MECHANICAL PROPERTIES OF INTERCELLULAR JUNCTIONS OF HEPATOCYTES IN INBRED MICE AND PREDISPOSITION TO SPONTANEOUS HEPATOMA

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Data showing that predisposition to tumors depends on inherited features of the tissue systems of the body have recently been obtained [8, 9]. In the study of intercellular junctions in the lung tissues of inbred lines of mice, negative correlation was found between the adhesiveness of the cells in the tissue and the frequency of spontaneous and induced tumors in it [4]. It has been suggested that genetically determined strength and stability of intercellular interaction in a tissue determines its predisposition to tumors.

The object of this investigation was to test the validity of these ideas for the liver. The adhesiveness of hepatocytes was measured in inbred lines of mice differing in their frequency of spontaneous hepatomas. In addition, the mechanical strength of intercellular junctions was studied at the ultrastructural level in two lines of mice differing maximally in the adhesiveness of their hepatocytes.

EXPERIMENTAL METHOD

Mice of seven lines (A/Y, Balb/cY, CBA/Y, C3HA/Y, C3H/Y, C57Bl/6Y, and CC57Br/Y) aged one month were used. Adhesiveness was measured by means of a micromanipulator by Coman's method [4, 7]. Adhesiveness was measured in three or four mice of each line, and 20 cells were chosen in each liver.

TABLE 1. Adhesiveness of Hepatocytes and Frequency of Spontaneous Liver Tumors in Mice of Inbred Lines

Line of mice	Sex	Adhesiveness, mg/cell (M ± m)	Frequency of spontaneous liver tumors in mice aged			Source
			12 months	14–15 months	22–24 months	
C57Bl/6Y	♂	0,160±0,005	0/75			o.d.
					<0,4%	[3]
Balb/cY	♂	0,142±0,002	0/20			o.d.
CC57Br/Y	♂	0,127±0,002	0/20			o.d.
				<1%		[3]
A/Y	♂	0,113±0,006	0/32			o.d.
			0/230			[5]
C3H/Y	♂	0,088±0,005	0/5			o.d.
				27%		[3]
C3HA/Y	♂	0,078±0,005	0/19	0/14	2/20=10%	o.d.
					28,3%	[2]
	♂	0,056±0,002		5/9=55,5%	11/21=52,5%	o.d.
	♂	0,056±0,002	35,2%	68,5%		[1]
CBA/Y	♀	0,053±0,002			3/29=17%	o.d.
			5,3%	11,5%	14,6%	[1, 2]

Legend. o.d.) Our data – number of animals with spontaneous liver tumors; in the batches of mice which were used to measure the adhesiveness of the hepatocytes.

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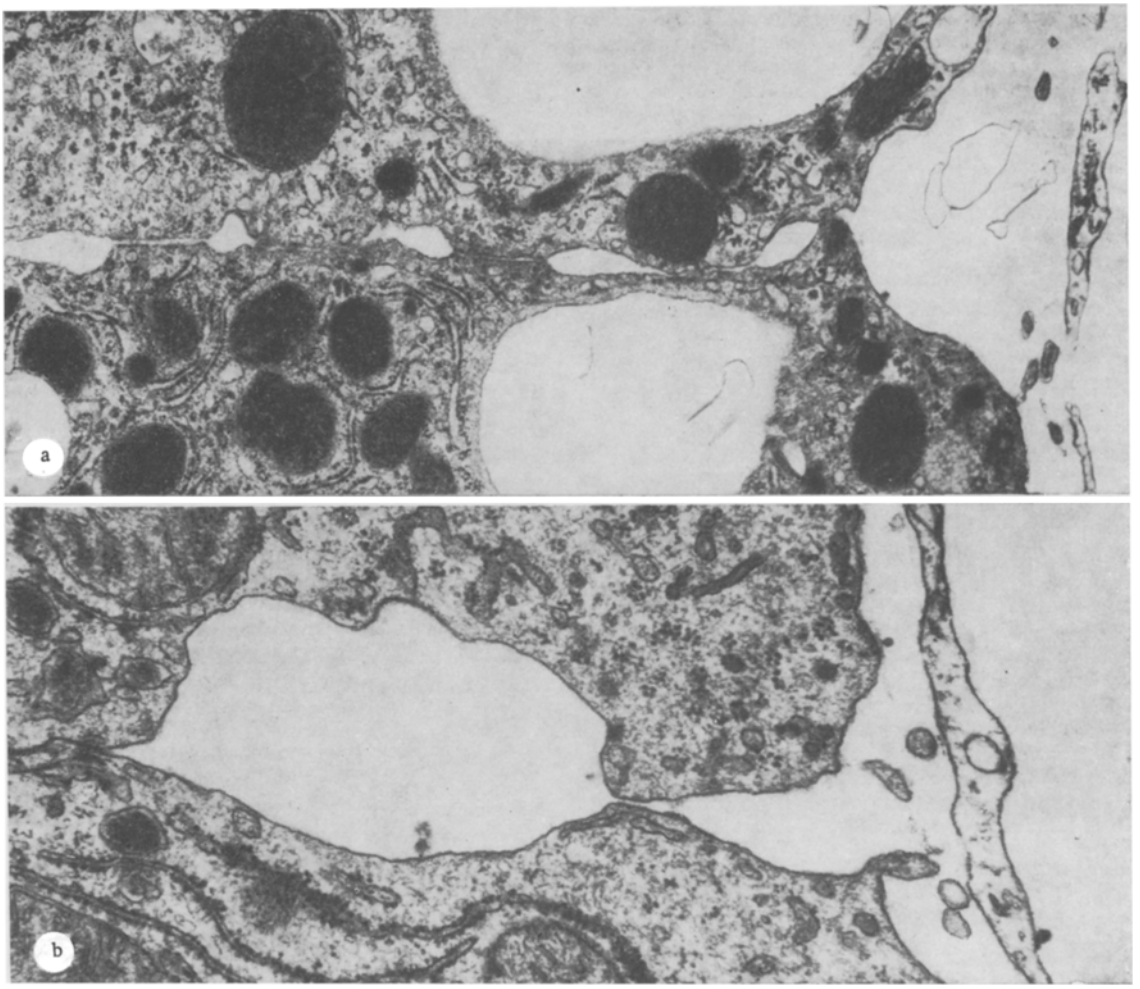

Fig. 1. Destruction of unspecialized areas of junctions between hepatocytes in C57Bl mice under the influence of increased hydrostatic pressure: a) action of pressure of 30 mm Hg for 15 sec, 19,800×; b) 20 mm Hg, 60 sec, 35,000×.

The mechanical properties of the intercellular junctions were studied at the ultrastructural level on CBA and C57Bl males by Ushakov's method. The liver was perfused in situ with Hank's solution, initially under the pressure of 10 mm Hg for 30 sec in order to remove blood from the liver, and later with a hydrostatic pressure in the blood stream of 20-30 mm Hg for 15-60 sec. Without changing the pressure, the Hanks's solution was replaced by a 2.5% solution of glutaraldehyde (4°C). The pieces were postfixed with 1% OsO_4 solution, stained with 1.5% uranyl acetate solution, and embedded in Araldite M. Ultrathin sections were stained with lead citrate. The linear dimensions of the junctions and the angles formed by the membranes were estimated by means of a scaling grid, projected on the microscope screen. Six mice of each line were studied. Two pieces of liver were investigated from each animal. Twenty sections were examined from each piece of liver. Measurements were made on five random sections from each piece, at a distance of not less than 10 μ from each other.

EXPERIMENTAL RESULTS

The adhesiveness of the hepatocytes in mice aged one month and data in the literature on the frequencies of spontaneous hepatomas in mice of these lines are given in Table 1, from which it will be clear that the rule found previously for the lung is valid also for the liver. In CBA, C3H, and C3HA mice, in which spontaneous hepatomas were found at the age of 12-24 months, the adhesiveness of the hepatocytes was already significantly lower at the age of one month than in animals of more resistant lines: C57Bl and Balb/c,Y. Because of the absence of data in the writers' laboratory on the frequency of spontaneous hepatomas in the animals of the various lines studied, it was impossible to determine the coefficient of correlation between these parameters.

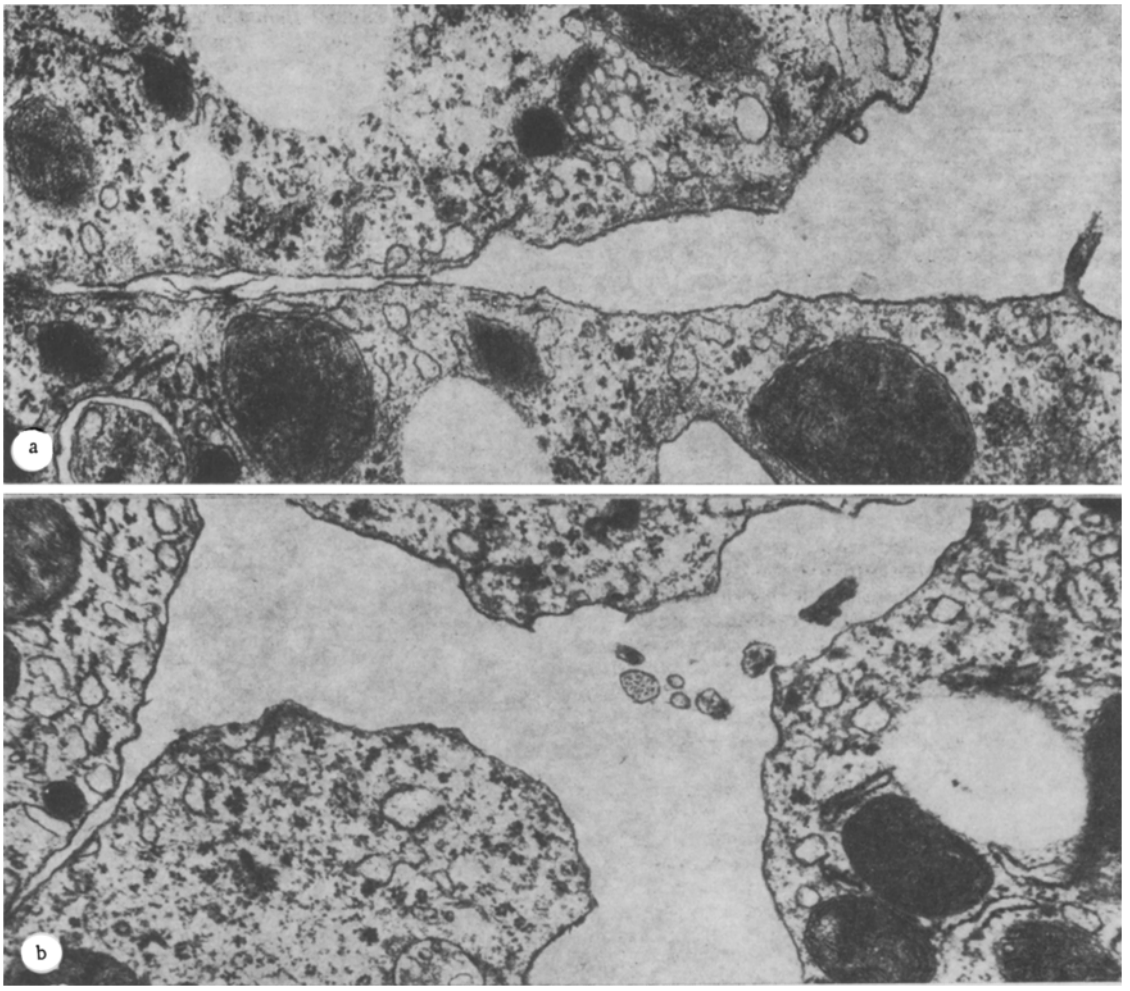

Fig. 2. Destruction of unspecialized areas of junctions between hepatocytes in CBA mice under the influence of increased hydrostatic pressure: a) action of pressure of 30 mm Hg for 15 sec, 43,200×; b) 20 mm Hg, 60 sec, 33,000×.

The adhesiveness of hepatocytes did not differ significantly in male and female CBA mice (Table 1), although their frequency of spontaneous hepatomas was different. This indicates that endogenous humoral factors, with whose action the observed sex differences are linked [6], either exhibit their action at other levels or affect adhesion of cells at an older age.

Increased hydrostatic pressure causes separation of the contacting surfaces of the hepatocytes, initially in those areas of the junctions where no specialized structures are observed in the intact tissue, and membranes of neighboring cells run parallel to each other 150-200 A apart. Under these circumstances heterogeneity of these areas of contact is observed: the areas of separation are often interrupted by regions of between 0.05 and 1 μ in which the original distance between the membranes is preserved. We called these highly adhesive regions (HAR; Fig. 1a). The separated surfaces of the hepatocytes close to the HAR form different angles. The size of the angles depends on the disuniting force which can maintain the preserved area of contact and on the viscoelastic properties of adjacent regions of the cell surfaces.

Different lines of mice differ in the degree of heterogeneity of the unspecialized regions of the junctions. For instance, for every 20 μ of the outline of the cell surface separated as a result of exposure to a pressure of 30 mm Hg for 15 sec in line C57Bl, there are 20.5 ± 3.7 (mean deviation) HAR, compared with 6.4 ± 2.1 HAR in CBA mice. The angle of HAR in C57Bl was 55.3 ± 8.0° (Fig. 1a) and in CBA mice 29.2 ± 4.1° (Fig. 2a). The difference between the number of HAR and the size of the angles in CBA and C57Bl mice was statistically significant ($P < 0.001$). A pressure of 20 mm Hg for 60 sec caused the appearance of wide intercellular spaces in C57Bl mice, interrupted in places by HAR (Fig. 1b). Under these conditions HAR were not preserved in CBA mice (Fig. 2b). Under the pressures used, no separation was observed in specialized structures of the junctions (in desmosomes, zones of adhesion, gap and dense junctions).

The difference in the number of HAR found between hepatocytes of CBA and C57Bl mice agrees in magnitude with the difference in adhesiveness between these cells. It can therefore be tentatively suggested that the degree of heterogeneity of unspecialized regions of junctions largely determines the differences found in adhesiveness of cells in inbred lines of mice.

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