binations of wild second chromosomes from natural populations. The mean number of eggs laid by heterozygous females was 30.3 ± 1.3 per day (number of females = 52), and the survival from egg to adult was 69.9 ± 1.8 percent. Both means are very similar to those obtained in the four experimental populations at the last testing in August 1967.

Dobzhansky and Spassky (*3*) have found improvements in the viability and in the rates of the development in strains of *D. pseudoobscura* homozygous for second and fourth chromosomes. The strains were kept in mass cultures for 50 generations. It seems, however, that the changes obtained were less uniform compared to the changes in the fecundity and in the egg-to-adult survival found in my experiment. With Zelikman's (*4*) "intensity of reproduction" used as a parameter of the betterment of fitness (the mean number of progeny per female per interval of time), it appears that in Borrego 71 populations, the fitness was improved by a factor of more than 14.

The genetic raw materials which were utilized by the natural selection to increase the fitness of the experimental populations arose probably by spontaneous mutations. According to Mukai (*5*), the rate of spontaneous mutations causing minor changes in the viability is very high, about 20 times greater than the rate of mutation to recessive lethals. Although the mutation process generates raw materials from which adaptive changes are constructed by selection, it also brings forth a multitude of poorly adapted variants. Since in the experimental populations the percentage of the eggs surviving to give adult flies is very small, the opportunity for the selection to operate must have been considerable.

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Exercise: Effects on Hexokinase Activity in Red and White Skeletal Muscle

Abstract. *Single bouts of exercise increase hexokinase activity in red and white skeletal muscle of guinea pigs. Multiple bouts of exercise cause twofold increases. In contrast to other enzymes associated with glycolysis, hexokinase activity is higher in red than in white skeletal muscle.*

Strenuous exercise can result in adaptations in the activities of certain enzyme systems providing the energy for muscle contraction. However, little is known about the amount of exercise required to produce such adaptations, the time course for their appearance, or how long they persist. Likewise, no comparisons of the effects of exercise on red and white skeletal muscle have been reported although red and white skeletal muscle have different metabolic characteristics, showing a reciprocal relationship of glycolytic and mitochondrial enzymes (*1*). Type I, or red muscle fibers, have a high capacity for oxidative metabolism with strong activities of Krebs cycle and electron transport enzymes, whereas type II, or white muscle fibers, have high rates of glycolysis with high activities of glycolytic enzymes and phosphorylase. We have measured hexokinase activity in both red and white skeletal muscle and compared effects of single and repeated bouts of exercise.

Three groups of male guinea pigs (mixed strains) weighing, on the average, 600 g were studied for 3 weeks. The sedentary group was not exercised; the exercised group ran once for 30 minutes and was killed 48 hours later; and the trained group ran for 30 minutes every other day for 21 days and was killed 48 hours after the last training session. All exercise was performed on a treadmill driven at 1.9 km/hour in a room maintained at 21°C. When the animals were killed a superficial quadriceps muscle, corresponding to the vastus lateralis of rats, was removed and frozen at −80°C. This muscle possesses distinguishable red and white segments which were shown histochemically to be composed mainly of type I and type II fibers, respectively.

Frozen muscles were later thawed and homogenized in a cold (1° to 4°C) medium containing in final concentration 50 m*M* tris(hydroxymethyl)aminomethane buffer, 1 m*M* ethylenediaminetetraacetate, 15 m*M* K_2SO_4, 6 m*M* $MgCl_2$, and 10 m*M* mercaptoethanol at *p*H 7.8. The homogenates were centrifuged for 45 minutes at 105,000*g*, and the resulting supernatants were used directly for enzyme assay.

Enzyme activity was determined spectrophotometrically, by a modification of the method used by Sharma *et al.* (*2*), by measuring the rate of formation of reduced nicotinamide-adenine dinucleotide phosphate (reduced NADP) at 340 nm. The reaction was started by the addition of a small volume of supernatant to a cuvette containing in final concentration 50 m*M* tris buffer, 0.75 m*M* NADP, 3.0 m*M* adenosine-5′-triphosphate, 8.0 m*M* $MgCl_2$, 1 m*M* glucose, and excess glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase at *p*H 7.5. The rate of increase of optical density was halved to correct to micromoles of glucose-6-phosphate formed, and a molar absorbancy index for reduced NADP of 6.22×10^6 cm²/mole was used. Reduced NADP formed during the assay was stable. The concentration of soluble protein in the respective supernatants was determined by the biuret method (*3*).

The 3-week training program resulted in approximately twofold increases in total hexokinase activity of both red and white skeletal muscle (Table 1). Differences were also manifest in the

Table 1. Hexokinase activities in red and white skeletal muscle from sedentary, exercised, and trained guinea pigs. Results are given as nanomoles of glucose-6-phosphate formed at 37°C per minute per gram of tissue or per milligram of soluble protein in the 105,000*g* supernatants. Values represent group means and standard errors with the numbers of animals in parentheses.

Group	Red muscle		White muscle	
	Unit/g muscle	Unit/mg protein	Unit/g muscle	Unit/mg protein
Sedentary (9)	188 ± 16*	5.08 ± .58*	135 ± 12*	4.64 ± .40*
Exercised (9)	281 ± 15	8.12 ± .55	211 ± 13	6.96 ± .46
Trained (10)	341 ± 24*	9.22 ± .80*	290 ± 29*	8.90 ± .75*

* Differences between sedentary and trained significant at $P = .05$.

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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.