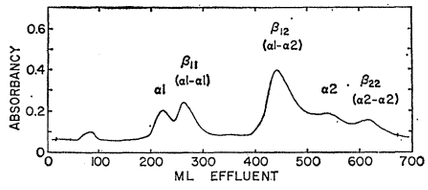

Fig. 2. Elution pattern of approximately 60 mg of denatured collagen from human skin extracted with 5*M* guanidine after salt and acid extractions. The denatured collagen was chromatographed on carboxymethylcellulose at 40°C. Absorbancy was measured at 230 mμ.

matography of guanidine-extracted samples from rat, guinea pig, and human (infant) skin. Measurements of the areas under the peaks demonstrated that these samples consistently contained 70 to 80 percent β-components. Acid-extracted collagens from the same species usually contain 50 to 60 percent but never more than 67 percent β-components. In addition to the expected peaks representing the α1 and α2 single chains and β_{12} and β_{11} double chains, a small peak consistently appeared after that of the α2 chain (Fig. 2). Protein from this peak was chromatographed a second time. The isolated protein had the same sedimentation coefficient and the same mobility during acrylamide-gel electrophoresis (*7*) as the β_{11}- and β_{12}-components. Its amino acid composition was identical to that of the α2 chain (Table 1). It must therefore be the covalently linked dimer of the α2 chain and is designated β_{22} (*8*). Its chromatographic behavior is consistent with this identification. The small amount of γ-component seen in the ultracentrifuge has been tentatively identified on chromatographs as part of the leading edge of the β_{12} peak (Fig. 2).

Since each collagen molecule consists of three chains, a purely intramolecular cross-linking process would yield a maximum of 67 percent β-components. In addition, a dimer of two α2 single chains could not form since the preponderance of evidence indicates that there is only one α2 chain per molecule (*2*). For these reasons we conclude that guanidine-extracted gelatin is obtained in part from intermolecularly cross-linked collagen.

That guanidine preferentially extracts this fraction can be explained as follows. Neutral salt and dilute acid solutions do not denature collagen in the cold. Therefore, these solvents tend to extract single collagen molecules but not covalently linked aggregates. However, 5*M* guanidine is a strong denaturing agent and could extract β-components from aggregates by first unfolding the chains. Preliminary results of isotope-incorporation experiments indicate that the intermolecularly cross-linked fraction extracted with guanidine does not necessarily represent collagen which is biologically older than the acid-extractable fraction. That is, inter- and intramolecular cross-linking may occur concurrently as part of a single process.

Bakerman and Hersh (*9*) have also found a collagen fraction that contained largely β-components when examined in the ultracentrifuge, but their conclusion that the collagen molecule must therefore contain a whole number of β-components does not necessarily follow if intermolecularly cross-linked components were extracted. For example, two collagen molecules could give rise to three β-components as a result of one intermolecular and two intramolecular cross-links.

Our results emphasize that the usual extraction procedures do not solubilize intermolecularly cross-linked collagen. Since the absolute and relative amounts of inter- and intramolecular cross-linking would appear to be important determinants of the properties of collagenous tissues, a knowledge of both the type and degree of cross-linking present is necessary for the interpretation of studies on the relationship of collagen structure to function.

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5. The denaturing action of 5*M* guanidine on collagen is indicated by measurements of optical rotation. In 0.15*M*, *p*H 4.8 acetate $[\alpha]_{313}$ of native rat skin collagen is −2300° at 5°C and changes to −825° at 45°C. When the solution is also 5*M* in guanidine, $[\alpha]_{313}$ is −844° at 5°C and does not change significantly with an increase in temperature.
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Eserine and Amphetamine: Interactive Effects on Sleeping Time in Mice

Abstract. *The sleeping time of mice given pentobarbital was found to be significantly shortened by the injection of eserine or amphetamine. When both eserine and amphetamine were given with pentobarbital the sleeping time, though much shortened, was significantly longer than with the addition of amphetamine alone. Though possessing an analeptic effect of its own, eserine appears to antagonize the analeptic effect of amphetamine.*

Amphetamine has been shown to antagonize the electroencephalographic synchronization produced by barbiturates (*1*) and to have strong analeptic effects on laboratory animals injected with pentobarbital (*2*). Eserine (physostigmine), though antagonistic to barbiturate-produced electroencephalographic synchronization (*1*), is usually considered to have no, or very little, effect on behavior. Since both drugs have been demonstrated to have an effect on the mesodiencephalic reticular formation, which in turn is known to have an influence on an animal's state of arousal, it was considered of interest to determine whether eserine would antagonize pentobarbital in some behavioral parameter as it does electroencephalographically and whether it

Table 1. Effect on sleeping time of injecting pentobarbital (PB), 60 mg/kg; amphetamine (AA), 2 mg/kg; and eserine, 0.1 mg/kg.

Group	Injections			Sleeping time* (min)
	1st	2nd	3rd	
1	PB	Saline	Saline	86 ± 3.8
2	PB	AA	Saline	52 ± 1.8
3	PB	Eserine	Saline	59 ± 2.7
4	PB	AA	Eserine	61 ± 2.6

* Mean ± standard error.

Table 2. Statistical results between groups (Student's *t*-test).

Group	Group 2	Group 3	Group 4
1	< 0.01	< 0.01	< 0.01
2		$< .05$	$< .05$
3			$> .05$

would alter the analeptic effect of amphetamine.

We measured the sleeping time of 80 female mice of the same age which had been kept in groups of 10 per cage prior to the injection of drugs. The animals, each weighing between 22 and 28 g, were divided at random into four groups as shown in Table 1. We gave each animal three injections intraperitoneally in rapid succession, taking a total time of less than 15 seconds, then placed them in individual containers. Twenty animals per day were used so that all animals were injected within ½ hour and at the same time each day. Care was taken to include animals from each group in each day's experiment. As soon as the animals were completely anesthetized they were placed on their backs. The first two times they righted themselves, they were immediately turned on their backs again. Sleeping time was taken as the time from completing the third injection till the animal had righted itself for the third time.

The results summarized in Table 1 indicate that eserine (salicylate) does have an analeptic effect on mice injected with pentobarbital (compare groups 1 and 3). However, eserine also antagonized the analeptic effect of amphetamine on mice injected with pentobarbital (compare groups 2 and 4). A summary of the statistical significance between groups is shown in Table 2. A separate experiment demonstrated that neostigmine had no effect on pentobarbital sleeping time.

It has been reported that eserine and amphetamine act on different cells to produce their characteristic electroencephalographic desynchronization (*3*), and there is evidence that adrenergic and cholinergic drugs have opposing effects on some cells in the midbrain (*4*). In the light of these findings the results reported here are interpreted as indicating that eserine and amphetamine act on separate neuronal pools, the activation of either one resulting in an early arousal from pentobarbital sleep. Eserine, however, either through those cells activated by it or by direct action, has an inhibitory influence on the amphetamine-activated neuron pool.

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Procaine Action: Antagonism by Adenosine Triphosphate and Other Nucleotides

Abstract. *Appropriate concentrations of adenosine tri-, di-, and monophosphate antagonize the depressant action of procaine on isolated nerve. Some calcium ion is required in the external solution in order for these nucleotides to produce their maximum effects. When applied either to normal or sodium deficient nerves, the nucleotides do not increase action-potential amplitude.*

The actions of ATP (*1*) and other nucleotides on excitable cells have been under investigation in this laboratory. These compounds antagonize the neural effects of calcium deficiency in the presence of about one-hundredth the normal external calcium-ion concentration (*2*). Evidence indicates that some calcium is needed to form complexes with the nucleotides in external solution, and these complexes presumably are bound to certain membrane sites. While our experiments were in progress, it was reported that a fivefold increase in the external calcium-ion concentration opposes the depressant action of procaine on nerve cells (*3*). In view of a possible interaction between calcium and nucleotides in the neuronal membrane, it seemed important to determine whether the procaine effect is also antagonized by nucleotides.

The isolated sciatic nerve of the frog, *Rana pipiens*, was used at room temperature (about 22°C) in Ringer solution (*p*H 7.2) containing $110.88 \times 10^{-3}M$ NaCl, $2.0 \times 10^{-3}M$ KCl, $1.8 \times 10^{-3}M$ $CaCl_2$, $0.1 \times 10^{-3}M$ NaH_2PO_4, and $2.0 \times 10^{-3}M$ $NaHCO_3$. All drugs were dissolved in this Ringer solution and then added to the nerve chamber. The addition of nucleotides to the Ringer caused a decrease in *p*H, and the *p*H was readjusted to 7.2 by adding tris or NaOH; the experimental results were the same regardless of which base was used. When relatively high nucleotide concentrations were prepared ($> 10 \times 10^{-3}M$), tris was always used for *p*H adjustment because high concentrations of sodium ion antagonize procaine action (*4*). The *p*H of the nerve bath was periodically checked during the course of an experiment, and it was never found to vary by more than 0.1. Monophasic action potentials were recorded in air at periodic intervals by conventional techniques. Stimulus voltage and duration was supramaximal for the rapidly conducting A fibers, and the stimulus was applied once every 2 seconds. The entire length of nerve was exposed to test solutions, the segments lying across both the stimulating and recording electrodes. The organic phosphates used were the highest purity obtainable (*5*), and calcium contamination was at most 0.2 percent. Except for AMP, all phosphates were used as sodium salts; AMP was the free acid. The EDTA was the disodium salt in a $5 \times 10^{-3}M$ concentration.

Curves of dose-response and time-action were determined for procaine dissolved in normal and in EDTA-Ringer solutions. For the purpose of testing antagonism of procaine action by nucleotides, we selected a procaine dose which produces close to maximum depression of action potential amplitude, but does not completely abolish it; this dose is $5 \times 10^{-3}M$ (Table 1). After immersing a nerve for 30 minutes in $5 \times 10^{-3}M$ procaine, application of ten times the control stimulus voltage and duration failed to increase the action potential amplitude.

The results of the nucleotide antagonism experiments are summarized in Table 1. In normal Ringer, adenine nucleotides prevent the depressant action of procaine. However, the effect