jor components were normal C_{25}, C_{27}, and C_{29} alkanes. The source or age of the spores, within the limits of our study, appeared to have little influence on the type of pattern obtained for *U. maydis*. When leaves adjacent to the infected area, the cob material, and the remaining unaltered seeds of the corn host were analyzed in the same manner, the hydrocarbon patterns were different from those of *U. maydis*. Uninfected seeds, leaves, and cob material of fresh corn obtained from two different sources provided almost identical hydrocarbon patterns that differed from those of host and of *U. maydis* spores.

On the other hand, *U. nuda* (Fig. 1C) yielded approximately the same quantity of hydrocarbon material (58 ppm) as did *U. maydis*, but the distribution was markedly different: the first population of alkanes ranged from C_{25} to C_{29}; the preponderance ranged from C_{31} to C_{37}, with C_{35}—the major contributor—representing approximately 18 percent of the total weight of hydrocarbons. As determined by gas chromatography of standards and partly by mass spectrometry, the major components were normal alkanes having odd numbers of carbon atoms. The chromatographic pattern of *U. nuda* indicated the presence of considerably more branched-chain alkanes than in *U. maydis*, on the basis of identification inferred from their respective retention times.

The predominant hydrocarbon present in *S. reiliana* (Fig. 1B) is *n*-nonacosane, which comprises over 34 percent of the total weight of hydrocarbons; *S. reiliana* yielded the largest quantity of hydrocarbons of the three spores studied: 146 ppm by weight.

Although the extraction procedure was rather rigorous, no attempt was made to disrupt the individual spores; therefore the hydrocarbons were assumed to be principally on the surface. As for the function of this outer hydrocarbon, it may serve as a protective covering to assist in controlling internal water balances and to resist microbial attack—as has been suggested for higher plants (*11*). Such mechanisms could help to explain the excellent environmental resistance of most fungal spores.

For several species of higher plants, the hydrocarbon distribution patterns are fairly consistent (*2, 12*) and may be of taxonomic significance. The positive identification of a given fungus, especially if several closely related types possess similar physical or biologic properties, is often difficult and time-consuming. It would seem that the readily distinguishable hydrocarbon patterns that we observed may offer a method of chemotaxonomic differentiation similar to that proposed for higher plants. Although these preliminary data suggest such a possibility, one should use caution in invoking such an hypothesis without consideration of the possible influences of variations in environment and host metabolism.

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Carbamylation and Binding Constants for the Inhibition of Acetylcholinesterase by Physostigmine (Eserine)

Abstract. *The kinetic treatment and experimental procedures which have been used to evaluate the binding and phosphorylation rate constants of organophosphate inhibitors were applied to the carbamate inhibitor eserine. The binding constant* K_I *and carbamylation rate constant* k_{2c} *for the inhibition of acetylcholinesterase by eserine were successfully evaluated.*

Wilson and co-workers (*1*) have proposed the following scheme for the reaction of carbamate inhibitors (I) and esterases (E):

$$\mathrm{I} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{E.I} \xrightarrow{k_{2c}} \mathrm{E}' \xrightarrow{k_3} \mathrm{E} + \text{carbamic acid} \qquad (1)$$

(E.I) is a reversible complex whose formation is controlled by the equilibrium affinity constant $K_I = (k_{-1}/k_1)$ and (E′) is the carbamylated active site of the esterase. Carbamylation is controlled by the carbamylation rate constant k_{2c} and decarbamylation by the rate constant k_3. The methods used have not permitted separate evaluations of K_I or k_{2c} to be made but have given a measure of the overall rate of formation of E′ through k'_2 where $k'_2 = (k_{2c}/K_I)$.

The significance of carbamylation rates as compared to binding seemed of interest since eserine has been regarded simply as a reversible competitive inhibitor having great affinity for the active site of cholinesterases (*2*).

Experimental techniques have been developed for the determination of K_a, the affinity constant, and k_{2p}, the phosphorylation constant for the inhibition of acetylcholinesterases by organophosphates, where K_a is precisely equivalent to K_I and k_{2p} is analogous with k_{2c} (*3*). The kinetic treatment used is based on the assumption that $k_3 = 0$ in scheme 1. The reaction is then assumed to be irreversible. But experimentally this condition has only to be approximated, and it is sufficient if k_{2c} or $k_{2p} \gg k_3$.

Since the mechanism of the carbamate reaction is considered to be identical with that of organophosphates, it seemed of interest to see if the kinetic treatment and experimental procedures giving k_{2p} and K_a would also apply to carbamates and give k_{2c} and K_I. Eserine was chosen for historical reasons and because of its solubility.

The experimental design and interpretation of results were based on the equation

$$\frac{[I]\ \Delta t}{2.3\ \Delta \log v} = \frac{[I]}{k_{2c}} + \frac{1}{k'_2} \qquad (2)$$

where $[I]$ is the concentration of eserine and $(\Delta t/2.3\ \Delta \log v)$ is the first-

order rate constant at constant [I].

Partially purified preparations of bovine erythrocyte acetylcholinesterase were obtained from the Sigma Chemical Co. Solutions of eserine and acetylcholinesterase (0.5 ml of each) were incubated for various times (t) at 25°C, pH 7.6, with 6 mM sodium phosphate buffer in the specially designed reaction vessel (*3*).

The velocity (v) of the substrate reaction catalyzed by the esterase remaining uninhibited after incubation for a specified time (t) was measured on a radiometer pH-stat. In each experiment sufficient acetylcholinesterase was used to hydrolyze 1.29 μmole of acetylcholine per minute at 25°C and pH 7.6 before inhibition; 3 mM acetylcholine was used, and no salts were added.

The curves of log (v) plotted against (t) were sensibly linear (Fig. 1). The slopes were calculated by regression analysis and gave the values ($2.3\ \Delta \log v/\Delta t$) and their standard errors (Table 1). These were used to construct the graphs of ($[I]\ \Delta t/2.3\ \Delta \log v$) plotted against [$I$] according to Eq. 2. The points fell on a straight line with significant slope and intercept values (Fig. 2), an indication that this treatment is valid for carbamates as well as for organophosphate inhibitors. The slope of the statistically fitted line in Fig. 2 gave $1/k_{2c}$, the intercept of the [I] axis gave $(-K_I)$, and the intercept on the ordinate gave $(1/k'_2)$.

Table 1. The eserine concentration [I] and corresponding ($2.3\ \Delta \log v/\Delta t$) values (plus or minus the standard error) from which K_I and k_{2c}, the binding and carbamylation rate constants, respectively, for inhibition of acetylcholinesterase, were calculated by the method of Wilkinson (*4*). Inhibition was at 25°C, pH 7.6, in 6mM sodium phosphate buffer.

$[I]$ $10^{-6}M$	$(2.3\ \Delta \log v/\Delta t) \pm$ SE (min^{-1})
0.20	0.57 ± 0.005
1.0	2.31 ± 0.07
2.0	3.96 ± 0.14
2.5	4.78 ± 0.13
5.0	6.72 ± 0.12
7.5	7.36 ± 0.26
10.0	8.05 ± 0.20

$K_I = 3.33 \pm 0.26 \times 10^{-6}M$
$k_{2c} = 10.8 \pm 0.34$ min^{-1}
$k'_2 = 3.25 \times 10^6 M^{-1}$ min^{-1}

The values for [I] and ($2.3\ \Delta \log v/\Delta t$) (Table 1) were used to calculate k_{2c} and K_I and their standard errors according to the method of Wilkinson (*4*). The K_I value for eserine and acetylcholinesterase was $3.33 \pm 0.26 \times 10^{-6}M$; k_{2c} was 10.8 ± 0.4 min^{-1}.

Winteringham and Fowler (*5*) have determined k_3 for eserine and bovine erythrocyte acetylcholinesterase at 37°C to be 2.81×10^{-2} min^{-1}. If Q_{10} is 2, k_3 would be 1.2×10^{-2} min^{-1} at 25°C. The assumption that $k_{2c} \gg k_3$ appears to be valid since $k_{2c} = 916\ k_3$ according to this calculation.

The k_{2p} of diisopropyl phosphorofluoridate (DFP) and acetylcholinesterase was 40.7 ± 1.4 min^{-1} at 25°C, pH 7.6 (*3*), so it would seem that phosphorylation and carbamylation constants are, at least in these instances, of the same order of magnitude. Eserine became bound to acetylcholinesterase much more tightly than did DFP whose K_a was $1.17 \pm 0.10 \times 10^{-3}M$. For this reason the bimolecular velocity constant k'_2 of eserine of $3.25 \times 10^6 M^{-1}$ min^{-1} was higher than the comparable value of the bimolecular velocity constant, k_i, of DFP which was $3.5 \times 10^4 M^{-1}$ min^{-1}. In this sense eserine inhibited acetylcholinesterase about 100 times more rapidly than DFP did.

Our results are consistent with those of Wilson and co-workers (*1*) and indicate that carbamates inhibit through a carbamylation step. They are also consistent with a recent study made by O'Brien (*6*) who used diisopropylphenyl N-methylcarbamate. The results indicate that, at least with eserine, a reversible complex of significance is formed which precedes carbamylation. Reiner and Simeon-Rudolf (*7*) found no evidence for the formation of a reversible complex between 1-naphthyl N-methylcarbamate, 2-isopropoxyphe-

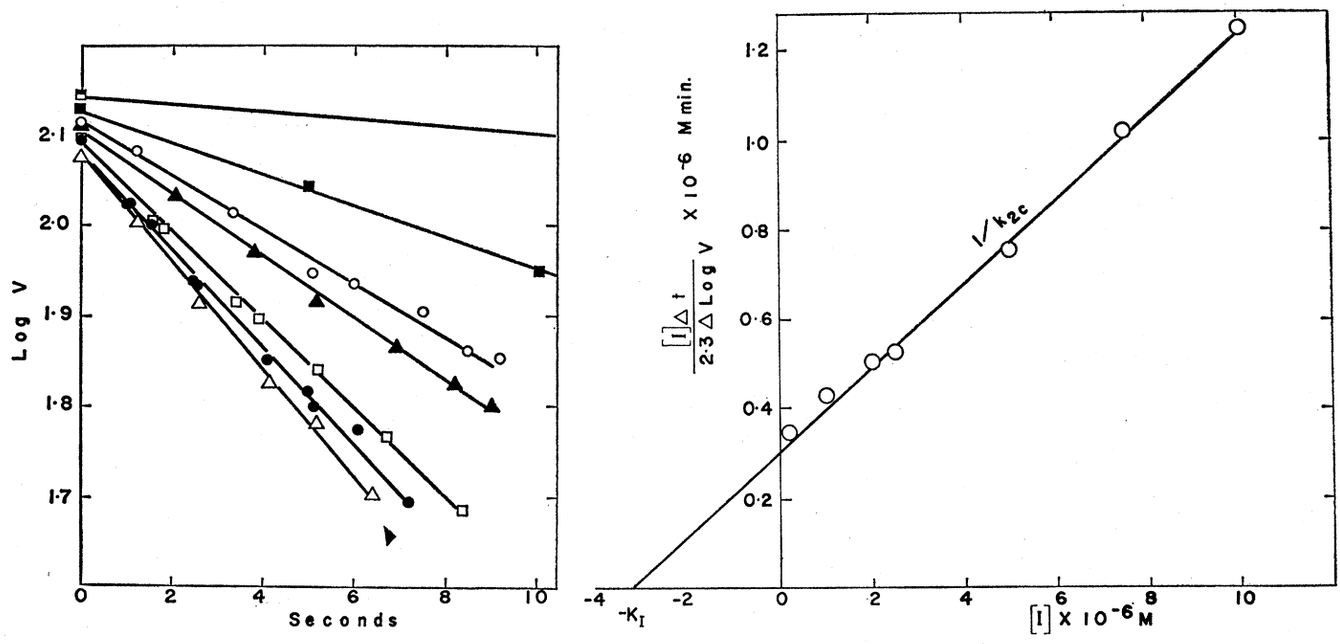

Fig. 1 (left). The curves of log v plotted against time at various concentrations of eserine: ▬ $2 \times 10^{-7}M$, ■ $1 \times 10^{-6}M$, ○ $2 \times 10^{-6}M$, ▲ $2.5 \times 10^{-6}M$, □ $5 \times 10^{-6}M$, ● $7.5 \times 10^{-6}M$, △ $1 \times 10^{-5}M$. At $2 \times 10^{-7}M$ timing began at 15.3 seconds, and the points could not therefore be plotted on the time scale chosen. Similarly, some of the times at $1 \times 10^{-6}M$ were beyond the chosen time scale. Fig. 2 (right). The eserine concentrations [I] plotted against the reciprocal of the first-order rate constants for each value of [I], ($[I]\ \Delta t/2.3\ \Delta \log v$) according to Eq. 2. The ($[I]\ \Delta t/2.3\ \Delta \log v$) values increased from 3.08 to $12.3 \times 10^{-5}M$ min or by a factor of four over the range of [I] values used, indicating the formation of a reversible complex of significance.

nyl *N*-methylcarbamate, phenyl *N*-methylcarbamate, and erythrocyte acetylcholinesterase, but they did not use the kinetics and experimental treatment employed in our work.

Evaluation of the k_{2c} and K_I values of other carbamates by the procedures used with eserine (*3*) may be possible provided that the limited solubilities exhibited by many permit values of $[I]$ approaching K_I to be realized.

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Pituitary Tumors in Mice after Prolonged Feeding of Synthetic Progestins

Abstract. *An enhanced development of pituitary tumors was observed in virgin female mice of the C57 Leaden strain following repeated oral administration of synthetic progestins. This finding appears to parallel the co-carcinogenic enhancement of mammary tumor development elicited in C3H mice treated repeatedly with progesterone.*

The observation of spontaneous pituitary tumors among inbred laboratory mice is relatively rare, although tumors, classed as chromophobe adenomas of the anterior pituitary, have been found in retired female breeders (*1–4*). Their occurrence in untreated mice two or more years old has led some experimental investigators to designate these pituitary tumors as a disease of senescence (*2*). Experimentally, the induction of tumors in the anterior pituitary follows long periods of hormonal imbalance, induced by ablation of a secretory target organ of the pituitary, by disruption of hypothalamic control of the pituitary, or by prolonged treatment with estrogen. Techniques for tumor induction include surgical, chemical, or irradiation-induced thyroidectomy, irradiation of the head or whole body, gonadectomy soon after birth, and repeated injections of estrogen (*2–4*). As for the latter, the induction of chromophobe adenomas of the adenohypophysis was first reported in 1936, following prolonged stimulation of mice with oestrin (*1*). More recent studies of pituitary tumor genesis induced by estrogen have been reviewed by Furth and Clifton (*2*) and Russfield (*3*). Progestins, by contrast, have not been associated with the induction of these tumors in the earlier literature. This report describes the development of pituitary tumors in mice fed orally-effective synthetic progestins.

Exogenous progesterone is a potent co-carcinogen for the chemical and viral induction of mammary tumors when administered repeatedly to C3H virgin female mice in order to keep them in an anovulatory state (*5*). Because of an appreciable incidence of spontaneous mammary tumors in untreated C3H females (*6*), a study was planned to test for effects of an orally active progestational agent in a mouse that does not develop spontaneous mammary tumors. The present study was made with virgin female C57 Leaden (C57L) mice. The animals, housed in groups of six per plastic cage, had unlimited access to Purina mouse chow and tap water for the duration of the experiment. For the phase of the study now reported, two commercially available synthetic progestins ("I," a mixture of approximately 50 parts Norethynodrel to 1 part Mestranol, and "II," a mixture of 50 parts Norethindrone to 1 part Ethynylestradiol) were obtained without binder and dissolved individually in peanut oil to obtain concentrations of 7 μg and 70 μg of I or II per 0.05 ml of solvent. Commencing when they were 13 weeks old, four groups of mice (24 mice per group) designated for bioassay of these solutions were fed 0.05 ml of one of the mixtures by gavage once a day, 5 days a week. Two control groups of mice were fed an equal volume of saline or peanut oil used as a solvent. A routine necropsy examination was made of all mice found dead during the course of the study. At first this did not include examination of the head in the absence of gross abnormalities. All tissues with gross pathologic changes were fixed in Bouin's solution for later histologic examination.

During the 81st to 84th week of the experiment four of ten mice found dead or moribund on routine examination in the 70 μg-I group had enlarged clitoral glands and grossly distended mammary ducts filled with a grayish-white secretion. When another mouse in the same group died, the head was examined to ascertain whether the activated mammary glands might be due to the presence of a pituitary tumor secreting prolactin. A soft, hyperemic, spheroid pituitary tumor (6 mm in diameter) was found; it had compressed, but apparently had not infiltrated, the surrounding brain. Examination (between the 84th and 89th week of the experiment) of the six remaining mice in the 70 μg-I group disclosed that they all had similar tumors, as did some mice of the other three groups given progestin (Table 1).

Seven of 15 surviving control animals were killed concurrently for comparison with those fed the progestin mixtures. None had pituitary tumors or mammary ducts distended by secretion. The eight surviving controls were killed after the 90th week, ending the experiment. In two of these mice early neoplastic foci were found. In one mouse treated with saline, a dark red, cyst-like area less than 1 mm in diameter was found in the anterior pituitary; a similar 1-mm area was found in one mouse treated with peanut oil (Table 1).

There were no gross changes in the mammary glands, adrenals, or thyroids of the two controls to suggest excessive secretion of any pituitary hormone. By contrast, the mammotropic nature of the pituitary tumors in the groups treated with progestin was apparent grossly, as evidenced by a secretory state of the mammary glands in most of the hosts of the pituitary tumor. The mammary glands that were not grossly secretory in a few hosts were moderately prominent, although ductal distention was not unusual. Despite the mammotropic effect of these pituitary tumors, none of the mice had mammary cancers.

Grossly, the mammotropic pituitary