*Mutation Research*, 275 (1992) 97–110
© 1992 Elsevier Science Publishers B.V. All rights reserved 0921-8734/92/$05.00


MUTAGI 09086

# Effect of neonatal exposure to 5-bromo-2′-deoxyuridine on life span, estrus function and tumor development in rats – an argument in favor of the mutation theory of aging?

Vladimir N. Anisimov and Galina Yu. Osipova

*Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, St. Petersburg, Russia*

(Received 18 November 1991)
(Revision received 30 March 1992)
(Accepted 1 April 1992)

*Keywords:* 5-Bromo-2′-deoxyuridine; Reproductive system function; Chromosome aberrations; (Rat)

## Summary

Outbred LIO rats were exposed to subcutaneous injections (3.2 mg) of a synthetic analogue of thymidine, 5-bromo-2′-deoxyuridine (BrdUrd), on days 1 and 3, or days 1, 3, 7 and 21 of postnatal life. The mean life span decreased by 31% and 38% in male and by 14% and 27% in female rats that received 2 and 4 injections of BrdUrd, respectively, in comparison to untreated controls. The opening of the vagina was delayed, whereas age-related changes in the length of the estrous cycle and in the incidence of persistent estrus and/or anestrus were observed earlier in BrdUrd-injected female rats than in untreated ones. Inhibition of compensatory ovarian hypertrophy induced by hemiovariectomy at the age of 3 months was found in females exposed neonatally to BrdUrd as compared to untreated rats, while the uterus weight increase induced by the administration of human chorionic gonadotropin was similar in both control and BrdUrd-treated infantile rats. These data suggest that exposure to BrdUrd in early life impairs pituitary gonadotropic function in female rats. It was also shown that neonatal administration of BrdUrd to rats doubles the incidence of chromosome aberrations in peripheral blood lymphocytes in comparison to controls and is followed by a dose-related increase in tumor incidence. Our observations on the decrease in mean and maximum life span, acceleration of age-related changes in reproductive system function, increase in chromosome aberration and tumor incidence and decrease in tumor latency in rats exposed to BrdUrd in early life suggest that this model could be used as a model of accelerated aging and that some of the results can be interpreted as arguments in favor of the mutation theory of aging.

Correspondence: Dr. V.N. Anisimov, Chief, Laboratory of Experimental Tumors, N.N. Petrov Research Institute of Oncology, Leningradskaya Str. 68, Pesochny-2, St. Petersburg 189646, Russia.
Tel.: (812) 237-86-07; Fax: (812) 237-89-47.

*Abbreviations:* BrdUrd, 5-bromo-2′-deoxyuridine; COH, compensatory ovarian hypertrophy; FSH, follicle stimulating hormone; HCG, human chorionic gonadotropin.

According to current concepts, DNA of somatic cells is a primary 'substrate of aging', and aging itself develops as a result of interaction of various endogenous and exogenous damaging agents with genetic material (Ames, 1989; Kirkwood, 1989; Vijg, 1990; Harman, 1991). Among theories of aging based on the suggestion that DNA is the target for damages, the leading one is a theory of somatic mutations, according to which aging is a consequence of the gradual accumulation of stochastic mutations in the genome of somatic cells resulting in its instability (Burnet, 1974; Strehler, 1986; Cutler and Semsei, 1989; Kirkwood, 1989; Vijg, 1990). DNA damage is likely to be critical for carcinogenesis as well (Singer and Grunberger, 1983; Ames, 1989; Anisimov, 1989, 1991; Cutler and Semsei, 1989; Lutz, 1990).

Some in vitro and in vivo effects of the thymidine analogue 5-bromo-2'-deoxyuridine (BrdUrd), widely used in biological studies, suggest that BrdUrd could serve as an adequate agent for the investigation of the role of selective DNA damage both in aging and in carcinogenesis. Thus, it has been reported both to enhance (Stockdale et al., 1981) and to inhibit (Bannigan and Langman, 1978; Tappscott et al., 1989) cell differentiation, and inhibit DNA synthesis and cell replication in vivo (Weghorst et al., 1991) and in vitro (Morris et al., 1989). BrdUrd induces viruses in cell culture (Lieber and Todaro, 1973) and teratogenic effects in mice and hamsters (DiPaolo, 1964; Ruffolo and Ferm, 1965). Since it is incorporated exclusively into cellular DNA in the place of thymidine, BrdUrd is widely used for measuring the rate of DNA synthesis (Lewis and Swenberg, 1982) and sister-chromatid exchange (Perry and Evans, 1975).

BrdUrd is mutagenic in cellular systems (Freese, 1963; Varshaver et al., 1980), produces second generation mutants in the eukaryotic alga *Volvox cartesi* (Kirk et al., 1987), and appears to have a miscoding effect in cell-free systems (Trautner et al., 1962). Being incorporated into replicating DNA in place of thymidine, BrdUrd may occur not only in the usual keto form but also in an enol tautomeric form (Fishbein et al., 1970), which forms hydrogen bonds with guanine instead of adenine, the normal pair for thymidine and 5-bromouracil. Assuming that there is no 5-bromouracil repair in rat DNA (Lindahl, 1982; Likhachev et al., 1983b), and as BrdUrd after incorporation into DNA pairs with guanine when present as the enol tautomer, base pair substitution mutations are expected to occur (GC → AT and AT → GC transitions) during subsequent DNA replication (Auerbach, 1978; Davidson et al., 1988).

Experiments with *D. melanogaster* (Potapenko et al., 1982) have shown that supplementation of BrdUrd in nutrition medium is followed by a reduction of insect life span. Craddock (1981) observed a dose-dependent shortening in the life span of rats exposed to BrdUrd in early life. However, few animals were treated with the agent groups and there was no untreated control in her study nor were any data presented on either tumor development or any biomarker of aging. In our previous experiments we have shown that the nucleoside analogue, BrdUrd, has carcinogenic potential and increases the susceptibility to the effects of some carcinogens and tumor promoters (Napalkov et al., 1989; Anisimov and Osipova, 1991, 1992a, b, c; Osipova and Anisimov, 1991). However, no biomarkers of aging were studied in these works. Among biomarkers of aging some parameters of survival and reproductive system function (time of sexual maturity, pattern of age-related changes of estrus function and time of switching it off) could be evaluated by non-invasive methods and monitored during the life span of the animals (Sacher, 1980; Dubina et al., 1984; Hochshield, 1989). In the present experiments we report the results of a study on the effects of neonatal administration of BrdUrd on survival parameters, tumor development and the functional status of the reproductive system of rats.

## Materials and methods

### *Chemicals*

BrdUrd, from Sigma Chemical Co. (USA), 100% pure, was stored at +4°C. HCG, from Serva Chemical Co. (USA) (2500 IU per vial) was stored at +4°C.

### *Animals*

Outbred male and female LIO rats from the Animal Department of the N.N. Petrov Research

Institute were used. The mean life span of both males and females of this strain is about 23 months, and the maximum life span 35 months; the spontaneous tumor incidence is 27% in males and 47% in females. These data as well as data on karyogram, survival pattern, and specific tumor incidence, localization and variability have been given elsewhere (Anisimov et al., 1989).

After mating and detection of pregnancy rats were kept in individual polypropylene cages until delivery. The offspring were kept with the dam for 4–5 weeks in the same housing conditions. Then, animals were kept 6–7 per cage. Animals received standard laboratory chow (Likhachev et al., 1983a; Baranova et al., 1986) and tap water ad libitum.

*Experiments*

BrdUrd was ex tempore dissolved in distilled water and injected subcutaneously on days 1 and 3, or on days 1, 3, 7 and 21 of life in a volume of 0.2 ml at the single dose of 3.2 mg/rat. Some rats born at the same time were not treated and served as controls.

Starting from the age of 3, 6, 9, 12, 15, 18 or 21 months, daily vaginal smears were cytologically studied for 30 days in 30 randomly selected female rats exposed to 4 injections of BrdUrd and in 30 untreated females. Animals were kept under observation until natural death or were killed when moribund.

In two additional sets of experiments the effect of BrdUrd upon regulation of the gonadotropic function of the pituitary was studied. Female rats exposed to 4 injections of BrdUrd and untreated ones at the age of 3 or 9 months were subjected to hemiovariectomy under ether anesthesia; after a week the animals were killed with ether vapor. Compensatory ovarian hypertrophy (COH) was estimated as the ratio of the weight increase of the ovary which was left in situ at the operation to the weight of the removed one, per 100 g of body weight, in percent. There were 10 animals in each hemiovariectomized group. In another experiment, control rats and female rats exposed to 4 injections of BrdUrd, starting at the age of 35 days were subcutaneously treated for 3 consecutive days with HCG in a daily dose of 0.5 IU, dissolved in 0.1 ml normal saline, or with solvent only. 24 h after the last injection of HCG animals were killed with ether vapor and the body and uterus weights were measured.

*Cytogenetic study*

Cytogenetic exmination was carried out in 15 female rats neonatally exposed to 4 injections of BrdUrd and 15 untreated control rats at the age of 3 months. Blood samples of 0.5–0.6 ml were taken from the tail vein and blood cultivation and chromosome preparations were performed as described elsewhere (Monakhov, 1988). In order to obtain an enhanced yield of second mitoses, the samples of rat blood were incubated for 70 h in medium containing 80% of Eagle medium, 20% of standard bovine serum, 100 units/ml of penicillin and 100 units/ml of streptomycin. This approach makes it possible to reveal both the stable chromosomes and the genomic lesions that developed during passage through two consecutive mitotic cycles at the time of cultivation. The cell suspension was fixed according to Islam and Levan (1987), and chromosome preparations were routinely produced. As mitogen we used concanavalin A (Calbiochem, USA) at a final concentration of 60 $\mu$g/ml of medium. All visible structural and numerical chromosomal aberrations (CsA) were examined under the light microscope in 50 Giemsa-stained metaphase plates from each rat.

*Pathohistological examination*

All animals that died or were killed were autopsied. The tumors found at necropsy were evaluated as 'fatal' or 'incidental' according to the IARC recommendations (Gart et al., 1989). At the autopsy the liver, kidney, spleen and all macroscopically abnormal organs were fixed in 10% neutral formalin. Routine histological treatment of 5–7 $\mu$m thick sections was followed by staining with hematoxylin and eosin and subsequent microscopic examinations. The tumors were classified according to the IARC classification (Turusov and Mohr, 1990).

*Statistics*

The experimental results were statistically processed according to the IARC recommendations (Gart et al., 1989). The statistical significance of

TABLE 1

AGE-RELATED DYNAMICS OF BODY WEIGHT OF LIO RATS EXPOSED NEONATALLY TO 4 INJECTIONS OF BrdUrd

| Treatment | Age (months) | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 6 | 9 | 12 | 18 |
| *Males* | | | | | | |
| Control | 118 ± 1.7 | 213 ± 2.7 | 358 ± 4.4 | 374 ± 4.7 | 428 ± 3.2 | 402 ± 5.1 |
| BrdUrd | 93 ± 1.1 * | 151 ± 2.1 * | 296 ± 4.9 * | 324 ± 4.4 * | 375 ± 5.5 * | 346 ± 8.8 * |
| *Females* | | | | | | |
| Control | 112 ± 1.5 | 184 ± 2.9 | 259 ± 4.7 | 291 ± 4.4 | 336 ± 4.5 | 347 ± 5.7 |
| BrdUrd | 85 ± 1.3 * | 136 ± 2.6 * | 217 ± 4.5 * | 255 ± 4.5 * | 303 ± 5.2 * | 309 ± 8.1 * |

* The difference with the sex-matched control is significant: $p < 0.05$.

any apparent increase in carcinogenic effect with regard to BrdUrd was assessed by combining the test for fatal and incidental tumors (Gart et al., 1989). In other places, Student's *t*-test and Fisher's test for equality of proportions were used as appropriate (Goubler, 1978). An IBM PC/AT computer was employed for statistical processing of the data.

## Results

### *Effect of BrdUrd on body weight and survival of the rats*

Both male and female rats exposed to 4 neonatal injections of BrdUrd that survived for more than 2 months, manifested a delay in body weight gain in comparison to control; the body

TABLE 2

LIFE SPAN AND TUMOR INCIDENCE IN RATS NEONATALLY TREATED WITH BrdUrd

| Treatment | Number of rats at the start | Life span (days) | | | Life span of fatal tumor-free rats (M ± SEM) | Effective number of rats [a] | Number of tumor-bearing rats | | Age of first fatal tumor detection (days) | Total number of tumors |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SEM | Median | Maximum | | | Total (%) | Fatal (%) | | |
| *Males* | | | | | | | | | | |
| Control | 59 | 773 ± 23.2 | 833 | 1095 | 761 ± 24.4 | 59 | 11 (18.6) | 5 (8.5) | 863 | 16 |
| BrdUrd, ×2 | 24 | 536 ± 34.9 * | 512 | 862 | 522 ± 13.5 ** | 22 | 9 * (40.9) | 1 (4.5) | 216 | 13 |
| BrdUrd, ×4 | 116 | 476 ± 16.4 ** | 489 | 910 | 419 ± 16.7 ** | 99 | 36 ** (36.4) | 22 * (22.2) | 295 | 55 |
| *Females* | | | | | | | | | | |
| Control | 39 | 752 ± 24.1 | 730 | 1013 | 743 ± 25.9 | 39 | 14 (35.9) | 4 (10.3) | 520 | 21 |
| BrdUrd, ×2 | 28 | 646 ± 34.1 * | 641 | 834 | 642 ± 39.0 * | 27 | 13 (48.1) | 5 (18.5) | 421 | 23 |
| BrdUrd, ×4 | 112 | 551 ± 15.1 ** | 602 | 853 | 547 ± 16.2 ** | 104 | 56 * (53.8) | 23 *** (22.1) | 159 | 84 |

[a] The number of rats surviving until the discovery of the first tumor in the experiment (males and females separately).
The difference with the sex-matched control is significant: * $p < 0.05$; ** $p < 0.01$; *** $0.05 < p < 0.06$.

weight of BrdUrd-treated rats was lower than in controls during the whole period of observation (Table 1). The estimated difference in body weight of control and BrdUrd-treated rats between 6 and 18 months of age exceeded 50 g in males and 30 g in females. In some cases a loss of teeth, an inhibition of skin keratinization and baldness at the site of injection of BrdUrd were observed.

The data presented in Table 2 show that administration of BrdUrd in early life was followed by a significant and dose-proportional decrease of the mean, median and maximum life span of both male and female LIO rats. The mean life span of the animals that died without any fatal tumor, as discovered at autopsy, was reduced in comparison to sex-matched controls as well. A significant shift to the left of the survival curves was observed in groups of male and female rats neonatally exposed to 2 or 4 injections of BrdUrd in comparison to untreated animals (Fig. 1). The aging rate of populations of rats exposed to 4 injections of BrdUrd and untreated rats was calculated according to Gompertz' equation: $R = R_0 \cdot \exp(\alpha t)$, where R = mortality; $R_0$ = mortality at time (t) = 0; $\alpha$ = constant, evaluated as the left part of the equation: $\Delta R/\Delta t = R_0 \cdot \alpha \cdot \exp(\alpha t)$ (Gavrilov and Gavrilova, 1991). The parameter was shown to be increased in males ($0.0229 \pm 0.00074$ days$^{-2}$ and $0.0049 \pm 0.00033$ days$^{-2}$, respectively) and females ($0.0024 \pm 0.00077$ days$^{-2}$ and $0.0047 \pm 0.00021$ days$^{-2}$, respectively).

*Effect of BrdUrd on non-tumor and tumor pathology in the rats*

A number of cases of non-tumorous lesions of internal organs, mainly the kidney, have been observed in rats neonatally treated with BrdUrd. Thus, the relative size of kidneys was sometimes decreased in comparison to control ones. Histologically, the convoluted tubules in kidneys of rats exposed to BrdUrd were enlarged and dilated to form micro- and macrocysts and were lined with eosinophilic epithelial cells which either tightly filled the lumen of tubules or protruded into it. These cystic lesions were observed in 21.9% of males and 55.7% of females treated 4 times with BrdUrd, compared to 5.0% and 2.5% in control animals, respectively ($p < 0.001$). Cases of a de-

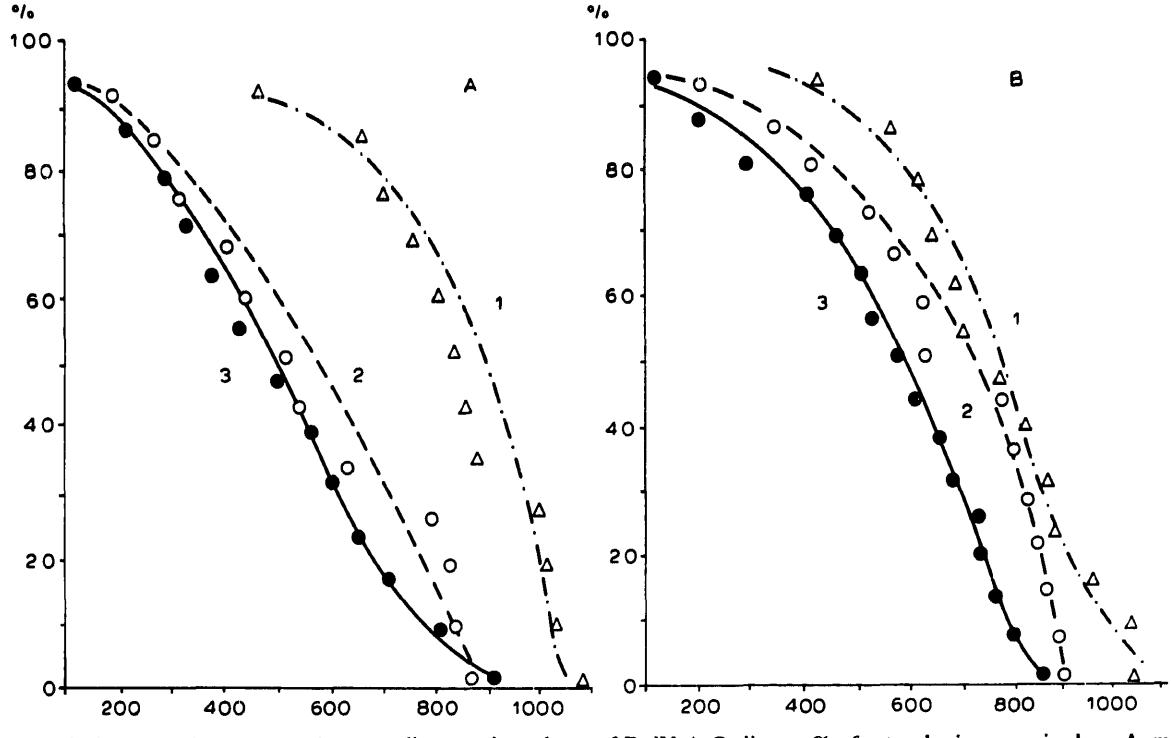


Fig. 1. Survival curves of rats exposed neonatally to various doses of BrdUrd. Ordinate, % of rats; abscissa, age in days. A, males; B, females. 1, control; 2, BrdUrd×2; 3, BrdUrd×4. Symbols represent every third animal.

crease in the relative size of the liver histologically manifesting spongiosis, and decreases in spleen size accompanied by declines in the size of follicles and fibrosis of stroma, and atrophy of the testicles have been registered in BrdUrd-exposed rats.

Two neonatal injections of BrdUrd were followed by a significant increase in total tumor incidence in male but not in female rats, while in rats exposed to 4 injections of the thymidine analogue the incidence of both total and fatal tumors was increased in comparison to untreated animals (Table 2). Compared to controls, the first tumors were discovered earlier and the tumor yield curves were significantly shifted to the left in groups of male and female rats exposed to any dose of BrdUrd (Fig. 2).

Pathological examination of the discovered tumors has shown that exposure to BrdUrd slightly increased the incidence of spontaneous tumors characteristic of LIO rats: pituitary and thyroid adenomas, malignancies of the hematopoietic system and mammary fibroadenomas (in females). However, the few mesenchymal kidney tumors, testicular Leydigomas, and somewhat benign and malignant uterine tumors discovered at autopsy in BrdUrd-treated rats were not seen in animals of the control group. These tumors practically never develop spontaneously in this rat strain (Anisimov et al., 1989) and could be attributed to the agent. Comprehensive data on the incidence, localization and histology of the tumors in the rats exposed to BrdUrd and in controls will be published elsewhere (Anisimov and Osipova, 1992).

*Cytogenetic study*

The mean level of spontaneous chromosome aberrations in peripheral blood lymphocytes of 15 control female rats at 3 months of age was evaluated as $5.3 \pm 0.4$ per hundred cells, whereas the level reached $11.6 \pm 0.6$ per hundred cells in 15 rats neonatally exposed to 4 injections of BrdUrd at the same age ($p < 0.01$).

*Effect of BrdUrd on sexual maturation and estrus function in female rats*

The mean age of sexual maturation, studied by registration of the day of the vagina opening, was estimated to be $57 \pm 0.9$ days in control rats and

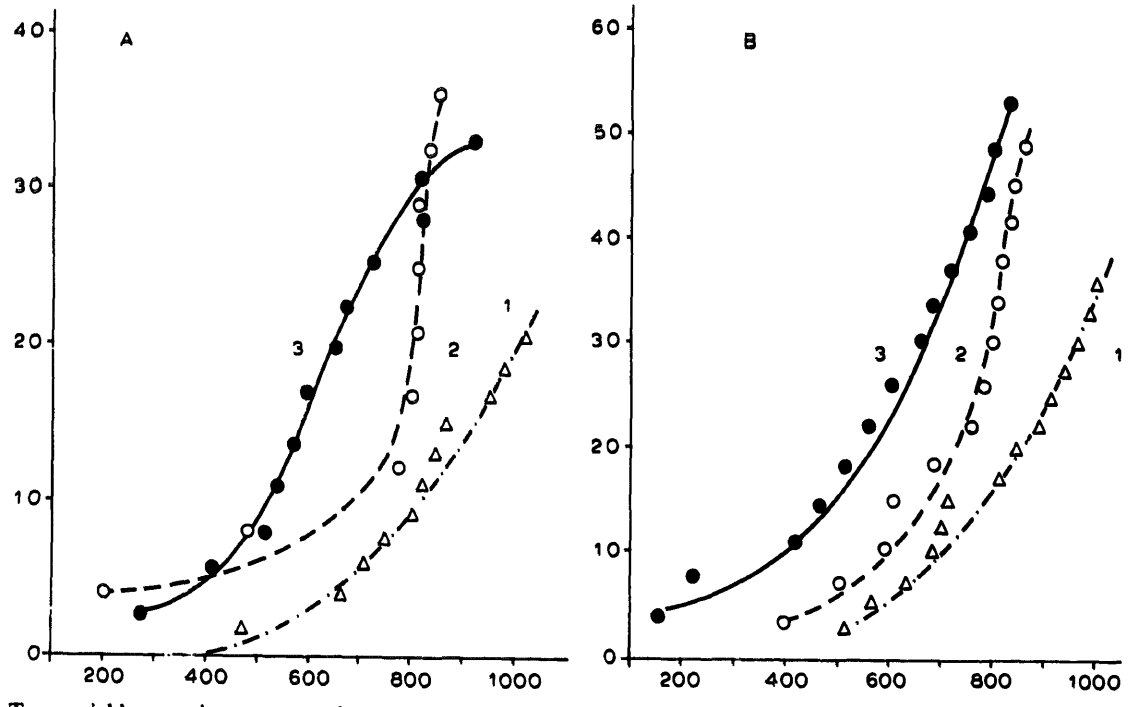


Fig. 2. Tumor yield curves in rats exposed neonatally to various doses of BrdUrd. Ordinate, % of tumor-bearing rats; abscissa, age in days. A, males; B, females. 1, control; 2, BrdUrd×2; 3, BrdUrd×4.

71 ± 1.3 days in animals neonatally exposed to 4 BrdUrd injections ($p < 0.001$). Daily cytological study of the vaginal smears showed an age-related decrease in the number of animals with regular estrus cycles in both control and neonatally BrdUrd-treated females. Thus, among 30 monitored control rats the estrus cycle was evaluated as regular in 90% of cases at the age of 3 months and in 37% at the age of 21 months, while in BrdUrd-exposed females the corresponding values were 60% and 30%. Although 3 of the 30 studied control females manifested an irregular estrus cycle at the age of 3 months, no cases of persistent estrus or anestrus were registered at this age in the group. At the age of 15 months these disorders of estrus function were detected in 27%, and at the age of 21 months in 47% of control females. At the same time, in 3-month-old rats neonatally exposed to 4 injections of BrdUrd persistent estrus and/or anestrus was present in 9% of cases, at the age of 15 and 21 months in 39% and 70%, respectively. The dynamics of age-related changes of the incidence of regular estrus cycles and their disturbances (persistent estrus plus anestrus) are given in Fig. 3.

It is worthy of note that the life-table analysis of rat mortality estimated as R from the Gompertz equation shows a reduction of the life span and an increase in the slope of the mortality

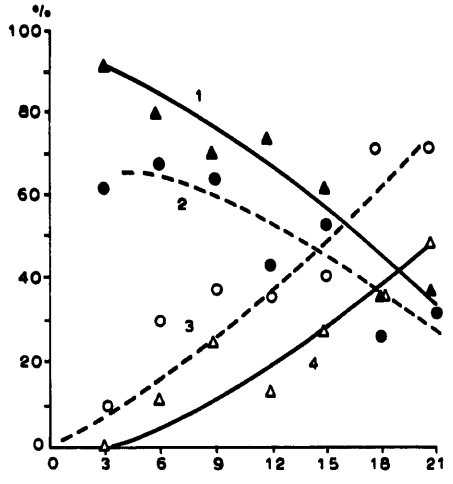


Fig. 3. Age-related dynamics of estrus function in female rats neonatally exposed to 4 injections of BrdUrd. Ordinate, % of animals; abscissa, age in months. 1, control, regular estrus cycle; 2, BrdUrd, regular estrus cycle; 3, BrdUrd, persistent estrus + anestrus; 4, control, persistent estrus + anestrus.

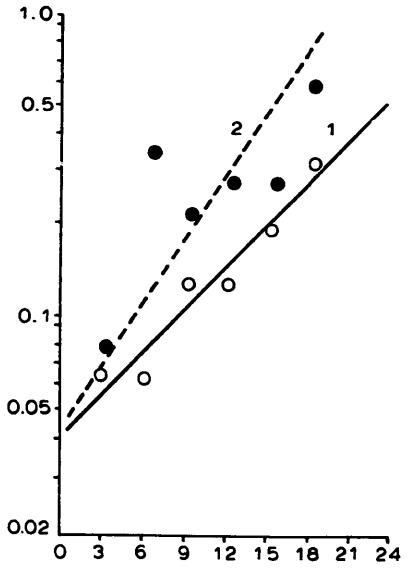


Fig. 4. Mortality of female rats neonatally exposed to BrdUrd and manifesting disturbances of estrus function (persistent estrus + anestrus). Ordinate, mortality (R), log scale; abscissa: age in months. 1, control; 2, BrdUrd.

curve of females neonatally exposed to BrdUrd and manifesting persistent estrus and/or anestrus, in comparison to control females (Fig. 4).

The results of daily cytological study of vaginal smears of BrdUrd-exposed and non-exposed female rats were analyzed in relation to the ratio of phases of the estrus cycle (diestrus : proestrus : estrus : metaestrus) and to the length of the estrus cycle. Only data from each full normal estrus cycle (where all phases were detected) were selected for calculations. The analysis failed to show any differences in the ratio of estrus cycle phases between the two groups. The mean length of the estrus cycle decreased from the age of 3 months to 6–9 months and increased with age in control rats. At the same time, in rats neonatally exposed to BrdUrd changes in the length of the estrus cycle were delayed between 3 and 9 months and developed early after the age of 9 months (Fig. 5).

*Effect of BrdUrd on compensatory ovarian hypertrophy in hemiovariectomized adult rats*

Hemiovariectomy of 3-month-old control rats were followed a week later by a 55.9 ± 1.3% increase in the weight of the remaining ovary,

whereas the COH value was only 1.1 ± 5.0% ($p < 0.001$) in rats neonatally treated with BrdUrd and subjected to surgery at the same age. However, the values of COH in BrdUrd-exposed and non-exposed rats subjected to hemiovariectomy at the age of 9 months were similar: 14.4 ± 1.9% and 12.3 ± 1.6%, respectively, $p > 0.05$ (Fig. 6).

*Effect of HCG on uterus weight of infantile rats exposed neonatally to BrdUrd*

To identify the possible mechanism of COH disturbance in rats exposed neonatally to BrdUrd, HCG or saline was injected to immature BrdUrd-exposed and non-exposed female rats at the age of 35 days. The mean body weight of BrdUrd-treated females at the age of 35 days was 56 ± 1.3 g, whereas in control females it was 90 ± 2.9 g ($p < 0.001$). The mean uterus weight in saline-injected BrdUrd-treated rats was 35.1 ± 3.3 mg/100 g body weight, and in controls 67.6 ± 1.5 g/100 g body weight ($p < 0.05$). However, the response of the uterus to injections of HCG was the same in both groups: uterine weight increased by 2.76 and 2.66 times, respectively.

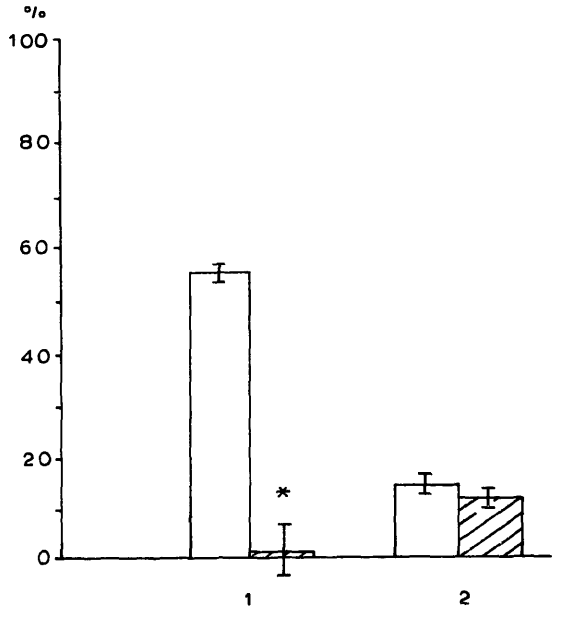


Fig. 6. Compensatory ovarian hypertrophy in female rats neonatally exposed to 4 injections of BrdUrd and subjected to hemiovariectomy at different ages. Ordinate, value of COH (%). White columns, control; shaded columns, BrdUrd. 1, 3-month-old rats; 2, 9-month-old rats. The difference with the value of COH in 3-month-old control rats is significant, * $p <$ 0.001.

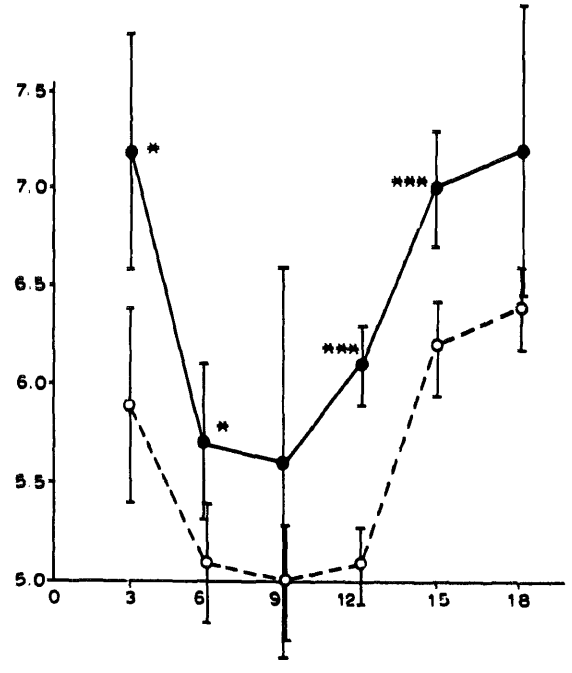


Fig. 5. Length of the estrus cycle in female rats neonatally exposed to 4 injections of BrdUrd. Ordinate, length of the estrus cycle in days; abscissa, age in months. White circles, control; black circles, BrdUrd. Values are means ± SEM. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

## Discussion

In comparison with DNA changes that have a high miscoding potential, such as $O^6$-alkylguanine or $O^4$-alkylthymine (Singer and Grunberger, 1983), the presence of 5-bromouracil in DNA results in a very low probability of miscoding (Davidson et al., 1988; Kaufman, 1988). However, in contrast to the alkylated bases which undergo intensive repair (Pegg, 1990), 5-bromouracil is not a substrate for DNA repair enzymes and thus persists over a long period (Likhachev et al., 1983b; Ward et al., 1991). It was shown in vitro that BrdUrd-induced mutagenesis in mammalian cells is dependent upon the perturbation of endogenous deoxycytidine metabolism, that mutations could arise from misincorporation of BrdUrd into DNA, driven by the unbalanced deoxynucleoside triphosphate pools available for

DNA synthesis, and that mutagenesis under such conditions results primarily in GC → AT transitions (Davidson et al., 1988; Kaufman, 1988). Using an in vitro shuttle vector system Davidson et al. (1988) have revealed a very high degree of sequence specificity for the BrdUrd mutagenesis. Thus, BrdUrd-induced GC → AT transitions occurred almost exclusively in sequences with two adjacent guanine residues, and in about 90% of the cases the guanine residue involved in mutation was the one in the more 3′ position.

In our recent experiments (Osipova and Anisimov, 1992) we observed the development of malformations and the burst of spontaneous tumorigenesis in offspring of rats exposed neonatally to BrdUrd and mated at the age of 3 months. In our joint pilot work with Dr. J.M. Rice (NCI, Frederick, MD) the paraffin sections of 3 mesenchymal kidney tumors developed in one male and two female rats from the present experiment and exposed to 4 injections of BrdUrd were studied with the polymerization chain reaction and in each case point mutations in the Ki-*ras* protooncogene were detected (unpublished data). All these results suggest the in vivo mutagenesis induced by administration of the agent in early life.

It is worthy of note that at present no direct evidence is available of BrdUrd-induced in vivo mutations. The hypoxanthine-phosphoribosyltransferase (HPRT) system (Morley et al., 1983) could be used for detection of in vivo mutations induced by BrdUrd in peripheral blood lymphocytes. However, only a limited number of cell types can be analyzed and the detected mutations may not always reflect mutation frequencies in the tissue of origin (Featherstone et al., 1989). A recently described system including transgenic mice harboring multiple copies of a bacteriophage lambda shuttle vector, provided with the LacZ gene as a target for mutagenesis (lambda-gt10LacZ), shows great promise as an in vivo model for studying spontaneous mutation frequencies and the relation of DNA damage and repair to mutagenesis, carcinogenesis and aging in different organs and tissues (Gossen et al., 1989, 1991).

It was revealed that BrdUrd considerably increases the number of chromosomal aberrations in cells and sister-chromatid exchanges in vitro (Ray, 1986; Kondrashova, 1988) and in vivo (Wilmer and Soares, 1980; Maier et al., 1983). In our experiment neonatal exposure of rats to 4 injections of BrdUrd led to a doubling of the number of chromosomal aberrations in peripheral blood lymphocytes at the age of 3 months, which is in agreement with the above-mentioned data and also suggests the mutagenic potential of BrdUrd in vivo.

As we observed in our experiment, neonatal administration of BrdUrd to rats led to a dose-dependent reduction of their mean life span and acceleration of the aging rate (Table 2, Fig. 1). The mean life span of males and females was decreased by 31% and 14%, respectively, in rats exposed to 2 injections of BrdUrd (total dose 6.4 mg per rat) and by 38% and 27%, respectively, in rats exposed to 4 injections (12.8 mg of BrdUrd), in comparison to control rats. In general, our data are in agreement with those reported by Craddock (1981). She observed a dose-dependent shortening of the life span of neonatally BrdUrd-exposed rats, but there were only 6–15 animals in each treated group and there was no untreated control.

Long-term addition of BrdUrd to the nutrient medium also shortened the life span of *D. melanogaster* in proportion to the dose of the nucleoside analogue, and sensitized them to the life span reducing effect of irradiation in doses of 125–500 Gy (Potapenko et al., 1982). Analysis of the character of the survival curves of the population allowed the authors to suggest that DNA is a primary substrate of the observed shortening of the life span of the insects and that it is similar to natural aging. However, in this study early mortality of the animals could be induced by metabolic disturbances in the cell, due to free (non-DNA-incorporated) BrdUrd, and also, the toxic effect of bromine (BrdUrd solution decomposes by the light emitter bromine) was not taken into account. The influence of the above-mentioned factors upon the results of the study can be reduced by pulse administration of BrdUrd into the medium.

Is the life span reduction induced by BrdUrd due to its specific effect on the aging process or is it a consequence of the toxicity of the agent? Being administered to adult mice or rats, BrdUrd

unincorporated into DNA is rapidly driven out of cells and undergoes catabolism down to uracil and bromidium ion in the liver (Kriss and Reveszf, 1962). It is difficult to deny any early and late toxic effects of neonatal administration of BrdUrd in rats which could reduce their life span. Thus, neonatal exposure of rats to 4 injections of BrdUrd at a single dose of 3.2 mg per animal was followed by increased mortality from the age of 1 week to weeks 2–4 of life and mortality was decreased to the control level in 1–1.5 weeks after the last injection of BrdUrd, i.e., the period of persistence in the body of one of its degradation products, bromine (Osipova and Anisimov, 1992). Some toxic effects of BrdUrd were observed in the present experiment: delays in body weight gain and in the age of opening of the vagina and an age-related decrease in the length of the estrus cycle during the period of maturation (Fig. 3), a decrease in mean body weight during the whole period of observation (Table 1) and a decrease in the relative size of some organs in rats treated neonatally with BrdUrd could be evaluated as a consequence of the toxicity of the agent as well. A hepatotoxic effect of infused BrdUrd was registered in dogs (Wollner et al., 1987).

A delay in differentiation and development of microcystic lesions in salivary glands were observed during the first 2 weeks of life in rats neonatally treated with BrdUrd (Fukushima and Barka, 1976), as well as a delay in the postnatal development of the testes and a weakening of spermatogenesis (Barasch, 1977). The development of macro- and microcysts in the kidneys observed previously (Napalkov et al., 1989) and in the present study could be regarded as an effect of BrdUrd on the differentiation of kidney cells. In the same model long-term persistence of neonatally administered BrdUrd was shown in kidney DNA (Likhachev et al., 1983b) and, with the help of monoclonal antibodies, in the renal cortex (Ward et al., 1991). The development of mesenchymal kidney tumors in neonatally BrdUrd exposed rats (Napalkov et al., 1989; present data) expressed, as mentioned above, point mutations in the Ki-*ras* protooncogene. The fact is, some of these effects could depend on the mutagenic potential of the nucleoside analogue.

Calculation according to the Gompertz equation of the mortality parameters for both untreated and neonatally BrdUrd-treated rats surviving to the age of 2 months showed an acceleration of the aging rate under the influence of the thymidine analogue. The results of monitoring estrus function suggested the acceleration of the natural age-related switching-off of reproductive function in female rats neonatally exposed to BrdUrd. This process was gradual and included not only an earlier increase in the incidence of estrus cycle disturbances (persistent estrus and/or anestrus) in BrdUrd-treated rats in comparison to untreated ones, but also an earlier increase than in controls in the mean length of normal estrus cycles (Figs. 2 and 3). It is well known that the natural age-related switching-off of reproductive function is manifested in rats by persistent estrus, repeated pseudopregnancies and anestrus (Aschheim, 1976) caused by age-related changes in the central chains of the hypothalamo-pituitary-ovarian axis (Aschheim, 1976; Dilman and Anisimov, 1979; Finch and Landfield, 1985). The induction of persistent estrus by neonatal androgenization or by different agents and manipulations at adult age (for review see Anisimov, 1987) is accompanied by some signs of premature aging and tumor development (Anisimov, 1972, 1987). Data presented in Fig. 4 suggest that a population of female rats neonatally exposed to BrdUrd and manifesting persistent estrus and/or anestrus shows an aging rate that is higher than in the control group. The induction of persistent estrus by autotransplantation of the ovary into the tail of ovariectomized adult female rats exposed in utero (during their mother's pregnancy) to *N*-nitrosomethylurea or 7,12-dimethylbenz[*a*]anthracene produced a tumor promoting effect (Alexandrov and Anisimov, 1976; Alexandrov et al., 1989). The induction of persistent estrus by this method in adult rats neonatally exposed to 4 injections of BrdUrd increased the incidence of ovarian tumors and of malignant tumors of the uterus in comparison to rats exposed to BrdUrd or the surgery alone (Napalkov et al., 1989).

We used the COH test to discover the possible mechanism of premature switching-off of the estrus function in female rats neonatally exposed to BrdUrd. It is well known that hemiovariectomy

leads to a primary decrease in the level of estrogen in the organism followed by hypertrophy of the ovary that was left at surgery, due to an increase in secretion of follicle stimulating hormone (FSH) by the pituitary (Benson et al., 1969). Our data on the age-related decrease in the value of COH in control females (Fig. 6) are in agreement with other observations and are a result of the age-related increase in the threshold of sensitivity of the hypothalamo-pituitary system to feedback control by estrogens (Dilman and Anisimov, 1979). In female rats subjected to neonatal administration of BrdUrd and hemiovariectomy at the age of 3 months there was no COH in comparison to high values in control animals (Fig. 6). This result could extend on disturbances of function of the central chains of the hypothalamo-pituitary-ovarian axis or on resistance of the remaining ovary to stimulation by endogenous FSH. We tested the response of the ovaries of infantile females to exogenous HCG and failed to find any difference between control and neonatally exposed BrdUrd-exposed female rats. Thus, these data suggest disturbances in the central regulation of gonadotropic function of the pituitary in BrdUrd-treated animals. This conclusion is in agreement with some other data on premature aging-related changes in the length of the estrus cycle and switching-off of estrus function in BrdUrd-exposed females that were discussed above.

Assuming a fairly even level of BrdUrd incorporation into the DNA of various tissues of neonatal rats and long-term persistence in them (Margison et al., 1980; Likhachev et al., 1983b; Ward et al., 1991), those cell populations with the greatest proliferative acivity will be more likely to undergo malignant transformation. Since exposure to BrdUrd has been shown to have dramatic effects on cellular functions including cell differentiation, inactivation of regulatory genes or master switch (Kaufman, 1988; Tapscott et al., 1989) and proliferation (Weghorst et al., 1991), it may be important for any chronic influence on specific sensitive tissues and cells. In the present study as well as in earlier reports (Napalkov et al., 1989) neonatal administration of BrdUrd produced a slight increase in total tumor incidence and a shortening in tumor latency. The majority of developed tumors were spontaneous neoplasias characteristic for the rat strain used; however, tumors of the gonads, kidney, uterus and some other locations never observed in untreated LIO rats (Anisimov et al., 1989) were also discovered at autopsy of BrdUrd-treated rats. These data provide additional evidence to the conclusion (Napalkov et al., 1989) that a single perturbation of DNA contributes substantially not only to the induction of cell transformation in vitro (Barrett et al., 1978), but also to carcinogenesis in vivo.

It is worthy of note that neonatal administration of BrdUrd increases the susceptibility of animals to subsequent exposure to genotoxic and non-genotoxic carcinogens and promoters: *N*-nitrosomethylurea, total-body X-ray irradiation, urethane, estradiol-benzoate and persistent estrus syndrome induced by autotransplantation of the ovary into the tail of ovariectomized rats, with the tumors developing mainly in the target tissues for the agents administered after BrdUrd exposure (Napalkov et al., 1989; Anisimov and Osipova, 1991, 1992a,b,c). Since BrdUrd enhances the sensitivity of cells to X-rays (Dawer et al., 1971), it is used in the therapy of cancer patients (Szybalsky, 1974). These data, the results of above-mentioned experimental works and the implications of intravenous infusions of BrdUrd to study the proliferative activity of human cancers (Christov et al., 1991; Miller et al., 1991) suggest the potential health risk for patients treated with this agent.

Thus, the data presented suggest that neonatal administration of the thymidine analogue BrdUrd leads to some phenomena which could speculatively be considered as arguments in favor of the mutation theory of aging. The suggestion that genetic instability leading to improper gene regulation represents a primary mechanism common in both aging and cancer and the fact that both these phenomena may arise from a common set of genetic alterations (Ames, 1989; Cutler and Semsei, 1989), together with data on molecular events that are sufficient for carcinogenesis in senile DNA (Anisimov, 1991), could give a theoretical background for the interpretation of the results of our experiments. At the same time, there are many possible explanations, both genetic and epigenetic, for the reduction in life

span caused by treatment of newborn animals with BrdUrd (Craddock, 1981). Our and other available data cannot deny the role of toxic and some metabolic effects in shortening the life span and there is no direct evidence that BrdUrd induces mutations in different organs and tissues. As was rightly stressed by Vijg (1990), in order to test the hypothesis that DNA mutations in somatic cells are a major cause of aging, it is necessary to quantitate DNA sequence changes in the organism as a function of age, characterize the age-related organ- and tissue-specific variation in spectra of DNA sequences, and at least, correlate these spectra of DNA defects to specific aging phenomena, such as age-related diseases, including cancer. At present we face more questions than we can answer.

## Acknowledgements

The authors are grateful to Prof. N.P. Naplakov for his unflagging interest in the work, Dr. A.J. Likhachev for discussion and valuable suggestions, I. Mikhailova and E. Solomatina for technical assistance.

## References

Alexandrov, V.A., and V.N. Anisimov (1976) Peculiarities of transplacental carcinogenic effect of *N*-nitrosomethylurea in persistent-estrous rats, Vopr. Onkol., 22, 98–101.

Alexandrov, V.A., I.G. Popovich, V.N. Anisimov and N.P. Napalkov (1989) Influence of hormonal disturbances on transplacental and multigeneration carcinogenesis, in: N.P. Napalkov, J.M. Rice, L. Tomatis and H. Yamasaki (Eds.), Perinatal and Multigeneration Carcinogenesis, IARC Sci. Publ. 96, IARC, Lyon, pp. 35–49.

Ames, B.N. (1989) Endogenous DNA damage as related to cancer and aging, Mutation Res., 214, 41–46.

Anisimov, V.N. (1971) Blastomogenesis in rats with persistent estrus, Vopr. Onkol., 16, 93–95.

Anisimov, V.N. (1987) Carcinogenesis and Aging, Volumes 1&2, CRC Press, Boca Raton, FL.

Anisimov, V.N. (1989) Age-related mechanisms of susceptibility to carcinogenesis, Semin. Oncol., 19, 10–19.

Anisimov, V.N. (1991) Effects of factors prolonging life span on carcinogenesis, Ann. NY Acad. Sci., 621, 373–384.

Anisimov, V.N., and G.Yu. Osipova (1991) Carcinogenesis induced by neonatal exposure to 5-bromo-2′-deoxyuridine and subsequent administration of estradiol benzoate in BALB/c mice, Vopr. Onkol., in press.

Anisimov, V.N., and G.Yu. Osipova (1992a) Carcinogenesis induced by combined neonatal exposure to 5-bromo-2′-deoxyuridine and subsequent total-body X-ray irradiation in rats, Carcinogenesis, in press.

Anisimov, V.N., and G.Yu Osipova (1992b) Dose-response carcinogenesis induced by neonatal exposure to BrdUrd in LIO rats, Vopr. Onkol., in press.

Anisimov, V.N., and G.Yu. Osipova (1992c) Two-step carcinogenesis induced by neonatal exposure to 5-bromo-2′-deoxyuridine and subsequent administration of urethan in BALB/c mice, Cancer Lett., in press.

Anisimov, V.N., G.B. Pliss, M.G. Iogannsen, I.G. Popovich, K.P. Romanov, A.S. Monakhov and T.K. Averyanova (1989) Spontaneous tumors in outbred LIO rats, J. Exp. Clin. Cancer Res., 8, 254–262.

Aschheim, P. (1976) Aging in the hypothalamic-hypophyseal-ovarian axis in the rat, in: A.V. Everitt and J.A. Burgess (Eds.), Hypothalamus, Pituitary and Aging, C.C. Thomas, Springfield, IL, pp. 376–418.

Auerbach, C. (1976) Mutation Research. Problems, Results and Perspectives, Halsted Press, London.

Bannigan, J., and J. Langman (1978) The cellular effect of 5-bromodeoxyuridine on the mammalian embryo, in: D. Neubert (Ed.), Role of Pharmacokinetics in Prenatal and Perinatal Toxicology, George Thieme, Stuttgart, pp. 545–557.

Baranova, L.N., K.P. Romanov and V.A. Yamshanov (1986) Study of levels of benzo[*a*]pyrene and *N*-nitrosamines in the food of laboratory animals, Vopr. Onkol., 5, 54–57.

Barasch, J.M. (1977) The effect of 5-bromodeoxyuridine on the postnatal development of the rat testis, J. Exp. Zool., 200, 1–8.

Barrett, J.C., T. Tsutsui and P.O.P. Ts'o (1978) Neoplastic transformation induced by a direct perturbation of DNA, Nature (London), 274, 229–232.

Benson, B.S., S. Sorrentino and J.V. Evans (1969) Increase in serum FSH following unilateral ovariectomy in the rat, Endocrinology, 84, 369–374.

Burnet, F.M. (1974) Intrinsic Mutagenesis: A Genetic Approach to Aging, Wiley, New York, NY.

Craddock, V.M. (1981) Shortening of the life span caused by administration of 5-bromodeoxyuridine to neonatal rats, Chem.-Biol. Interact., 35, 139–144.

Christov, K., K.L. Chew, B.-M. Ljung, F.W. Waldman, L. Duarte, W.H. Goodson, H.S. Smith and B.H. Mayall (1991) Proliferation of normal breast epithelial cells as shown by in vivo labeling with bromodeoxyuridine, Am. J. Pathol., 138, 1371–1380.

Cutler, R.G., and I. Semsei (1989) Development, cancer and aging: possible common mechanisms of action and regulation, J. Gerontol., 44, 25–34.

Davidson, R.L., P. Broeker and C.R. Ashman (1988) DNA base sequence changes and sequence specificity of bromodeoxyuridine-induced mutations in mammalian cells, Proc. Natl. Acad. Sci. USA, 85, 4406–4410.

Dawey, W.C., L.E. Stone, H.H. Miller and R.E. Gilback (1971) Radiosensitization with 5-bromodeoxyuridine in Chinese hamster cells X-irradiated during different phases of the cell cycle, Radiat. Res., 47, 624–688.

Dilman, V.M., and V.N. Anisimov (1979) Hypothalamic

mechanisms of aging and specific age pathology. I. Sensitivity threshold of hypothalamo-pituitary complex to homeostatic stimuli in the reproductive system, Exp. Gerontol., 14, 161–174.

Di Paolo, S.A. (1964) Polydactylism in the offspring of mice injected with 5-bromodeoxyuridine, Science, 145, 501–503.

Dubina, T.L., V.A. Dyudnikova and E.V. Zhuk (1983) Biological age and its estimation. II. Assessment of biological age of albino rats by multiple regression analysis, Exp. Gerontol., 18, 5–18.

Featherstone, T., P.D. Marshall and E.J. Evans (1987) Problems and pitfalls in assessing human T-lymphocyte mutant frequencies, Mutation Res., 179, 215–230.

Finch, C.E., and P.W. Landfield (1985) Neuroendocrine and autonomic functions in aging mammals, in: C.E. Finch and E.L. Schneider (Eds.), Handbook of the Biology of Aging, 2nd edn., Van Nostrand Reinhold, New York, NY, pp. 645–691.

Fishbein, L., W.G. Flamm and H.L. Falk (1970) Mode of action and types of mutations induced by chemicals, in: L. Fishbein, W.L. Manus and H.L. Falk (Eds.), Chemical Mutagens, Academic Press, New York, NY, pp. 13–17.

Freese, E. (1963) Molecular mechanisms of mutation, in: J.H. Taylor (Ed.), Molecular Genetics, Part I, Academic Press, New York, NY, pp. 207–269.

Fukushima, M., and T. Barka (1976) The effect of 5-bromodeoxyuridine and isoproterenol on the postnatal differentiation of rat submandibular gland, Am. J. Anat., 147, 159–182.

Gart, J.J., D. Krewsky, P.N. Lee, R.E. Tarone and J. Wahrendorf (1986) Statistical Methods in Cancer Research. Vol. III: The Design and Analysis of Long-Term Animal Experiments, IARC Sci. Publ. 79, IARC, Lyon.

Gavrilov, L.A., and N.S. Gavrilova (1991) The Biology of Life Span: A Quantitative Approach, Harwood, New York, NY.

Gossen, J.A., W.J.F. de Leeuw, C.H.T. Tan, E.C. Zwarthoff, F. Berends, P.H.M. Lohman, D.L. Knook and J. Vijg (1989) Efficient rescue of integrated shuttle vectors from transgenic mice: a model for studying mutations in vivo, Proc. Natl. Acad. Sci. USA, 86, 7971–7975.

Gossen, J.A., W.J.F. de Leeuw, A. Verwest, P.H.M. Lohman and J. Vijg (1991) High somatic mutation frequencies in a LacZ transgene integrated on the mouse X-chromosome, Mutation Res., 250, 423–429.

Goubler, E.V. (1978) Calculating Methods of Analysis and Identification of Pathological Processes, Meditsina, Moscow.

Harman, D. (1991) The aging process: major risk factor for disease and death, Proc. Natl. Acad. Sci. USA, 88, 5360–5363.

Hochschild, R. (1989) Improving the precision of biological age determinations. Part I. A new approach to calculating age, Exp. Gerontol., 24, 289–300.

Islam, M.Q., and G. Levan (1987) A new fixation procedure for improved quality G-bands in routine cytogenetic work, Hereditas, 107, 127–130.

Kaufman, E.R. (1988) The role of deoxyribonucleotide metabolism in 5-bromo-2'-deoxyuridine mutagenesis in mammalian cells, Mutation Res., 200, 149–155.

Kirk, D.L., G.J. Baran, J.F. Harper, R.J. Huskey, K.S. Huson and N. Zagris (1987) Stage-specific hypermutability of the *reg* A locus of *Volvox*, a gene regulating the germ-soma dichotomy, Cell, 48, 11–24.

Kirkwood, T.B.L. (1989) DNA, mutation and aging, Mutation Res., 219, 1–7.

Kondrashova, T.V. (1988) The role of 5-bromodeoxyuridine in induction of SCE by X-irradiation of human lymphocytes during presynthetic stage of mitotic cycle, Radiobiology, 28, 125–127.

Kriss, J., and L. Reveszf (1962) The distribution and fate of bromodeoxyuridine and bromodeoxycytidine in the mouse and rat, Cancer Res., 22, 254–265.

Lewis, J.G., and J.A. Swenberg (1982) The effect of 1,2-dimethylhydrazine and diethylnitrosamine on cell proliferation and unscheduled DNA synthesis in target and nontarget cell populations in rat liver following chronic administration, Cancer Res., 42, 89–92.

Lieber, M.M., and G.J. Todaro (1973) Spontaneous and induced production of endogenous type-C virus from a clonal line of spontaneously transformed BALB/3T3, Int. J. Cancer, 11, 616–627.

Likhachev, A.J., H. Oshima, V.N. Anisimov, A.I. Ovsyannikov, S.Y. Revskoy, L.K. Keefer and E.J. Reist (1983a) Carcinogenesis and aging. II. Effect of aging on interaction of methyl(acetoxymethyl)nitrosamine with DNA in rats, Carcinogenesis, 8, 967–973.

Likhachev, A.J., L. Tomatis and G.P. Margison (1983b) Incorporation and persistence of 5-bromodeoxyuridine in newborn rat tissue DNA, Chem.-Biol. Interact., 46, 31–38.

Lindahl, T. (1982) DNA repair enzymes, Annu. Rev. Biochem., 51, 61–87.

Lutz, W.K. (1990) Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis, Mutation Res., 238, 287–295.

Maier, P., B. Weibel and G. Zbinden (1983) The mutagenic activity of 5-bromo-2'-deoxyuridine (BrdUrd) in vivo in rats, Environ. Mutagen., 5, 695–703.

Margison, G.P., A.J. Likhachev and L. Tomatis (1980) Incorporation of 5-bromodeoxyuridine into DNA in newborn tissues, Chem.-Biol. Interact., 30, 297–303.

Miller, M.A., C.M. Mazewski, N. Yousuf, Y. Sheikh, L.M. White, G.A. Yanik, D.M. Yams, B.C. Lumpkin and A. Raza (1991) Simultaneous immunohistochemical detection of INdR and BrdU infused intravenously to cancer patients, J. Histochem. Cytochem., 39, 407–414.

Monkahov, A.S. (1988) Cytogenetic and blastomogenic effects of calcium-45 and iodine-131 administered to rats, Exp. Onkol., 4, 27–30.

Morley, A.A., K.J. Trainor, R.S. Seshadri and R.G. Rayal (1983) Measurement of in vivo mutations in human lymphocytes, Nature (London), 302, 155–156.

Morris, S.M., L.J. McGarrity, O.E. Domon, W.G. Hinson and P.L. Kodell (1989) Flow cytometric analysis of bromo-

deoxyuridine-induced inhibition of cell proliferation in the human teratocarcinoma-derived cell line, P3, Environ. Mol. Mutagen., 14, 107–112.

Napalkov, N.P., V.N. Anisimov, A.J. Likhachev and L. Tomatis (1989) 5-Bromodeoxyuridine-induced carcinogenesis and its modification by persistent estrus syndrome, unilateral nephrectomy, and X-irradiation in rats, Cancer Res., 49, 318–323.

Osipova, G.Yu., and V.N. Anisimov (1991) Carcinogenesis induced by neonatal administration of 5-bromo-2′-deoxyuridine and subsequent exposure to *N*-nitrosomethylurea in rats, Vopr. Onkol., 37, in press.

Osipova, G.Yu., and V.N. Anisimov (1992) Phenotype manifestation of isolated DNA damage induced by neonatal administration of 5-bromo-2′-deoxyuridine in rats and their offspring, Teratogen. Carcinogen. Mutagen., in press.

Pegg, A.E. (1990) Mammalian $O^6$-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents, Cancer Res., 50, 6119–6129.

Perry, P., and H.J. Evans (1975) Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange, Nature (London), 258, 121–125.

Potapenko, A.I., A.D. Akifiev and V.I. Ivanov (1982) Radiation-induced shortening of life span of *D. melanogaster*. Report 2. Sensibilizing effect of 5-bromo-2′-deoxyuridine, Radiobiology, 22, 318–322.

Ray, M. (1986) Distribution of sister chromatid exchanges in chromosomes of normal Chinese hamster and its cell lines exposed to BrdUrd and MMC, Cytobios, 45, 77–84.

Ruffolo, P.R., and V.H. Ferm (1965) The embryonal and teratogenic effects of 5-bromodeoxyuridine in the pregnant hamster, Lab. Invest., 14, 1547–1553.

Sacher, G.A. (1980) Biomedical correlates of aging, in: S.G. Haynes and M. Feinleib (Eds.), Epidemiology of Aging, NIH Publ. 80-969, US Government Printing Office, Washington, DC, pp. 51–55.

Singer, B., and D. Grunberger (1983) Molecular Biology of Mutagens and Carcinogens, Plenum, New York, NY.

Stockdale, F., K. Okasaki, M. Nameroff and M. Holzer (1964) 5-Bromodeoxyuridine: effect on myogenesis in vitro, Science, 146, 533–535.

Strehler, B.L. (1986) Genetic instability as the primary cause of human aging, Exp. Gerontol., 21, 283–319.

Szybalsky, W. (1974) X-ray sensitization by halopyrimidines, Cancer Chemother. Rep., 58, 539–557.

Tapscott, S.J., A.B. Lassar, R.L. Davis and H. Weintraub (1989) 5-Bromo-2′-deoxyuridine blocks myogenesis by extinguishing expression of MyoD1, Science, 245, 532–534.

Trautner, T.A., M.N. Swartz and A. Kornberg (1962) Enzymatic synthesis of deoxyribonucleic acid. X. Influence of bromouracil substitution on replication, Proc. Natl. Acad. Sci. USA, 48, 449–455.

Turusov, V.S., and U. Mohr (Eds.) (1990) Pathology of Tumours in Laboratory Animals. Volume I – Tumours of the Rat, 2nd edn., IARC Sci. Publ. 99, IARC, Lyon.

Varshaver, N.B., M.I. Marshak, L.V. Gorbunova, L.L. Lukash and N.I. Sapiro (1980) The synergistic effect of SV40 and BUdR on induction of gene mutations and chromosomal aberrations in Chinese hamster cells, Mutation Res., 70, 351–364.

Vijg, J. (1990) DNA sequence changes in aging: how frequent, how important?, Aging, 2, 105–123.

Ward, J.M., J.R. Henneman, G.Y. Osipova and V.N. Anisimov (1991) Persistence of 5-bromo-2′-deoxyuridine in tissues of rats after exposure in early life, Toxicology, 70, 345–352.

Weghorst, C.M., J.R. Henneman and J.M. Ward (1991) Dose response of hepatic and DNA synthesis rates to continuous exposure of bromodeoxyuridine (BrdU) via slow-release pellets or osmotic minipumps in male B6C3F1 mice, J. Histochem. Cytochem., 39, 177–182.

Wilmer, J.L., and E.R. Soares (1980) Sister chromatid exchange in vivo in mice: 1. The influence of increasing doses of bromodeoxyuridine, Environ. Mutagen., 2, 35–42.

Wollner, I.S., C.A. Knutsen, K.A. Ulrich, C.E. Chrisp, S.E. June, J.C. Andrews, M.J. Tuscan, P.L. Stetson and W.D. Ensminger (1987) Effect of hepatic arterial yttrium-90 microsphere administration alone and combined with regional bromodeoxyuridine infusion in dogs, Cancer Res., 47, 3285–3290.