and FU interfered with the DNA (virus) to RNA (intermediate) to DNA (virus) sequence. Instead of the expected cycle, these pyrimidines caused accumulations of abnormal RNA-staining material which inhibited virus maturation. Removal of this "fraudulent" RNA-staining matrix with RNAase unmasked DNA-staining immature virus. These observations suggested that FU and FO did not interfere with RNA or DNA synthesis *per se* but did interfere with development of intact virus through inhibition of protein synthesis.

When chlortetracycline HCl was added to the nutrient medium at the "red-ball stage" of psittacosis virus, maturation of the inclusion was arrested and virus was not recoverable from such cultures(6). While chlortetracycline and FU interfered with virus replication, the mechanism of each was different: the former appeared to block the mobilization of an RNA-staining matrix, while the latter interfered with the normal biosynthetic sequence by formation of an abnormal intermediate compound.

Summary. Cytochemical changes associated with psittacosis virus infection of tissue cultures were studied by acridine orange staining and fluorescence microscopy. Normal virus development was interrupted by the addition of 5-fluorouracil and 5-fluoroorotic acid which caused the accumulation of "fraudulent" RNA. It is suggested that the substituted pyrimidines interfered with development of intact virus through inhibition of protein synthesis.

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Permeability of Ehrlich Ascites Cells to Adenine.* (26147)

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Data on the permeability of cell membranes to a considerable number of non-electrolytes are available(1,2). Relatively little is known of the permeabilities of cells to the purines and pyrimidines although nucleic acid metabolism has been a major area of investigation for over a decade.

The present paper reports our work on permeability of Ehrlich ascites cells to adenine.

Methods and materials. The Ehrlich ascites used was a hypotetraploid line carried in Swiss albino mice by weekly intraperitoneal injections of 0.1 ml of ascites. For experiments, 6 day ascites was collected into 50 ml Krebs-Ringer bicarbonate (KRB) containing 0.2 mg heparin. The resulting suspension was diluted with an equal volume of

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distilled water to hemolyze the contaminating erythrocytes and centrifuged to collect the cells. After one wash with KRB, the cells were resuspended in KRB. Suspensions which had not been subjected to osmotic hemolysis were used in some experiments. The two types of preparations did not differ in adenine permeability.

The permeability constant was determined at 4°C and at 37°C. All procedures were carried out in a cold room at 4°C except for the incubations at 37°C. For the determinations at 4°C, a cell suspension and a solution of adenine in KRB were mixed and at various times aliquots were taken and centrifuged at top speed in a Phillips-Drucker 703 centrifuge. Time of contact between cells and adenine was taken to be the time from mixing until the centrifuge reached top speed. For the studies at 37°C, the cell suspension and adenine were put into separate arms of a Heinicke reaction vessel which was then gassed with 5% carbon dioxide in air and stoppered. After a preincubation of 4-5 minutes at 37°C, the contents of the 2 arms were mixed. At the end of the incubation, the vessels were chilled rapidly in ice water, taken to the cold room and samples were centrifuged to separate cells from the suspending medium.

The supernatant fluids were extracted by adding 4 ml of 95% ethanol to one ml of supernatant and centrifuging. The resulting precipitates were washed with 5 ml of 75% ethanol containing .09% NaCl. The extract and wash were combined and diluted to an appropriate volume with 75% ethanol. The adenine was determined spectrophotometrically(3).

In some experiments the cell pellets were extracted with 75% ethanol containing .09% NaCl, the extracts were dried, dissolved in water and spotted on Whatman No. 1 paper for chromatography. Ascending paper chromatograms were run in a solvent consisting of isoamyl alcohol—5% Na_2HPO_4 (9:21).

On all samples, cytocrits, dry weight of cells and total water in pellets from 2 ml of suspension and cell counts were determined. The volume of extracellular fluid trapped in the pellets was read from a calibration curve

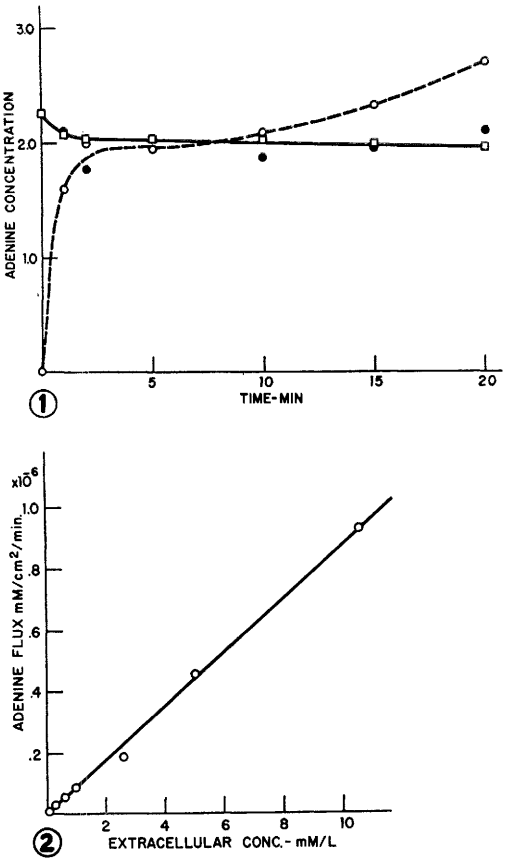

FIG. 1. Kinetics of penetration of adenine into Ehrlich ascites cells. $-\square-$ c_e in mM/l; $-\bigcirc-$ c_i in mM/kg H_2O, calculated from change in c_e; $-\bullet-$ c_i calculated from adenine found in pellets.

FIG. 2. Dependence of adenine flux on avg extracellular concentrations.

of the sucrose space of pellets of Ehrlich ascites cells obtained with our standard technic(4).

The amount of adenine entering the cells was determined by difference from the drop in extracellular concentration during the incubations. In some experiments the total pellet adenine was determined from paper chromatograms of the pellet extracts.

Results and discussion. Fig. 1 shows the kinetics of entrance of adenine into Ehrlich ascites cells at 37°C. Within a few minutes the *calculated* intracellular concentration plateaus at a value slightly below the extracellular but then rises to exceed the extracellular by 20 minutes. Since intracellular concentration is calculated from the amount of adenine

disappearing from the extracellular fluid this might be explained by a slow metabolism of adenine. However, the adenine in the pellets was also determined directly by paper chromatography of the pellet extracts, elution of the adenine spots with ammonia water (pH 10.5) and determination of adenine spectrophotometrically. The adenine found in the one and 5 minute pellet samples was unaccountably high. There was an increase in adenine recovered in the pellets from 10 to 20 minutes suggesting that the increase in the calculated intracellular concentration might be partly a true increase and not entirely due to a metabolic conversion of the adenine. This might be explained if the adenine slowly accumulated in some compartment of the cell, perhaps one with a partition coefficient against water greater than one for adenine. The data in Fig. 1 suggest that adenine enters the cell by a simple diffusion mechanism. Further evidence for this was obtained by measuring the amount of adenine entering the cells in the first 2 minutes of incubation at various extracellular concentrations of adenine. Fig. 2 shows that the flux entering the cells is a linear function of average extracellular concentration. This is the expected result if only a simple diffusion is involved in this process. This finding makes it unlikely that any carrier or active transport system is involved in the entrance of adenine into Ehrlich ascites cells, although it does not unequivocally rule these out. The slope of this line gives a kp of $1.9 \cdot 10^{-4}$ cm/min at 37°C.

Permeability constants were determined by measuring the fall in extracellular concentration in the first 10 minutes at 4°C and in the first 2 minutes at 37°C. The extracellular concentration changed less than 15% in these experiments; usually this change was less than 10%. Thus it was assumed that intracellular concentration was given by $c_i = (c_e)\left[1 - e^{\frac{-kpA}{Vi}t}\right]$ where kp is permeability constant, A/Vi is ratio of cell surface area to intracellular water volume, and (c_e) is the average extracellular concentration during the period involved. Intracellular concentration was determined by difference from the change in extracellular concentration. Table I gives the results. The kinetics of adenine uptake in the first 5 minutes shown in Fig. 1 would fit a kp of about 2.10^{-4} cm/min. The data in Table I may be expected to have an error of as much as 50% because of the inherent difficulty in measuring a small quantity by difference between two large quantities. The data do show that the kp at 37°C is in the range 10^{-4} to 2.10^{-4} cm/min and that it is about 5 fold greater at 37°C than at 4°C.

TABLE I. Permeability of Ehrlich Ascites Cells to Adenine.

Temp., °C	kp, cm/min.	Temp., °C	kp, cm/min.
4	$2.3 \cdot 10^{-5}$	37	$.6 \cdot 10^{-4}$
4	$1.9 \cdot 10^{-5}$	37	$1.9 \cdot 10^{-4}$
4	$2.6 \cdot 10^{-5}$	37	$1.0 \cdot 10^{-4}$*
37	$1.0 \cdot 10^{-4}$	37	$.6 \cdot 10^{-4}$†
37	$1.0 \cdot 10^{-4}$	37	$.8 \cdot 10^{-4}$

* In presence of glucose at 100 mg %.
† *Idem* at 200 " .

Summary. Adenine enters Ehrlich ascites cells by simple permeability. The permeability constant for adenine is in the range 10^{-4} to 2.10^{-4} cm/min at 37°C and 2.10^{-5} cm/min at 4°C.

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