erythrocytes one which cannot be overlooked is the autoimmune processes that accompany malignant growth, and which take place invariably with the participation of erythrocytes. The role of hormonal and neurotrophic disturbances connected with the development of neoplasms in metabolic changes leading to echinocytosis must also be studied. Investigation of the problems listed above will help not only to discover the causes of the change in configuration of the erythrocytes, but also to shed light on some other aspects of the pathogenesis of tumor growth.

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EFFECT OF AGE, CASTRATION, AND PREGNANCY ON CARCINOGENESIS INDUCED IN CBA MICE BY 1,2-DIMETHYLHYDRAZINE

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When injected subcutaneously in a dose of 8 mg/kg weekly into female CBA mice, 1,2-dimethylhydrazine (DMH) induced the development of tumors of the intestine, anal region, uterus, and liver. When DMH was injected into mice aged 12-13 months the appearance of sarcomas of the uterus was observed earlier (at 8 weeks) and the incidence of tumors of the anal region rose more rapidly than in mice aged 3 months. In mice receiving DMH against the background of repeated pregnancies, a statistically significant decrease in the frequency of sarcomas of the uterus was observed (10.3% compared with 48.3% in nonpregnant mice); pregnancy did not affect the frequency of tumors of other organs. Castration had no significant effect on the time of appearance or the frequency of tumors in all situations.

KEY WORDS: 1,2-dimethylhydrazine; tumors; aging; castration; pregnancy.

A definite role in the carcinogenic effect of 1,2-dimethylhydrazine (DMH) is ascribed to the effect of this carcinogen on the neuroendocrine system and the associated hormonal-metabolic disturbances [2, 3]. In rats, DMH induces predominantly intestinal tumors, and castration changes their localization in different segments of the large intestine [6]. In CBA mice, besides intestinal tumors, in a high percentage of cases DMH induces epithelial tumors in the anal region, sarcomas of the uterus, and hemangioendotheliomas of the liver [7].

The object of the present investigation was to study the effect of factors such as age, castration, and pregnancy on carcinogenesis induced by DMH in CBA mice.

EXPERIMENTAL METHOD

Female CBA mice obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, received subcutaneous injections of DMH dissolved in distilled water in a dose of 8 mg/kg once a week for 30 weeks. The experimental mice were divided into four groups: young virgin, castrated, pregnant, and old virgin. The first three groups consisted of 30 mice aged 3 months and weighing 19-23 g. The group of old mice consisted of 50 animals aged 12-13 months and weighing 30-45 g. Bilateral castration was carried out 2 weeks before the begin-

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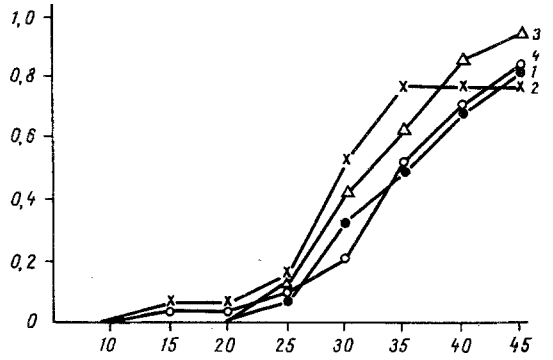

Fig. 1. Frequency of tumors in the anal region: 1) young, 2) old, 3) pregnant, 4) castrated mice. Abscissa, time after beginning of DMH administration (in weeks); ordinate, cumulative probability of appearance of tumors (determined by Kaplan and Meyer's method [11], allowing for probability of appearance of tumors in mice dying without neoplasms in this situation throughout the period of observation; the final values of probability in this figure do not therefore coincide with the frequency of tumors given in Table 1).

TABLE 1. Frequency of Tumors Induced in CBA Mice by 1,2-Dimethylhydrazine

Group of mice	Total number of mice*	Number of mice with tumors of								other organs
		uterus		anal region		intestine		liver		
		abs.	%	abs.	%	abs.	%	abs.	%	
Young	29	14	48,3	23	79,3	21	72	11	38	4 b
Old	46	21	45,6	34	74	32	70	16	34,7	11 c
Castrated	29	12	41,4	22	75,8	20	69	14	48,2	2 d
Pregnant	29	3	10,3	25	86,2	20	69	15	51,7	3 e
Control	65†	2	3,1	—	—	—	—	14 a	21,5	12 f

*Surviving until appearance of first tumor (13 weeks)
†Mice surviving over 1 year
a Hepatomas
b Two granulosa-cell tumors of the ovary, two angiomas of the ovary
c Eight granulosa- ell tumors of the ovary, two angiomas of the ovary, one adenoma of the ovary
d Two adenomas of the lungs
e Two angiomas of the ovary, one adenoma of the lung
f Seven adenomas of the lung, five granulosa-cell tumors of the ovary

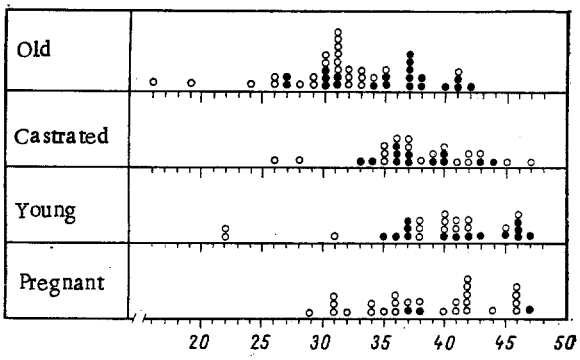

Fig. 2. Times of death of mice with sarcomas of the uterus. Filled circles denote mice with sarcomas of the uterus, unfilled circles mice without sarcomas of the uterus. Numbers indicate time after beginning of DMH administration (in weeks).

ning of injection of DMH. One group of mice was kept together with the control males; these mice had from 2 to 6 pregnancies with lactations during the period of DMH administration. Observation was kept on the mice until they died naturally or were killed in an agonal state. The last experimental mice died 47 weeks after the beginning of DMH administration. Observation on the control mice receiving distilled water subcutaneously was kept until they died naturally (over 24 months). Pieces of organs were fixed in 10% formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin. The frequency of the tumors was determined as the ratio of the number of animals with tumors to the total number of animals surviving until appearance of the first tumor (13 weeks after the beginning of DMH administration). Differences in frequency were assessed by the χ^2 method. Since the appearance of tumors in the anal region could be observed visually, the increase in their frequency was represented graphically by a method enabling the probability of development of tumors in mice dying without tumors in this particular situation throughout the period of observation to be taken into account.

EXPERIMENTAL RESULTS

The frequency of tumors observed in the different groups is given in Table 1.

Tumors in the anal region appeared sooner in the groups of old and castrated mice (Fig. 1). The increase in the frequency of tumors in old mice in the course of two intervals (26-30 and 31-35 weeks) was more rapid than the young of all groups, and the differences were statistically significant ($P < 0.01$). Toward the end of the period of observation the differences between the groups were equalized.

The first sarcomas in the old mice were found at autopsy 8 weeks earlier than in the young mice (Fig. 2). After 31 weeks, when seven tumors had already been found in the group of old mice, they were still absent in the young mice. This fact cannot be attributed to the increased mortality of the old mice during this period, for tumors of the uterus in this group began to be palpable correspondingly earlier than in the other groups. In other words, there was a true increase in the rate of development of sarcomas of the uterus in the old mice. The final frequency of sarcomas of the uterus in young, old, andcastrated mice was equalized.

It should be noted that the young mice lived longer and the last five sarcomas were found in them at a time when all the animals in the group of old mice had already died. This fact (given the equal final frequency of tumors) is also evidence of the higher rate of sarcoma development in the uterus of the old mice.

Fewest sarcomas of the uterus developed in the pregnant mice, namely 10.3%, which is significantly ($P < 0.001$) fewer than in the other groups. Two tumors of the uterus were found in the control group in mice which died at the ages of 23 and 26 months.

Tumors of the intestine also were found earlier in the old mice which died, but this was perhaps due to the earlier death of the animals inthis group. The final frequency of intestinal tumors was the same in all groups (Table 1). There were some differences (but not statistically significant) in the frequency of hemangioendotheliomas of the liver.

The results of the investigation showed that aging and pregnancy had some effect on the development of tumors induced by DMH. As far as aging is concerned, data in the literature of its effect on carcinogenesis are contradictory: Both acceleration [8, 10] and inhibition [11] of development of experimental tumors in old animals and also no difference compared with young animals [9, 12] have been observed. It followed from the results of the present investigation that aging does not affect the appearance of tumors induced by DMH in certain organs

(the liver) but accelerates their development in others (uterus, anal region and, possibly, intestine). As was stated above, there were considerable variations in the weight of the old mice (from 30 to 54 g). The heavier mice thus received larger absolute doses of the carcinogen. However, there were no significant differences between the group of old mice weighing 30-39 g (29 mice) and the group weighing 40 g or more (17 mice), although the very first tumors (of the anal region and uterus) in fact appeared in animals weighing 40 g or more.

The marked decrease in the frequency of sarcomas of the uterus in mice receiving the carcinogen during pregnancy can evidently be explained by hormonal influences on the uterus rather than by the character of DMH metabolism in the liver, for the frequency of tumors in other situations in this group was unchanged (Table 1).

Clinical experience shows that among patients with sarcoma of the uterus there is always a high percentage of nulliparous women or of mothers who have few children [4, 5]. Although the mechanisms of the inhibitory action of pregnancy on the development of uterine sarcomas in women and experimental animals may be different, the coincidence of the facts merits attention.

According to one report [1] sarcoma of the uterus developed in rats receiving nitrosamides during pregnancy, but not in their offspring. It is difficult to compare these findings with our own observations not only because carcinogens with different mechanisms of action were used on animals of different species, but also because in the investigations cited there was no group of nonpregnant females to which the carcinogen was given at the same age as the pregnant animals.

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