of features characteristic of epithelium, but had an ultrastructure similar to that of macrophages, located in the submucosal layer of the bladder.

The correlation found between the increase in the number of dark cells (macrophages) in the urothelium of the rats receiving FCA and delay of the development of papillomatous hyperplasia in these animals is in harmony with evidence for the carcinoinhibitory action of macrophages activated by injection of FCA [5]. Injection of FCA in the late stages of carcinogenesis was not accompanied by hyperplasia of the nonepithelial dark cells, possibly on account of the immunodepressive effect of the developed bladder carcinoma.

The results are evidence that among the dark cells found in tumors of the urothelium, epithelial and nonepithelial cells must be distinguished. Whereas the former, like dark cells of the parenchyma of many organs [3], serve as a unique indicator of the functional activity of the tissue, including tumor tissue [1], the latter evidently perform protective functions and have a different prognostic significance.

Injection of levamisole in these experiments had no morphologically detectable effect on carcinogenesis in the urinary bladder. However, it did affect the results of subcutaneous transplantation of the tumor. None of the tumors taken from animals receiving levamisole took successfully in the recipients, by contrast with the 100% take in the other groups. These results agree with evidence of the carcinoinhibitory action of levamisole and, in particular, of its action in preventing metastatic spread of the tumor [4]. The mechanism of this action requires further study.

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INHIBITION OF THE CARCINOGENIC EFFECT OF 7,12-DIMETHYLBENZ(a)ANTHRACENE IN FEMALE RATS BY BUFORMIN, PHENYTOIN, PINEAL POLYPEPTIDE EXTRACT, AND L-DOPA

V. N. Anisimov, M. N. Ostroumova, and V. M. Dil'man

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7,12-Dimethylbenz(a)anthracene (DMBA) induces adenocarcinoma of the mammary gland in female rats [11]. Meanwhile, after administration of DMBA to an animal, significant hormonal and metabolic disturbances arise [1, 3, 4, 8]; these disturbances characterize the syndrome of cancrophilia, i.e., the sum of the metabolic conditions promoting proliferation of somatic nonlymphoid cells and depressing cellular immunity [5].

The object of this investigation was to study the effect on the carcinogenic action of DMBA of the antidiabetic drug buformin, the antiepileptic drug phenytoin, pineal polypeptide extract (PPE), and the catecholamine precursor L-dopa, which exert their action on different systems of the body that may participate in the formation of the cancrophilia syndrome. However, all these substances likewise possess one common property, namely they lower the threshold of sensitivity of the hypothalamo-hypophyseal complex to homeostatic signals

Laboratory of Endocrinology and Laboratory of Experimental Tumors, Professor N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

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TABLE 1. Effect of Various Preparations on Tumors Induced by DMBA in Female Rats

Treatment	Number of rats	Number of rats receiving tumors	Total number of tumors	Mean latent period of all tumors (days)	Neoplasms of mammary gland						Other neoplasms		
					adenocarcinomas			fibroadrenomas and fibromas			number of rats		
					number of rats	number of tumors	mean latent period, days	number of rats	number of tumors	mean latent period, days	carcinoma of skin and Tsirbal's gland	leukemia	thyroid adenoma
Control	37	36 (97,3%)	52	102±11	30 (81,1%)	43	95±6	3 (8,1%)	3	81±44	4	1	1
Buformin	33	18* (54,5%)	24	165±22	12* (36,4%)	15	112±12	3 (9,1%)	5	326±22*	2	2	—
Phenytoin	38	27* (71,0%)	38	129±17	21* (55,3%)	31	104±20	3 (7,9%)	4	177±112	4	2	1
PPE	35	28* (80,0%)	33	182±15	9* (25,7%)	10	86±89	12* (34,3%)	14	216±21*	5	4	—
L-dopa	20	10* (50,0%)	19	107±12	5* (25,0%)	9	106±23	5 (25,0%)	7	108±18	—	3	—
Buformin + L-dopa	21	13* (61,9%)	21	115±14	4* (19,0%)	6	127±43	7* (33,3%)	12	115±10	1	2	—

*Reliable difference from control ($P < 0.05$).

[6]. Since raising of the threshold of sensitivity of the hypothalamus to homeostatic signals is the key element in the neuroendocrine program of development, aging, and formation of age pathology (and, in particular, the cancrophilia syndrome) [4, 5], it was considered essential to study and compare the possible antitumor effects of these substances.

EXPERIMENTAL METHOD

Experiments were carried out on 184 female rats reared at the "Rappolovo" nursery, Academy of Medical Sciences of the USSR. The animals were divided into six groups and, starting from the age of 2 months, they were given 1 ml of tap water or 1 ml water containing one of the following substances: buformin 5 mg/day (Adebit, from Chinoin, Hungary), phenytoin 7.5 mg/day (diphenylhydantoin), L-dopa 20 mg/day (3,4-dihydroxyphenylalanine, as Levodopa from KPKA, Yugoslavia), or a combination of buformin and L-dopa in these same doses, 5 times a week per os through a tube, and they also received acetic acid PPE [6, 9] in a dose of 0.5 mg/day subcutaneously. Three weeks after the beginning of injection of these substances all animals were given six injections, at weekly intervals, of a water–lipid emulsion containing 1.5 mg DMBA (from Fluka, Busch, Switzerland), into the caudal vein. The total dose of the carcinogen was 9 mg per animal. Administration of the drugs listed above continued during injections of the carcinogen and after their end, until death of the animals. All neoplasms discovered were studied microscopically after appropriate histological treatment. The numerical data were subjected to statistical analysis by Student's t-test and the chi-square test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all the substances used significantly inhibited the tumor effect: the frequency of discovery of all neoplasms and of adenocarcinoma of the mammary gland was reduced. Both buformin and PPE increased the mean latent period of detection of all neoplasms significantly. Buformin and phenytoin did not affect the frequency of development of fibroadenomas of the mammary gland, whereas L-dopa and PPE increased the frequency of their discovery. The latent period of the fibroadenomas, incidentally, was significantly longer in the groups of rats receiving PPE and buformin than in the control. Combined administration of buformin and L-dopa did not lead to significant summation of the effects of the two agents. Meanwhile, some parameters (frequency and histological type, latent period) indicate predominance of the effect of L-dopa when used in conjunction with buformin. None of the substances used had any significant effect on the frequency of tumors in other situations.

Several facts may play an important role in the mechanism of the anticarcinogenic effect of the antidiabetic biguanides and, in particular, of buformin. These include their ability to inhibit gluconeogenesis, to increase the fibrinolytic activity of blood and, perhaps, to intensify thyroid function [8]. It is important to note that antidiabetic biguanides have a normalizing effect on the feedback mechanism in the reproductive and thyroid systems disturbed after administration of DMBA [1, 3], and also that they abolish manifestations of immunodepression which develop both during chemical carcinogenesis [7] and in patients with cancer and during natural aging [5].

It is difficult to determine the mechanism of the antitumor action of phenytoin unambiguously. On the one hand there are data showing the carcinogenicity of phenytoin [12]. However, on the basis of observations that phenytoin raises the level and increases the metabolism of biogenic amines in the hypothalamus, lowers the threshold of sensitivity of the hypothalamus to estrogens, and lowers the level of insulin and glucocorticoid hormones in the blood [4, 6, 10], it has been used to inhibit carcinogenesis. Phenytoin did not affect the frequency of liver tumors induced in rats by 2-acetylaminofluorene, but reduced the number of tumors arising per animal [14]; the authors cited attach definite importance, in the anticarcinogenic effect of phenytoin, to its ability to stimulate activity of the microsomal enzymes of the liver, which metabolize carcinogens. Levo [13], who observed the inhibitory effect of phenytoin on the carcinogenic action of urethane in mice, attaches great importance to the action of phenytoin on the immune surveillance system.

An important factor in the mechanism of the antitumor action of pineal polypeptide extract may be its ability to lower the threshold of sensitivity of the hypothalamus to the action of estrogens [6] and also its normalizing effect on lipid and carbohydrate metabolism and its immunostimulant effect [9].

The antitumor effect of L-dopa is usually linked with its inhibitory action on secretion of prolactin-inhibiting factor and, correspondingly, on the secretion of prolactin itself, which together with estrogens plays an important role in the induction of mammary gland carcinoma by polycyclic hydrocarbons [15]. At the same time, we have shown that L-dopa also abolishes the ability of carcinogenic polycyclic hydrocarbons

to raise the thresholds of hypothalamic sensitivity to estrogens [2], a matter of great importance, in particular, in the maintenance of steroid homeostasis.

The use of substances with different points of application in their action on the body yet, nevertheless, having the property of preventing the development of the cancrophilic syndrome, thus has an inhibitory effect on the appearance of malignant mammary gland tumors induced by DMBA.

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