SYNTHESIS OF DNA AFTER PARTIAL HEPATECTOMY WITHOUT CHANGES IN THE LIPID AND GLYCOGEN CONTENTS OF THE LIVER*

Joseph F. Simek**, Fred Rubin, and Irving Lieberman
Department of Anatomy and Cell Biology, University of Pittsburgh, School of Medicine, Pittsburgh, Penna.

Received February 8, 1968

Partial hepatectomy is not immediately followed by a rise in liver DNA synthesis. Rather there is an initial preparative period (13 h in the rats used in these studies) during which many alterations in liver chemistry occur. To study the mechanisms that control hepatic DNA synthesis, it is essential to distinguish between the changes that are and are not requisite for the entry of the liver cell into the replicative period. This report shows that two prereplicative changes that have received a good deal of attention, the accumulation of neutral lipid (Ludewig et al., 1939) and the fall in glycogen (Stone, 1935), may be blocked without preventing the subsequent synthesis of DNA.

EXPERIMENTAL PROCEDURE

Female albino rats, obtained locally, were used when they weighed about 100 g. Partial hepatectomy refers to the removal of 67% of the liver. DNA synthesis was measured with ^{3}H-thymidine (2 μC) and neutral lipid (Folch et al., 1957; Van Handel and Zilversmit, 1957) and glycogen (Good et al., 1933; Seifter et al., 1950)

* This work was supported by grants CA 06663 and HD 40-11 from the National Institutes of Health, U.S. Public Health Service.

** Permanent address: Department of Physiology, Faculty of Medicine, Charles University, Hradec Kralove, Czechoslovakia.

were determined by the usual methods. DNA was estimated with diphenylamine (Burton, 1955). Perfusions were made in the tail vein from 20 ml hypodermic syringes fixed in a horizontal position in stainless steel plates with a moving carriage assembly acting on the plungers to deliver a constant flow of 1 ml/h.

RESULTS AND DISCUSSION

That the levels of neutral lipid and glycogen begin to change immediately after partial hepatectomy is shown in Fig. 1. As the figure shows, the changes were completed before the end of the 13 h preparative period.

Continuous perfusion of glucose (1 mmole/h) into partially hepatectomized rats blocked the changes in the lipid and glycogen contents of the residual liver but not the rise in DNA synthesis

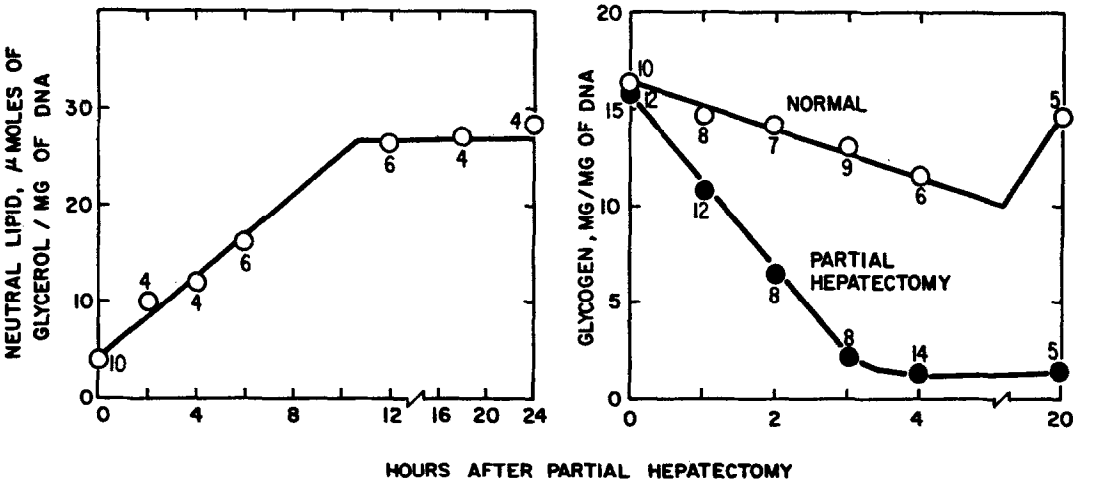

Fig. 1. The accumulation of neutral lipid and the fall in glycogen in liver as a function of time after partial hepatectomy. Liver samples were removed at the indicated times after partial hepatectomy. Neutral lipid and glycogen were measured in each liver sample and the values shown represent the averages of the individual results obtained with 4 to 14 animals as indicated.

(Table I).[1] These results indicate that the accumulation of neutral lipid and the disappearance of glycogen *per se* are not obligatory for entry of the liver cell into the replicative period.

TABLE I

THE EFFECT OF GLUCOSE ON THE INCREASE IN NEUTRAL LIPID, THE DECREASE IN GLYCOGEN, AND THE RISE IN DNA SYNTHESIS AFTER PARTIAL HEPATECTOMY

Partially hepatectomized rats were continuously perfused (1 ml/h) with glucose (1 M) or Ringer's solution, as shown, beginning immediately after the operation. Normal rats were similarly perfused. The perfused animals were not fed whereas those that were not perfused received food and water *ad libitum*. After 21 h, all the animals were given ^{3}H-thymidine in the tail vein and liver samples were taken 1 h later. Each liver sample was used to estimate the radioactivity incorporated into DNA and the levels of neutral lipid and glycogen. The values shown are the averages of the individual results obtained with 6 to 8 rats and the ranges are given in the parentheses.

Operation	Perfusate	DNA synthesis	Neutral lipid	Glycogen
		cpm/mg DNA	μmoles glycerol/mg DNA	mg/mg DNA
None	None	150 (70-200)	3.0 (2.4-3.9)	21 (19-25)
	Ringer's	210 (140-260)	5.3 (4.0-6.5)	
	Glucose	120 (90-140)	3.4 (3.2-4.8)	19 (16-22)
Partial hepatectomy	None	7300 (6200-11800)	37 (20-53)	3.7 (2.9-5.9)
	Ringer's	5800 (4900-7300)	24 (16-32)	
	Glucose	4300 (2400-8100)	5.2 (3.6-8.1)	17 (14-20)

1) As Table I shows, glucose caused a small decrease in the radioactivity incorporated into DNA. The decrease resulted from a brief delay in the onset of the replicative period rather than from an inhibition of DNA synthesis.

Most of the hepatic alterations known to occur during the preparative period, for example, the rise in the specific activity of RNA polymerase (Busch et al., 1962; Tsukada and Lieberman, 1965; Pogo et al., 1966) and the increased rates of synthesis in vivo of ATP (Ove et al., 1967), protein (Majumdar et al., 1967), and ribosomes (Chaudhuri et al., 1967), would seem to be etiologically related. Thus, these changes can also be induced in intact animals by acute stress (Feigelson et al., 1962; Ove et al., 1967; Majumdar et al., 1967) (the intraperitoneal injection of Celite or water-insoluble cortisone).[2] Celite (5 mg/100 g rat) and cortisone (Cortone acetate, Merck Sharp and Dohme, 10 mg/100 g rat), however, produced no change in the fat and glycogen contents of normal rat liver, suggesting that these changes form a second group of alterations. It remains to be seen whether any of the changes of the first group are obligatory for DNA synthesis.

REFERENCES

Burton, K., Biochem. J., 61, 473 (1955).
Busch, S., Chambon, P., Mandel, P., and Weill, J. D., Biochem. Biophys. Res. Commun., 7, 255 (1962).
Chaudhuri, S., Doi, O., and Lieberman, I., Biochim. Biophys. Acta, 134, 479 (1967).
Feigelson, P., Feigelson, M., and Greengard, O., Recent Progr. Hormone Res., 18, 491 (1962).
Folch, J., Lees, M., and Stanley, G. H. S., J. Biol. Chem., 226, 497 (1957).
Good, C. A., Kramer, H., and Somogyi, M., J. Biol. Chem., 100, 485 (1933).

2) Although partial hepatectomy and acute stress cause several similar changes to occur in liver, it should be emphasized that there are important differences between the two stimuli. Thus, the liver changes after partial hepatectomy, including the rise in DNA synthesis, occur in adrenalectomized rats. Celite, on the other hand, has no effect in adrenalectomized animals nor does it stimulate DNA synthesis in intact animals.

Ludewig, S., Minor, G. R., and Hortenstine, J. C., Proc. Soc. Exp. Biol. Med., 42, 158 (1939).
Majumdar, C., Tsukada, K., and Lieberman, I., J. Biol. Chem., 242, 700 (1967).
Ove, P., Takai, S., Umeda, T., and Lieberman, I., J. Biol. Chem., 242, 4963 (1967).
Pogo, A. O., Allfrey, V. G., and Mirsky, A. E., Proc. Nat. Acad. Sci., U.S.A., 56, 550 (1966).
Seifter, S., Dayton, S., Novic, B., and Muntwyler, E., Arch. Biochem., 25, 191 (1950).
Stone, C. S., Jr., Arch. Surg., 31, 662 (1935).
Tsukada, K., and Lieberman, I., J. Biol. Chem., 240, 1731 (1965).
Van Handel, E., and Zilversmit, D. B., J. Lab. Clin. Med., 50, 152 (1957).