hybridization and is taken to indicate 100 percent homology; that is, if we assume that the chloroplast rRNA is coded for by plastid, not nuclear, DNA. The blue-green algae tested provide a graded degree of hybridization from 30 percent to 9 percent. The percentage hybridization obtained with chloroplast DNA and *Gloeocapsa alpicola* rRNA indicated homology of 47 percent. *Chlorogloea fritschii* and *Nostoc muscorum* rRNA possessed identical homology to chloroplast DNA of 32 percent. The rRNA from seven species of bacteria all showed a significantly lower degree of homology to chloroplast DNA than did blue-green algae rRNA. Of the bacteria examined, the RNA of *Rhodomicrobium vannielii* exhibits the highest affinity to chloroplast DNA. This organism is a photosynthetic bacterium whose fine structure is similar to certain blue-green algae (*15*). When a 50-fold excess of choroplast rRNA was included with the blue-green algae rRNA, the hybridization of all species of blue-green algae RNA to chloroplast DNA was reduced to about the background level due to random nonspecific RNA attached to a washed blank filter. This result indicated that the rRNA from blue-green algae was hybridized to specific sites on the chloroplast DNA.

Chloroplast DNA has been shown to hybridize with chloroplast rRNA but not with cytoplasmic rRNA (*16*), and our results confirm this. An examination of the interaction of cytoplasmic and chloroplastic nucleic acids has been presented by Ingle *et al.* (*17*), who have shown that higher plant chloroplast rRNA will bind to both chloroplast and nuclear DNA, whereas cytoplasmic RNA does not bind effectively to chloroplast DNA. The interspecies hybridization of rRNA and DNA in *Myxobacter* (*2*) and *Bacillus* species (*18*) indicate that considerable interspecies homology exists, presumably a reflection of the conserved sequences of the ribosomal cistrons (*19*).

Our data indicate a significant degree of homology between rRNA from species of blue-green algae and chloroplast DNA of *E. gracilis*. The species exhibiting the greatest degree of hybridization, *Gloeocapsa alpicola*, has a polyunsaturated fatty acid distribution most similar to that of chloroplasts from *E. gracilis* (*20*). Examination of other chloroplast DNA for homology with blue-green algal rRNA is desirable, as well as measurements on interplastid hybridization. An implication of our hybridization data is that the cistrons for rRNA synthesis in certain blue-green algae and *Euglena* chloroplasts are similar, and, with respect to this macromolecule at least, a meaningful degree of relatedness may be observed.

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Activities of Sodium and Potassium Ions in Epithelial Cells of Small Intestine

Abstract. *Intracellular molar Na^+ activity (a_{Na}), measured with cation-selective glass microelectrodes, in epithelial cells of isolated bullfrog small intestine immersed at 26°C in a sodium sulfate Ringer solution containing mannitol was 0.0144 ± 0.0031 (average value plus or minus standard deviation). The corresponding K^+ activity (a_K) was 0.0854 ± 0.0060. Combination of these values with previous estimates of intracellular Na^+ and K^+ concentrations under identical conditions indicated that a substantial fraction of the cellular Na^+ is in an osmotically inactive state. When the cells were exposed to a Ringer solution in which 26 millimoles of mannitol per liter were replaced by 3-O-methyl glucose, highly significant decreases in a_K and a_{Na} were observed. The decrease in a_K was proportionately larger than the corresponding decrease in a_{Na}.*

The Na^+ gradient hypothesis (*1*) is an attractive model for coupled transport of Na^+ and organic solutes in small intestine and other tissues (*2*). For the interactions observed between Na^+ transport and that of sugars and neutral amino acids in epithelial cells of small intestine, this hypothesis and its experimental basis may be summarized as follows (*2*). When isolated small intestine is immersed in normal Ringer solutions, net movement of sugars and amino acids from lumen to cell against a concentration gradient depends on the presence of Na^+ in the mucosal medium. Conversely, net mucosal to serosal Na^+ transport across the tissue and its electrical correlates, transmural potential difference (PD) and short circuit current, are dramatically increased when actively transported sugars or amino acids are added to the luminal medium. At constant mucosal Na^+ concentration, these increases are saturable functions of the luminal concentration of sugar or amino acid. According to the Na^+ gradient hypothesis, the energy needed for net accumulation of sugars or amino acids across the brush border of the cells is derived from concomitant movement of Na^+ ions into the cells in the direction of their activity gradient. This gradient is conserved by the operation of a Na^+ pump in the lateral-serosal cell membrane which depends directly on metabolic energy and which can effect net movement of Na^+, against an electrochemical gradient, from the cell interior to the serosal medium. Current models for this process postulate that simultaneous translocation of Na^+ and

sugars or amino acids across the luminal membrane involves a ternary complex between Na^+, organic solute, and a common membrane carrier (*1, 2*). The hypothesis requires that, under given conditions, the direction of net sugar or amino acid transport across the luminal membrane be governed by the orientation, with respect to the cell interior and the mucosal medium, of the Na^+ activity gradient across this membrane.

Although much evidence supports the Na^+ gradient hypothesis as a mechanism for Na^+-dependent solute transfer in small intestine (*2*), a number of observations are difficult to reconcile with it (*3*). A cardinal difficulty (*2*) in any conclusive evaluation of this hypothesis is uncertainty about the exact distribution and activity of intracellular solutes, particularly Na^+. Attempts to equate the true Na^+ gradient across the mucosal membrane to the ratio between the luminal Na^+ concentration and the apparent intracellular Na^+ concentration, C_{Na} (obtained by dividing the amount of intracellular Na^+ by the total water content of the cell), are subject to several major uncertainties. First, chemical determinations of C_{Na} in tissues exposed to media high in Na^+ are drastically affected by relatively small errors in the measurement of extracellular space. This is reflected in the variance of reported values for C_{Na} (*4–6*). Second, the assumption that all the cell water acts as a solvent for intracellular ions is open to question in many cell species (*7–9*). Third, it is well established that, in a variety of cells, C_{Na} does not accurately reflect the true intracellular Na^+ activity (*7–13*). These difficulties can be largely circumvented by direct determination of intracellular ionic activities with ion-selective microelectrodes (*7–13*). As far as we know, such measurements have not yet been reported for epithelial cells of small intestine. This report demonstrates the application of this technique to the measurement of Na^+ and K^+ activities (a_{Na} and a_K) in these cells under control conditions and in the presence of an actively transported nonmetabolized sugar analog, 3-*O*-methyl glucose.

Sheets of small intestine from adult bullfrogs (*Rana catesbeiana*) were mounted at 26°C between identical oxygenated sodium sulfate Ringer solutions (*p*H 7.2) in a perfusion chamber which permitted (i) continuous monitoring throughout the experiment of transmural PD, (ii) impalement of individual epithelial cells with microelectrodes and measurement of their membrane potentials, and (iii) rapid changes of the mucosal or serosal medium or both during the experiment (*14*). Mannitol was used to make the medium isosmotic with normal frog Ringer solution containing sodium chloride.

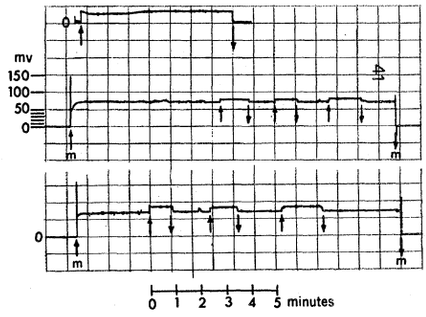

Fig. 1. Upper tracing: Recording of a mucosal membrane potential from a single epithelial cell of bullfrog small intestine (electrode resistance, 140 megohms). Middle and lower tracings: Six successive recordings obtained in the same experiment with a cation-selective microelectrode before (middle trace) and after (lower trace) addition of 3-*O*-methyl glucose. In all three tracings, an upward deflection following impalement indicates increasing negativity of the cell interior with respect to the (grounded) mucosal medium; (↑) indicates the time of impalement and (↓) the time of withdrawal of the electrode from the cell. In the two lower tracings the letter *m* under the arrows indicates the times of insertion of the electrode into and its withdrawal from the mucosal medium.

The techniques used for measuring transmural PD and microelectrode potentials are described elsewhere (*14*). A high input impedance (greater than 10^{14} ohms) Keithley 610B electrometer connected to the electrode holder by a shielded cable was used as an amplifier with cation-selective microelectrodes. Transmural PD and microelectrode potentials were displayed on separate Digitec 401P microvoltmeters and recorded simultaneously on two channels of a Brush Mark 240 recorder. Open tip microelectrodes were filled with 3*M* KCl, had tip resistances between 50 and 150 megohms, and had tip potentials less than 5 mv (results were accepted only if the tip resistance remained within this limit). Cation-selective glass microelectrodes were modifications of Hinke's (*10*) original design, made from Corning NAS 27-4 K^+-sensitive glass, calibrated as previously described (*11*), and had tip diameters about 0.5 μm and exposed tip lengths of 2 to 3 μm.

When transmural PD became steady, three to six impalements of individual cells were made with an open tip microelectrode. This was then replaced in turn by each of a pair of cation-selective electrodes, and three to six impalements of different cells were made with each of these. A pair of electrodes with different selectivity coefficients, $k_{K\text{-}Na}$ (*10–13*), was used in each experiment. In one experiment the electrode pair had $k_{K\text{-}Na}$ values of 0.677 and 0.823. The mean (± standard error) $k_{K\text{-}Na}$ values in the other seven experiments were 0.244 ± 0.013 for the more K^+-selective electrodes and 0.441 ± 0.050 for the less K^+-selective electrodes. The $k_{K\text{-}Na}$ ratios of the electrode pairs in individual experiments ranged from 1.44 to 2.42. Nicolsky's equation (*12*) was used to compute a_{Na} and a_K from the average potential recorded with each of the three microelectrodes in individual experiments. For this purpose, potential values were taken from the Digitec voltmeter.

In four experiments the tissue was first equilibrated with the control medium and the sequence of potential measurements described above was performed. Both the mucosal and serosal media were then replaced simultaneously with solutions containing 26 m*M* 3-*O*-methyl glucose instead of an equivalent amount of mannitol. When the expected increase in transmural PD (*14*) had occurred and this parameter had reached a new steady state, the above sequence of intracellular potential measurements was repeated in reverse with the same three microelectrodes. Impalements were made under ×100 magnification through the mucosal surface of the cells.

Figure 1 shows some potentials recorded during a single experiment. This figure is included to illustrate the quality of the records obtained. The upper tracing shows that the membrane potentials recorded in this study met the criteria for acceptability previously established with electrodes filled with 1*M* K^+-citrate (*14*). The magnitudes of these potentials were similar to those previously obtained under identical conditions (*14*), and, as anticipated from earlier studies, 3-*O*-methyl glucose induced a significant decrease ($P < .05$) in the mucosal membrane potential (E_m) in experiments where sufficient penetrations were made before and after its addition to permit statistical evaluation.

The middle and lower tracings in Fig. 1 show that, after a cell was impaled with a cation-selective microelectrode, the potential changed rapidly

and then remained virtually constant until the electrode was withdrawn. For the recordings shown in Fig. 1, the average change in the control potential following insertion of the electrode was about 10 mv. The corresponding value after adding 3-*O*-methyl glucose was about 15 mv. Under both conditions the intracellular potential was negative with respect to the mucosal medium. However, it should be emphasized that, with cation-selective microelectrodes, the magnitude and orientation of the intracellular potential with respect to the mucosal medium depend on $k_{K\text{-}Na}$ for the electrode as well as on a_{Na} and a_K.

Table 1 is a summary of the a_{Na} and a_K values we obtained. The average a_{Na} value under control conditions (control 1), when combined with the mean value previously reported for C_{Na} under identical conditions (*5*) indicates (despite the uncertainties inherent in the estimation of C_{Na}) that in these cells, as in most cell species so far studied (*7–13*), a relatively large fraction of the intracellular Na^+ is not recorded as osmotically active Na^+ in the cytoplasm surrounding the cation-selective microelectrode. The pooled data of Armstrong *et al.* (*5*) give an average of 29 ± 7 mmole per kilogram of cell water for C_{Na} ($n = 16$). Combining this with the mean value for a_{Na} shown in Table 1 (control 1), one obtains an a_{Na}/C_{Na} ratio of 0.5. In the presence of 3-*O*-methyl glucose the corresponding values are 27 ± 7 ($n = 8$) for C_{Na} and 0.45 for a_{Na}/C_{Na} (Table 1). These values are considerably lower than those predicted on the assumption that all the intracellular Na^+ is in "free" solution in the cytoplasm (*15*) and provide direct experimental support for the widely held opinion (*2*) that estimates of the Na^+ gradient based on C_{Na} alone are subject to serious error.

When control 1 or control 2 is compared with 3-*O*-methyl glucose (Table 1), a highly significant ($P < .001$) decrease in a_{Na} is found. Csáky and Esposito (*6*) and Armstrong *et al.* (*5*) did not observe any significant effect of 3-*O*-methyl glucose on C_{Na} in isolated bullfrog small intestinal epithelia immersed in sodium sulfate media, but due to the uncertainties in these estimates of C_{Na}, and since relatively small changes in the absolute value of a_{Na} could easily be masked by the comparatively large thermodynamically "inactive" fraction of cell Na^+ suggested by the low a_{Na}/C_{Na} values reported herein, we feel that, despite the small number of observations involved, the decrease in a_{Na} observed in the presence of 3-*O*-methyl glucose is probably real (*16*).

Table 1. Sodium and potassium activities, a_{Na} and a_K, in epithelial cells of isolated bullfrog small intestine immersed in sodium sulfate Ringer solutions—effect of 26 m*M* 3-*O*-methyl glucose. Average values and standard deviations are shown for the number of intestinal segments indicated by the first figure in parentheses. The second figure gives the total number of activity determinations (that is, sets of three impalements—see text) for each condition. Control 2 shows the control data (without 3-*O*-methyl glucose) for those experiments in which 3-*O*-methyl glucose was subsequently added. These results are also included in the overall values designated control 1.

Conditions	a_{Na}	a_K
Control 1 (8 : 37)	0.0144 ± 0.0031	0.0854 ± 0.0060
Control 2 (4 : 16)	0.0149 ± 0.0014	0.0850 ± 0.0040
3-*O*-Methyl glucose (4 : 15)	0.0121 ± 0.0014	0.0644 ± 0.0045

Table 1 shows that there is a relatively large and highly significant ($P < .001$) decrease in a_K following exposure of the cells to 3-*O*-methyl glucose (no significant differences in a_{Na} or a_K were found when control 1 of Table 1 was compared to control 2). This agrees with the observations of Csáky and Esposito (*6*) and Armstrong *et al.* (*5*) that 3-*O*-methyl glucose induces significant decreases in C_K under similar conditions and can be ascribed to the same cause, dilution of "free" cytoplasmic K^+ by osmotic entry of water into the cells. However, the following considerations suggest that the observed decrease in C_K (*4–6*) may lead to an overestimate of the increase in "solvent" water in the cytoplasm under these conditions. When a_K in the absence or in the presence of 3-*O*-methyl glucose (Table 1) is compared with C_K (*5*) under identical conditions (86 ± 7 and 54 ± 5 mmole per kilogram of cell water; $n = 16$ and 8, respectively), a_K/C_K (1.0 without and 1.2 with 3-*O*-methyl glucose) is higher than the predicted K^+ activity coefficient for the bathing medium (*15*). Hinke *et al.* (*7, 8*) consistently found a_K/C_K ratios greater than 1 in single fibers from depressor muscles of the barnacle *Balanus nubilus* during immersion in isosmotic Ringer solutions and interpreted these as indicating the presence of a significant fraction of nonsolvent water in the fibers. Assuming that β_K, the fraction of "bound" or sequestered fiber K^+, is negligible and that the molal activity coefficient of myoplasm is equal to that of the external medium, McLaughlin and Hinke (*8*) showed that such activity data can be used to estimate solvent water and "bound" Na^+ in myoplasm. Since these estimates agree well with values based on other techniques, and since there is good evidence for the presence of substantial amounts of ordered water in various cells (*7–9*), we suggest as a working hypothesis that the high a_K/C_K ratios obtained from our data reflect the presence of a significant fraction of nonsolvent water in epithelial cells of small intestine. If it is assumed (*7–9*) that β_K is approximately 0 in the cytoplasm and that g_c, the cytoplasmic osmotic coefficient (*15*), is equal to g_m, the osmotic coefficient of the medium, estimates of the fractions of "solvent" water (α) and "bound" Na^+ (β_{Na}) in these cells can be obtained from the equations

$$\alpha = (g_m \cdot C_K)/a_K \quad (1)$$

$$\beta_{Na} = 1 - (\alpha \cdot a_{Na})/(g_m \cdot C_{Na}) \quad (2)$$

We determined g_m from cryoscopic measurements. Inserting the value obtained (0.83), together with the appropriate values for a_{Na}, C_{Na}, a_K, and C_K, into Eqs. 1 and 2, one obtains $\alpha = 0.84$ and $\beta_{Na} = 0.50$; that is, under control conditions about 16 percent of the cell water and about half the cell Na^+ appear to be sequestered in one or more regions of low osmotic activity. The corresponding values for cells exposed to 3-*O*-methyl glucose are $\alpha = 0.70$ and $\beta = 0.62$ (*17*).

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16. Although Na^+ influx from lumen to cell is increased in the presence of 3-*O*-methyl glucose (*2*), an increase in a_{Na} under these conditions seems unlikely because of the concomitant increase in cell water (*5*, *6*). The observed decrease in a_{Na} (Table 1) is easily interpreted in terms of the energy requirement (w) for net Na^+ transport from the cell interior to the serosal medium. For the reversible transfer of 1 gram equivalent of Na^+ under steady state open circuit conditions

$$w = RT \ln (a_{Na}^{o}/a_{Na}) + E_s F \quad (3)$$

where a_{Na}^{o} is the extracellular Na^+ activity, E_s is the serosal membrane potential, and R, T, and F have their usual meanings. If it is assumed that the supply of metabolic energy and its coupling to Na^+ "pumping" are unaffected by the actively transported organic solute, the known decrease in E_s under these conditions (*14*) would permit the maintenance of a lower steady state a_{Na} since the other parameters of Eq. 3 are unchanged. The increased short circuit current (under conditions where this reflects net transcellular Na^+ transport) induced by actively transported solutes (*2*) is also consistent with a decrease in a_{Na}. "Short circuiting" means abolishing $(E_s - E_m)$, the transmural PD. Hence, in this condition, E_m can be substituted for E_s in the electrical work term of Eq. 3. Since E_m is significantly reduced by actively transported solutes (*14*), the electrical work required for net Na^+ transport from the cell interior to the serosal medium will be correspondingly deduced. Thus, for a given energy input one can expect an increased transcellular movement of Na^+.

17. Although these estimates are consistent with those reported for the same parameters in other cells (*7*), they must be regarded as highly tentative and subject to further experimental verification. Both the assumptions implicit in Eqs. 1 and 2, that is, $g_c = g_m$ and $\beta_K \sim 0$, are open to question. In particular, the second assumption which maximizes the value of α obtained from Eq. 1, may not be true for all experimental conditions (*11*).
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Messenger RNA Structure: Compatibility of Hairpin Loops with Protein Sequence

Abstract. *Examination of the amino acid sequences of human cytochrome c and the α-chain variant of human hemoglobin Constant Spring has revealed the possibility for base-paired hairpin loops in the messenger RNA's for these proteins. A similar analysis of the bacteriophage R17 coat protein suggests an additional unobserved loop in the R17 RNA. If such loops are present in messenger RNA's generally, it would suggest that DNA has more than one stable base-paired conformation.*

A number of RNA's appear to possess secondary structure in the form of base-paired hairpin loops. Transfer RNA's (tRNA) are the most familiar examples (*1*), but 5*S* ribosomal RNA of *Escherichia coli* has these properties (*2*) as does an RNA of unknown function from human cells infected with adenovirus 2 (*3*). Most surprising, however, has been the demonstration of several loops in the segment of RNA that codes for the coat protein of R17 bacteriophage (*4*). These hairpin loops imply that the base sequence of the messenger RNA (mRNA) is dictated by its ability to form stable base-paired loops, as well as by the functional tertiary structure of the coat protein. At first glance this combination of restriction on mRNA structure might seem incompatible; however, the redundancy in the genetic code permits extensive alteration of base sequence to achieve base-pairing without greatly altering the amino acid sequence. If occasional unpaired bases or shifts in the register such as those that occur in R17 RNA (*4*) are permitted, the potential for loop formation is further increased.

We have, with the aid of a computer program, examined several proteins with the object of discovering regions compatible with base-paired loops in the corresponding mRNA. The process involves translating the protein amino acid sequence into an RNA sequence that includes all the redundancies in the genetic code. This RNA sequence is compared with itself in reverse polarity in all possible linear registers. Base comparisons are recorded as a match if they are complementary, a potential match if one of the redundancies permits base-pairing, and no match if there is no possibility for complementary pairing. Potential loops on the computer printout are recognized by long runs of matches or potential matches, with few gaps or register shifts. The program thus eliminates all regions where loops cannot occur but does not uniquely predict the existence of loops, only the possibility for loops.

Our analysis of the R17 coat protein has revealed all the internal loops in the mRNA observed experimentally by Sanger (*4*). In addition, several loops suggested by the program were shown to be spurious upon examination of the known base sequence. One of the interesting results of analysis of the R17 coat protein is the indication of a loop in the mRNA coding for residues 12 to 23, a region of R17 RNA that has not yet been sequenced. This region is presented in Fig. 1a along with the RNA base sequence predicted, provided that there is a base-paired loop in this region. Twelve previously undefined bases in this sequence are predicted by this analysis, leaving only one unspecified base at an unpaired position at the closed end of the loop.

The spatial restrictions for packing RNA in the R17 bacteriophage capsule might represent a strong selective pressure for the formation of base-paired loops. Such restrictions would not exist for mRNA's in bacteria and higher organisms. The loops may have a more general function such as the stabilization of RNA; therefore, we have examined human cytochrome c (*1*) and the recently described chain termination variant (Constant Spring) of the α chain of human hemoglobin (*5*). There are several amino acid sequences in these proteins which are compatible with base-paired loops in their mRNA. Selected regions are illustrated in Fig. 1b for cytochrome c and Fig. 1c for the hemoglobin α chain.

The regions chosen have particular theoretical importance. Cytochromes c from a diversity of organisms possess a sequence of 11 invariant amino acids (*1*). The evolution of a loop in the RNA coding for this region might tend to stabilize the amino acid sequence. Two possible loops for this region are presented in Fig. 1b. The first example is in a "1-1" register, that is, the first base of triplet codons on opposite sides of the loop are aligned. The second example is in a "2-2" register. In general, loops with "3-3" registers are considered less likely since they do not take full advantage of the redundancy in the third position of most codons.

The discovery of a chain termination variant for the α chain of human hemoglobin (*5*) has enabled us to examine a portion of the hemoglobin messenger not normally translated into protein. The additional 31 amino acids found in this variant represent a segment of mRNA whose base sequence has been free to evolve toward the formation of