EXPERIMENTAL RESULTS

The results in Table 1 show that 15 min after injection of LCCS into the animals MI rose. After 30 min a distinct fall of MI was observed, which was most marked after 45 min; after 60 min inhibition of entry of the thymocytes into mitosis still continued, but was weaker than at the previous time of the experiment (Table 1). After 90 min MI of the thymocytes of animals receiving LCCS returned to the control level (Table 1).

The duration of the G_2-phase of the mitotic cycle of mouse thymocytes is known to be about 45 min [2, 5]. On the basis of the fact that delay of entry of cells into mitosis under the influence of LCCS develops over a period of 30-60 min and is reversible in character, it can be concluded that the preparation used gives a G_2-chalone effect, expressed as a specific action on the passage of thymocytes from the G_2-phase into mitosis.

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AGE CHANGES IN IMMUNE CYTOSIS OF MOUSE CELLS WITH STREPTOLYSIN-O-INDUCED CYTOGENETIC DISTURBANCES

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KEY WORDS: streptolysin-O; mutagenesis; immune cytolysis

With age the number of cytogenetically changed cells in man and animals increases [6, 10], as also does the sensitivity of the cells to the action of mutagens [7, 9]. It was shown previously [3] that in connection with these age changes there is a very close relationship between the state of the T system of immunity and the level of cytogenetic disturbances in patients with influenza and in mice immunized with measles vaccine. It has been suggested that since the immune system exercises control over genetic homeostasis in vivo [2], when the function of the immune system declines with age, accumulation of cytogenetically changed cells must evidently take place on account of both spontaneous and induced mutagenesis. Meanwhile it is logical to suppose also that the observed effects may be due to weakening of immune antiviral defense during aging, and this may lead to the accumulation of virus-induced cytogenetically changed cells in the body.

The aim of this investigation was to answer the question whether age differences exist in the control of genetic homeostasis during infectious mutagenesis. Experiments were carried out on the basis of a method of studying cytolytic activity of lymphocytes relative to cytogenetically changed cells in culture, developed previously [4].

EXPERIMENTAL METHOD

Streptolysin O, a toxin of *Streptococcus haemolyticus* [5], was used as the mutagenic factor. Pure-line BALB/c mice, closely inbred for five generations, were used. The mice were aged 1-10, 180-190, and 360-380 days. After decapitation of the mice the kidneys were removed and cell cultures prepared from them by the usual method [1]. The cultures were "infected" with streptolysin-O in a dose of 0.1 ml of the standard dilution to 1 ml of culture medium. A standard solution of the preparation was used. After contact with the culture for 8 h the streptolysin-O was washed off with medium 199, and 24 h later splenic

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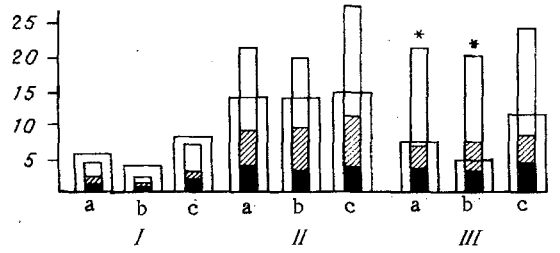

Fig. 1

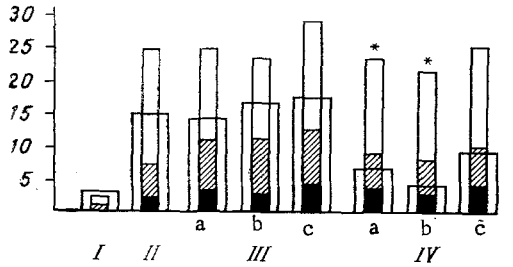

Fig. 2

Fig. 1. Number (in %) of cells with cytogenetic disturbances in cultures of kidney cells, infected with streptolysin-0, and obtained from newborn (a), middle-aged (b), and old (c) mice, before and after injection of splenic lymphocytes from syngeneic animals. I) Intact cultures; II) cultures after injection of streptolysin-0; III) cultures after addition of streptolysin-0 and syngenic lymphocytes. Wide columns denote cells with altered number of chromosomes, narrow columns — cells with structural chromosomal aberrations (black part indicates exchanges, obliquely shaded — chromosomal breaks, unshaded — chromatic breaks). Asterisk indicates significant difference ($p \leq 0.05$) compared with experimental control (II).

Fig. 2. Number (in %) of cells with cytogenetic disturbances in cultures, infected with streptolysin-0, of kidney cells obtained from newborn mice after addition of splenic lymphocytes from syngeneic and nonsyngeneic animals. I and II) The same as in Fig. 1; III) culture after addition of streptolysin-0 and nonsyngeneic lymphocytes; IV) the same, and syngeneic lymphocytes. Lymphocyte obtained from newborn (a), middle-aged (b), and old (c) mice. Remainder of legend as to Fig. 1.

lymphocytes obtained from syngeneic and nonsyngeneic mice of different ages, were added to the culture. The recommendations of Novikov and Novikova [8] were followed during isolation and purification of the lymphocytes. The number of cells with chromosomal aberrations and the total number of cells in a monolayer of the culture flask were analyzed 12 h after introduction of the lymphocytes into the cultures. The experiment and control were repeated five times and in each case 100 metaphases were analyzed. The data were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

A significantly higher level of cytogenetic disturbances was observed in cultures of kidney cells obtained from old mice than in corresponding cultures obtained from newborn and, in particular, middle-aged mice (Fig. 1). Addition of streptolysin-0 in all cases induced a rise of the level of cytogenetic disturbances in the cultures. The number of cells with structural chromosomal aberrations in cultures obtained from the kidneys of newborn mice was increased from 4.8 ± 0.6% (intact control) to 23.6 ± 1.7%, in cultures from kidneys of old mice from 7.8 ± 0.9 to 28.3 ± 1.1%. The corresponding changes in the number of cells with changes in the number of chromosomes were: 6.1 ± 0.7 and 14.3 ± 1.1, 4.4 ± 1.0 and 14.5 ± 0.8, and 8.9 ± 1.2 and 15.3 ± 1.4%, respectively (in all cases $p < 0.01$). Addition of splenic lymphocytes obtained from syngeneic mice of the same age to cultures "infected" with streptolysin-0 did not affect the number of cells with structurally disturbed chromosomes but significantly reduced the number of aneuploid cells in cultures obtained from newborn and middle-aded mice (by 52 and 85%; in both cases $p < 0.01$). In cultures obtained from old mice the number of aneuploid cells was reduced but not significantly. In cases of an antimutagenic effect of the lymphocytes, the density of the monolayer was reduced and cell debris appeared in the culture medium.

In the other series of experiments splenic lymphocytes from nonsyngeneic (noninbred) mice were added to cultures of kidney cells obtained from newborn mice, after "infection" with streptolysin-0. No decrease was found in the number of cells with cytogenetic disturbances. Addition of syngeneic lymphocytes from old animals reduced the number of cells with cytogenetic disturbances by 10-15% ($p > 0.05$) and from middle-aged mice by 82-94% ($p < 0.01$; Fig. 2).

The results are thus evidence that splenic lymphocytes from mice of different ages give different cytolytic effects against cells cytogenetically modified by streptolysin-O. A weaker cytolytic effect was exhibited by lymphocytes of old animals, and in our opinion this can be attributed to the long preservation of a high level of cells with cytogenetic disturbances induced in man and mice by infectious factors [3, 5]. At the same time, our results show that even in cell cultures obtained from mice of different ages, the spontaneous and induced levels of cytogenetically aberrant cells differ, evidently due to ontogenetic differences in the control of genetic homeostasis at the subcellular level and, in particular, differences perhaps in activity of the DNA-repair systems.

Splenic lymphocytes, it will be noted, eliminate only aneuploid cells and do not change the number of cells with structural chromosomal disturbances. Structural chromosomal disturbances evidently do not change the antigenic structure of the cell membrane and these cells cannot be "recognized" and eliminated by effector cells.

Thus, a considerable increase in the number of cytogenetic aberrations is observed in cultures of kidney cells, obtained from mice of different ages, and "infected" with streptolysin-O. On the addition of syngeneic splenic lymphocytes from newborn and middle-aged animals to these cultures, the number of aneuploid cells present in them is significantly reduced. Splenic lymphocytes of old mice under similar conditions did not cause a significant decrease in the number of cells with cytogenetic disturbances. No antimutagenic cytolytic activity likewise was observed in lymphocytes obtained from nonsyngeneic mice.

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COMPARATIVE ABILITY OF EARLY AND LATE CFU-S TO RECOVER AFTER SUBLETHAL RADIATION DAMAGE

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High ability to repair after sublethal radiation damage is a property of splenic colony-forming units (CFU-S). This property is possessed by CFU-S forming colonies both in an endogenous test and on transplantation of bone marrow into an irradiated recipient [10]. We know that CFU-S constitute a heterogeneous class of cells, which produce early ($CFU\text{-}S_{7\ days}$) and late ($CFU\text{-}S_{12\ days}$) colonies in the spleen of irradiated recipients [8, 9]. These two subpopulations of CFU-S differ not only in the time of formation of macroscopically visible colonies, but also with respect to certain other characteristics: their self-maintaining ability [3-7, 11], their response to cycle-specific agents [4-7], position in the cell cy-

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