out that the competitive "cell for cell" theory used to explain the virus-interference phenomenon has not been rigorously proved to occur in experimentally infected animals, since this explanation is based on bacterial-virus systems and the chick embryo-influenza system. Experiments are in progress to determine whether certain animal virus-interference systems are actually due to competition for susceptible host cells between two viruses.

Note added in proof: The RIP is highly specific under the experimental conditions shown in Table 1, nine bacterial species and nine viruses being tried as well as various other substances. All gave no interference. The RIP is also independent of the time that the challenge dose is given, provided that it is after the protective dose but not longer than about 10 days after the protective dose (*1*).

Recent results in animals, using a neurotropic virus system, have strongly indicated that in this case interference cannot be due to a saturation of susceptible cells by the protective dose. These results so far are very similar to those described here for the RIP.

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Potassium and Sodium Balance in Mammalian Red Cells

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Estimations performed by flame spectrophotometry (*1*) of the red cell potassium and sodium content for nine mammals (man, baboon, rabbit, rat, horse, sheep, ox, cat, and dog) showed considerable species variation, whereas the chloride and calculated bicarbonate values were more constant (Table 1). The results confirm values by chemical methods for some species previously reported (*2, 3*). The individual red cell : plasma concentration ratios ranged from 1.5 to 32 for potassium and 0.1 to 0.9 for sodium; the red cell : plasma chloride and bicarbonate ratios were 1.2 to 1.6. The mechanism of distribution of potassium and sodium is, therefore, different from that of chloride or bicarbonate.

Blood specimens from the various species were collected in tubes containing heparin powder of low sodium content and were spun immediately under oil; the supernatant plasma, buffy coat, and superficial red cells were removed within 15 min of sampling to control cell-plasma ion and fluid exchange. The packed red cells were resuspended in an equal volume of:

1) One-percent NaCl-glucose-phosphate buffer, *p*H 7.6, at room temperature; a steady state with minimal electrolyte variation and normal glycolysis occurred for 6 to 12 hr (*1*).

2) Buffer with or without glucose and refrigerated at 2° to 7°C for 3 to 7 days; glycolysis was minimal; in accord with chemical concentration gradients, potassium diffused out and slightly more sodium in, with resultant cell swelling (Table 2*a*).

3) Cells from the refrigerated buffer were resuspended in isotonic saline-glucose-potassium, 5 milliequivalents per liter phosphate buffer solutions (*p*H, 6.8–8.0), in volume equivalent to that removed, and incubated at 37°C for 6 to 8 hr; restoration of glycolysis was associated with sodium extrusion slightly greater than potassium influx, with correction of cell volume and content (Tables 2*b* and 3).

Samples were removed periodically for potassium, sodium, and chloride estimations in the whole suspension and fluid medium, with calculation of the red cell values from determination of the red cell water and the packed cell volume corrected for trapped intercellular fluid (*1*). The hematocrit values were used to calculate water shifts in the system.

From chloride values and *p*H determinations of the fluid medium and red cell hemolysate, a Donnan ratio, r, $[Cl^-]_e/[Cl^-]_i = [H^+]_i/[H^+]_e$, calculated in log

Table 1. Ionic patterns of mammalian red cells; mean values. The notations [K], . . . denote milliequivalents per liter of red cell or plasma water.

Species and No: estimated	Red cells			Plasma			Ratios		
	[K]	[Na]	[Cl]	[K]	[Na]	[Cl]	$\frac{[K]_i}{[K]_e}$	$\frac{[Na]_i}{[Na]_e}$	$\frac{[Cl]_e}{[Cl]_i}$
Man (120)	136	19	78	5.0	155	112	27.4	0.16	1.44
Baboon (56)	145	24	78	4.7	157	115	30.8	.15	1.48
Rabbit (15)	142	22	80	5.5	150	110	25.4	.15	1.38
Rat (36)	135	28	82	5.9	152	118	23.0	.18	1.44
Horse (8)	140	16	85	5.2	152	108	25.0	.11	1.27
Sheep (18)	46	98	78	4.8	160	116	9.6	.61	1.49
Ox (28)	35	104	85	5.1	150	109	6.8	.69	1.28
Cat (5)	8	142	84	4.6	158	112	1.7	.90	1.33
Dog (28)	10	135	87	4.8	153	112	2.1	.88	1.44

Table 2. Concentration ratios in human red cells (*a*) refrigerated for 6 days, followed by (*b*) reincubation with glucose at *p*H 7.6 for 8 hr.

	(*a*) 4°C				(*b*) 37°C	
Hours	0	48	96	144	4	8
*p*H, fluid medium	7.35	7.20	7.25	7.25	7.50	7.40
*p*H, cells	7.20	7.10	7.10	7.15	7.35	7.25
Hematocrit	48.4	50.2	52.8	54.6	52.8	49.2
$[K]_i/[K]_e$	130	18	12	7	14	25
$[Na]_i/[Na]_e$	0.12	0.30	0.38	0.42	0.28	0.17
$[Cl]_e/[Cl]_i$	1.4	1.3	1.3	1.2	1.3	1.4
log $[H]_i/[H]_e$	0.15	0.10	0.15	0.10	0.15	0.15
log $[Cl]_e/[Cl]_i$	.14	.11	.12	.08	.11	.14

$[K]_i$, . . . denote concentration of red cell ion, milliequivalent per liter of cell water.
$[K]_e$, . . . denote concentration of suspension ion, milliequivalent per liter of water.

Table 3. Incubation of refrigerated human red cells with glucose and at different *p*H values for 8 hr at 37°C.

Initial *p*H, fluid	6.80	7.00	7.20	7.40	7.60	8.00
Final *p*H, fluid	6.70	6.95	7.10	7.30	7.40	7.70
Final *p*H, cells	6.70	6.90	7.00	7.15	7.25	7.50
$[K]_i/[K]_e$	19	21	22	25	25	27
$[Na]_i/[Na]_e$	0.25	0.25	0.20	0.16	0.17	0.15
$[Cl]_e/[Cl]_i$	1.1	1.2	1.3	1.4	1.4	1.6
log $[H]_i/[H]_e$	0	0.05	0.10	0.15	0.15	0.20
log $[Cl]_e/[Cl]_i$	.04	.08	.11	.16	.14	.20
Glucose utilization, mg/lit red cells per hour	165	190	230	250	280	295

form, obtained with high correlation. The bicarbonate and *p*H ratios, calculated from gas estimations on some samples by the method of Yeomans and Stueck (*4*), showed fair correspondence. Hence, the distribution of chloride, bicarbonate, and hydrogen ions in mammalian red cells accords with simple diffusion. The results for human red cells are presented in Tables 2 and 3. The Donnan ratio for chloride ranged from 1.5–1.7 at *p*H 7.7 to 1.0–1.1 at *p*H 6.7 and was independent of the metabolic status of the red cells. The high cell chloride at low *p*H resulted only from the reduced buffering capacity of the nondiffusible cell hemoglobin (isoelectric point, *p*H 6.6–6.8) and organic phosphate. Harris and Maizels (*5*) report that human red cells, refrigerated for 4 to 8 wk, show chloride and cation ratios approaching 1, that is, diffusion equilibrium.

The potassium and sodium ratios were dependent on the degree of cell glycolysis (Tables 2 and 3). With decreasing *p*H, some inhibition of glycolysis occurred at 37°C with diminished sodium extrusion and potassium uptake by red cells (Table 3). The extrusion of sodium is an active process (*6*). Apart from the dog and cat red cell, the simultaneous influx of potassium is either carrier conditioned or an active process requiring energy (*7*). It does not conform to the criterions given by Ussing (*8*) for passive or exchange diffusion. The electric potential of the Donnan ratio is about 10 to 15 mv and could not produce more than a twofold concentration ratio between red cells and plasma. Since the red cell in heparinized plasma is almost a perfect osmometer (*3, 9*) and ^{42}K exchanges completely by a single rate constant (*10, 11*), it is improbable that sufficient potassium could exist in bound form to account for potassium accumulation by diffusion.

Thus, the process of potassium accumulation in red cells contrasts with the diffusion process in nerve and muscle (*12*). Here, high intracellular nondiffusible anions (proteins, organic phosphates, and so forth) and low chloride yield $[Cl^-]_e/[Cl^-]_i$ and $[K^+]_i/[K^+]_e$ ratios of 20 : 1 or greater with resting electric potentials of 50 to 100 mv. This negative force provides steep electrochemical gradients for the passive exchange diffusion of positive potassium ions following active sodium extrusion.

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