hibited by hypocholesteremic agents found to be active in humans they may be useful for screening compounds inhibiting sterol or fatty acid synthesis.

Summary. The hypocholesteremic agents, triparanol and benzmalecene, inhibit the multiplication of the sterol-synthesizing phytoflagellates, *Ochromonas danica*, *O. malhamensis*, *Euglena gracilis*, and the non-sterol-synthesizing ciliate, *Tetrahymena pyriformis*. The inhibition of multiplication of the *Ochromonas* species may be prevented by cholesterol, ergosterol, farnesol, squalene, lauric acid, oleic acid, linoleic acid, and linolenic acid. Oleic acid and linolenic acid were most effective.

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Permeability of Ehrlich Cells to Uracil, Thymine and Fluorouracil.* (27126)

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Ehrlich ascites cells(1) and human erythrocytes(2) are highly permeable to adenine. Little is known about the permeabilities of cells to the pyrimidines although the work of Bosch *et al.*(3) and Heidelberger *et al.*(4) suggests that uracil and fluorouracil readily enter Ehrlich ascites cells. This paper reports our studies on the permeability of Ehrlich ascites cells to uracil, thymine and fluorouracil.

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. Obviously bloody ascites was discarded. Faintly pink ascitic fluid containing small numbers of erythrocytes was accepted for use. The resulting suspension was diluted with an equal volume of KRB and centrifuged to collect the cells. After one wash, the cells were resuspended in KRB. Osmotic hemolysis(1) was not used.

Details of the technics utilized have been described(5,6). Experiments were conducted at 5°C and 39°C. Heinicke reaction vessels were used. Three ml of cell suspension was placed in one arm and 3 ml of a solution of pyrimidine in KRB in the other arm. Glucose was not added to the medium because it was found that uracil and uridine were metabolized at a considerably higher rate than

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when it was omitted. The experiments at 5°C were carried out in a cold room. The contact time between cells and pyrimidine was taken to be the time from mixing until 2 minutes after the start of centrifugation of the sample, 2 minutes being the time required to form a firm pellet by centrifugation. For the experiments at 39°C the reaction vessels were gassed with 5% carbon dioxide in air and stoppered. After temperature equilibration in the water bath, the contents of the 2 arms were mixed and the incubations were carried out. The vessels were plunged into ice water, taken to the cold room, and samples were taken for centrifugation as rapidly as possible.

In all cases one ml of supernatant fluid and the pellet from 2 ml of final cell suspension were taken for analysis. The pyrimidines were determined spectrophotometrically on barium acetate-ethanol extracts which had been alkalinized by addition of concentrated NH_4OH. The latter was added to shift the absorption spectrum of the pyrimidines into the 275-300 mμ region.

The quantities measured on each sample were: B—the total amount of pyrimidine in the pellet; c_e—extracellular concentration; w_t—total water in the pellet. The extracellular water retained in the pellet was assumed to equal the sucrose space of pellets prepared by a standard procedure. A calibration curve relating extracellular water in the pellet (w_e) to the pellet volume was determined and used for all experiments. Intracellular concentration was calculated on the assumption that the intracellular pyrimidine was uniformly distributed in intracellular water, $c_i = B - c_e w_e / w_t - w_e$.

Results and discussion. In Fig. 1 is shown the relation between intracellular concentration of uracil attained after an incubation of 9 minutes at 5°C, and average extracellular concentration. The change in extracellular concentration during the incubation period was approximately 6% of its initial value. The linear dependence of intracellular concentration on extracellular concentration suggests that a simple diffusion is involved. The permeability constant was calculated from the integrated diffusion equation

$$c_i = (c_e)\left[1 - e^{-k_p \frac{A}{w_i} t}\right].$$

In this equation, k_p is the permeability constant, A/w_i is the ratio of cell surface area to intracellular water and (c_e) is the average extracellular concentration during period of incubation. Over 99% of each of the pyrimidines used would be in the nonionized form in the extracellular space (pH 7.1-7.2) and in the intracellular space† so that no account need be taken in the calculations of pH gradient effects. The surface area was calculated from the pellet volume, cell number, and extracellular space of the pellets with the assumption that the ascites cells are uniform spheres. The mean value of A/w_i from 19 experiments was 4.69×10^{-3} cm^{-1} with a standard error of the mean of $.05 \times 10^{-3}$. The surface area of the Ehrlich ascites cells has small folds and ridges(7) so that the above asumption is probably not valid. It is nonetheless a convenient operational definition. The cellular parameter $k' = k_p \frac{A}{w_i}$ is also given for each experiment. From the experiment shown in Fig. 1, one obtains for uracil, $k_p = 1.2 \times 10^{-5}$ cm/min and $k' = .053$ min^{-1} at 5°C. Corresponding experiments on thymine and fluorouracil gave linear graphs as good as Fig. 1 and the values $k_p = 3.2 \times 10^{-5}$ cm/min, $k' = .15$ min^{-1} for thymine and $k_p = 1.1 \times 10^{-5}$ cm/min, $k' = .054$ min^{-1} for fluorouracil.

Fig. 2 shows the time course of uracil entry into Ehrlich cells at 39°C and total uracil recovered from 2 ml of the suspension. Intracellular concentration at zero time is from a sample which was not incubated at 39° but was subjected to the same handling in the cold as the other samples. This shows how much uracil enters the cells during the time of handling in the cold room at the initial gradient across the cell mmebrane. The error due to this would be much less in the other samples since the gradient across the cell membrane is practically zero by one minute

† In Ehrlich ascites cells incubated under the same conditions as were used in the present experiments, we have obtained intracellular pH's of 6.5 - 6.8 from glass electrode measurements on frozen-thawed cells and from drug distribution studies.

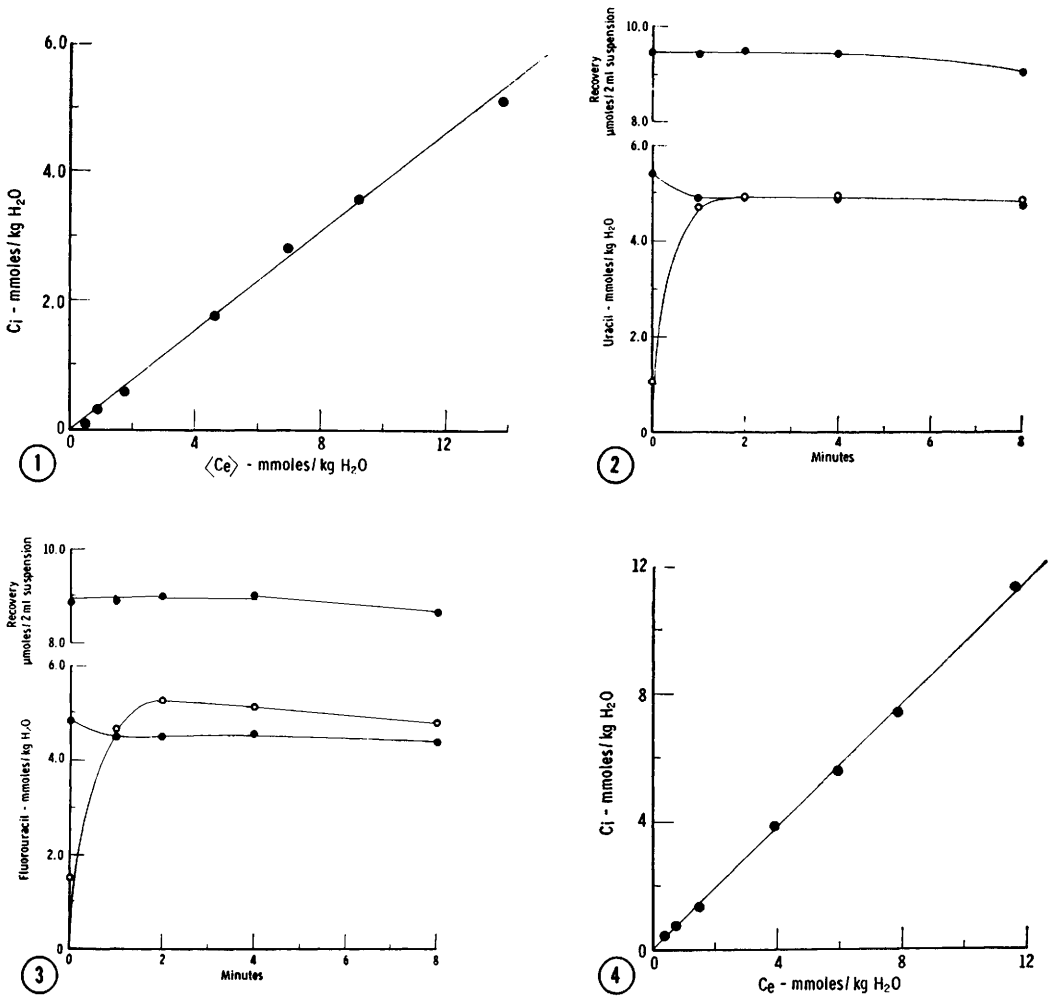

FIG. 1. Concentration dependence of uracil entry into Ehrlich cells at 5°C. Incubation for 9 min., 5°C. c_i intracellular concentration. $\langle c_e \rangle$ avg extracellular concentration.

FIG. 2. Time dependence of uracil entry into Ehrlich cells at 39°C. Extracellular concentration —●—. Intracellular concentration —○—.

FIG. 3. Time dependence of fluorouracil entry into Ehrlich cells at 39°C. Extracellular concentration —●—. Intracellular concentration —○—.

FIG. 4. Concentration dependence of uracil entry into Ehrlich cells at 39°C. c_i intracellular concentration after 0.5 min. incubation at 39°C. c_e extracellular concentration at end of incubation.

of incubation. The figure shows that uracil enters the intracellular space rapidly and apparently attains a diffusion equilibrium. The total uracil recovered falls slightly after the first 4 minutes of incubation. This probably represents metabolism of the uracil. The corresponding experiment with thymine gave results almost identical to those obtained with uracil. With fluorouracil the intracellular concentration attained was slightly higher than the extracellular (Fig. 3). This may indicate that there is some solubility of fluorouracil in non-aqueous compartments of the cell or some weak binding to cell structures but a 10% error in the estimate of the extracellular water of the pellets could explain this equally well. Fig. 4 shows results of an experiment on the concentration dependence of uracil entry at 39°C. This experiment involved an incubation at 39°C for one-half minute and a handling time of 8½ minutes in the cold. As shown, the

uracil has practically attained a diffusion equilibrium in this time. Similar results were obtained with thymine and fluorouracil. Because of the rapidity of entrance into the cells at 39°C it was difficult to measure accurately the k_p for these compounds. However, one can estimate from the experiments that the k_p's at 39°C fall in the range 10^{-3} to 10^{-4} cm/min.

Schanker and Tocco(8) have reported studies on intestinal absorption of uracil and thymine which they interpret as evidence for an active transport of these compounds. If the cells of the intestinal epithelium have as high a permeability to these compounds as do the Ehrlich ascites cells, an active transport would be unlikely. In that case, their findings might be explained by a high permeability for thymine and uracil and a saturation of metabolic pathways with increasing concentration.

Summary. Uracil, thymine and fluorouracil enter Ehrlich ascites cells by diffusion. The permeability constants at 5°C were $1.2 \cdot 10^{-5}$ cm/min for uracil, $3.2 \cdot 10^{-5}$ cm/min for thymine and $1.1 \cdot 10^{-5}$ cm/min for fluorouracil. Accurate measurements could not be obtained at 39°C because of the rapidity of the equilibration of the intracellular space but estimates of 10^{-3} to 10^{-4} cm/min for the permeability constants at 39°C were obtained.

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Effect of Magnesium Ions on Removal of Radiostrontium from Rats.* (27127)

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In recent years the fear of increased dietary contamination with radioactive Sr-90 from "fall-out" has stimulated considerable effort towards finding a safe and effective means of decreasing the body burden of radioactivity. Different experimental approaches have been utilized in the search, *i.e.*, chelating agents(1); alterations in salt content of the diet(2); dilution with non-radioactive carrier(3); the acidotic salt NH_4 Cl (4); and Vit. D_3(5). Relatively toxic amounts of some of these substances, *i.e.*, Vit. D and NH_4 Cl, must be administered to be effective. A certain degree of success has been obtained by increasing the calcium content of the diet, depending on how soon after ingestion of isotope the dietary change was made(3). In general, chelating agents have proven unsatisfactory following chronic ingestion of isotope(6).

Magnesium ion, a potent hypercalciuric agent(7,8), recently has been shown to increase urinary Ca-45 regardless of whether the nuclide was ingested one or 60 days before(9). Since strontium resembles calcium in its affinity for bone and in its elimination from the body, magnesium was tested for its ability to increase the elimination of radioactive strontium by rats. The following experiments demonstrate that oral or parenteral ad-

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