of an *in vitro* assay for this and other murine leukemia viruses.

Summary. Cell cultures grown in modified NCTC 109 medium and infected with the Rauscher leukemia virus showed CPE when subcultured in Eagle's basal medium. Non-infected control cultures treated in a similar manner showed no CPE. The CPE in infected cultures included increased cytoplasmic eosinophilia in the form of either eosinophilic granules or inclusion bodies, cytoplasmic vacuolization, and marked variation in the size, shape, and intensity of staining of cell nuclei.

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Effects of Sugars on Potential Difference Across Wall of Small Intestine of Rodents. (30133)

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It seems well established that the potential difference (PD) across the wall of the small intestine depends on the presence of glucose on the mucosal side(1,2,3). Furthermore the short-circuit current across the intestinal wall was also dependent upon the mucosal glucose, and the results imply that the active transport of glucose is intimately related to the electrical phenomena, which, in turn reflect the active transport of Na ions(4). However these observations were made only with the small intestine of the rat. In view of the fact that there is a difference among species of animals with regard to intestinal handling of different sugars, it seemed worthwhile to investigate the effect of various sugars on the PD with different animal species. For this purpose the small intestines of the rat, the golden hamster, and the guinea pig were tested with glucose, galactose, fructose, sorbitol, and 3-0-methyl glucose in relation to PD.

Method. Experimental animals used were female Wistar strain rats which weighed between 100 and 180 g, female golden hamsters which weighed between 80 and 110 g, and guinea pigs of either sex which weighed between 300 and 500 g. Animals were fasted overnight prior to experiment except that water was available *ad libitum*.

The experimental procedure was similar to that described previously(2), except that a Keithley electrometer amplifier (#603) was used for determination of PD and the experiments were carried out at 33°C. The animal was anesthetized lightly with ether and a portion of the middle segment of the small intestine (10 to 15 cm in length) was excised and made into an everted sac. Glucose free Ringer's fluid (0.6 to 1.0 ml) was deposited in the sac (serosal side) and this fluid remained therein throughout the experiment. The sac was immersed in about 250

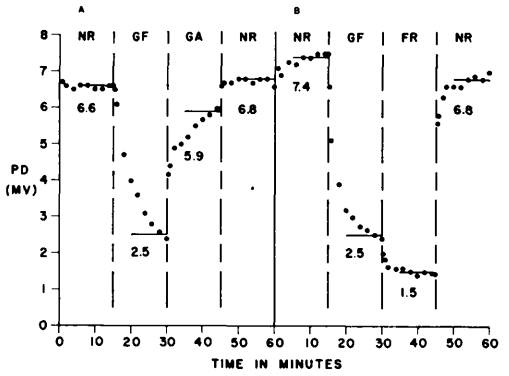

FIG. 1. Effect of sugars on PD in hamster small intestine. NR, GF, GA, FR indicate application of normal Ringer's fluid, Ringer's fluid without glucose, Ringer's fluid containing galactose, or fructose at concentration of 11.1 mM. *Ordinate* indicates PD in mv and *abscissa* time in min. The most representative values for each treatment are shown in Figure.

ml of Ringer's fluid of various sugar composition successively with 15-minute intervals. The PD between the inside and outside of the sac was led through saturated KCl agar bridges connected to calomel electrodes and was determined with the electrometer.

The composition of the normal Ringer's fluid was as follows: NaCl 125 mM, KCl 5 mM, $NaHCO_3$ 10 mM, NaH_2PO_4 1.2 mM, $CaCl_2$ 1.4 mM and glucose 11.1 mM. The solution was equilibrated with a gas mixture of 5% CO_2 and 95% O_2 bubbled into the mucosal solution. The pH was about 7.0. Glucose was depleted from the normal Ringer to make glucose-free Ringer and no adjustment was made for the change in osmotic pressure. Modified Ringer's fluid was made by substituting glucose with D-galactose, D-fructose, D-sorbitol, and 3-0-methyl D-glucose at the concentration of 11.1 mM.

Results. The PD obtained when the normal Ringer's fluid was applied on the mucosal side was 8.7 ± 1.4 mv for the rat, 6.7 ± 1.2 mv for the golden hamster, and 5.9 ± 1.0 mv for the guinea pig, the serosal side being positive. These values are the mean and s.d. of 27, 25, and 27 values for each species obtained at 15 minutes after the start of the experiment. The PD showed slight differences in magnitude among species. With intestine from the rat and hamster, the PD stayed approximately at the initial level for one hour or more, so long as glucose was present on the mucosal side. With intestine from the guinea pig the PD was not maintained with such regularity and during a one-hour period it declined steadily to 80 to 90% of the initial value.

Fig. 1 shows a typical result obtained with hamster intestine. In Fig. 1A the mucosal solution was changed successively with normal Ringer's, glucose-free Ringer's, galactose-Ringer's, and again normal Ringer's fluid. In Fig. 1B fructose Ringer's was used instead of galactose-Ringer's. In each treatment the PD assumed steady characteristic values, which were obtained by inspection as shown in the Figure. These values are compiled with rats and hamster in 4 series of experiments, each comprising 6 experimental runs. The mean and s.e. are shown in Table IA and IB. The recovery of the PD at the final application of normal Ringer's fluid was complete for rats and hamster, although the intestine had been exposed to glucose-free and modified Ringer's for 30 minutes. The results with rats were similar to those already reported(4), except that in the case of 3-0-methyl glucose a small but

TABLE I. PD Across Intestinal Wall Affected by Application of Sugars (mv).

Test sugar	Treatment NR	GF	TS	NR
	A. Rat			
GA	8.9 ± .5	3.9 ± .5	7.6 ± .7	8.8 ± .6
FR	9.6 ± .2	3 9 ± .4	2.1 ± .3	8.3 ± .2
SO	7.9 ± 1.0	3.1 ± .5	1.3 ± .2	7.2 ± .7
MG	8.6 ± .3	3.8 ± .2	4.9 ± .2	8.3 ± .3
	B. Golden Hamster			
GA	6.8 ± .4	2.6 ± .2	6.2 ± .3	7.1 ± .3
FR	7.5 ± .6	2.9 ± .3	1.6 ± .2	6.8 ± .5
SO	6.1 ± .5	2.3 ± .3	.9 ± .2	5.6 ± .4
MG	6.6 ± .2	2.4 ± .1	2.4 ± .2	6.3 ± .2
	C. Guinea Pig			
GA	6.0 ± .3	3.8 ± .3	5.1 ± .3	4.9 ± .1
FR	5.7 ± .5	4.2 ± .3	4.6 ± .3	6.2 ± .5
SO	6.1 ± .4	4.7 ± .5	3.3 ± .4	4.8 ± .3
MG	6.1 ± .5	5.1 ± .6	4.1 ± .6	5.1 ± .6

Mean and S.E. of the PD are shown. In each row 6 experiments are performed. The intestine was treated successively with NR, the normal Ringer's fluid, GF, glucose free Ringer's fluid, TS, Ringer's fluid containing test sugar, and finally again the normal Ringer. Test sugar are either GA, galactose, FR, fructose, SO, sorbitol, or MG, 3-0-methyl glucose as indicated in the left column.

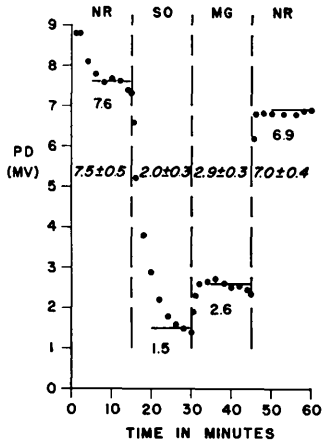

FIG. 2. Effect of 3-0-methyl glucose compared with that of sorbitol in hamster intestine. NR, SO, MG indicate application of normal Ringer's fluid, Ringer's fluid containing sorbitol or 3-0-methyl glucose at concentration of 11.1 mM. *Ordinate* indicates PD in mv, and *abscissa* time in min. The most representative values for each treatment are shown in Figure. The mean and s. e. of 6 similar experiments are shown in italics.

definite recovery from the glucose-free condition was detected. Barry *et al* also noted the effect of 3-0-methyl glucose(3).

The results with hamster intestine were almost identical with those of rat intestine, except that with 3-0-methyl glucose the PD neither rose nor decreased from the glucose-free level. This finding, however, may be interpreted as a beneficial effect of 3-0-methyl glucose, not the absence of it. Sorbitol or fructose induced a further reduction from the glucose-free level, due presumably to the osmotic effect. If 3-0-methyl glucose is ineffective to the same extent as these two, one must expect also a reduction of the PD. The absence of this reduction must indicate enhancement of the PD, however small it may be. To prove this point a series of experiments was performed in which the hamster intestine was exposed to sorbitol Ringer's instead of glucose-free Ringer's followed by 3-0-methyl glucose Ringer's. A typical result is shown in Fig. 2, where the mean and s.e. of 6 similar experiments are shown in italics. There is clearly a recovery through the transition of 2 sugars.

The results with the guinea pig intestine were somewhat different from those described above as shown in Fig. 3A and 3B, and Table IC. Table IC indicates the mean and s.e. of 6 similar experiments for each series of test sugar applications. The effect of glucose depletion was observed but was much smaller than with the former 2 species; and recovery at the final application of glucose was not complete. A noteworthy difference is the effect of fructose. At the transition from glucose-free Ringer's to fructose-Ringer's there was a dip in the PD which usually lasted for about one minute, then there was an increase above the glucose-free level. This increase, however, was not maintained during fructose application, but the PD gradually declined to such an extent that at the end of 15 minutes the PD was lower than at the glucose-free status. Furthermore at the application of normal Ringer's the PD promptly increased over the initial level, although the enhancement was not sustained. Therefore it was difficult to assign a value representing the steady value of the PD at these stages and the values listed in Fig. 3B and Table IC under the heading of fructose and final normal Ringer's were obtained at the highest level at these stages and were essentially different from other values. Further difference was the absence of the effect of 3-0-methyl glucose. There seems to be no significant difference between the effect of sorbitol and 3-0-methyl glucose on the PD.

Discussion. The results presented here

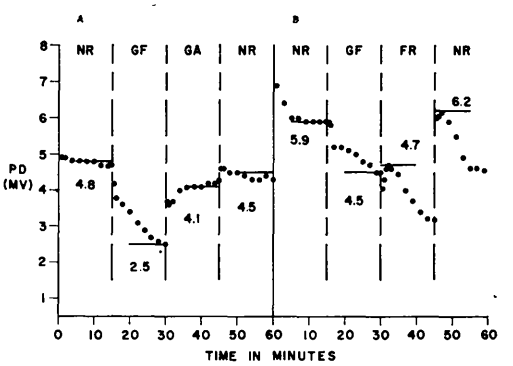

FIG. 3. Effect of sugars on PD in guinea pig small intestine. NR, GF, GA, FR indicate application of normal Ringer's fluid, Ringer's fluid without glucose, Ringer's fluid containing galactose, fructose at concentration of 11.1 mM. *Ordinate* indicates PD in mv and *abscissa* time in min. The most representative values for each treatment are shown.

seem to support the hypothesis propounded in earlier reports(2,4) that the PD across the intestinal wall requires the presence of actively transported sugars, at appropriate concentrations on the mucosal side. The sugars tested in this study can be classified according to their modes of transport and metabolism in intestinal mucosa(5). In the intestines of rats and hamsters, glucose and galactose are actively transported and metabolized, while fructose is not actively transported but metabolized. On the other hand 3-0-methyl glucose is actively transported but not metabolized. Finally sorbitol is neither actively transported nor metabolized. The fact that in rat and hamster intestines the PD is supported by glucose, galactose, and 3-0-methyl glucose though to various degrees, but not by fructose and sorbitol indicates clearly the relevance of the active transport of sugars, not of the metabolism, on the maintenance of the PD. The effect of 3-0-methyl glucose shown here seems to give unequivocal support to this thesis, since it is not metabolized and there was no metabolizable substance in the serosal and mucosal solutions.

The importance of glucose on the mucosal side of the intestine for fluid transfer was demonstrated in the rat(6); however, in hamster intestine a sizable fluid transport was observed in the absence of glucose(7). As for the PD there is no difference between the two species with their dependency on sugars.

The small intestine of the guinea pig differs somewhat from that of the rat and hamster with respect to transport of sugars. Glucose and galactose are transported actively(8). It has been shown that fructose is largely converted into glucose, which is actively transported and accumulated in the serosal solution, although only fructose is applied on the mucosal side(8). This conversion is either absent or very small in the rat(9). Therefore the enhancement of the PD with fructose in guinea pig intestine, however transitory it may be, can be correlated with the conversion of fructose to glucose. Subsequent decline of the PD cannot be explained in this simple way. As there is no report on absorption of 3-0-methyl glucose in the guinea pig intestine, it is not possible to correlate the mode of transport and the effect on the PD. However, failure to support the PD as shown here might imply that this sugar is not actively transported in the small intestine of the guinea pig.

Summary. 1. The potential difference across the wall of the middle section of the small intestine was determined with rats, golden hamsters, and guinea pigs *in vitro*. The value obtained when normal Ringer's fluid was applied to the mucosal side was 8.7 $\pm$ 1.4 mv for the rat, 6.7 $\pm$ 1.2 mv for the golden hamster, and 5.9 $\pm$ 1.0 mv for the guinea pig, the serosal side being positive. 2. The potential difference was affected by sugars applied on the mucosal side. With rats and hamsters, the PD was enhanced by glucose, galactose, and 3-0-methyl glucose but not by fructose or sorbitol. 3. The potential difference was supported by glucose and galactose in the guinea pig intestine. Fructose gave a transitory enhancement in the potential difference, but 3-0-methyl glucose and sorbitol had no effect.

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