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.

KENNETH L. ZIERLER
Department of Medicine, Johns Hopkins University, Baltimore, Maryland

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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.

C. E. DAWSON
Bears Bluff Laboratories, Wadmalaw Island, South Carolina

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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

sulfate, ammonium sulfate, and alumina gel. Methods for the extraction and purification of wild-type tryptophan synthetase have been previously described (*4, 10*). Evidence is presented suggesting that this activation may involve the dissociation of an enzyme-inhibitor complex.

As shown in Table 1, the amount of tryptophan synthetase present in mutant strain td_{24}, after activation, is about 30 percent of the amount present in the wild-type strain. Attempts to activate crude extracts of this mutant by heat, salt (NaCl) dissociation, acid, or dialysis have not been successful thus far.

It has been possible to inhibit the activated enzyme completely by adding back a 23 to 50 percent ammonium sulfate fraction, resuspended in 0.1*M* phosphate buffer at *p*H 7.8, or by using aliquots of the crude, unfractionated extract. The results of experiments on the effect of inhibitor concentration indicate that a linear relationship exists between inhibitor concentration and percentage inhibition, suggesting that a stoichiometric combination of enzyme and inhibitor may occur (*9*). A low dissociation constant for the complex is suggested, for no protective or competitive effect is exerted by the substrate or the coenzyme (*9*).

The inhibitor precipitates over a fairly wide concentration range of ammonium sulfate. The inhibitory fraction has the following properties: (i) stable to boiling; (ii) precipitated but not inactivated by 10 percent TCA; (iii) dialyzable; (iv) partially adsorbed on alumina gel; (v) not absorbed on charcoal; (vi) stable to 2-hour refluxing in concentrated HCl; (vii) partially precipitated by 95-percent ethanol; and (viii) stable to ashing. Whether the inhibitory material consists solely of one or more metals is not yet known, but there is evidence that metals may play a role in both the function and formation of tryptophan synthetase (*11*).

The inhibitor has been obtained from all of the *td* mutants (*12, 13*) examined, but attempts to activate the enzyme in strains other than td_{24} have proved unsuccessful to date. The inhibitor has also been obtained from several wild-type strains (*9*). The inhibitor-sensitivity of the mutant and wild-type enzymes has been compared at similar levels of specific activity, and the mutant enzyme is exceedingly more sensitive to the inhibitor (*9*). The inhibitor from *Neurospora* is also effective against tryptophan synthetase from *Azotobacter vinlandii* (*14*), but it has no effect on *Neurospora* alcohol dehydrogenase (*15*).

Table 1. Effect of fractionation on crude extracts of mutant td_{24} lacking tryptophan synthetase activity. Both strains td_{24} and 5256A were incubated at 25°C for 3 days. Strain td_{24} was grown in Fries minimal medium plus DL-tryptophan, while strain 5256A was grown in minimal medium alone. Mycelia were grown, harvested, extracted, fractionated, and assayed by methods already described (*4, 10*).

Strain	Tryptophan synthetase (unit/ml)	Protein (mg/ml)	Specific activity*
	Crude extract		
Mutant td_{24}	0.0	13.6	0.0
Wild-type 5256A	43.5	13.2	3.3
	Fractionated preparation		
Mutant td_{24}	76.0	10.0	7.6
Wild-type 5256A	196.0	9.4	20.9

* Specific activity = units of enzyme per milligram of protein.

These preliminary findings suggest, at least in the case of mutant td_{24}, that gene mutation has not prevented the formation of tryptophan synthetase. Rather it would seem that an active protein is formed which may immediately combine with a readily available inhibitor. This likelihood is also suggested by earlier work on tryptophan synthetase formation in *Neurospora* (*16*).

The fact that the inhibitory material is found in the wild-type as well as the mutant organism indicates that the material is a normal cell component. As has already been mentioned, the mutant enzyme appears to be considerably more sensitive to the inhibitor than is the wild-type enzyme. One interpretation of these results is that the td_{24} enzyme may be a structurally altered protein (*1, 17*) which has an extremely high affinity for some normally occurring cell constituent, forming with it, *in vivo*, an enzymatically inactive indissociable complex. One of the possibilities being considered is that the protein antigenically related to tryptophan synthetase in *td* mutants of *Neurospora* lacking the enzyme (*2, 4*) may be an enzyme-inhibitor complex. Perhaps, as Beadle contends (*18*), specific suppressor genes, effective in partially restoring tryptophan synthetase activity in certain *td* mutants (*13*), may function by controlling the level of an inhibitor, or possibly by creating an intracellular environment that favors dissociation of an enzyme-inhibitor complex.

The fact that tryptophan synthetase from the wild-type organism is much more resistant to the inhibitor *in vitro* (*9*) suggests either that the dissociation constant of the complex *in vivo* may be high or that a mechanism may exist in the normal cell for controlling complex formation. The significance of enzyme-inhibitor combinations as potential regulatory mechanisms in cellular metabolism has recently been pointed out by several workers (*19*).

SIGMUND R. SUSKIND
LORETTA I. KUREK
McCollum-Pratt Institute,
Johns Hopkins University,
Baltimore, Maryland

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7. Neurospora crassa, strain td_{24}, obtained from C. Yanofsky, Department of Microbiology, Western Reserve University School of Medicine. This mutant exhibits no crude extract tryptophan synthetase activity when grown at 25°C and has an absolute requirement for L-tryptophan at this temperature. At 33°C it forms trace amounts of enzyme and will grow slightly on minimal medium after 5 to 6 days' incubation (*8*). A serologically active, enzymatically inactive protein (CRM), closely related to the enzyme, is formed at both temperatures, but the amount formed is much greater at the higher temperature (*4, 9*).
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