EFFECT OF AGE ON THE INCORPORATION OF C^{14} THYMIDINE INTO DNA, CATALYSED BY SOLUBLE EXTRACT OF RAT LIVER. *

M. A. Mukundan **, Anima Devi and N.K. Sarkar,

Department of Biochemistry, Faculty of Medicine , Laval University, Quebec, Canada.

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While studying the effect of age from infancy to senility on variations of DNA and RNA concentrations (1) and nuclease activity (2) in rat liver, the desirability of investigating changes in the ability of rat liver to synthesise DNA with age was realised and undertaken. The immediate aim was elucidation of the factors that regulate levels of polymerase activity. Rats ranging from 3-4 weeks to 14-16 months old were used for this purpose. The animals were sacrificed by decapitation, the livers were immediately removed and chilled in ice, and a 20% homogenate was prepared in a Dounce homogenizer at $0^{o}C$ in a medium containing 0.35 M sucrose, 0.004 M $MgCl_2$ and 0.035 M $KHCO_3$. High speed supernatant fractions to be used as sources of polymerase activity were prepared by centrifuging the homogenates at 105,000 x g (Spinco, Model L) for 2 hours.

The incorporation of 2-C^{14} thymidine (2-C^{14} TMP was also used in some experiments) into primer DNA was studied in a system involving the use of high speed supernatant fractions prepared from the livers of young and old rats which contained Mg ions, triphosphates of the four deoxyribonucleosides (dGTP, dCTP, dTTP, dATP) with C^{14} thymidine

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**A graduate student in the Department of Biochemistry of this Faculty.

TABLE I

DNA-polymerase activity of rat liver supernatant fractions with two-stranded and heat-denatured DNA as primers

Age of rat	Two-stranded DNA	Heat denatured DNA	Two-stranded DNA	Heat denat DNA
	Total count/min./ml.		Ca^{++} (added)	
3 weeks old (A)	2108	684	2955	997
11-12 months old (B)	750	1756	1097	2610
mixtures of supernatants of the livers of rats A and B	calculated 1429 observed 832	dalculated 1220 observed 1640		

Conditions of the experiment: The incubation mixture in a total volume of 0.4 ml contained 5 m μ moles each of dGTP, dATP, TTP and 2 C14-thymidine (10,000 c.p.m.), 2 mM of mercaptoethanol, magnesium acetate 8.0 μ moles, Tris-acetate 75 μ moles (pH 8.5), 350 μ g of calf-thymus DNA, Ca^{++} 25 μ moles, 2.5 mg of enzyme protein and 0.1 ml of water. Incubation was at 37°C for 1 hour and the DNA-polymerase assay was carried out by the disk procedure (7).

or C^{14} TMP, and a primer DNA. In some experiments Ca^{++} as well as Mg^{++} was added. The conditions of these experiments are given at the bottom of Table I. The results are summarized in Table I and Figs. 1 and 2.

In Table I and Fig.1 is shown the effect of age on the ability of rat liver to synthesise DNA when two-stranded DNA and heat denatured DNA are used as primers. It appears from Table I that the ability of the high speed supernatant fractions of the livers of young rats to incorporate C^{14} into two-stranded primer DNA is considerably higher than that of old rats. Furthermore the ability to synthesise DNA

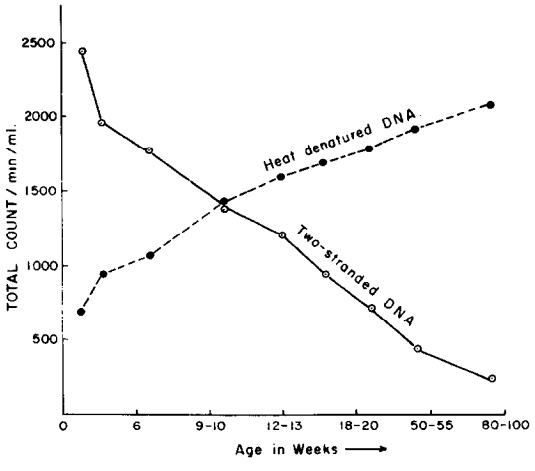

FIG. 1. EFFECT OF AGE ON DNA POLYMERASE ACTIVITY IN RAT LIVER.

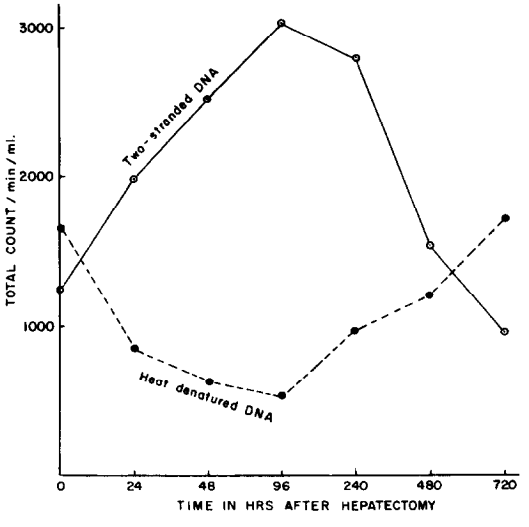

FIG. 2. DNA-POLYMERASE ACTIVITY IN REGENERATING RAT LIVER AT DIFFERENT INTERVALS AFTER HEPATECTOMY.

Figs. 1 and 2:

Conditions of the experiment are the same as described in Table I.

from two-stranded primer as can be seen from Fig.1 decreases from birth through maturity, while the ability to use heat-denatured DNA as a primer, although very low in young rats, increases steadily with age to the point where extracts of old rat livers can use heat- denatured DNA as the primer to the extent of 70-75%, compared to the priming ability of two-stranded DNA, when the high speed supernatant fraction of young rat liver (3-5 weeks old) is used as enzyme source. It is also of some interest to note that using the livers of rats of 4-6 months old, the incorporation of thymidine into primer DNA is the same whether or not the primer DNA is two-stranded or heat-denatured DNA, so that two-stranded DNA and heat-denatured DNA can both act equally well as primers using rats of this age.

In contrast to this, the ability of the high speed supernatant fraction of regenerating rat liver to use two-stranded DNA as primer increases at first steadily after hepatectomy, reaching a maximal level on the 4th day after operation (96 hrs), and then declines steadily until it finally returns to the original level of thymidine incorporation between the 26th and 30th day after operation. The ability to use heat-denatured DNA as a primer on the other hand decreases at first after hepatectomy, then shows a steady increase, and finally returns to the original level of activity on the 20th to 30th day.

The results from Table I also show that if a mixture of equal volumes of the supernatant fraction of young and old rat livers serves as the source of polymerase, quite unexpected results are obtained. When two-stranded DNA is used as a primer, a 40-50% drop in the incorporation of C^{14} into the primer DNA is observed; but if heat-denatured DNA is used as a primer, a 40-50% increase in the incorporation of C^{14} into primer DNA is noted.

The activity of the polymerase depends on Mg ions, and among the metals tested (Ca^{++}, Mn^{++} and Co^{++}) none can replace Mg^{++} to any

appreciable extent. The activity obtained when any one of these metals is used alone may be due to the presence of traces of Mg^{++} in the high speed supernatant fraction. Of the three metals in question, only Ca^{++} has appreciable ability to enhance the incorporation of C^{14} into primer DNA, if added together with Mg^{++}. Addition of EDTA inhibits the polymerase, depending on the concentration used. It should also be noted that the effects of metals are independent of the nature of the primer used.

DISCUSSION

A reduced ability of supernatant liquid obtained by centrifuging the homogenate of liver from normal rats of unspecified age to synthesise DNA has previously been noted by Davidson et al. (3); these authors have also noticed that addition of this supernatant to the high-speed supernatant fraction of regenerating rat liver, inhibited the polymerase activity of the latter. Based on these observations they suggested the presence of an inhibitor of DNA synthesis in normal rat liver. Bollum and Potter (4) also observed a low incorporation of tritiated thymidine into two-strand primer DNA in the system involving the use of high speed supernatant fraction of old rat liver. The results presented here showed a regular decrease in the ability of rat liver to synthesise DNA with increasing age when the two-stranded DNA acts as a primer. Our results may also indicate the presence of an inhibitor of DNA synthesis in the supernatant fraction of old rat liver but they suggest that such an inhibitor may not act directly on DNA polymerase rather might block the "mechanism" which initiates the stepwise breakdown of hydrogen bonds of the two-stranded DNA.

It is very interesting to note that in the systems where two-stranded DNA acts best as a primer, heat-denatured DNA does not act so well and vice versa. Of the various interpretations of these findings that can be offered, the mechanism suggested by Meselson and Stahl (5) and slightly

modified by Sarkar et al (6), involving the idea of progressive separation of the two strands of DNA molecule due to stepwise breakdown of hydrogen bonds with concomitant DNA synthesis, seem to be adequate to explain the results. Sufficient evidence was presented to show that the separation of the two strands and synthesis of DNA occur simultaneously. It has been suggested by the authors that there is a "mechanism" which, under conditions of the experiment, can initiate the stepwise breakdown of hydrogen bonds between the complementary base pairs from one end or two ends of the DNA molecule, so that the incoming bases (deoxynucleotides) can form hydrogen bonds with the hydrogen bonded bases of the chain as the initial stage of DNA synthesis. Our results indicate that such a "mechanism" either disappears slowly with age or is progressively blocked by the gradual appearance of an inhibitor in the liver with age.

SUMMARY

The ability of rat liver (high speed supernatant fraction) to synthesise DNA when two stranded DNA acts as a primer, decreases steadily with age whereas it increases progressively if heat-denatured DNA is used as a primer. The ability of the hepatectomized rat liver to synthesise DNA also increases after hepatectomy with time when two-stranded DNA acts as a primer but on and after 4th day of hepatectomy, this activity begins to drop and continues to decline thereafter until it reaches the level of activity observed before hepatectomy. On the other hand, if heat-denatured DNA is used as a primer the activity at first decreases, then increases steadily until it reaches the level of activity observed before hepatectomy. The reaction depends on Mg^{++} and cannot be replaced by any other metal, its action can however be stimulated by adding Ca^{++} but not by any other metal.

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