THE EFFECT OF PARTIAL HEPATECTOMY ON THE RIBONUCLEIC ACID POLYMERASE OF RAT LIVER

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Weiss (1960) described, in the liver of normal rats, a nuclear enzyme which polymerizes nucleoside triphosphates into RNA*. We used his techniques, slightly modified, to ascertain if and how partial hepatectomy affected this enzyme.

Material and methods. In each experiment, 2 to 4 rats were hepatectomized according to Higgins and Anderson (1931). One to 48 hours later, the regenerating livers were removed, the nuclei were prepared according to Chauveau (1956) in 2.2 M sucrose, washed once in 0.25 M sucrose, lysed during 10 minutes in 0.05 M Tris-phosphate buffer pH 7.4, centrifuged and suspended in the same buffer. This extract was used as enzyme.

The incubations lasted 10 minutes at 37°C. Each sample contained in a final volume of 0.25 ml (in µmoles) : Tris-phosphate buffer pH 7.5 25 ; Mg^{++} 7.5 ; 2-mercaptoethylamine 0.25 ; KCl 15 ; NaF 5 ; C^{14}-ATP 0.25 ; nonlabeled GTP, UTP and CTP, each 0.25 ; enzyme (2 mg. of proteins). All incubations were run in duplicate. Incubation was stopped with trichloroacetic acid, the precipitate dissolved in sodium-ammonium phosphate M (Canellakis 1960), reprecipitated and counted in a Packard

*Abbreviations. RNA : ribonucleic acid ; DNA : deoxyribonucleic acid ; Tris : tris(hydroxymethyl)aminomethane ; ATP, UTP, GTP and CTP : adenosine triphosphate, uridine triphosphate, guanosine triphosphate and cytidine triphosphate respectively.

Tricarb scintillation counter. In some experiments, nonlabeled UTP was replaced with UTP-α-P^{32}, C^{14}-ATP with C^{12}-ATP and NaF and KCl were omitted. The omission of three nonlabeled nucleotides always resulted in practically no incorporation. Results are expressed as µµmoles of radioactive nucleotide incorporated in 10 minutes per mg. of proteins.

Results. In all, 32 experiments were performed with C^{14}-ATP, including 9 on normal rats, 6 at 18 hours and 5 at 12 hours after hepatectomy. In the liver of normal rats, the enzymic activity was 78 µµmoles/mg., with a standard deviation of ±21. The fact that the assay on controls gave reproducible results led us to believe that the method which Weiss devised as preparative could also be used for quantitative comparisons in various physiological conditions.

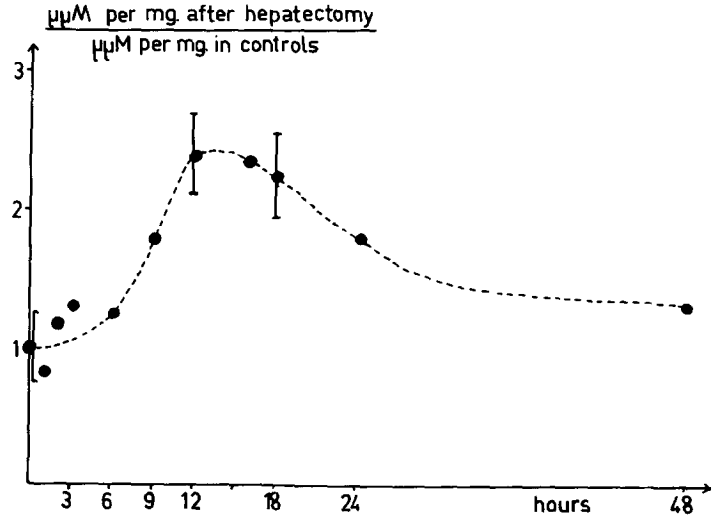

Figure 1. Variation of activity of RNA polymerase after hepatectomy.
Ordinates show the ratio of activity per mg. of proteins in the liver of hepatectomized rats to that of controls. The brackets show standard deviation where calculated.

Figure 1 shows the variations when taking the activity in normal livers (78 µµmoles/mg.) as 1. The values up to 6 hours

after partial hepatectomy fall in the range of normal variations. Then begins a rather sharp increase. The maximum activity seems to be at 12 hours (184 ±22 μμmoles/mg., or a 2.37-fold increase), but the activity is still high at 16 hours (169 μμmoles/mg.) and 18 hours (174 ±31 μμmoles/mg., or a 2.24-fold increase). The average activity 48 hours after removal of part of the liver is 100 μμmoles/mg., in the upper range of normal values. Using UTP-α-P^{32}, the normal value (7 experiments) is 210 ±40 μμmoles/mg. ; 18 hours after partial hepatectomy the average value (6 experiments) is 451 ±36 μμmoles/mg. or a 2.15-fold increase, very similar to the 2.24-fold increase observed with C^{14}-ATP. The more important incorporation of UTP than ATP must reflect the constitution of the newly formed RNA.

Discussion. We checked that the observed increase in activity is not due to decreased nucleases by incubating enzymes from normal and hepatectomized livers with bentonite (Brownhill 1959). On the other hand, we excluded by mixed incubations that this increase could be caused by the loss of an inhibitor (or nucleases) : the incorporation of the labelled triphosphate by a mixture of liver extracts from control and hepatectomized rats was either identical with the sum of isolated incubations or differed at the most by 10 per cent from this value. On paper radiochromatograms of alkaline hydrolysates of RNA synthesized in the presence of UTP-α-P^{32}, radioactivity was associated with all 4 nucleotides ; after alkaline hydrolysis of RNA synthesized in the presence of C^{14}-ATP, only 5 per cent of the radioactive label was recovered as adenosine ; finally, with RNA labelled with UTP-α-P^{32}, release of radioactive P by snake venom was parallel to that of inorganic P, ruling out a simple end-attachment. Thus our observations tend to suggest that RNA polymerase might have physiological importance, since we see

its measurable activity increase in conditions where we know the nuclear RNA synthesis to be enhanced (Welling 1960). Conversely, in the kidney, where the synthesis of RNA is known to be much less important, the activity measured in the same conditions is 20 times less than that in the liver. Since the enzyme requires DNA, the newly formed RNA might be messenger RNA which brings information to the cytoplasm for the synthesis of proteins. However it must be kept in mind that messenger RNA is usually considered highly unstable (Jacob and Monod 1961, Gros 1961, Hayashi and Spiegelman 1961), while synthesis of proteins in regenerating liver proceeds at an increased rate long after the activity which we measured returns to normal : thus thymidylate kinase and DNA polymerase, which have a small activity in normal liver, show a maximum 48 hours after hepatectomy (Bollum 1959, Hiatt 1960).

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References

Bollum, F.J., and Potter, V.R., Cancer Research, 19, 561 (1959).
Brownhill, T.J., Jones, A.S., and Stacey, M., Biochem. J., 73, 434 (1959).
Canellakis, E.S., and Herbert, E., Biochim. Biophys. Acta, 45, 133 (1960).
Chauveau, J., Moulé, Y., and Rouiller, C., Exptl. Cell Research, 11, 317 (1956).
Gros, F., Hiatt, H., Gilvert, W., Kurland, C.G., Risebrought, R.W., and Watson, J.D., Nature, 190, 581 (1961).
Hayashi, M., and Spiegelman, S., Proc. Nat. Acad. Sci., 47, 1564 (1961).
Hiatt, H., and Bojarski, T.D., Biochem. Biophys. Research, Communs., 2, 35 (1960).
Higgins, G.M., and Anderson, R.M., Arch. Path., 12, 186 (1931).
Jacob, F., and Monod, J., J. Mol. Biol., 3, 318 (1961).
Weiss, S.B., Proc. Nat. Acad. Sci., 46, 1020 (1960).
Welling, W., and Cohen, J.A., Biochim. Biophys. Acta, 42, 181 (1960).