate	50	47 (94,0%)	38 (76,0%)*	26 (52,0%)	14 (28,0%)	14 (28,0%)	5 (10,0%)	123	2,6±0,1	319±13
4-	NEU + thymalin	52	47 (90,4%)	34 (65,4%)	15 (28,8%)***	22 (42,3%)	13 (25,0%)	5 (9,6%)	105	2,2±0,1**	355±13***
5-	NEU + epithalamin	56	49 (87,5%)*	33 (58,9%)	19 (33,9%)***	15 (26,8%)*	7 (12,5%)**	3 (5,4%)	93	1,9±0,1***	359±12***
6-	NEU + hemalin	49	46 (93,9%)	32 (65,3%)	28 (57,1%)	15 (30,6%)	15 (30,6%)	6 (12,2%)	125	2,7±0,2	305±12
7-	NEU (control 2)	55	55 (100%)	33 (60,0%)	31 (56,4%)	16 (29,1%)	16 (29,1%)	7 (12,7%)	135	2,5±0,1	282±10
8-	NEU + phenformin	46	38 (82,6%)**	20 (43,5%)*	18 (39,1%)*	12 (26,1%)	10 (21,7%)	5 (10,9%)	73	1,9±0,1***	291±12
9-	NEU ± succinic acid	61	57 (93,4%)	38 (62,3%)	33 (54,1%)	17 (27,9%)	13 (21,3%)	9 (14,8%)	139	2,4±0,1	277±10

Legend. No significant difference was found in the development of neoplasms between males and females, and the data are therefore pooled. a) Animals surviving until the day of appearance of the first neoplasm. b) Index calculated as mean number of tumors per tumor-bearing rat. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with corresponding control.

that thymalin, epithalamin, and phenformin inhibited the development of neoplasms of the nervous system and kidneys, induced transplacentally by NEU in rats, to some extent. It should be noted that all three preparations have similar mechanisms of action.

Selenium and retinoids are nowadays regarded as the most promising substances for preventing tumor development [8, 11]. However, in the present experiment these anticarcinogenic agents had no significant effect on the transplacental carcinogenic effect of NEU (Table 1). Compared with control 1, the frequency of brain tumors was 15% higher in rats receiving retinol acetate ($P < 0.05$), whereas selenium caused a statistically significant increase in the multiplicity of spinal cord tumors. There are indications in the literature of a possible anticarcinogenic action of succinic acid and hemalin [3, 5]. In the present experiments these substances likewise did not inhibit the transplancental carcinogenic effect of NEU (Table 1).

In animals of all groups compared no significant differences were found in the morphological characteristics of the neoplasms. Among brain and spinal cord tumors, oligodendrogliomas and mixed oligoastrocytomas were found most frequently. Many trigeminal nerve neurinomas developed in the peripheral nervous system. Neoplasms of the kidneys are classified as mesenchymal tumors. In rats of the breed used, neoplasms of the nervous system and kidneys are virtually never found spontaneously [2]. Among other tumors mainly solitary neoplasms of organs of the endocrine and reproductive systems were observed, in agreement with the spontaneous spectrum of tumors in rats of this particular breed [2].

Little information is yet available on the effect of anticarcinogenic factors on development of nonepithelial neoplasms. In the present investigation, of the seven different anticarcinogenic agents used during the period of promotion of carcinogenesis, only three had a weak inhibitory action on the development of neurogenic neoplasms and mesenchymal tumors of the kidneys. These results agree to a certain extent with the view that it is difficult to inhibit transplacental carcinogensis [10]. There is no doubt that the search for effective inhibitors of transplacental carcinogenesis must be continued.

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