specific activities (units per milligram of soluble protein) of the same tissues. The amounts of protein soluble at 105,000*g* in the red or white muscle were not different as a result of training. Although the effect of a single exercise bout on hexokinase activity was not as significant as the effect of training, there is good indication of increases which are maintained for 48 hours after the single exercise bout (Table 1). Data not included here indicate that similar increases in hexokinase activity occur in animals killed immediately after a single 30-minute run.

The activities of some enzymes in skeletal muscle do increase with exercise (*4*, *5*), and an earlier investigation (*6*) suggested that hexokinase activity in rat skeletal muscle might increase with training. However, only general inferences are possible regarding the relationship between the amount of exercise and change in enzyme activity, the latent period between exercise and change in enzyme activity, and the length of time a change persists. Our findings indicate that exercise of sufficient intensity results in increases in hexokinase activity which appear immediately and are maintained for at least 48 hours. Previous studies showed that increased activities of several oxidative enzymes persist for at least 24 hours (*4*), whereas increased creatine phosphokinase activity returns to control values 72 hours after exercise (*5*).

The increases in hexokinase activities, whether due to the activation of some inactive form or to the synthesis of new enzyme, result in an increased capacity for glucose phosphorylation which may facilitate the synthesis of glycogen. We find that exercising guinea pigs for 30 minutes results in a marked depletion of muscle glycogen stores, and that following exercise there is a supercompensation in glycogen storage which is apparent after 48 hours (*7*). This supercompensation phenomenon has also been observed in humans (*8*).

An interesting, unexpected result of this study was the consistent finding of greater hexokinase activities in red than in white skeletal muscle. This, to our knowledge, is the first such demonstration for an enzyme associated with glycolysis.

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Artificial Membranes: A New Type of Cell for Measuring Diffusional Resistance

Abstract. *A novel technique allows determination of membrane diffusivities and eliminates the complications of the fluid resistance to mass transfer on either side of the membrane. Results on the permeability of a dialysis membrane of the type used in artificial kidneys agree with previous data and are obtained in much shorter time. The measured activation energy for diffusion demonstrates that the transport of salt through the membrane occurs by the same mechanism as the diffusion of salt in water, but that only 10 percent of the membrane surface is effective.*

Interest in the use of artificial membranes for a number of processes, as those used in water desalination and artificial kidneys, has necessitated techniques for evaluating the diffusional properties of such membranes. The usual technique involves a dialysis cell in which the liquid in the chambers is stirred violently in an attempt to eliminate the diffusional resistance in the field adjacent to the membrane.

The effectiveness of this stirring is open to controversy. Lane and Riggle (*1*) claim that the liquid resistance is removed at a speed of 600 rev/min in their apparatus. However, as improved membranes with higher permeabilities are developed, the errors due to the liquid resistance increase and higher speeds are required. The effect can ultimately never be overcome by stirring alone. Leonard and Bluemle (*2*) have used a "Wilson plot" method that involves many measurements at different speeds of stirring with extrapolation to infinite speed. Their data indicate that with dialysis membranes the permeability of the membrane is about 20 percent greater than that measured at 600 rev/min.

Our report concerns a new type of cell, which, while more complex to construct, allows absolute determination of the membrane permeability alone, with little or no stirring. The method involves rotation of the membrane separating the two compartments. The critical factor in the apparatus is the design of the disk or membrane holder, since it must form a liquid-tight seal while rotating. The disk consists of a plexiglass ring glued to the inside of a bored-out pulley for a timing belt, as shown in Fig. 1. A second ring, also shown, screws to the face of the first, clamping the membrane firmly between the two. Positive sealing of the membrane between the faces is accomplished with O-rings; the exposed diameter of the membrane, available for transport, is 1 inch (2.5 cm). The disk assembly is then fitted into the vertical support section shown in Fig. 2. Matching faces in the disk and support section are cut with a 3-deg taper that allows the disk to seal while rotating. The disk is held in place against the support by a plexiglass face plate that seals against the disk by means of a greased O-ring, and the face plate is screwed up against the disk with just enough pressure to allow sealing without excessive wear of the ring. Solution compartments, each with a volume of about 250 ml, are screwed to the face plate and support section, on either side of the disk, respectively, and sealed with an easily removed silicone adhesive. The disk is rotated by a timing belt that is inserted through a slot in the support assembly. The assembled apparatus is shown in Fig. 3.

With this apparatus, rotational speeds up to 55 rev/min can be maintained indefinitely with no leakage. Above 55 rev/min wear on the O-rings became excessive during runs of the usual time.

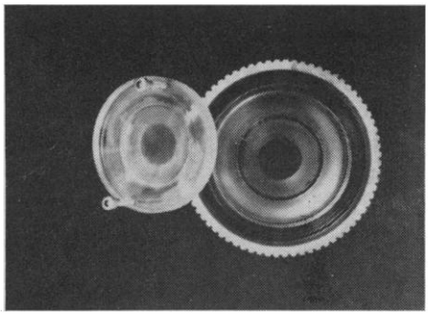

Fig. 1. Rotating membrane holder and clamp plate.

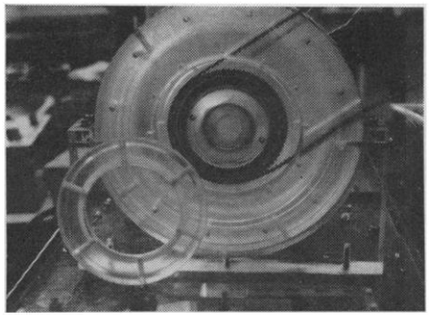

Fig. 2. Rotating disk mounted in support section with screw-on face plate shown at lower left.

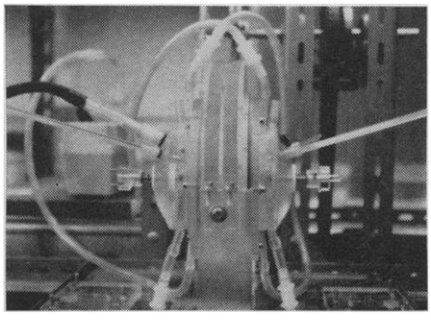

Fig. 3. Side view of assembled apparatus.

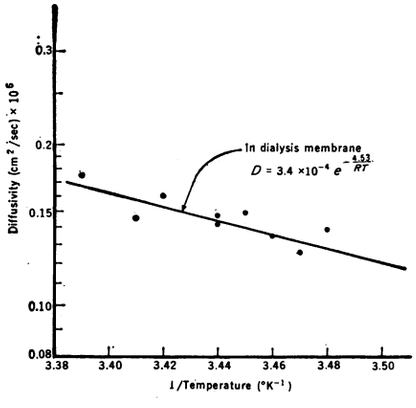

Fig. 4. Diffusivity of sodium chloride in dialysis membrane. R, gas constant in kilocalories per mole per degree Kelvin; T, temperature in degrees Kelvin; and e, the base of the natural system of logarithms.

Also, a temperature control system is necessary to maintain isothermal conditions, because of the friction produced by the rotating seals. Plexiglass proved to be a poor choice of material for construction of this apparatus, since above about 30°C thermal expansion can cause the disk to lock because of the close tolerances required. An improved version of the apparatus will eliminate these problems by using teflon O-rings and a material with greater thermal stability.

The theoretical basis of the technique is that the diffusional field adjacent to a rotating disk in a fluid medium is one-dimensional and the field structure can be obtained analytically for a variety of boundary conditions. This system has been exploited in many studies of ion diffusion, heat transfer, and diffusional kinetics (*3*). For our apparatus, the resistance in the fluid can be calculated a priori, if one knows the dimensions of the system, rotational speed of the disk, and properties of the fluid, including the diffusivity of the solute species in the liquid. In the experimental system, edge effects are known to cause some error; therefore, we checked the system with a known solute system—the dissolution of benzoic acid from the disk surface (*4*). The result for the liquid mass transfer coefficient (k_l) (permeability of solute in liquid film) is given as

$$k_l = 0.544 \frac{D}{r} \left(\frac{r^2 \omega}{\nu} \right)^{1/2} \left(\frac{\nu}{D} \right)^{1/3}$$

where D, r, ω, and ν are the solute diffusivity, radius of the membrane, speed of the disk, and kinematic viscosity of the liquid, respectively. This relationship gives an estimate to ± 1 percent. Because the resistances to diffusion through the liquid films and membrane are in series, the membrane permeability can then be obtained from

$$1/K = (1/k_l)_{left} + 1/k_m + (1/k_l)_{right}$$

where K is the permeability measured for the whole system and k_m the permeability of the membrane itself.

Figure 4 gives the diffusivity of sodium chloride through a dialysis membrane that differs from that used by Leonard and Bluemle only in thickness. The diffusivity is calculated from the permeability by multiplying the latter by the membrane thickness.

The permeability for our membrane at 37°C, as obtained from this data, is 15×10^{-3} cm/min. Leonard and Bluemle obtained, after correcting for the difference in membrane thickness, a value of 17×10^{-3}, which is in good agreement since the thickness of the swollen membrane is difficult to determine to ± 10 percent. In a comparison of the number of runs (based on their statements) required for their technique to allow accurate extrapolation with the single run necessary in our technique, the new method represents a saving of about 10 hours in evaluating a single membrane at a single temperature. They report that, even when the rate of stirring is high, the fluid film contributes 20 percent to the overall resistance; therefore, for such membranes the effect is important and our technique is of real value.

Figure 4 indicates that the activation energy for diffusion of sodium chloride through this membrane is essentially the same as in water, on the order of 4.5 kcal/mole. We can conclude that the membrane does not represent an added energy barrier to diffusion of sodium chloride, which implies that the solute does not undergo any chemical reaction or combination within the membrane. However, since the diffusion constant itself is only 10 percent of that in water, the lack of an energy barrier implies that only 10 percent of the membrane area is available for diffusion.

This technique should prove applicable to various types of membranes, particularly for those with high permeability. We have already applied the technique with promising results to ion-exchange membranes (*5*). Measurements of permeability of natural membranes should be possible, if the membrane can be obtained in the form of a small flat disk.

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