Summary. The lipid composition of *Trypanosoma ranarum* was determined. The cells contained traces of monoglycerides and about equal amounts of di- and triglycerides. Free fatty acids and "fast acting" sterols, mostly in free form, were present. The sterol was isolated and identified as ergosterol. The implications of these findings are discussed.

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Effect of D_2O on Uptake in Yeast.* (29636)

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It has been shown that deuterium oxide (D_2O) inhibition of the rates of growth and glucose catabolism in yeast were similar(1). The rates of these functions are dependent, to some extent, on rate of uptake of substrate into the cell. Thus, the possibility existed that D_2O effects on growth and glucose catabolism were due to uptake inhibition. In this report, the effects of D_2O on uptake of glucose, potassium and phosphate are described.

Materials and methods. Unless otherwise stated, the haploid yeast strain and growth medium were the same as those described before(1). For uptake experiments, cells were grown to mid-log phase, washed free of growth medium, suspended in distilled water and starved for 3 hours with vigorous aeration. After the starvation period, cells were washed and resuspended in 0.02 M tris buffer (pH 7.2) to a final concentration of approximately 25 mg per ml wet weight (5-7 mg dry weight). After temperature equilibration at 25°C, an equal volume of a solution containing per ml: 2 μmoles potassium as KCl, 2 μmoles phosphate as K_2HPO_4, 6 nc $P^{32}O_4^{\equiv}$, and 10 mg glucose was added. The cell suspensions were incubated at 25°C with constant shaking and at time intervals indicated in Fig. 1 and 2, 5 ml samples taken. Cells and medium were separated by centri-

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fugation and both cells and supernatant saved for analysis. The cells were washed twice with ice cold distilled water and held in an ice bath until analyzed. For D_2O experiments, the same procedures were used except that all solutions were constituted with 99% D_2O prepared as described before(1).

Potassium and glucose uptake were determined by assaying the suspending medium by flame photometry and the glucostat reagent (Worthington Biochemical Co.) respectively. Phosphate uptake was followed by determining the P^{32} content of washed cells in a gas flow proportional counter. Radioactivity was converted to μmoles phosphate from the specific activity of the suspending medium.

Glucose and sorbose transport were studied independent of catabolism according to the procedures described by Cirillo, Wilkins and Anton(2). These experiments utilized a respiration deficient mutant derived from the strain used in the experiment described above. In this mutant strain the inhibitor (iodoacetic acid) blocked glycolysis, and respiration was genetically blocked. Thus, the experiments could be carried out in air, rather than under nitrogen. Cells were suspended in 5 mM iodoacetic acid (IAA) adjusted to pH 7.2 with KOH. The concentration of IAA used was sufficient to completely block glycolysis since no CO_2 was evolved in Warburg experiments and no growth occurred. After temperature equilibration at 25°C an equal volume of a 10% sugar solution in 5 mM IAA was added. At indicated intervals (Fig. 3), samples were removed and the cells washed with an ice cold 5 mM IAA, 1 mM uranyl nitrate solution at pH 4.0. After washing, the cells were suspended in 5 ml of water and intracellular sugar extracted by boiling the cells for 10 minutes. Glucose was determined with the glucostat reagent and sorbose by the method of Dische and Devi(3). Results are reported as mg sugar per ml cell water. Cell water was taken as 47% of the packed cell volume(2).

All experiments were repeated at least 3 times.

Results. Uptake curves for potassium, phosphate, and glucose in D_2O and H_2O are

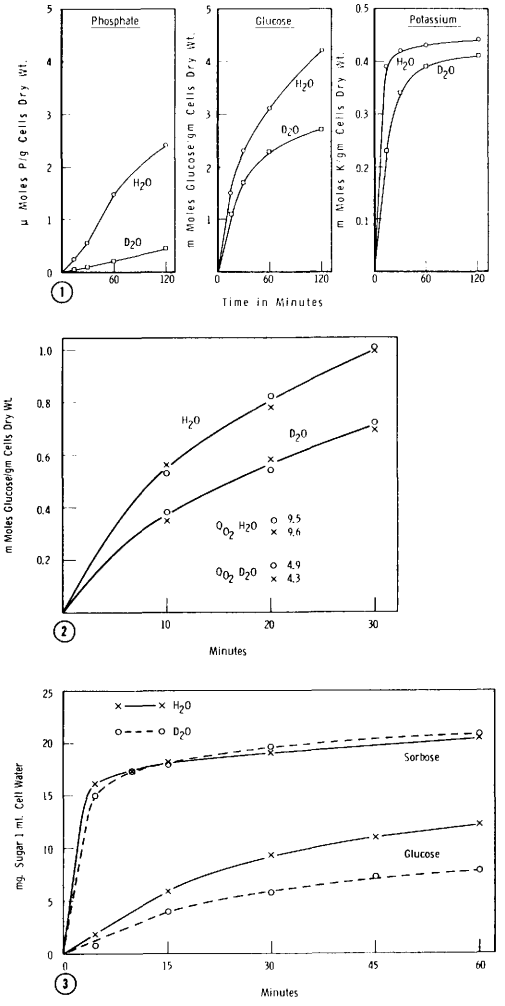

FIG. 1. Effect of 99% D_2O on uptake of glucose, potassium, and phosphate. Cells were suspended in 0.01 M tris buffer at pH 7.2. Cell concentration was 2.5 mg dry wt. per ml, temperature 25°C, substrate concentrations/ml were phosphate, 1 μmole, potassium, 3 μmoles, and glucose, 5 mg.

FIG. 2. Uptake of glucose (×) and deuterioglucose (○) in H_2O and 99% D_2O. Other conditions as in Fig. 1 except glucose concentration was 3.5 mg per ml. Q_{O_2} values expressed as μl O_2/mg/hr.

FIG. 3. Effect of 99% D_2O on glucose and sorbose transport in respiration deficient yeast cells suspended in 5 mM IAA. Cell concentration was 0.1 ml packed cells per 5 ml solution. Sugar concentration was 5%, temperature, 25° C, pH 7.2.

shown in Fig. 1. Uptake rates calculated during linear uptake show that phosphate transport was inhibited 75%, glucose 35%, and potassium 45%. Uptake of potassium and phosphate are dependent on glucose catabolism(4,5), which is inhibited 50% by D_2O

(1). Since deuterium inhibition of glucose catabolism and potassium uptake were nearly the same, it seems likely that reduced potassium uptake was due to the slower rate of glucose catabolism in D_2O, rather than a direct effect on the transport process. In the case of phosphate, this explanation does not suffice, since the D_2O effect on uptake was half again as large as the effect on the rate of glucose catabolism. Phosphate is thought to enter the cell *via* oxidative phosphorylation associated with glyceraldehyde 3-phosphate dehydrogenase(5). However, this enzyme is part of the catabolic sequence, so that at most uptake could only be inhibited 50%. Possibly there are other deuterium sensitive pathways or steps in phosphate transport which enhance the inhibitory action of D_2O on uptake.

Since uptake of ordinary glucose was inhibited by D_2O, it was of interest to study uptake of fully deuterated glucose in D_2O and H_2O environments. Results presented in Fig. 2 show that uptake of glucose and deuterio-glucose was the same in H_2O and D_2O and that the inhibitory effect of deuterium on uptake was the same for both forms of glucose. Also shown in Fig. 2 are Q_{O_2} values for the 2 forms of glucose and it is evident that deuterated glucose was catabolized at the same rate as ordinary glucose in H_2O and D_2O. It is clear, then, that neither uptake inhibition nor inhibition of glucose catabolism were due to a mixed isotope environment as has been reported for deuterium effects on growth of bacteria(6). Also, inhibition of uptake was the same whether the cells had previously been grown in D_2O or H_2O media, indicating that deuterium incorporation into the organic mass of the cell was not a factor in these experiments.

As mentioned earlier, phosphate and potassium are taken up by the yeast cell only during glucose catabolism. Sugar uptake, however, can be followed independent of metabolic activity. Cirillo *et al*(2) and others have shown that when glucose catabolism is inhibited with IAA, sugar accumulation inside the cells can be measured if the external sugar concentration is sufficiently high. In Fig. 3 are shown results of experiments with IAA-treated cells in 5% glucose solutions. In D_2O, transport was inhibited 30% (during linear uptake) which is the same as was obtained with cells able to catabolize glucose (Fig. 1). It would appear, then, that D_2O inhibition of glucose uptake was due to interference with the transport process rather than inhibition of catabolism. The data also indicate that the previously reported inhibition of glucose catabolism(1) was not entirely due to uptake inhibition, since catabolism was reduced 50% compared to 30% reduction in uptake. The difference is presumably due to isotope effects on enzyme reaction rates.

Present data on the mechanism of glucose transport in yeast indicate that the process is carrier mediated, and that transport across the cell membrane is by facilitated diffusion (7). The postulated carrier is stereospecific, but is not specific for glucose since a variety of non-metabolizable monosaccharides such as sorbose are transported by the same carrier(7). Heavy water could inhibit transport by interfering with formation and dissociation of sugar carrier complexes, by slowing the rate at which the complexes diffuse across the cell membrane, or both. In any event, it would be expected that deuterium effects on transport of other sugars would be similar to effects on glucose transport. That this was not the case is shown in Fig. 3, where it can be seen that sorbose transport was insensitive to D_2O. This finding clearly shows that diffusion of sugar carrier complexes was not affected by D_2O. The data also suggest that either transport of glucose and sorbose is by different mechanisms, or, assuming a common carrier for both sugars, glucose and sorbose combine with carrier differently and only the former process is sensitive to D_2O. Presently, it is not possible to choose between these alternatives, since no information is available on the nature of the sugar carrier. Consideration was also given to the possibility that hexokinase was involved in glucose transport. However, in accord with the findings of Katz(8), no isotope effect on this enzyme reaction was observed, indicat-

ing that glucose phosphorylation was not a factor in uptake inhibition.

Discussion. Previously it was shown that the rates of growth and glucose catabolism of yeast were affected immediately when cells were transferred from H_2O to D_2O or from D_2O to H_2O, whereas the half time for equilibration was at least 10 minutes(1). From the results presented here, it is possible to account for the rapid response to changing the isotopic composition of the suspending medium. The glucose, and to a lesser extent, phosphate uptake processes were shown to be sensitive to D_2O inhibition independent of metabolic effects. Since transport systems are located on or near the cell surface, uptake would be affected almost immediately when cells are transferred to D_2O or H_2O, resulting in an abrupt change in the rates at which glucose and phosphate enter the cell. This, in turn, would cause a rapid alteration in the rates of both growth and glucose catabolism. In addition to affecting rates of growth and metabolic activity, it is likely that uptake inhibition would cause physiological imbalances within the cell and thus lead to further metabolic disturbances.

Summary. Deuterium inhibited uptake of glucose, potassium, and phosphate. Potassium uptake inhibition was probably due to the depressed rate of glucose catabolism in D_2O. Glucose and phosphate uptake inhibition was due to direct isotope effects on the transport processes. Uptake and the effects of D_2O on uptake were the same for deuterio-glucose as for ordinary glucose. Results of experiments on the effects of D_2O on sorbose and glucose transport indicated that these sugars enter the cell by different mechanisms. It was concluded that the immediate effects of D_2O in depressing the rates of growth and glucose catabolism were due to inhibition of glucose and phosphate uptake.

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Identification of the Antiviral Substances in Nasal Secretions. (29637)

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Over the years, investigations of local immunity of the respiratory tract have led to a search for antibodies or antibody-like activity in respiratory secretions. Substances capable of inhibiting proliferation of viruses have been described repeatedly in respiratory mucus(1) although the nature of the inhibiting substances has not been clearly elucidated. Primarily on the basis of experiments showing very little anti-influenzal activity when incubation of nasal secretions and virus was carried out at 4°C, Burnet *et al*(2) suggested that the virus inactivating substance was different from serum antibody. Francis studied nasal antibodies to influenza in humans and found a positive correlation with serum titer, and certain biochemical and serologic characteristics which suggested the active material to be similar to serum antibody(3). Fazekas St. Groth found that influenzal immunization of mice produced a parallel response of serum and nasal antibodies(4). He also demon-