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The intracellular potential of regenerating liver

During the past decade, several alterations in the properties of the cell membrane have been described in systems characterized by comparatively frequent cell division when compared to the similar nongrowing tissue. Among these changes are an increase in surface charge density[1,2], a decreased permeability to sucrose[3], and a decreased permeability to potassium and sodium[4,5]. The conclusion that, with growth, there is a decrease in membrane permeability to potassium has been based partly on an increase in the concentration gradient of potassium coupled with no increase in the ionic flux. Similar changes might result from an increased binding of potassium in a growing cell even though data from the previous work on potassium exchange in normal liver and 48-h regenerating liver was interpreted as showing little increase in potassium complexing[5].

The present study was designed to compare the membrane potential of normal rat liver parenchymal cells to that of regenerating liver 48 h after a subtotal hepatectomy under the same conditions used for the previous flux studies[5]. Two groups of male Sprague–Dawley rats were used. In one group, two thirds of the liver was removed; 48 h later the animals were killed by a blow on the head. The second group of animals, with normal liver, underwent no operative procedure but were killed in a similar manner. In both groups, the liver was removed and tissue slices approx. 0.3 mm in thickness were cut and put into flasks containing 15 ml of a Krebs–Henseleit solution containing glucose and 5.0 mequiv/l of potassium. The flasks were gassed with O_2–CO_2 (95:5, v/v), then shaken in a water bath at either 35° or 15°

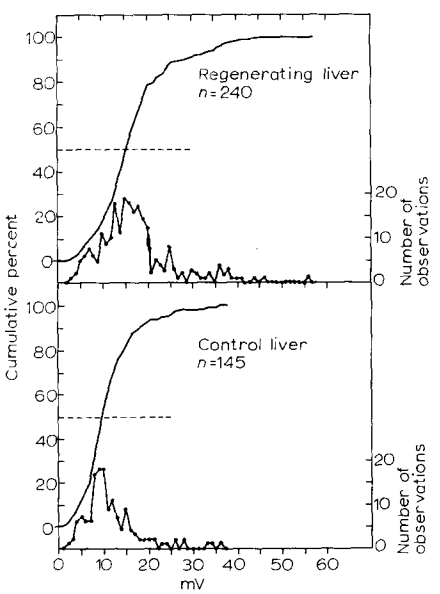

Fig. 1. Membrane potential, 15°. ·····, median value. *n* is the number of observations. The left ordinate gives the cumulative percentage of observations at or below a given value of the intracellular potential.

for 80 min. This period of incubation was necessary to bring the slices into a steady state with respect to sodium and potassium. Following this preliminary incubation, a slice was removed, placed between grids, and suspended within a special chamber containing the same incubation fluid for the micro puncture studies. The chamber was kept at a given temperature by use of a heating stage. O_2–CO_2 (95:5, v/v) was bubbled through the chamber continuously.

To measure the membrane potential, liver cells were impaled with glass capillary micro-electrodes filled with 3 M KCl. Electrodes with resistances between 20 and 50 mΩ were used. The electrodes were connected to a conventional cathode follower preamplifier through Ag–AgCl electrode. The potentials were displayed on a cathode ray oscilloscope and photographed.

A parallel series of experiments was done in which a tracer amount of radio-iodinated serum albumin was added to the incubation fluid as an indicator of the extracellular fluid volume. After similar incubation periods, the slices were removed, blotted, and weighed in tared tubes. They were dried overnight, weighed again, and then digested in 0.5 M LiOH. Each sample was analyzed for potassium, sodium and radio-iodinated serum albumin. These data permitted a calculation of the intracellular concentration of each ion from a knowledge of the total water, total sodium and potassium, extracellular water and extracellular ionic concentration.

The values of the potential difference across the membrane of both normal and regenerating liver cells at 15° are plotted in Fig. 1. The horizontal line indicates the median value of the membrane potential for each tissue. The mean intracellular concentrations of sodium and potassium at 35° and at 15° are listed in Table I together with the median membrane potentials at the given temperature and the potassium potential calculated from the intracellular–extracellular ratios of this ion. It is apparent that only at 15° there is a significant difference between the median membrane potential of normal liver cells and of regenerating liver cells.

From the observed membrane potential and the extracellular concentration of potassium, an intracellular concentration of potassium may be calculated with the assumption that the entire potential is due to potassium. This value will divide the total cell potassium into two fractions, one termed "free" and the other "bound".

TABLE I

	Normal liver		*Regenerating liver*	
	35°	*15°*	*35°*	*15°*
Intracellular concentration, mequiv/l of intracellular water				
K^+	98 ± 1.1	18.8 ± 0.7	152 ± 1.7	56.1 ± 2.4
Na^+	143 ± 2.8	172 ± 3.3	81.7 ± 3.3	140.1 ± 4.0
Median membrane potential, mV	28	10	29	16
Calc. K^+ potential, mV	77.5	32.5	89.0	58.8
Ratio: $\frac{\text{Membrane potential}}{K^+ \text{ potential}}$	0.36	0.31	0.33	0.28

Over the range of values in this experiment, the bound potassium is a linear function of the total in spite of the difference in tissue and in incubation conditions.

The median membrane potential at 35° reported here is similar to other reports. LI AND MCILWAIN[6] reported a mean intracellular potential of *minus* 35 mV in guinea pig liver slices incubated *in vitro* at 37.5°. PENN[7] found a potential difference of 35–45 mV for mouse liver cells *in situ*, but only 15–25 mV *in vitro*. From the equation based on Goldman's constant field theory, using these data, and with the knowledge that 32% of the intracellular sodium is not exchangeable under these conditions, the ratio P_{Na^+}/P_{K^+} for normal liver at 35° will vary from 0.11 for a P_{Cl^-}/P_{K^-} of 0.45 to 0.25 for a P_{Cl^-}/P_{K^+} of 1.0. From measurements on rat liver *in situ*, SHANNE and CORABOEUF[8] calculated a P_{Na^+} of 0.3.

In the present study, the measured potential difference is from 0.28 to 0.36 that of the calculated potassium potential difference. Under these experimental conditions, the slices gain sodium and chloride, so some nonspecific shunting is possible. At 15°, the ratio of the intracellular potassium concentrations of the two tissues is greatest and the resulting difference in potential becomes significant. This demonstrated difference between the measurements and the constancy of the fraction of "inactive" potassium, would seem to rule out increased potassium complexing as the sole cause of the previously reported increase in intracellular potassium in a growing tissue, although it does not exclude an increase due to the Donnan effect.

It must be concluded that the permeability characteristics of the cell membrane of nonexcitable tissues can change. This change is reflected in the behavior of the cell. A decrease in permeability is associated with both normal and abnormal growth. In those tissues examined the change seems to occur very early in the growth cycle. It is not clear, however, whether the control of cell growth is mediated through the permeability characteristics of the membrane, or whether the changed permeability is a secondary consequence of cell growth.

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