2. A. A. Boyden, *Am. Naturalist* 77, 234 (1943).
3. R. A. McCabe and H. F. Deutsch, *Auk.* 69 No. 1, 1 (1952).
4. This work was aided by grants from the Penrose Fund of the American Philosophical Society and the C. F. Kettering Foundation. We thank John Johnson, Miami Valley Hospital, Dayton, Ohio, for the Analytrol scannings, and James McMahon and Don Rawlings for their assistance.
5. A. F. Carr, *Handbook of Turtles* (Comstock, Ithaca, N.Y., 1952).
6. J. W. Crenshaw, Ph.D. dissertation, University of Florida (1955).
7. Additional evidence for the differentiation of turtle groups may be found by the analysis of the free amino acids in the serum. Preliminary experiments, in which paper chromatography was used, have indicated that certain amino acids may be absent in individual species. Two of these amino acids have been tentatively identified as lysine (deficient in *scripta*) and alanine (deficient in *nelsoni*). Further work on this aspect is in progress.
8. R. J. Block, E. L. Durrum, G. Zweig, *Paper Chromatography and Paper Electrophoresis* (Academic Press, New York, 1955).
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Increase in Resting Membrane Potential of Skeletal Muscle Produced by Insulin

Although the mechanism of action of insulin is not known, evidence is accumulating to suggest that it alters the rate at which certain substances enter skeletal muscle. Stadie, Haugaard, and Vaughan (*1*) showed that insulin affixes itself firmly to muscle, perhaps to the muscle membrane. Levine, Goldstein, Huddlestun, and Klein (*2*) demonstrated increased movement of glucose and related hexoses into muscle in eviscerated animals, and Park and Johnson (*3*) confirmed the phenomenon in the rat. Fisher and Lindsay (*4*) made essentially the same observation in the isolated, perfused rat heart. These observations do not distinguish between an effect of insulin on muscle membrane and an effect on a hypothetical hexose transport system.

Recently use has been made of the fact that aldolase, an intracellular enzyme, diffuses from muscle incubated under a variety of conditions (*5*). Alterations in the rate of diffusion of aldolase are attributable to changes in membrane permeability; no transport mechanism need be invoked. Insulin increased the rate at which aldolase diffused from isolated rat muscle; this is presumptive evidence that insulin altered membrane permeability.

The sum of these observations suggests that insulin becomes associated intimately with the muscle membrane, perhaps deforming it to alter permeability. If this is true, the association between insulin and muscle membrane might lead to altered electrical properties of the membrane. From the well-known effect of insulin on serum potassium concentration, it was suspected that insulin might hyperpolarize the muscle membrane.

Insulin administered to the intact animal causes a decrease in concentration of potassium in serum owing at least in part to movement of potassium from extracellular fluid into muscle. The consequent increase in ratio of activities of intracellular and extracellular potassium should lead to hyperpolarization of the muscle membrane. However, there is no satisfactory explanation for the movement of potassium induced by insulin. It was conceivable that, rather than producing intracellular migration of potassium, which then caused a change in membrane potential, insulin might act by first hyperpolarizing the membrane; this, in turn, would lead to intracellular migration of potasium, impelled by the new potential difference.

For these reasons the effect of insulin on resting membrane potential of isolated rat skeletal muscle was examined (*6*). Insulin caused hyperpolarization of the membrane.

The test system was the peroneus longus muscle of the rat. The muscle was excised by freeing it gently and cutting its tendons without transection of muscle fibers. In young rats this muscle weighs about 25 mg and exhibits stable resting membrane potentials for several hours. The muscle was placed at rest length in an appropriate perfusion system containing balanced buffered saline-glucose-HCO_3 solution and K^+ at 4.7 milliequivalents per liter; it was then gassed with 95 percent O_2, 5 percent CO_2. Intracellular puncture was performed with KCl-filled microelectrodes of impedance of approximately 15 megohms. Signals were led through conventional circuitry and displayed on a cathode-ray oscilloscope at a scale of 25 mv/in. After the muscle had been probed widely and it had been determined that resting potentials were stable, insulin was added to the system; resting membrane potentials were measured from approximately the 20th to the 60th minute thereafter. In several experiments, as a control on changes with time, potentials were measured either in the absence of insulin or in its presence but not in both circumstances.

In 207 impalements in six muscles in the absence of insulin, resting membrane potential was 70 ± 0.7 mv (standard error of the mean; S.E.M.). In 320 impalements in six muscles in the presence of insulin, 0.1 to 0.3 unit/ml, resting membrane potential was 75.4 ± 0.5 mv (S.E.M.). Insulin produced an increase of 5.4 ± 0.89 mv (S.E.M.), a highly significant difference by t test ($t = 6$, $P \ll 0.0001$). Data from four paired experiments appear in Table 1. Insulin produced hyperpolarization, highly significant by t test, in all pairs.

The mean increase in membrane potential was 8 percent. Were this the result of movement of potassium from perfusion fluid to intracellular fluid, the ratio of activity of potassium inside muscle to that outside would be required to increase by about 50 percent, in accordance with the equation

$$\frac{E_R''}{E_R'} = \frac{\ln (K)_i''/(K)_o''}{\ln (K)_i'/(K)_o'} = 1.08$$

where E_R is the resting membrane potential, $(K)_i$ is the activity of potassium inside the cell, and $(K)_o$ is its activity outside the cell; single primes refer to control muscles and double primes to muscles after exposure to insulin. If the true mean increase were as small as two standard errors less than the observed mean increase in resting potential, the ratio of activities of potassium would have had to increase by about one-third.

Potassium concentration of perfusion fluid was, by design, held constant owing to the large volume of fluid, approximately 150 ml, compared to the small mass of muscle. Analysis of potassium in perfusion fluid by flame photometry demonstrated that its concentration was indeed constant.

Both peronei longi from a rat were removed. One muscle was placed in 150 ml of insulin-free perfusion fluid and gassed simultaneously with its mate in which membrane potentials were measured and which was exposed to insulin. At the end of the experiment the muscles were analyzed for potassium. In five pairs of muscles $(K)_i$ increased by 1, 14, 25, 27 and 44 percent. The greatest increase occurred in a muscle in which membrane potential increased by 14 per-

Table 1. Hyperpolarization of muscle membrane produced by insulin.

Insulin concentration (units/ml)	Fibers measured (No.)	Resting membrane potential*			
		Control (mv)	Insulin (mv)	Difference (mv)	P†
0.1	135	69.4 ± 1.6	75.4 ± 1.1	5.9 ± 1.9	< 0.01
0.3	124	70.6 ± 1.8	76.3 ± 1.0	5.7 ± 2.1	< 0.01
0.1	90	61.5 ± 1.0	68.5 ± 1.2	7.0 ± 1.6	< 0.001
0.1	58	71.7 ± 1.9	81.3 ± 1.5	9.6 ± 2.6	< 0.001

* Potentials are given as mean ± standard error of the mean. † P is probability that the difference measured occurred by chance, estimated by t test.

cent. In this instance, an increase of more than 100 percent is required to account for the hyperpolarization. In no case could the rise in intracellular potassium account alone for the observed increase in resting membrane potential, and the average increase at the end of 1 hour's exposure to insulin was less than half that theoretically required to cause the hyperpolarization.

These data, with earlier data on the effect of insulin on aldolase efflux, are interpreted to indicate that insulin can act by its association with muscle membrane and that the insulin-membrane complex results in spatial changes in the barrier to diffusion, increasing membrane permeability and simultaneously increasing the potential difference across the membrane. In response to increased potential difference across the membrane, potassium moves into muscle toward a new equilibrium ratio of concentrations.

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References and Notes

1. W. C. Stadie, N. Haugaard, M. Vaughan, *J. Biol. Chem.* 199, 729 (1952).
2. R. Levine *et al.*, *Am. J. Physiol.* 163, 70 (1950).
3. C. R. Park and L. H. Johnson, *ibid.* 182, 17 (1955).
4. R. B. Fisher and D. B. Lindsay, *J. Physiol. (London)* 131, 526 (1956).
5. K. L. Zierler, *Am. J. Physiol.* 185, 1 (1956); *ibid.*, in press; *J. Clin. Invest.* 36, 938 (1957).
6. This work was performed under a contract between the Office of Naval Research, Department of the Navy, and Johns Hopkins University (NR 113-241) and further supported by a grant from the Muscular Dystrophy Associations of America, Inc. The assistance of William J. Sullivan, who constructed much of the apparatus and shared in the conduct of the experiments, is acknowledged gratefully.

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Balanus Fouling of Shrimp

Fouling of commercial crabs (*Callinectes sapidus*) and lobsters (*Homarus americanus*) by various species of barnacles (*Balanus*) is a common occurrence (*1*) but the presence of maturing sessile barnacles on shrimp is noteworthy. This report is based on observation of four *Balanus*-fouled white shrimp (*Penaeus setiferus*) taken from the inshore waters of Mississippi and South Carolina during the winter of 1957.

The single Mississippi specimen (*2*) was collected at the mouth of Ocean Springs Harbor, Biloxi Bay, on 17 February. This was a 90-mm male carrying five small (less than 2-mm basal diameter) unidentified *Balanus*. The barnacles were attached along the mid-dorsal line of the fourth, fifth, and sixth abdominal segments.

The South Carolina specimens were

Fig. 1. *Penaeus setiferus* (119-mm female) with 4-mm *Balanus amphitrite niveus* attached to the first abdominal segment.

taken from the Edisto River system on 2, 11, and 25 March. These shrimp, two males (125 mm and 150 mm) and one female (119 mm) each carried a single barnacle. The female (Fig. 1) and smaller male were each fouled with a 4-mm (basal diameter) *Balanus amphitrite niveus* Darwin on the first abdominal segment. The barnacle on the female was located 1 mm to the right of the mid-dorsal line, whereas the attachment site on the male was 2 mm to the left. The remaining shrimp carried a 9-mm *Balanus improvisus* Darwin dorsolaterally on the fifth abdominal segment with the left edge of its base on the mid-dorsal line (*3*).

Smith (*4*) showed that, at Miami, *B. amphitrite niveus* attained a size of 4 mm in 13 days during February, and McDougall (*5*) indicated that some individuals of *B. improvisus* attain, in December and January, a size of 13 mm in 42 days at Beaufort, N.C. Gunter and Geyer (*6*) gave data showing a minimal winter growth rate for *B. improvisus* of 0.13 mm per day off the Louisiana coast. No data are available on the winter growth of *Balanus* in South Carolina, but it is reasonable to assess minimal growth periods of 10 and 25 days for the 4 mm and 9 mm *Balanus* found on local shrimp. The age of the Mississippi barnacles is estimated at about 2 weeks.

Since fouling can become established only during interecdysal periods, the balanoids developed between the previous molt and time of capture. None of the shrimp showed signs of imminent shedding. Winter growth of shrimp is minimal (*7*), and fouling by maturing barnacles is probably confined to this period of reduced molting frequency.

The capture of four fouled shrimp from the Atlantic and Gulf coasts within a short space of time suggests that careful observation of winter shrimp catches may reveal numerous instances of this association between *Balanus* and *Penaeus*. Analysis of the growth of attached *Balanus* might yield information on winter molting frequencies of individual shrimp.

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References and Notes

1. D. P. Henry, *U.S. Fish Wildlife Service Fishery Bull.* 89, 443 (1954); R. W. Dexter, *Ecology* 36, 159 (1955).
2. This specimen was kindly supplied by Gordon Gunter, director of the Gulf Coast Research Laboratory, Ocean Springs, Miss.
3. These specimens are retained in the reference collections of Bears Bluff Laboratories.
4. F. G. W. Smith, *Biol. Bull.* 90, 51 (1946).
5. K. D. McDougall, *Ecol. Monographs* 13, 321 (1943).
6. G. Gunter and R. A. Geyer, *Publs. Inst. Marine Sci., Univ. of Texas* 4, 37 (1955).
7. M. J. Lindner and W. W. Anderson, *U.S. Fish Wildlife Service Fishery Bull.* 106, 555 (1956).

14 August 1957

Enzyme-Inhibitor Complex in a Tryptophan-Requiring Mutant of Neurospora crassa

Numerous reports indicate that gene mutations can cause the loss of specific enzymatic activities (*1*). It is important, from both a genetic and a biochemical standpoint, to know whether such mutant cells continue to synthesize enzymatically inactive molecules structurally related to the enzyme. The presence of a serologically active protein closely related to the enzyme tryptophan synthetase has been demonstrated in a number of allelic tryptophan-requiring mutants of *Neurospora crassa* which lack the enzyme (*2–4*). Similar results have also been found in *Escherichia coli* (*5*).

The present study (*6*), in which a temperature-sensitive, tryptophan-requiring mutant of *Neurospora crassa* (*7–9*) was employed, indicates that highly active preparations of tryptophan synthetase can be obtained from *inactive* crude extracts of this mutant when the crude extracts are purified by using protamine