AGE DIFFERENCES IN DNA REPLICATION IN THE INTACT ALBINO RAT LIVER

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One cause of the slowing of cell growth and renewal observed during aging [2, 7] may be a change in the temporal parameters of replication of intracellular DNA. Age changes in DNA structure, changes in the activity and other properties of DNA-polymerases, and a decrease in the incorporation of labeled precursor into DNA confirm this suggestion [2]. It was shown previously on a model of the regenerating liver that the rate of DNA replication is reduced in rats during aging, in connection with slowing of the conversion of single-stranded intermediates into the double-helical structure in old animals [3].

It was decided to investigate this process in the intact liver of adult and old animals in order to ascertain whether the temporal parameters of DNA replication change during aging or whether these changes are induced during stimulation of cell proliferation.

EXPERIMENTAL METHOD

Noninbred female albino rats aged 6 months (170-250 g) and 24 months (300-400 g) were used. [^{3}H]-Thymidine (from Izotop, USSR, specific radioactivity 11-19 Ci/mmole, concentration 1 mCi/ml) was injected into the jugular veins 10, 30, 70, and 120 min before decapitation in a dose of 4 μCi/g body weight. Allowing for the diurnal rhythm of mitosis, the animals were killed in the morning. The liver was frozen in liquid nitrogen. Nucleic acids were isolated in medium containing 6% p-aminosalicylic acid, 2% sodium dodecyl sulfate, and 1 mM EDTA, with a lysis temperature of 60°C, and the sample was deproteinized with a mixture of 88% phenol, 12% m-cresol, and 0.01% hydroxyquinoline [3, 4]. According to the published data [4], this method leaves the DNA fragments and its secondary structure intact. The aqueous phase was subjected to continuous dialysis against 10-15 liters of 0.01 M Na-phosphate buffer to an optical density E_{270} of the dialysate of 0.05, ensuring freedom from contamination with phenol. To investigate the secondary structure of the DNA formed during the presence of the radioactive label in the animal, the sample ($E_{260} = 200$) was applied to a 3 × 20 cm column with hydroxyapatite in 0.001 M K-phosphate buffer at $E_{260} = 1$. Elution was carried out with a linear gradient of 0.05-0.35 M K-phosphate buffer in a volume of 400 ml. Fractions, each measuring 6 ml, were sedimented in the presence of RNA-carrier (the total quantity of nucleic acids did not exceed 120 μg per sample) on Synpore (Czechoslovakia) filters with a pore diameter of 1.5-2.5 μ. Radioactivity was determined on an SL-30 counter (France). The content of intracellular radioactive label (acid-soluble) in the liver perfused with physiological saline was determined by precipitation of 1 ml homogenate with 1 ml 1 N perchloric acid, followed by counting the radioactivity in 0.1 ml of supernatant in 5 ml of dioxan scintillator.

The results were assessed as the ratio between the radioactivity of the first peak (molarity of elution 0.10-0.16 M K-phosphate buffer) corresponding to single-stranded DNA [5] to the total radioactivity of the first and second peaks (molarity of elution 0.22-0.24 M), as specific radioactivity of double-stranded DNA, as specific radioactivity of total DNA (the radioactivity of total DNA per unit of double-stranded DNA), and as radioactivity of single-stranded DNA per unit of double-stranded DNA (the relative radioactivity of single-stranded DNA). The fraction of acid-insoluble radioactive material not bound with hydroxyapatite (in % of the total acid-insoluble radioactivity) and its specific radioactivity, and fractional incorporation of total DNA (the radioactivity of DNA as a percentage of the total radioactivity of the part of the organ from which this DNA was isolated), were also determined [6]. Statistical analysis was carried out by nonparametric methods using Wilcoxon's criterion [1].

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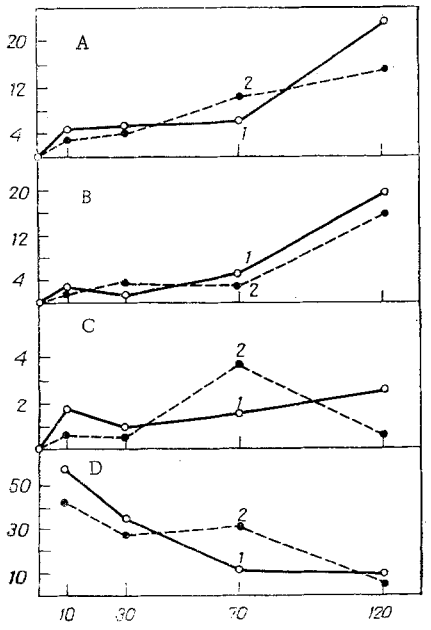

Fig. 1

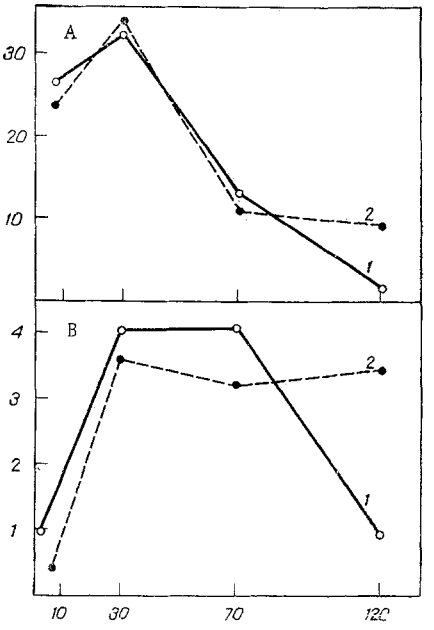

Fig. 2

Fig. 1. Specific radioactivity of total DNA (A) and of double-helical DNA (B), relative radioactivity of single-stranded DNA (C), and ratio of radioactivity of single-stranded labeled DNA to radioactivity of total DNA (D) in liver of 6-month-old (1) and 24-month-old (2) rats. Abscissa, duration of labeling (in min); ordinate: A) specific radioactivity of total DNA (in cpm/mg double-stranded DNA $\cdot 10^{-3}$), B) specific radioactivity of double-helical DNA (in cpm/mg double-helical DNA $\cdot 10^{-3}$), C) activity of single-helical DNA fraction (in cpm/mg double-helical fraction of DNA $\cdot 10^{-3}$), D) ratio (in %) of radioactivity of single-helical labeled DNA to combined radioactivities of single- and double-helical DNA.

Fig. 2. Ratio of radioactivities of labeled radioactive material not bound with hydroxyapatite and total acid-insoluble fraction (A), and specific radioactivity of material not bound with hydroxyapatite (B) in livers of 6-month-old (1) and 24-month-old (2) rats. Abscissa, duration of labeling (in min); ordinate: A) ratio (in %) of radioactivity of material not bound with hydroxyapatite to total acid-insoluble radioactivity, b) specific radioactivity of material not bound with hydroxyapatite (in cpm/mg DNA/ml $\cdot 10^{-2}$).

EXPERIMENTAL RESULTS

Analysis of incorporation of [^{3}H]thymidine into DNA (Fig. 1A, B, C) shows that significant age differences were found only for incorporation of the label into single-stranded newly synthesized DNA (Fig. 1C). In the early stages (10-30 min after injection of labeled thymidine) the process of incorporation of label into single-stranded DNA took place more intensively in animals aged 6 months than in the older animals (after 10 min, 53.5 and 39.9%, respectively, more single-stranded DNA was found in the 6-month-old than in the 24-month-old rats; Fig. 1D). The relative radioactivity of single-stranded DNA at this time also was significantly higher in the 6-month-old animals (Fig. 1C). Incorporation of the label into single-stranded DNA did not reach a maximum in the old animals until the 70th minute, i.e., the process of labeling single-stranded newly synthesized DNA took place less intensively with time in the 24-month-old rats (Fig. 1C, D). Delay of labeling of single-stranded DNA in old animals also is revealed by the fact that their relative radioactivity of single-stranded DNA rose significantly from the 10th to the 70th minutes after injection of [^{3}H]thymidine; whereas in adults, on the other hand, there was a tendency for it to decrease at this time (Fig. 1C).

Short oligonucleotide fragments of labeled DNA not bound with hydroxyapatite, and, like single-stranded DNA of the first peak, consisting of a replicative intermediate product [8-10], were incorporated into double-stranded DNA of the adult and old animals with approximately the same intensity until the 70th minute after injection of the label, although until the 120th minute after injection of [^{3}H]thymidine they were significantly fewer in number in the adults than in the old animals (Fig. 2A), on account of a significant decrease in the specific radioactivity of this material in the 6-month-old animals in the interval between the 70th and 120th minutes

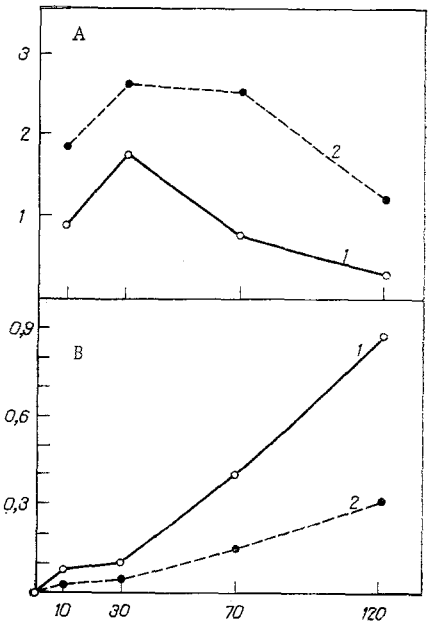

Fig. 3. Level of intracellular acid-soluble radioactivity (A) and fractional incorporation of DNA (B) in liver of 6-month-old (1) and 24-month-old (2) rats. Abscissa, duration of labeling (in min); ordinate: A) acid-soluble fraction (in cpm/mg tissue $\cdot 10^{-6}$), B) fractional incorporation (radioactivity of DNA in % of total radioactivity of that part of the organ from which this DNA was isolated).

(Fig. 2B). This effect can evidently be explained on the grounds that significantly less of the low-molecular-weight acid-soluble precursors is found at these times in adult animals (Fig. 3A) than in old animals, and for that reason the possibility cannot be ruled out that by the 120th minute the reserves of precursors for synthesis of this material in 6-month-old rats are deficient. Other possible causes may include the higher rate of incorporation of precursors into high-molecular-weight DNA or their more rapid degradation in adult animals.

Incorporation of label into total and double-stranded DNA was essentially identical (Fig. 1A, B) in the animals of both age groups (no signicant age differences were found at any of the times studied).

Conversion of single-stranded labeled DNA into double-stranded DNA thus took place more intensively in adult animals on account of the slowing of conversion of single-stranded into double-stranded DNA in the old animals. Since double-stranded DNA accounts for the main bulk of the DNA, as a result of the very small contribution of single-stranded DNA the age differences are not reflected in the labeling curves of double-stranded and total DNA. Comparison of the kinetics of incorporation of label into fractions of single- and double-stranded DNA in the intact and regenerating liver [3] of the adult and old rats shows that stimulation of proliferation affects the degree of manifestation of age changes in DNA replication in a specific manner, namely by increasing it. In fact, significant age differences were found in the kinetics of labeling of both single-stranded DNA and newly synthesized double-stranded DNA in the regenerating liver, whereas in intact tissue age changes were found only in the character of incorporation of label into single-stranded DNA.

On the whole, in intact tissue also DNA synthesis took place more intensively in the adults than in the old animals, for the efficiency of incorporation of label into their DNA was significantly higher at all times of the investigation (Fig. 3B), on account of a higher level of intracellular acid-soluble radioactivity in the old animals (Fig. 3A).

Conversion of single-stranded newly synthesized DNA into double-stranded DNA is thus slowed in the intact liver of old rats and the efficiency of DNA labeling (fractional incorporation of DNA) is also lower in old animals. However, the change in conversion of single-stranded newly synthesized DNA into double-stranded is more marked in the regenerating liver of old rats than in the intact liver. This is evidence that stimulation of cell proliferation is responsible for the greater intensity of the age changes in DNA replication.

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CHRONOBIOLOGICAL STUDIES OF THE INTESTINAL EPITHELIUM IN THE ACUTE PHASE OF OPISTHORCHIASIS

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Opisthorchiasis causes lesions of varied severity in organs of the gastrointestinal tract [3, 8-11]. In the acute phase of opisthorchiasis, a gastroenterocolitic type of course of the disease is distinguished by clinicians [1, 5, 10].

The involvement of the gastrointestinal tract as a functional system in the pathological process in opisthorchiasis may be associated with changes in the kinetics of the epithelium and with disturbances of regeneration and adaptation in it. Structural disturbances in the organs are preceded by deviations at the time level [2]. Hence, the need for a study of the diurnal rhythm of mitosis in the gastric and intestinal epithelium as an indicator of cell renewal and of the regenerative capacity of the tissues.

The object of this investigation was to analyze the diurnal rhythm of mitotic activity of the intestine of the jejunum, ileum, and cecum in the acute phase of experimental opisthorchiasis.

EXPERIMENTAL METHOD

Experiments were carried out on 64 sexually mature golden hamsters (32 experimental and 32 control) weighing 88.35 ± 2.16 and 85.55 ± 2.01 g respectively. The acute phase of opisthorchiasis was reproduced by introducing 50 viable metacercariae, isolated from a freshly killed ide, into the pharnyx of the animals. The animals were decapitated after open ether anesthesia on the 30th day after infection at 9 a.m., 3 and 9 p.m., and 3 a.m. Pieces of jejunum, ileum, and cecum were fixed in 10% neutral formalin and embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin and eosin, by Van Gieson's method, and by the PAS reaction. The number of mitoses was counted in 1000 cells in the region of the intestinal crypts – the zones where cambial cells which are the sources of regeneration are situated [6, 7, 12]. The characteristics of mitotic activity studied included the following: mitotic index (MI), mean diurnal value of MI ($M \pm m$), the coefficient of diurnal rhythm (CDR) as described by Krotov and Lugovoi [4], the index of diurnal adaptation (IDA), and the coefficient of functional synchronization (CFS) [2].

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