Research into the mechanism of action of ODC has been hampered by the difficulty of purifying the enzyme. In an attempt both to purify ODC and demonstrate an interaction between ODC and RNA polymerase I, the polymerase was coupled to an activated Sepharose column which was used to purify ODC more than 1000-fold. This ODC preparation was then used to explore the possible direct effects of ODC on the kinetics of the RNA polymerase I assay.

We used male Sprague-Dawley rats weighing 100 to 125 g. The activity of ODC was determined by measuring the release of $^{14}CO_2$ from D,L-[1-^{14}C]ornithine [5.3 mc/mmole; see (*6*)] as described (*7*). The activity of RNA polymerase I was determined by measuring the incorporation of [^{3}H]uridine triphosphate (18.6 c/mmole) into acid-insoluble RNA in the presence of 1.8 μg/ml of α-amanitin (*8*). For some experiments we used ^{14}C-labeled adenosine triphosphate, [8-^{14}C]ATP (45 mc/mmole) and [γ-^{32}P]ATP (4.8 c/mmole).

The RNA polymerase I was isolated from rat liver nuclei by the method of Jänne *et al.* (*9*). The active fractions eluted from diethylaminoethyl (DEAE)-Sephadex were pooled and dialyzed for 8 hours against 100 volumes of 0.1*M* $NaHCO_3$ (*p*H 8.3) buffer containing 0.5*M* NaCl. This preparation (approximately 30 mg of protein of which over 60 percent was estimated to be RNA polymerase I) was then coupled to 5 g of cyanogen bromide–activated Sepharose (Pharmacia).

Ornithine decarboxylase was isolated from the livers of rats injected intraperitoneally with 3-isobutyl, 1-methylxanthine (40 μmole/kg, in 0.9 percent saline with ethanol, 5 : 1) 4 hours prior to being killed. This induced a 50- to 100-fold increase in ODC activity (*5*). The preparation was subjected to acetic acid precipitation (*p*H 4.3) and centrifuged for 10 minutes at 50,000*g*. The pellet was resuspended in 0.01*M* phosphate buffer and adjusted to *p*H 6.0 with 2*M* dibasic sodium phosphate before being recentrifuged. The supernatant was immediately chromatographed through DEAE-cellulose. The active fractions were pooled and applied to the RNA polymerase I affinity chromatography column. This column was washed with 0.1*M* ornithine in the homogenizing buffer and the flow stopped for 2 hours. Enzyme activity was eluted in the void volume of the column when the flow was reinitiated. This procedure for the purification of ODC resulted in a 1000- to 3000-fold purification of the enzyme (Table 1). The ability to purify ODC to this extent with an RNA polymerase I affinity column suggests a physical interaction between ODC and RNA polymerase I.

Table 2. Effect of a highly purified preparation of ODC on the ratio of [γ-^{32}P]ATP to [8-^{14}C]ATP incorporated into RNA in an RNA polymerase I assay. The activity of RNA polymerase I was measured in isolated nuclei in the presence and absence of 10 μU of ODC. The reaction mixtures differed from those described in that 0.075 μmole each of guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, 0.048 μmole of ATP, 0.002 μmole of [γ-^{32}P]ATP, and 0.02 μmole of [8-^{14}C]ATP were present in each assay. Each point represents the mean ± standard error for five separate determinations.

Amount of label incorporated (pmole)		Ratio of ^{32}P to ^{14}C
^{32}P	^{14}C	
Control experiment		
0.88 ± 0.06	520 ± 32	0.0017
Assay with added ODC activity		
1.18 ± 0.04*	575 ± 25	0.0021

*Data differ from control ($P < .005$)

The addition of the purified ODC preparation to an RNA polymerase I assay increases the initial rate of the reaction and the time for which the reaction is linear, as well as restoring linearity to a reaction that has plateaued (*5*, *7*). These results strongly suggest that ODC serves as an initiation factor. To further test this concept, RNA polymerase I was assayed with [γ-^{32}P]ATP and [8-^{14}C]ATP. The addition of purified ODC to these assays resulted in the increased incorporation of both isotopes (Table 2). The increase in the incorporation of γ-^{32}P, however, was greater than that of the ^{14}C, indicating that the presence of ODC results in increased initiation of RNA chains.

The purification of ODC by means of an RNA polymerase I affinity chromatography column is evidence of a physical interaction between these two proteins. These data, in conjunction with the stimulation of RNA polymerase I activity by the purified ODC preparation, which seems to be the result of increased initiation, strongly support the hypothesis that the labile protein which regulates RNA polymerase I activity is ornithine decarboxylase.

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16 July 1976; revised 3 September 1976

Human Babesiosis

Healy *et al.* (*1*) reported an outbreak of human babesiosis on Nantucket Island and suggested that the localization of the disease might be due to the prevalence of *Babesia microti* infection in mice on the island. Both *Babesia* protozoa and Rocky Mountain spotted fever (RMSF) rickettsiae are transmitted by ixodid ticks and are enzootic in the tick's host population. An additional factor involved in their maintenance is the transovarial transmission of the pathogens from adult ticks to their offspring (*2*). As recently as 1967, Hoogstraal (*3*) reemphasized the relationship, which has been commonly disregarded, between the presence of the vector tick and disease.

The major vector of RMSF is the tick *Dermacentor variabilis* (*4*). In Massachusetts, the focus of RMSF is in the lower Cape Cod area (*5*). This is because the mix of high-density *D. variabilis* and human populations there produces the maximum risk of tick "bite" and not because of local differences in the level of RMSF rickettsiae in the tick host complex (*6*). Preliminary screening of *D. variabilis* from inland Massachusetts has indicated the presence of RMSF rickettsiae (*7*). The risk of RMSF in South Carolina was

shown not to be a function of the frequency of RMSF rickettsiae in the tick population (*8*). The ticks *Ixodes dentatus* and *I. scapularis* also carry RMSF rickettsiae (*2, 9, 10*) and use the same rodent hosts as *D. variabilis* (*11*). Another factor in the widespread distribution of RMSF rickettsiae is the common occurrence on migratory birds of the immature stages of *I. dentatus* and *I. scapularis* (*12*). The dynamics of the biological complex involved in a tick-borne epizootic will result in the spread of the pathogen through the species range of the tick vectors.

In Massachusetts, the ticks suspected as vectors of babesiosis are *D. variabilis* and *I. scapularis*, the only local ticks which attack humans. High infestations of the spring and summer tick, *D. variabilis*, are restricted to the Cape Cod area, while low infestations are usual in the rest of eastern Massachusetts (*13*). The fall and winter tick, *I. scapularis*, is restricted to the immediate area of Cape Cod and the islands (*14*). The immature stages of both species use the mice *Microtus pennsylvanicus* and *Peromyscus leucopus* as hosts. There is no reason to exclude an established *Babesia* enzootic from the species range of either *D. variabilis* or *I. scapularis*. The comparison of Nantucket to Muskeget by Healy *et al.* (*1*) is invalid as the vector ticks are absent (*15*) on Muskeget because of the lack of adult hosts to maintain a tick population.

Why then did babesiosis first occur on Nantucket and not on the lower Cape Cod area, the region of maximum human and tick interaction in Massachusetts? The recent occurrence of babesiosis and its subsequent frequency on Nantucket (*1*) indicate a change in the characteristics of *B. microti* to both increased human virulence and increased adaptability to its normal rodent or tick hosts. The new strain would appear to be displacing the original one. Local strain differences are known for *Babesia* spp. (*2*). That the virulent strain of *B. microti* has spread is shown by the cases of babesiosis in 1975 on both Martha's Vineyard and Long Island, New York (*1, 9*). The frequency of babesiosis on Nantucket also indicates that *Babesia* is more common in ticks than RMSF rickettsiae. It is probable that this virulent strain will spread south along the Atlantic flyway during the fall bird migration. The area of maximum risk for RMSF during the *D. variabilis* season would be the area of maximum risk for babesiosis. This region includes Suffolk County on Long Island and extends from the coastal plain of Maryland through the piedmont of Virginia and North Carolina into the Appalachian highlands of Georgia (*4*). During the *I. scapularis* season the area of maximum risk would extend south from Virginia into Florida, where *I. scapularis* replaces *D. variabilis* as the dominant pest species (*16*) and fall and winter cases of RMSF become more common than in the North Atlantic states (*17*). The possible spread of babesiosis would result in a major public health problem.

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12 May 1976

McEnroe's comment introduces a comprehensive theory concerning the epidemiology of human babesiosis by critically citing our recent report. We reported that certain rodents serve as reservoir hosts for this zoonosis on Nantucket Island. Observations were limited to that island and to Muskeget, a nearby islet that was included because it was virtually uninhabited and usually devoid of hosts for potential vector ticks. (We are puzzled by McEnroe's statement that a comparison of these localities is "invalid.")

Our suggestion that the observed high prevalence in mice may have contributed to human infection on Nantucket is the focus of McEnroe's main criticism. He extends our wording when he cites us as suggesting that human disease is localized to Nantucket because more rodents are infected there than in other localities. Reasoning that the epidemiology of babesiosis is analogous to that of spotted fever, he argues that the prevalence of infection in vector or reservoir hosts is irrelevant to the frequency of transmission to man and that risk of human infection is determined solely by "risk of tick bite." We disagree with this opinion and restate the obvious, that prevalence of *Babesia* infection in reservoir mice may be causally related to prevalence in vector ticks and thereby may affect the prevalence of human disease.

McEnroe cites our data as evidence that a uniquely virulent strain of *Babesia microti* has arisen on Nantucket Island. Certainly, this may be the case, but we know of no rigorous evidence supporting such a view. He develops this theory and states that "it is probable that this virulent strain will spread" and "result in a major public health problem" involving densely populated regions of Long Island and the southeastern quadrant of the United States. The present state of knowledge does not permit such speculation.

McEnroe's reasoning concerning the spread and ultimate distribution of human babesiosis rests on the belief that *Dermacentor variabilis* is a suitable vector of *B. microti* and that the infection is maintained by transovarial transmission. However, he provides no documentation for these statements, and we know of none. Transstadial transmission by *Ixodes scapularis* has been reported (*1*). McEnroe assumes that the epidemiological features of babesiosis are precisely those of spotted fever. This assumption is premature.

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21 December 1976