A NUCLEAR MEMBRANE CHANGE AFTER PARTIAL HEPATECTOMY

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ABSTRACT

Partial hepatectomy (67 per cent extirpation) of the rat leads to a change in the membrane of liver nuclei (purified with citric acid) detectable as an increase in electrophoretic mobility. No change is detectable 2 hours after the operation, but between 2 and 6 hours about a 1.4-fold increase in mobility occurs after which the mobility becomes constant at the elevated level. Removal of only 10 per cent of the liver causes no detectable change in 6 hours. Bilateral adrenalectomy immediately before partial hepatectomy does not affect the development of the nuclear change. Actinomycin D and *p*-fluorophenylalanine, but not noradrenalin, ionizing radiation, or EDTA, suppress the increase in electrophoretic mobility. The level of actinomycin D required to block the nuclear membrane change is 6 times greater than that necessary to prevent the rate increase in hepatic RNA metabolism that follows removal of part of the liver. This discrepancy and the difference in the response to noradrenalin indicate that, at least initially, the nuclear membrane change and the change in the rate of RNA synthesis are independent processes. The inability of EDTA to block the nuclear membrane change shows that the Zn^{++} requirement for DNA replication is not related to the events that lead to the alteration in the electrokinetic properties of liver nuclei.

Partial hepatectomy causes a marked increase in the number of liver cells that synthesize DNA, but only after a period of about 15 hours (1). The presynthetic period is characterized by enhanced metabolic activity including changes in RNA and protein synthesis (2–8). It is clear that at least some of the synthetic reactions that occur during this time are essential for the subsequent formation of DNA (9, 4). Although little is known, it is reasonable to assume that these preparatory events finally result in the formation of suitable levels of the enzymes necessary to form DNA and in a physical change in DNA that allows it to be replicated (10).

Of great interest are the triggering devices, activated by removal of a portion of the liver, that cause the changes of the presynthetic period. To study the initial stimuli to hepatic regeneration, metabolic alterations have been sought that follow soon after the operation. Some of the liver changes that appear to begin immediately postoperatively are an increase in the activity of a nuclear and a nucleolar enzyme that synthesizes RNA[1], an increase in nucleolar RNA (5), an increased rate of turnover of RNA (3, 4), and an increase in the electrophoretic mobility of the intact cell (11).

Since many of these changes are associated with the nucleus, and especially in view of the increased postoperative electrophoretic mobility of the liver cells (11), studies have now been made of the electrokinetic properties of liver nuclei after partial

[1] Tsukada, K., and Lieberman, I., unpublished obserlished observations.

hepatectomy. The present report describes a postoperative increase in the electrophoretic mobility of liver nuclei and presents evidence that RNA and protein synthesis are requisite for this surface change. Evidence is also presented to show that the increases in the metabolism of liver RNA that follow partial hepatectomy are not requisite to the change in the surface properties of the liver nuclei.

Methods

Surgery was performed under ether anesthesia. Unless otherwise indicated, partial hepatectomy refers to the removal of 67 per cent of the liver (median and left laberal lobes) (12). The sham operation was performed in the same way except that no liver was excised.

To purify nuclei, the rat was decapitated and the liver was quickly removed, blotted, and immediately homogenized (10 strokes) in ice cold citric acid (0.1 M) in a Dounce homogenizer with a loose fitting pestle. The homogenate was layered over 5 volumes of 0.34 M sucrose, and the nuclei were sedimented by centrifugation (about 275 *g* for 10 minutes). The pellet, resuspended in citric acid, was homogenized again (20 strokes) and recentrifuged. To remove residual connective tissue, the purified nuclei were again suspended in citric acid and allowed to pass through an 80-mesh stainless steel wire gauze held at the mouth of a stainless steel press. Citric acid was removed by washing three times with a solution of sucrose (0.5 M) and sodium phosphate (0.005 M, pH 7.4). The washed nuclei were then suspended in the sucrose-phosphate solution and the velocities of their migration were immediately measured. The final preparation did not

TABLE I

Distribution of Electrophoretic Velocities of Individual Nuclei

Electrophoretic velocity	No. of nuclei observed for each of 8 rats after partial hepatectomy							
	0 hours				*6 hours*			
seconds	I	II	III	IV	I	II	III	IV
4.9–5.2	0	0	0	0	0	0	0	0
4.5–4.8	1	3	4	1	0	0	0	0
4.1–4.4	4	4	4	3	0	0	0	0
3.7–4.0	10	17	10	11	0	1	0	1
3.3–3.6	10	7	8	10	3	2	0	0
2.9–3.2	9	6	10	9	9	5	11	7
2.5–2.8	4	1	2	5	15	8	11	8
2.1–2.4	2	2	2	1	10	18	9	17
1.7–2.0	0	0	0	0	3	6	9	7
1.3–1.6	0	0	0	0	0	0	0	0
Mean velocity (seconds)	3.43	3.64	3.56	3.36	2.67	2.47	2.50	2.35
Over-all mean velocity (seconds)		3.50 ± 0.64				2.50 ± 0.43		
95 per cent confidence limit of over-all means		3.40–3.60				2.45–2.57		

Each column of electrophoretic velocities represents the observations made with one rat. The nuclei were purified and the electrophoretic measurements were made as described in Methods. To simplify the arithmetic, the statistical calculations were made from the electrophoretic velocity measurements (time required for a nucleus to move a distance of 40 μ).

MATERIALS AND METHODS

Materials

Unless otherwise indicated, male albino rats weighing about 80 gm were used. They were obtained locally and received food and water *ad libitum*. Orotic acid-6-^{14}C (5.4 μc per μmole) was supplied by the New England Nuclear Corp., Boston, and ^{3}H-thymidine (6.65 mc per μmole) by Schwarz Bioresearch Labs., Inc., Mount Vernon, New York. DL-*p*-fluorophenylalanine was obtained from the Nutritional Biochemicals Corporation, Cleveland, and noradrenalin (Levophed) from the Winthrop Laboratories. Actinomycin D was kindly supplied by Dr. Elmer Alpert, Merck Sharp and Dohme research laboratories, West Point, Pennsylvania.

appear to contain any intact cells, and no cytoplasmic fragments were found adherent to any of the nuclei.

The electrophoretic measurements[2] were made in a Northrop-Kunitz cell obtained from the Arthur H. Thomas Company, Philadelphia. The electrodes contained 2 per cent agar in 0.005 M sodium phosphate (pH 7.4) in their inner halves, and their outer portions were filled with 2 per cent $CuSO_4$. Copper wires were inserted into the $CuSO_4$ solution. The cell was held horizontally; the nuclei did not settle rapidly enough to constitute a serious problem. Standardization was made by measuring the electrophoretic mobility of rat erythrocytes (13, 14).

Measurements were made with 1 to 2 per cent suspensions of nuclei, at room temperature (24–25°C), and with a phase microscope (magnification 250) fitted with an ocular micrometer. The current used was 0.50 mamp at a field strength of 14.7 volts/cm. The field strength was calculated from the current, the specific resistance of the buffer, and the cross-sectional area of the cell. The velocity of the horizontal migration of the nuclei was measured in 0.5 M sucrose–0.005 M sodium phosphate (pH 7.4) over a distance of 40 μ with the aid of a stop watch whose large hand covered 360° in 10 seconds. Mobility was calculated as microns/second/volt/centimeter. Each value represents the average of 40 individual measurements, half of them made at the 20 per cent and half of them at the 80 per cent, stationary level. The direction of the current was reversed after every 5 to 10 measurements. Moving nuclei stopped immediately when the current was turned off, indicating the absence of detectable convection currents. About 2.5 per cent of the nuclei had a net positive charge; *i.e.*, their direction of movement was opposite that of the majority of the nuclei. Their velocities were omitted from the calculations of electrophoretic mobility. Their origin, perhaps from blood cells, was not determined.

Intravenous perfusions were administered 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 per hour.

The rate of liver RNA synthesis was measured as previously described (4). The rats were injected with 0.5 μc of ^{14}C-orotate and were killed by decapitation 7 minutes later. Isotope incorporation was estimated by a filter procedure involving washes with trichloroacetic acid, ethanol, and ether, and radioassay was carried out in a Packard Tri-Carb liquid scintillation spectrometer. DNA formation was estimated following intravenous injections of ^{3}H-thymidine (25 μc per rat). One hour after the injection, the rat was killed and a sample of liver was homogenized (10 per cent) in 0.25 M sucrose. Isotope incorporation was then measured exactly as for RNA synthesis. The omission of steps involving centrifugation and incubation in NaOH (15) did not affect the final result.

RESULTS

Nuclear Electrophoretic Mobility After Partial Hepatectomy

TIME AFTER PARTIAL HEPATECTOMY: No change in the electrophoretic mobility of the nuclei of the residual liver was detectable at 2 hours after partial hepatectomy, but between 2 and 6 hours an increase in mobility occurred after which the rate again became constant at the elevated level (Fig. 1). As the figure shows, the mobility at 6 hours was about 1.4 times greater than the initial value and reflected an increase in the net negative charge of the nuclear surface. Table I shows the distribution of the electrophoretic velocities (seconds of time required to move 40 μ) of the individual nuclei from 8 of the animals of Fig. 1. Half of the preparations were from rats killed immediately after partial hepatectomy, and the other half were from rats killed 6 hours later.

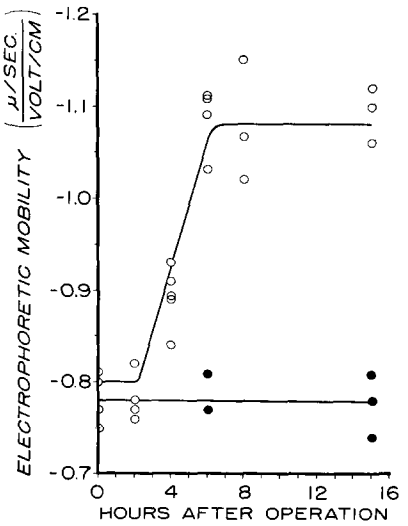

FIGURE 1 Nuclear electrophoretic mobility after partial hepatectomy. The liver nuclei were prepared in citric acid and their electrophoretic mobiliites were measured as described in Methods. Each point represents the result obtained with a single rat; the open circles, partially hepatectomized animals; the solid circles, sham-hepatectomized ones.

[2] The authors gratefully acknowledge their indebtedness to Dr. C. C. Brinton, Jr., for his invaluable instruction and guidance in making the electrophoretic measurements.

Not shown in Fig. 1 are the results that were obtained when a sucrose-phosphate solution of pH 7.0 instead of 7.4 was used. Tested with nuclei prepared 0 and 6 hours after partial hepatectomy. the difference in pH had no effect on the electrophoretic velocities.

REMOVAL OF 10 AND 67 PER CENT OF THE LIVER: To study the electrophoretic mobility of liver nuclei as a function of the amount of liver removed, the caudate lobe (10 per cent) was extirpated (Table II). The table shows that removal of only 10 per cent of the liver caused no detectable change in the surface properties of the residual nuclei as measured after 6 hours. In this connection it should be pointed out that removal of the caudate lobe involves a much more drastic handling of the liver than does the 67 per cent extirpation.

TABLE II

Nuclear Electrophoretic Mobilities After Removal of 10 and 67 Per Cent of Liver

Liver removed		Electrophoretic mobility	
Lobes	Per cent of total liver	μ/sec. volt/cm	Average
	0*	−0.80, −0.83	−0.82
Caudate	10	−0.78, −0.81	−0.80
Left lateral + median	67	−1.01, −1.14	−1.08

The rats were killed after 6 hours. Liver nuclei were prepared with citric acid and their electrophoretic mobilities were measured as described in Methods.

* Sham-hepatectomized.

ADRENALECTOMY: The adrenals do not play a role in the change in the nuclear surface properties that result from removal of part of the liver. Thus, as shown in Table III, the removal of both adrenals at the time of partial hepatectomy did not affect the increase in nuclear electrophoretic mobility that was measured 6 hours later.

Inhibitory Agents

ACTINOMYCIN D: Multiple injections of actinomycin D in an amount (2 μg per injection) that had no effect upon the rate of RNA synthesis in untreated animals did not prevent the increase in nuclear electrophoretic mobility (Table IV). However, a larger amount of the antibiotic, *i.e.*, one that reduced the rate of RNA formation in untreated animals by about 50 per cent (12 μg per injection) as shown in the table, blocked the postoperative electrokinetic change.

The relative resistance of the nuclear membrane change to actinomycin D distinguished it from the postoperative change in the rate of RNA synthesis. The latter change has been previously shown to be very sensitive to the antibiotic (4, 5) and, as the table shows, was completely suppressed by 2 μg injections of actinomycin D.

p-FLUOROPHENYLALANINE AND NORADRENALIN: At the levels tested, *p*-fluorophenylalanine, but not noradrenalin, blocked the increase in the electrophoretic mobility of the liver nuclei (Table V). Both compounds, on the other hand, inhibited the postoperative increase in the rate of RNA metabolism. The inability of the hormone to prevent the nuclear surface change, in contrast with its ability to suppress the altered rate of RNA metabolism, constituted a second difference between the two changes.

TABLE III

Lack of Effect of Adrenalectomy on Increase in Nuclear Electrophoretic Mobility After Partial Hepatectomy

	Electrophoretic mobility	
Operative treatment	μ/sec. volt/cm	Average
Sham hepatectomy	−0.78, −0.81	−0.80
Sham hepatectomy + adrenalectomy	−0.80, −0.82	−0.81
Partial hepatectomy	−1.10, −1.14	−1.12
Partial hepatectomy + adrenalectomy	−1.04, −1.14	−1.09

Bilateral adrenalectomy was carried out immediately before the sham or partial hepatectomy and the rats were killed 6 hours later. Liver nuclei were prepared with citric acid and their electrophoretic mobilities were measured as described in Methods.

Not shown are the results that were obtained with ionizing radiation. Whole-body irradiation (5,000 r) given immediately before the removal of two-thirds of the liver had no effect on the increase in the electrophoretic mobility that was measured 6 hours later.

EDTA: Using EDTA, a Zn^{++} requirement for passage through the period preceding DNA forma-

TABLE IV

Effect of Actinomycin D on Increase in Nuclear Electrophoretic Mobility and on the Rate of RNA Synthesis in the Livers of Normal, Sham-Hepatectomized, and Partially Hepatectomized Rats

Operative treatment	Actinomycin D μg/injection	Electrophoretic mobility μ/sec volt/cm	Electrophoretic mobility Average	Liver RNA cpm/gm liver, wet weight	Liver RNA Average
Sham hepatectomy	0	−0.82, −0.83	−0.83	4160, 4020, 4340	4173
	12	−0.82, −0.84	−0.83		
Partial hepatectomy	0	−1.04, −1.02	−1.03	8200, 9220, 8040	8487
	2	−1.14, −1.03	−1.09	5060, 5540, 4780, 3120	4625
	6	−0.89, −−0.91	−0.90		
	12	−0.80, −0.80	−0.80		
None	0	−0.76, −0.74	−0.75	4470, 4540	4505
	2			4690, 4220	4455
	6			3190, 3120, 3150	3153
	12			2000, 1830, 2360	2063

The indicated doses of actinomycin D were injected into the tail vein at 0, 2, and 4 hours postoperatively. The rats were killed 6 hours after the first injection and nuclear electrophoretic mobilities and liver RNA synthesis were estimated as described in Methods.

TABLE V

Effect of p-Fluorophenylalanine and Noradrenalin on Nuclear Electrophoretic Mobility and on the Rate of RNA Synthesis After Partial Hepatectomy

Operative treatment	Inhibitor	Electrophoretic mobiiity μ/sec volt/sec	Electrophoretic mobiiity Average	Liver RNA cpm/gm liver, wet weight	Liver RNA Average
Sham hepatectomy	none	−0.78, −0.80	−0.79	5340, 6160, 5800	5787
	p-fluorophenylalanine	−0.81, −0.79	−0.80		
	noradrenalin	−0.79, −0.78	−0.79	5340, 6840, 6620	6267
Partial hepatectomy	none	−1.02, −1.14, −1.14	−1.10	10,460, 11,960, 12,270	11,563
	p-fluorophenylalanine	−0.74, −0.80	−0.77		
	noradrenalin	−1.08, −1.03, −1.08	−1.06	5740, 4720, 6180	5547

p-Fluorophenylalanine was given into the tail vein: 22 μmoles at zero time, and 5.6 μmoles at 2 and at 4 hours postoperatively. Noradrenalin (200 μg of base) was injected, at zero time only, subcutaneously in the suprascapular region. The rats were killed 6 hours after first injection and the electrophoretic mobilities of liver nuclei and the liver RNA synthesis were estimated as described in Methods.

tion has been demonstrated for kidney (16, 10) and liver cells (15). It was of great interest to learn whether the need for the metal might be related to the events that result in the nuclear membrane change (Table VI). No Zn^{++} requirement for the membrane change could be demonstrated.

Effect of Other Factors

The size of the rat did not affect the electrophoretic mobilities of the liver nuclei. Thus, with nuclei prepared from rats weighing about 400 gm the average electrophoretic mobilities after the sham operation and 6 hours after partial hepatectomy were −0.76 and −1.09, respectively. Nuclei prepared from female rats behaved like those of male animals.

In the preparation of nuclei, increasing the number of cycles of homogenization in citric acid and centrifugation did not alter the mobility of the nuclei. With nuclei from sham-hepatectomized animals, prepared by the standard procedure (2 cycles) and after an additional 3 cycles, the mobilities were −0.78 and −0.78, respectively. With nuclei prepared 6 hours after partial hepatectomy, the comparable values were −1.04 and −1.10. Similarly, storage in 0.1 M citric acid (3°C) had no effect. With freshly prepared nuclei from a sham-hepatectomized animal and after storage for 4 days, the electrophoretic mobilities were −0.78 and −0.80, respectively. With nuclei from an animal 6 hours after partial hepatectomy, the comparable values were −1.10 and −1.15.

TABLE VI

Lack of Effect of EDTA on Nuclear Electrophoretic Mobility after Partial Hepatectomy

Operative treatment	EDTA	Electrophoretic mobility, μ/sec. / volt/sec.	Average	Liver DNA, cpm/gm liver, wet weight	Average
Sham hepatectomy		−0.79, −0.77	−0.78	3500, 2870, 4950	3,740
	+	−0.81, −0.82	−0.82	3230, 4220, 3490	3,647
Partial hepatectomy		−1.10, −1.13	−1.12	30,860, 31,520, 47,420	36,600
	+	−1.05, −1.07	−1.06	10,430, 4130, 4370	6,310

The rats were perfused intravenously with a neutral solution (1 ml per hour) containing Na-EDTA (0.05 M) and $MgCl_2$ (0.002 M) beginning immediately after the operation. Animals that did not receive EDTA were perfused with NaCl (0.15 M, 1 ml per hour). After 22 hours, some of the animals were used for the estimation of electrophoretic mobilities of liver nuclei and others received ^{3}H-thymidine. The radioactivity incorporated into DNA was measured as described in Methods.

DISCUSSION

During the time the liver cell of the partially hepatectomized rat is preparing to replicate its DNA, it undergoes quantitative and perhaps qualitative changes in the metabolism of its RNA (2–5), presumably in the absence of chemical alterations in the composition of its DNA. How are these changes initiated? One way might involve altering the availability of some of the DNA to serve as a template for RNA formation (17). A mechanism that may play an important role in achieving such alterations is the manipulation of the sizes of various micromolecular pools (17). In these terms, membrane changes can ultimately reflect themselves in the RNA and protein metabolism of the cell and may have special importance in the stage that precedes DNA synthesis. It is of interest to note in this connection that an increase in the liver pools of inorganic orthophosphate and Na^+ is detectable 5 minutes after partial hepatectomy.[3]

Several questions are raised by the observation that a change occurs in the electrophoretic mobility of liver nuclei after partial hepatectomy. One of these concerns the membrane structure of the

[3] Gingold, J. L., and Lieberman, I.; and Lieberman, I., and Kane, P., unpublished observations.

nucleus. Since the nucleus is surrounded by two membranes, it is of some interest to know the anatomy of the nuclei that were used for the electrophoretic measurements. Electron microscope studies (18, 19) have revealed that citric acid causes the complete removal of the outer membrane from all rat liver nuclei. In view of this and the fact that chemical or physical changes inside the nucleus cannot affect their electrokinetic properties (14), the altered mobilities would appear to reflect a chemical change in the nuclear membrane.

Other questions that arise include a consideration of the role the membrane change plays in the sequence(s) of reactions that results in DNA formation, the cause and chemical nature of the change, and its relationship to the other events that take place during the first postoperative hours.

The role of the nuclear membrane change during the DNA presynthetic period is unknown. Indeed, it should be emphasized that it is not even known at the present time that the nuclear change (or the cell surface one (11)) is requisite to the later entry of the liver cell into the period of DNA synthesis. To provide such evidence, it will be necessary either to relate nuclear poolsize changes to competency to form DNA or to find a specific inhibitor that blocks both the membrane change and DNA formation.

Only little is known about the cause of the nuclear change and its chemical nature. The ability of actinomycin D and *p*-fluorophenylalanine to suppress the change indicates that prior syntheses of RNA and protein, presumably an enzyme, are essential. Preliminary observations[4] on the chemistry of the change have suggested that it involves loss of some ethanol-soluble material that had decreased the net negative charge of the nuclear membrane. Thus, extraction with ethanol of nuclei prepared from livers of sham-hepatectomized rats raises their mean electrophoretic mobility to that of nuclei from partially hepatectomized animals. Similar treatment of nuclei from partially hepatectomized animals, on the other hand, is without effect.

More information is available about the relationship, or more correctly the absence of a relationship, between the nuclear membrane change and the changes in RNA metabolism that characterize the early period after partial hepatectomy. Inhibitors (noradrenalin and low levels of actinomycin D) that completely block the rate increases in RNA metabolism do not prevent the increase in the electrophoretic mobility of liver nuclei. If both of these changes are essential for the later replication of DNA, they must represent independent, parallel processes at least during the early part of the presynthetic stage.

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