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EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM

DNA - SYNTHESIS IN THE ISOLATED PERFUSED RAT LIVER

by

G. GERBER

1963

Directorate General for Research and Training
Department of Biology

Paper presented at the 47th Meeting
of the Federation of American Societies for Experimental Biology
Atlantic City - USA, 15-19 April 1963

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a maximal value (50-80 times that of normal liver) between 24 and 48 hours after partial hepatectomy and decreased at later times. The data on labelled nuclei paralleled those on specific activity of DNA. If the liver was perfused during a period of marked increase in DNA synthetic activity e.g. 18 to 24 hours after hepatectomy the increase in specific activity of DNA during the second period of the perfusion was markedly greater (up to 4 times), than during the first period. This observation as well as the found correlation in DNA synthesis between perfused liver and liver „in vivo", attests to the suitability of the perfused, partially hepatectomized liver for studies on DNA synthesis.

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DNA-SYNTHESIS IN THE ISOLATED PERFUSED RAT LIVER

SUMMARY

Liver of normal and partially hepatectomized rats was perfused according to the technique of Miller et al. (J. Exp. Med. 94,431, 1951). H^3-thymidine was added to the perfusate at the start of the experiment and again three hours later. A liverlobe was removed before the second addition of thymidine and at the end of the perfusion (4-5 hours after the start of the perfusion) DNA was isolated from the liversamples, and its specific activity was determined. Autoradiographs from liver sections were also prepared, and the number of labelled nuclei was counted.

Incorporation of H^3-thymidine into DNA was low in normal liver, but increased 8 hours after partial hepatectomy. It reached a maximal value (50-80 times that of normal liver) between 24 and 48 hours after partial hepatectomy and decreased at later times. The data on labelled nuclei paralleled those on specific activity of DNA. If the liver was perfused during a period of marked increase in DNA synthetic activity e.g. 18 to 24 hours after hepatectomy the increase in specific activity of DNA during the second period of the perfusion was markedly greater (up to 4 times), than during the first period. This observation as well as the found correlation in DNA synthesis between perfused liver and liver "in vivo", attests to the suitability of the perfused, partially hepatectomized liver for studies on DNA synthesis.

Synthesis of DNA has been studied extensively on a large variety of systems beginning with the total organism down to the single cells. However, little is still known about certain aspects of DNA synthesis in mammalian organs such as

a) the relationship between the amounts of precursormaterial *de novo* synthesized and that utilized for DNA synthesis or

b) the influence of variations in the precursor pools on rate of DNA synthesis and on concentration of DNA synthesizing enzyms.

These aspects lend themselves not easily to investigations either on total organisms because of the interference of other DNA synthesizing organs, or on single cells because the conditions of continuous cell replacement in tissue culture probably do not correspond the conditions in a mammalian organ.

However, some of these difficulties might be avoided by studying DNA synthesis in isolated perfused organs, in which nearly normal biochemical and physiological functions are maintained for a sufficient lenght of time. Thus we have investigated whether DNA synthesis remains intact in isolated perfused liver after partial hepatectomy and how H^3 labelled Thymidine is degraded under these conditions.

The liver of normal or partially hepatectomized rats was perfused with heparinized diluted rat blood according to the method of Miller et al. The equipment used by Miller has been modified slightly in so far as a different oxygenerator was used and the total equipment was housed in a commercial incubator.

A scheme of this apparatus is shown in Fig. 1.

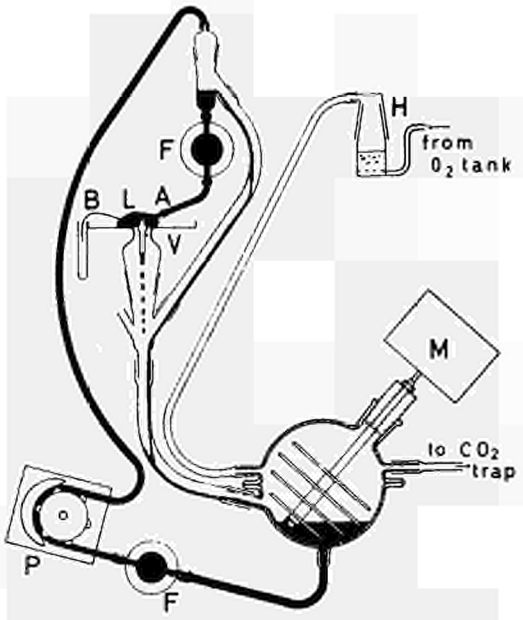

Diagram of the apparatus used for liver perfusion

A inflow cannula
B bile cannula
F filter
H humidifier
L liver
M motor
P pump
V outflow cann.

Fig. 1

At the beginning of the perfusion 50 μ C of H^3 Thymidine were added to the perfusate and a second equal dose was given after 3 hours.

The perfusion was maintained for $4\frac{1}{2}$ to 6 hours.

A liverlobe was removed after 3 hours before the addition of the second dose and another liversample was taken at the end of the perfusion. DNA was isolated from these samples by the method of Schneider and the specific activity of DNA was determined by means of the Disch reaction and counting of the radioactivity in a Tricarb Liquid Scintillation counter. Autoradiographs were prepared from tissuesection with liquid Ilford L4 emulsion.

Samples of the perfusate were also removed at regular intervals and total and nonvolatile radioactivity were determined.

In a number of these samples the total radioactivity of thymidine was also determined by isolation of the thymidinefraction on paperchromatograms after addition of carrier.

Fig. 2 shows data on specific activity of DNA in normal liver and at various time intervals after partial hepatectomy.

It should be pointed out that in order to render the small values of specific activities better visible beside the large ones, the specific activity has been plotted on a logarithmic scale. The two adjoining bars marked differently represent incorporation of thymidine during the first and second period of perfusion.

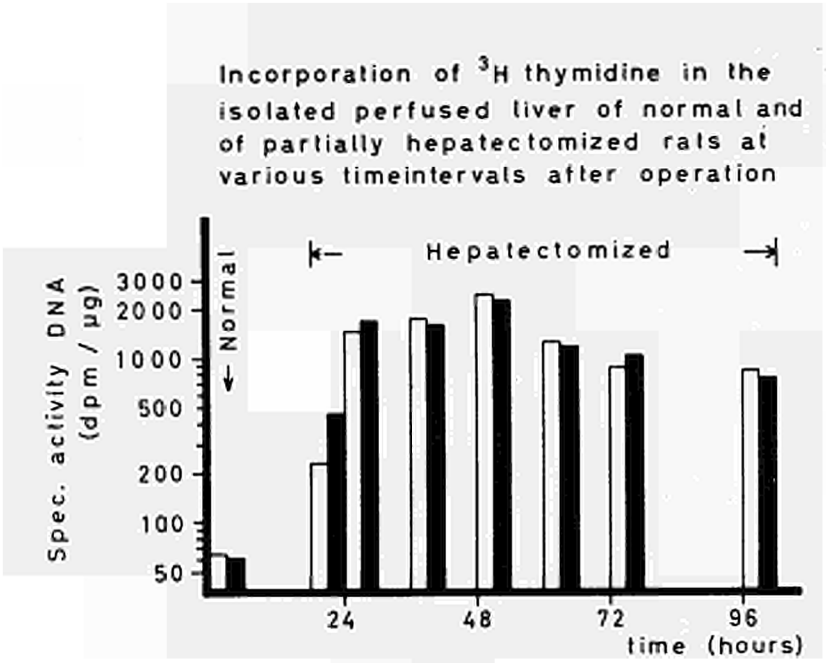

Fig. 2

As can be seen, only small quantities of thymidine are incorporated into normal liver, whereas DNA synthesis is increased from 18 hours on after partial hepatectomy. DNA synthesis attains a maximal value from 24 to 48 hours after partial hepatectomy and decreases again thereafter.

Only about 0.7 % of the radioactivity added as H^3 thymidine are incorporated into DNA of normal liver, in contrast to an incorporation of up to 13 % after partial hepatectomy. The incorporation into DNA of perfused liver corresponds thus to that observed in liver of intact rats after partial hepatectomy.

The isolated perfused liver maintains its ability to synthesize DNA for a considerable time as long as other biochemical functions are also intact and a sufficient flow can be obtained.

Not only remains incorporation of thymidine about the same as initially after three hours of perfusion but a considerable increase in DNA synthesis can be observed if the perfusion falls into a time when in intact rats DNA synthesis would rise rapidly as from 18 to 24 hours after partial hepatectomy.

Fig. 3 shows an autoradiograph of a tissuesection from a liver 36 hours after partial hepatectomy. The pattern of labelling of the cell nuclei seems indistinguishable from that in intact rats and mitoses are common. Futhermore specific activity of DNA and the numbre of labelled nuclei follow a paralell course with time after partial hepatectomy.

In another experimental series liverlobes from normal and hepatectomized perfused liver were removed at various intervals after addition of H^3 thymidine. The upper part of the Fig. 4 shows data on specific activity of DNA as a function of time after addition of thymidine to the perfusate whereas in the lower part corresponding data on total and nonvolatile radioactivity of the acid soluble fraction of liver are presented.

Incorporation of thymidine into DNA begins almost at once and is virtually complete after 1 to 2 hours but concurrently degradation of radioactive thymidine into volatile H_2O activity takes place. The latter reaction can be studied in more detail in the perfusate.

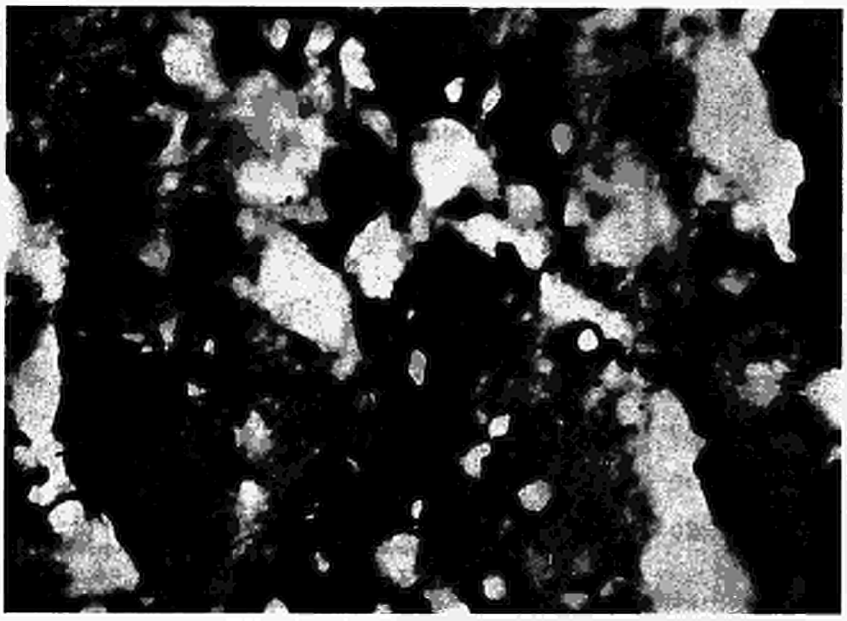

Fig. 3

Total and nonvolatile radioactivity as well as radioactivity of the thymidine in the perfusate from a normal liver are plotted as a function of time in Fig. 5. Already thirthy minutes after addition of H^3 thymidine more than 1/2 of its radioactivity has been converted into tritium-oxyde and radioactive thymidine disappears even more rapidly. If one compares inflowing and outflowing perfusate one finds that up to 30 % of the thymidine present is degraded during the flow through the liver. About 17 μg of thymidine per hour are catabolized in the liver under these conditions at concentration of ca 4μg/100 ml. If inactive thymidine at a concentration of 8 mg/100 μ l is added to the perfusate a considerable larger quantity of thymidine about 6 mg/hour per liver can be degraded. At the same time the total amount of radioactivity incorporated into DNA decreases to $\leqslant$ 0.5 % of the value from liver without addition of inert thymidine.

Thus, the concentration of the enzymes capable of degrading thymidine in the liver appears not to be the limiting factor for degradation of thymidine. This fact would explain the low concentrations of thymidine observed in the acid soluble fraction of liver. On the other hand, it does not appear that alterations in the catabolism of thymidine are responsible for the increased DNA synthesis of partially hepatectomized liver, since this catabolism does not differ markedly between normal and hepatectomized liver. Finally, it is noteworthy that this rapid catabolism of thymidine seems to be a rather unique property of the liver. Studies on perfused intestine and kidney as well as on eviscerated preparations have shown that only a negligible degradation of thymidine takes place in organs other than liver.

In summary, DNA synthesis and thymidine catabolism were studied in the isolated perfused liver of normal and hepatectomized rats. Incorporation of thymidine into DNA of perfused liver corresponds to that observed in intact rats after partial hepatectomy. Radioactive thymidine is degraded rapidly to volatile tritiumoxyde by the liver and amounts up to 10 μ g/hour per g liver can thus be catabolized.

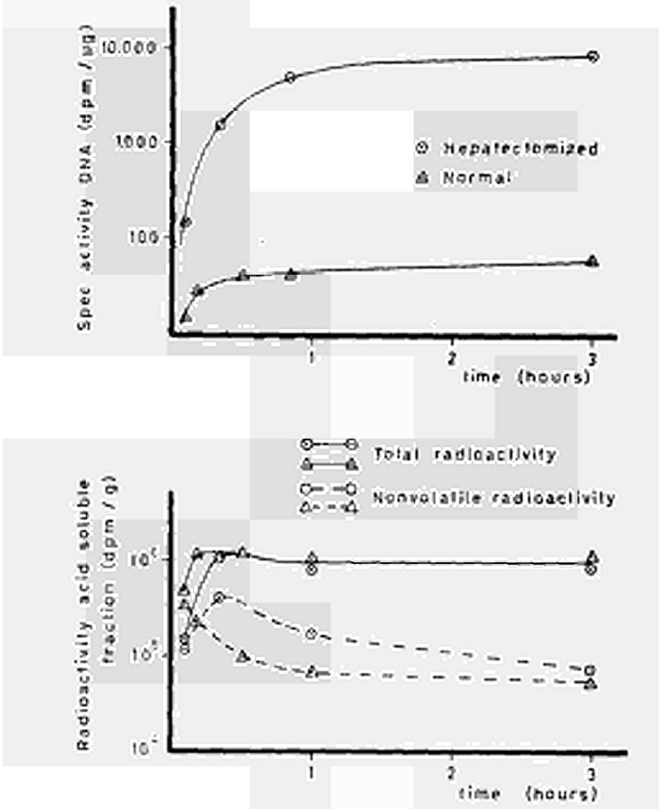
Spec activity DNA (dpm/µg)
10.000
1000
100
Hepatectomized
Normal
1
2
3
time (hours)
Total radioactivity
Nonvolatile radioactivity
Radioactivity acid soluble fraction (dpm/g)
10^2
10^1
10^0
1
2
3
time (hours)

Fig. 4

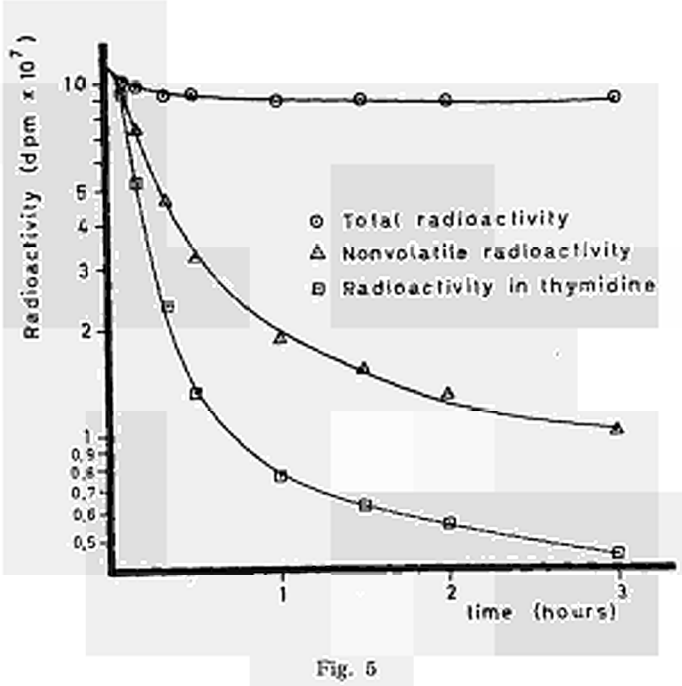
Radioactivity (dpm x 10^7)
10
5
4
3
2
1
0,9
0,8
0,7
0,6
0,5
Total radioactivity
Nonvolatile radioactivity
Radioactivity in thymidine
1
2
3
time (hours)

Fig. 5

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