THE SURFACE OF THE LIVER CELL AFTER PARTIAL HEPATECTOMY

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Received June 17, 1965

A change in the surface of the rat liver cell after partial hepatectomy, measured as an increase in electrophoretic mobility, was first reported in 1960 (1). Later, additional studies were made in the same (2) and another laboratory (3) but the chemical nature and the etiology of the surface alteration were not studied.

Evidence consistent with the following conclusions has now been obtained. 1) The rise in electrophoretic mobility of the liver cell after partial hepatectomy results from an increase in surface neuraminate; 2) The neuraminate is bound to a molecule that is soluble in 30-95% ethanol; and 3) The appearance of the neuraminate is dependent upon the synthesis of RNA and functional protein.

MATERIALS AND METHODS

Male albino rats, obtained locally, were used when they weighed about 80 g. Neuraminidase (Vibrio cholerae), N-acetylneuraminic acid (NANA), and N-acetylmannosamine were from General Biochemicals, muramic acid was from the Sigma Chemical Company, and actinomycin D was a gift from Merck Sharp and Dohme Research Laboratories.

Partial hepatectomy refers to the removal of 67% of the liver (4). The sham operation was performed in the same way except that no liver was excised. Unless otherwise indicated, liver samples were taken at 15 hours after the operations, and from sham-operated animals, only the 33% part

Table I

Effects of Neuraminidase on the Electrophoretic Mobilities of Liver Cells from Sham and Partially Hepatectomized Animals

The disaggregated liver cells were washed twice by centrifugation, once with Dulbecco's phosphate-buffered saline solution, and once with Tris-maleate (0.05 M, pH 6.0). After resuspension in the Tris-maleate solution (ca 1.5 x 10^6 cells/ml), the additions were made as indicated and the suspensions were incubated at 37° for 10 minutes with occasional shaking. At the end of this time, the cells were washed twice with a solution of sucrose (0.5 M) and sodium phosphate (0.005 M, pH 7.4) and electrophoretic measurements were made at once. "Control" refers to the liver cells from sham-operated animals, "Experimental", to the cells from partially hepatectomized animals.

Additions			Electrophoretic mobility	
Neuraminidase	Cations	Other	Control	Experimental
units/ml	μatoms/ml	μmoles/ml	$\frac{\mu/sec}{volt/cm}$	
0	0	0	-0.94*	-1.10*
	5 Ca^{++}		-0.94*	-1.12*
			-0.95	-1.11
3				-1.09
10			-0.92	-0.94
30			-0.89	-0.90
10	0			-1.11
10	5 Mg^{++}			-1.06
10	5 Ca^{++}	0.5 NANA		-0.94
		1.5		-1.09
		5.0		-1.12
		15 N-acetyl-mannosamine		-0.93
		15 pyruvate		-0.92
		15 lactose		-0.92
		5 muramate		-0.93
		5 muramate + 1.5 NANA		-1.11

* The cells were not incubated at 37°

of the liver was used for analysis.

The preparation of liver cells and the estimations of their electro-

phoretic mobilities were as described by Eisenberg, Ben-Or, and Doljanski (2) except that the rates of cell migration were estimated in 0.5 M sucrose - 0.005 M sodium phosphate (pH 7.4) and only 20 measurements were made on each sample. All the measurements were made on coded cell suspensions and each of the values shown is the average of the results obtained with 3 rats. The standard errors of the means ranged from $\pm$ 0.004 to $\pm$ 0.012.

NANA was measured with 2-thiobarbituric acid (5) and DNA by the method of Burton (6). Cell counts were made with a Levy counting chamber.

RESULTS

Effects of Neuraminidase - A preparation of Vibrio cholerae neuraminidase reduced the electrophoretic mobility of the liver cells from partially hepatectomized animals to the mobility of the cells from sham-operated animals[1] (Table I). The table shows some of the evidence that neuraminidase rather than a contaminating enzyme was responsible for altering the surface properties of the experimental cells. Thus, Ca^{++} was required (7) and NANA (1.5 μmoles/ml), but not related compounds, blocked the action of the enzyme preparation.

Additional evidence for the identity of the activity with neuraminidase was obtained by measuring the effects of the enzyme preparation on liver cells as a function of pH (Table II). In keeping with the properties of Vibrio cholerae neuraminidase (7), the enzyme preparation was active over a broad range of pH but its activity fell off sharply between pH 5.0 and 3.9 and between pH 7.0 and 7.8.

Neuraminidase released a slightly greater amount of NANA (per mg of cell DNA) from the cells of the partially hepatectomized animal than from the control cells. Thus, incubated at 37° without enzyme and with 30 units of neuraminidase, 0.03 and 0.16 μmole of NANA were released from

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1) Liver cells from normal rats behaved exactly as cells from sham-operated animals.

Table II

The Effects of Neuraminidase as a Function of pH on the Electrophoretic Mobility of Liver Cells from Partially Hepatectomized Animals

The treatment of the cells was the same as for Table I except that the pH of the Tris-maleate buffer was varied as shown. All the reaction mixtures received $CaCl_2$ (0.005 M) and neuraminidase (10 units/ml) was added as indicated.

Neuraminidase	pH	Electrophoretic mobility
		$\frac{\mu/sec}{volt/cm}$
-	3.9	-1.14
+		-1.13
-	4.4	-1.12
+		-1.01
-	5.0	-1.10
+		-0.95
-	6.0	-1.13
+		-0.95
-	7.0	-1.13
+		-0.95
-	7.8	-1.12
+		-1.08

the cells of the sham-operated animal, respectively, and the comparable values for the cells of the partially hepatectomized animal were 0.04 and 0.21 μmole.

Effect of Ethanol - Treatment of the liver cells from partially hepatectomized animals with 30% ethanol reduced their electrophoretic mobility to that of the cells from sham-operated animals (Table III). As the table shows, the electrokinetic properties of the control cells were only slightly affected by 30% ethanol. The table does not show the results of the measurements of NANA released by ethanol. From the control cells, 0.06, 0.08 and 0.18 μmole of NANA were extracted (per mg of cell DNA) by

Table III

Effect of Ethanol on the Electrophoretic Mobilities of Liver Cells From Sham and Partially Hepatectomized Animals

The cells were thrice extracted with the indicated concentrations of ethanol in water (0°, 2 ml/extraction). The extracted cells were then washed with a sucrose-sodium phosphate solution and electrophoretic measurements were made as described in "Materials and Methods". Treatment with neuraminidase (10 units/ml) was detailed in Table I. "Control" refers to the liver cells from sham-operated animals, "Experimental", to the cells from partially hepatectomized animals.

Ethanol	Electrophoretic mobility	
	Control	Experimental
%	$\frac{\mu/sec}{volt/cm}$	
0	-0.94	-1.13
0 (neuraminidase)	-0.91	-0.93
10		-1.05
20		-1.03
30*	-0.91	-0.90
95	-0.73**	-0.73**

* Similar results were obtained when the ethanol was diluted with phosphate-buffered saline solution.

** Many of the liver cells were motionless in the electric field and they were not considered in the calculation of electrophoretic mobility.

10, 20, and 30% ethanol, respectively. With the experimental cells, the comparable values were 0.11, 0.12, and 0.23 μmole.

Effects of Actinomycin D and p-Fluorophenylalanine - The cell surface change, as measured electrophoretically, was shown to be complete at about 6 hours after partial hepatectomy (2). Hourly (0 to 5 hours) intraperitoneal injections of actinomycin D and p-fluorophenylalanine

have now been found to block completely the cell surface change measured 6 hours after partial hepatectomy. The electrokinetic properties of liver cells from sham-operated animals were unaffected by the inhibitors. Thus, after injections of NaCl (0.15 M), actinomycin D (2 μg/hour), and p-fluorophenylalanine (15 μmoles/hour), the electrophoretic mobilities of liver cells from partially hepatectomized animals were -1.10, -0.95, and -0.93, respectively. The comparable values with cells from sham-operated animals were -0.94, -0.95, and -0.94.

DISCUSSION

The NANA that appears on the liver cell surface after partial hepatectomy seems to be bound to a molecule that is soluble in 30-95% ethanol. Although the nature of the ethanol-soluble molecule is not yet known, these observations are perhaps more suggestive of a mucoprotein than a ganglioside.

Both neuraminidase and 30% ethanol markedly reduce the electrophoretic mobility of liver cells from partially hepatectomized animals and have relatively little effect on the mobility of cells from sham-operated animals. This difference is not accompanied by a similarly large discrepancy in the amount of NANA released from the control and experimental cells. It must follow, therefore, that most of the released NANA was not on the cell surface or, for one of the reasons considered by Wallach and Esandi (8), its removal causes no alteration in the net surface charge of the cell. Removal of the NANA that appears after partial hepatectomy, on the other hand, leads to a loss of negative charge from the cell surface.

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