I. THE SYNTHESIS OF D- AND L-THREO- AND D- AND L-ERYTHRO-α-AMINO-β-HYDROXY-n-CAPROIC ACIDS

II. EXPERIMENTS ON THE PREPARATION OF α-AMINOALKANESULFONAMIDES

III. THE INFLUENCE OF NERVE IMPULSE SEQUENCE ON THE CONTRACTIONS OF DIFFERENT CRUSTACEAN MUSCLES

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Robert Train Adams

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ABSTRACT

The preparation of each of the four optical isomers of α-amino-β-hydroxy-n-caproic acid is described. The configurations of the four optically active amino acids have been deduced on the basis of evidence obtained from the reactions of their N-benzoyl-O-methyl derivatives with p-toluidine in the presence of papain and evidence obtained by oxidative degradation of one of the active α-amino-β-hydroxy-n-caproic acids to an optically active α-hydroxyvaleric acid. The reduction of α-benzamido-β-methoxy-n-caproic acid with lithium aluminum hydride is described.

Experiments directed toward the synthesis of aminomethanesulfonamide and α-aminoethanesulfonamide are reported.

The influence of nerve impulse sequence on the contractions of different crustacean muscles has been investigated by studying the effect of intercalating extra stimuli during stimulation at a low frequency and by studying the effect of changes in the pattern of the stimulating impulses. The effect of impulse-spacing on subsequent facilitation of the contraction has been examined. The results of these investigations are described. The significance of pattern-sensitivity in synaptic structures is discussed.

TABLE OF CONTENTS

I. THE SYNTHESIS OF D- AND L-THREO- AND D- AND L-ERYTHRO-α-AMINO-β-HYDROXY-n-CAPROIC ACIDS.*

Introduction

The α-amino-β-hydroxy-n-caproic acids are of interest in view of the occurrence in nature of compounds possessing contiguously substituted hydroxyl and amino groups. Such natural products as serine, threonine, sphingosine, dihydrosphingosine, chloromycetin, streptamine, and ephedrine fall within this classification.

The synthesis of the α-amino-β-hydroxy-n-caproic acids was undertaken as part of a program designed to develop model syntheses for dihydrosphingosine and its isomers (1), to provide starting materials which could be converted into substrates suitable for studying the kinetics and specificity of *in vitro* enzymatic reactions (1), and to prepare metabolic antagonists of the natural α-amino acids (2). The utility of the α-amino-β-hydroxy-n-caproic acids with respect to the second and third phases of this three-fold program is apparent in view of the fact that they are members of an homologous series which includes serine and threonine.

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* The prefixes threo and erythro define the relative configuration about the two asymmetric carbon atoms bearing the amino and hydroxyl groups; the letters D and L relate the configuration about the asymmetric carbon atom bearing the hydroxyl group with the configuration about the asymmetric carbon atom present in D- or L-lactic acid.

In connection with the first phase of the program, relating to development of model syntheses for dihydrosphingosine and its isomers, the present work is a sequel to the synthetic studies of Niemann, Benson, and Mead (loc. cit.), who prepared 1-amino-2,3-dihydroxy-n-hexane and 3-amino-1,2-dihydroxy-n-hexane. For the synthesis of the third member of this family of amino glycols in which the substituents occupy terminal and contiguous positions, viz., 2-amino-1,3-dihydroxy-n-hexane, various methods suggest themselves. Of these, we chose to study a preparative path which proceeded via an α-amino acid intermediate because of the relative ease of resolution of α-amino carboxylic acids by the enzymatic method developed by Bergmann and co-workers (3).

In this thesis are described the preparation of the four isomeric α-amino-β-hydroxy-n-caproic acids and the elucidation of their stereochemical configurations. A preliminary exploration of methods for conversion of the amino hydroxy acids to the corresponding amino glycols is also reported.

Discussion

Method of Preparation.

The synthesis of one of the racemic α-amino-β-methoxy-n-caproic acids was carried out by Abderhalden (4) by ammonolysis of the bromo acid resulting from mercuration (5, 6) in methanol solution of ethyl 2-hexenoate followed

by bromination of the addition compound and hydrolysis of the ester linkage. The amino methoxy acid was then converted to β-hydroxynorleucine by hydrolysis with concentrated hydrobromic acid. This method was applied by Abderhalden (loc. cit.) for the synthesis of β-hydroxyleucine, and by Abderhalden and Heyns (7) for the preparation of allothreonine and β-hydroxyvaline, the appropriate α,β-unsaturated ester being used in each case. It was later shown by Wood, Madden, and Carter (8) and by Carter and West (9-11) that for the prearation of threonine and allothreonine the mercuration and bromination reactions proceeded as well when carried out with the α,β-unsaturated acid as they did when the ester was used. Furthermore, by starting from the carboxylic acid, the necessity of hydrolyzing the α-bromo ester was avoided, thus eliminating one step in the preparation.

In our synthesis of β-hydroxynorleucine we followed the same general scheme as that used in the synthesis of threonine, proceeding over 2-hexenoic acid. The reactions involved in the preparation of a mixture of the diastereoisomeric α-amino-β-methoxy-n-caproic acids are summarized in the following chart.

$$n\text{-}C_3H_7\text{-}CHO + CH_2(COOH)_2 \xrightarrow{(C_5H_5N)} n\text{-}C_3H_7\text{-}CH{=}CH\text{-}COOH$$

$$n\text{-}C_3H_7\text{-}CH{=}CH\text{-}COOH + Hg(OAc)_2 + CH_3OH \longrightarrow \text{Addition Product}$$

$$\xrightarrow{KBr} \underline{n}\text{-}C_3H_7\text{-}CH(OCH_3)\text{-}CH(HgBr)\text{-}COOK \xrightarrow[(2)\ HBr]{(1)\ KBr + Br_2}$$

$$\underline{n}\text{-}C_3H_7\text{-}CH(OCH_3)\text{-}CHBr\text{-}COOH \xrightarrow{NH_3}$$

$$\underline{n}\text{-}C_3H_7\text{-}CH(OCH_3)\text{-}CH(NH_3^+)\text{-}COO^-.$$

Abderhalden (4) found it necessary to accept a very small recovery of product if any analytically pure amino acid were to be obtained by recrystallization of the crude mixture which resulted from the ammonolysis of α-bromo-β-methoxy-n-caproic acid. Having verified this fact, we used the crude amino acid directly for the preparation of α-benzamido-β-methoxy-n-caproic acid.

The mixture of α-benzamido acids which was formed was obtained as a viscous syrup which crystallized with difficulty. It was found that this reluctance to crystallize was to be ascribed only in part to the presence of diastereoisomeric forms of α-benzamido-β-methoxy-n-caproic acid. When the acylation was carried out on a relatively large scale, a significant quantity of D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid was found, although the presence of this substance in the product of small scale benzoylations was overlooked. That this material was indeed N-benzoyl-α-amino-β-hydroxy-n-caproic acid was proved by analysis, by conversion of it to an unsaturated azlactone, 4-n-butylidene-2-phenyl-5(4)-oxazolone, and by hydrolysis of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide, prepared from it, to L-erythro-α-amino-β-hydroxy-n-caproic acid.

The formation of α-benzamido-β-hydroxy-n-caproic acid was doubtless due to the presence of a small amount of water in the methanolic mercuric acetate solution with which the mercuration of 2-hexenoic acid was accomplished. It is to be noted that Carter and his collaborators (8, 12) carried out this type of reaction with equivalent quantities of mercuric oxide and glacial acetic acid in place of mercuric acetate, but made no mention of the formation of a hydroxy compound in addition to the desired methoxy compound.

After some difficulty it was found possible, through use of isopropyl ether, chloroform, and 1,4-dioxane, to obtain D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid, and D,L-erythro- and D,L-threo-α-benzamido-β-methoxy-n-caproic acids by fractional crystallization of the benzoylation product. No success was achieved in the attempted isolation of the threo form of the benzamido hydroxy acid. The overall yields of the three racemic acids based on n-butyraldehyde were: D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid, 1.2%; D,L-erythro-α-benzamido-β-methoxy-n-caproic acid, 14.8%; D,L-threo-α-benzamido-β-methoxy-n-caproic acid, 2.2%.*

The three racemic acids were resolved through a papain catalyzed selective conversion of the D-threo or the

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* The configurations of these intermediates, and of others to be described, are assigned on the basis of arguments presented in the section next succeeding (page 8).

TABLE I

Resolution of Derivatives of α-Amino-β-hydroxy-n-caproic Acid With Papain.

Racemate	M.P.	Resolution Prod.	M.P.	$[\alpha]_D$	Yield
D,L-Erythro-α-benzamido-β-hydroxy-n-caproic acid	177.5-178.3°	L-Erythro-α-benzamido-β-hydroxy-p-n-caprotoluide	224-225°	-27.2° (a)	68.5%
		D-Erythro-α-benzamido-β-hydroxy-n-caproic acid	164.5-165.0°	-34.1° (b)	76.5%
D,L-Erythro α-benzamido β-methoxy-n-caproic acid	111.6-112.0°	L-Erythro α-benzamido-β-methoxy-p-n-caprotoluide	211-212°	-14.0° (c)	50.3%
		D-Erythro-α-benzamido-β-methoxy-n-caproic acid	124-125°	-14.0° (d)	52.0%
D,L-Threo-α-benzamido-β-methoxy-n-caproic acid	120-121°	D-Threo-α-benzamido-β-methoxy-p-n-caprotoluide	176-177°	+17.3° (e)	83.0%
		L-Threo-α-benzamido-β-methoxy-n-caproic acid	151-152°	-51.4° (f)	72.4%

Notes:
(a) C = 3.42 w/v % in pyridine; t = 27.0°.
(b) C = 4.32 w/v % in absolute ethanol; t = 23.5°.
(c) C = 5.58 w/v % in pyridine; t = 25.0°.
(d) C = 3.42 w/v % in absolute ethanol; t = 25.0°.
(e) C = 9.79 w/v % in pyridine; t = 25.0°.
(f) C = 5.06 w/v % in absolute ethanol; t = 25.0°.

L-erythro acid to the corresponding p-toluide, the L-threo or D-erythro acid being recovered unchanged. The physical constants of the optically active acids and toluides and the yields obtained in the resolutions are summarized in table I.

The resolution products were converted to the free α-amino-β-hydroxy-n-caproic acids by acid hydrolysis,

TABLE II

Physical Properties of the Diastereoisomeric α-Amino-β-hydroxy-n-caproic Acids.

Configuration	M.P.	$[\alpha]_D$ in water	$[\alpha]_D$ in 6 N HCl	Yield
L-Erythro	203-205° (dec.)	-2.0° (a)	+27.1° (b)	95.8%
	203-204° (dec.)	-2.1° (c)		94.0%
D-Erythro	198-202° (dec.)	+2.0° (d)	-27.4° (e)	79.7%
L-Threo	185-188° (dec.)	-4.6° (f)	-18.5° (g)	95.0%
D-Threo	184-188° (dec.)	+4.6° (h)	+18.6° (i)	84.2%

Notes:
(a) From L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide; C = 5.87 w/v % in water; t = 22.0°.
(b) C = 2.47 w/v % in 6.07 N hydrochloric acid; t = 25.0°.
(c) From L-erythro-α-benzamido-β-methoxy-p-n-caprotoluide; C = 3.39 w/v % in water; t = 22.0°.
(d) From D-erythro-α-benzamido-β-hydroxy-n-caproic acid; C = 8.81 w/v % in water; t = 26.0°.
(e) C = 2.63 w/v % in 6.07 N hydrochloric acid; t = 25.0°.
(f) From L-threo-α-benzamido-β-methoxy-n-caproic acid; C = 3.50 w/v % in water; t = 25.0°.
(g) C = 2.28 w/v % in 6.03 N hydrochloric acid; t = 25.0°.
(h) From D-threo-α-benzamido-β-methoxy-p-n-caprotoluide; C = 3.46 w/v % in water; t = 25.0°.
(i) C = 2.42 w/v % in 6.07 N hydrochloric acid; t = 25.0°.

with 48% hydrobromic acid in the case of the methoxy compounds, and with 20% hydrochloric acid in the case of the hydroxy compounds. The L-erythro-α-amino-β-hydroxy-n-caproic acid was prepared both by hydrolysis of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide with 20% hydrochloric acid and by hydrolysis of the corresponding toluide of the methoxy series with 48% hydrobromic acid. The products from the two hydrolyses were identical. The physical constants of the diastereoisomeric α-amino-β-hydroxy-n-caproic acids and the yields obtained in the hydrolyses are summarized in table II.

Elucidation and Proof of Configuration.

A. Configurations of the α-Amino-β-hydroxy-n-caproic Acids.

The proof of the configurations of the four α-amino-β-hydroxy-n-caproic acids is based on two lines of experimental evidence: first, the stereochemical specificity of the enzymatic resolution, and second, the oxidative degradation of one of the amino acids to an optically active α-hydroxy acid.

The enzymatic synthesis of anilides and phenylhydrazides of acylated α-amino acids was shown by Bergmann and Fraenkel-Conrat (3) to exhibit antipodal specificity. Thus, when a racemic acylated α-amino acid was incubated at 40° with aniline or phenylhydrazine in the presence of papain and L-cysteine, in aqueous solution with the pH maintained at 4.6, the L form of the α-acylamino acid was converted to

the corresponding anilide or phenylhydrazide while the D form remained unchanged.

Latterly it was observed by Bennett and Niemann (13) that antipodal specificity was markedly reduced in the enzymatic synthesis of N-carbobenzoxy-o-fluorophenylalanylphenylhydrazide. A study by these investigators (14) of the structural features responsible for this decreased stereochemical selectivity showed that a reduction in the degree of specificity was characteristic of N-carbomethoxy-, N-carboethoxy-, and N-carbobenzoxyphenylalanines, but was not apparent in the case of N-carbobenzoxyalanine. Furthermore, a high degree of antipodal specificity was exhibited in the enzymatic resolution of N-acetyl- and N-benzoyl-D,L-phenylalanines.

The enzymatic resolution of the N-benzoyl-α-amino-β-hydroxy- and N-benzoyl-α-amino-β-methoxy-n-caproic acids then furnishes proof of the configuration about the alpha carbon atom of each resolution product. The two α-amino-β-hydroxy-n-caproic acids with specific rotations (in 6 N hydrochloric acid) of +27.1° and +18.6° (table II) possess the L configuration about their alpha carbons since they were derived by hydrolysis of enzymatically synthesized toluides. It therefore follows that the other two amino acids ($[\alpha]_D^{25}$ = -27.4° and -18.5° in 6 N hydrochloric acid) have the D configuration about their alpha carbon atoms since these two acids are enantiomorphs respectively, of

the first pair.

The configuration about the beta carbon atom of each of the four amino hydroxy caproic acids was shown by the same method utilized by Meyer and Rose (15) for elucidation of the structure of threonine. This procedure makes use of the observation of Dakin, Cohen, Daufresne, and Kenyon (16) that an α-amino acid may be oxidized by Chloramine-T to an aldehyde with one less carbon atom, according to the equation:

$$R\text{-}CH(NH_2)\text{-}COOH + (CH_3\text{-}C_6H_4\text{-}SO_2NCl)^- Na^+ + H_2O \longrightarrow$$
$$RCHO + CO_2 + NH_3 + CH_3\text{-}C_6H_4\text{-}SO_2NH_2 + NaCl \quad .$$

One of the α-amino-β-hydroxy-n-caproic acids ($[\alpha]_D^{25} = -18.5^\circ$ in 6 N hydrochloric acid) was oxidized by Chloramine-T to an optically active α-hydroxyvaleraldehyde. The aldehyde was not isolated, but was oxidized with bromine water to active α-hydroxyvaleric acid. The hydroxy acid was isolated as its barium salt, which proved to be levorotatory ($[\alpha]_D^{20} = -11.0^\circ$, C = 3.18 w/v % in water; $[\alpha]_D^{20} = -11^\circ$, C = 0.98 w/v % in water). A solution of barium α-hydroxyvalerate to which slightly more than the equivalent quantity of hydrochloric acid had been added gave a specific rotation, computed on the basis of α-hydroxyvaleric acid, $[\alpha]_D^{20} = -2.5^\circ$ (C = 0.81 w/v % for α-hydroxyvaleric acid in the presence of an equivalent amount of barium chloride in 0.067 N hydrochloric acid).

Levene and Haller (17) carried out a partial

resolution of α-hydroxyvaleric acid and proved, by chemical methods, the configurational series to which each of the optically active forms belongs. It was shown that L-α-hydroxyvaleric acid is that enantiomorph the barium salt of which is levorotatory. The barium L-α-hydroxyvalerate prepared by these authors had a specific rotation (at 20° C.) of -4.9° (C = 3.16 w/v % in water) while the specific rotation of L-α-hydroxyvaleric acid was positive ($[\alpha]_D^{20}$ = +1.5°; C = 12.7 w/v% in dilute hydrochloric acid solution in the presence of an equivalent amount of barium chloride). The direction of change of the specific rotation observed on converting the salt to the free acid was in agreement with the rule formulated by Levene, Mori, and Mikeska (18) to the effect that: "those acids which had the configuration of levo-lactic acid showed a change in rotation toward the right on passing from the unionized state (free acid) to the ionized state (salt). These acids were designated as D acids. 2-Hydroxy acids configurationally related to dextro-lactic acid suffered under identical conditions a change in rotation towards the left; they were designated as L acids."

As indicated earlier, we obtained a levorotatory solution on adding hydrochloric acid to a solution of the levorotatory barium α-hydroxyvalerate prepared by us. Our observation was made on a solution of much lower concentration than was that of Levene and Haller because of the small

quantity of barium L-α-hydroxyvalerate at hand. Since the α-hydroxyvaleric acid of Levene and Haller was partially racemized, and since it is known (19) that the rotation of a solution of a hydroxy acid may be affected to a marked degree by the addition of neutral salts, we consider that the specific rotation reported by Levene and Haller for L-α-hydroxyvaleric acid is an unreliable criterion for identification of the compound.

It having been shown that the α-amino-β-hydroxy-n-caproic acid with $[\alpha]_D^{25} = -18.5^\circ$ (in 6 N hydrochloric acid) was degraded to an α-hydroxyvaleric acid with a levorotatory barium salt, the rotation of which changed in the positive direction on addition of hydrochloric acid, the configuration about the beta carbon atom of the original amino acid must have been L. Inasmuch as the configuration about the alpha carbon was shown by the enzymatic reaction to be D, the amino hydroxy acid with $[\alpha]_D^{25} = -18.5^\circ$ is therefore L-threo-α-amino-β-hydroxy-n-caproic acid. It then follows that the enantiomorphic amino acid ($[\alpha]_D^{25} = +18.6^\circ$ in 6 N hydrochloric acid) is D-threo-α-amino-β-hydroxy-n-caproic acid. The remaining two amino acids, with specific rotations (in 6 N hydrochloric acid) of $+27.1^\circ$ and -27.4° must possess the erythro configuration since they are diastereoisomeric with the acids of the threo series. Since the acid with $[\alpha]_D^{25} = +27.1^\circ$ is known by the earlier argument to possess

the L configuration about the alpha carbon atom, it must then be L-erythro-α-amino-β-hydroxy-n-caproic acid while the fourth isomer ($[\alpha]_D^{25} = -27.4^\circ$) is the D-erythro compound.

B. Configurations of the α-Benzamido-β-hydroxy- and α-Benzamido-β-methoxy-n-caproic Acids and their Toluides.

The configurations about the alpha carbon atoms of the α-benzamido-β-methoxy-p-n-caprotoluides and the enantiomorphously related carboxylic acids (see table I), and of α-benzamido-β-hydroxy-p-n-caprotoluide and the carboxylic acid enantiomorphously related to it, are established by virtue of the antipodal specificity exhibited in the enzymatic resolution, as discussed earlier. Thus, the three toluides are known to have the L configuration at the alpha carbon while the three carboxylic acids must possess the D configuration around that carbon atom.

The configurations around the beta carbon atoms of these compounds, however, must be derived from the configurations of the α-amino-β-hydroxy-n-caproic acids prepared from them by hydrolysis. The configuration of each amino hydroxy acid and that of the derivative from which it was prepared must be the same provided that no net Walden inversion occurred during the hydrolysis. It was on this basis that the configurations of the various derivatives tabulated in table I (page 6) were assigned.

In the hydrolysis of the α-benzamido-β-methoxy-n-caproic acids (or their toluides), if a Walden inversion

were to occur its probable course would involve first a replacement of the hydroxyl group (resulting from hydrolysis of the methoxyl group) by a halogen atom, a substitution which would proceed with inversion (20)*.

It is apparent from the work of Carter and his collaborators (8-12) that replacement of the secondary hydroxyl group of threonine or allothreonine does not occur to a detectable extent in the hydrolysis of their O-methyl derivatives with 48% hydrobromic acid, nor does the cleavage of the methoxyl group proceed in such a manner as to give α-amino-β-bromo-n-butyric acid directly. In view of these facts it seems justifiable to conclude that no Walden inversion occurs during hydrolysis of the ether linkage.

By analogy with the hydrolysis of the O-methyl derivatives of threonine and allothreonine, the hydrolysis of the α-benzamido-β-methoxy-n-caproic acids (and their toluides) and of α-benzamido-β-hydroxy-n-caproic acid (and its toluide) will proceed without Walden inversion. We endeavored to obtain additional confirmatory evidence for this conclusion by attempting the methylation of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide and D-erythro-α-

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* Subsequent hydrolysis of the halide substituent in the presence of silver carbonate would proceed, it is expected, with retention of configuration (21) so that the net effect of the substitutive and hydrolytic processes would be an odd number of inversions.

benzamido-β-hydroxy-n-caproic acid.* The toluide proved to be unexpectedly resistant to methylation either with dimethyl sulfate or with methyl iodide and silver oxide. The benzamido hydroxy acid proved similarly resistant to methylation by dimethyl sulfate. In each attempted methylation the reaction proceeded incompletely and efforts to separate the O-methyl compound from the starting material were without avail.

The Reduction of α-Benzamido-β-methoxy-n-caproic Acid.

It was of interest in connection with the development of model syntheses for dihydrosphingosine to study various methods by which α-amino-β-hydroxy-n-caproic acid might be reduced, either directly or indirectly, to 2-amino-1, 3-dihydroxy-n-hexane. The requirements for a suitable method of conversion include not only that the reactions proceed in good yield, but that they proceed with little or no racemization.

Carter and Rockwell (22) carried out the conversion of D,L-threonine and D,L-allothreonine to the corresponding racemic 2-amino-1,3-dihydroxy-n-butanes by reduction of the methyl esters of the amino acids with hydrogen and Raney nickel. They observed no racemization in the case of threonine

* It will be recalled that L-erythro-α-amino-β-hydroxy-n-caproic acid was obtained both by hydrolysis of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide and of one of the two α-benzamido-β-methoxy-p-n-caprotoluides (see Table II). If the hydrolysis in the latter case proceeded without net Walden inversion, methylation of L-erythro-α-amino-β-hydroxy-p-n-caprotoluide should then give this same methoxy toluide.

methyl ester, but did in the case of the allothreonine ester.

We attempted the preparation of the benzyl thiol ester of D,L-erythro-α-benzamido-β-methoxy-n-caproic acid with a view to reducing this ester by a "reductive desulfurization." This method of reduction is attractive since it proceeds under mild conditions and has been shown to be unaccompanied by racemization when applied to thiol esters of N-acyl amino acids (23). We first attempted the preparation of the thiol ester of α-benzamido-β-methoxy-n-caproic acid by reaction of benzyl mercaptan with α-benzamido-β-methoxy-n-caproyl chloride, the latter being prepared by reaction of the acid with phosphorus pentachloride in acetyl chloride solution and used in the crude state. The product was a discolored reddish tar from which no pure substances could be obtained. An attempt to prepare the desired thiol ester alternatively by a direct acid-catalyzed esterification of the acid with benzyl mercaptan was similarly unsuccessful; the esterification product was a viscous yellow oil which resisted all efforts to purify or to provoke crystallization.

A preliminary experiment designed to effect the reduction of α-benzamido-β-methoxy-n-caproic acid with lithium aluminum hydride (24) was successful. This reduction was performed on a mixture of the D,L-threo and D,L-erythro benzamido acids since, at that time, the pure racemates were not at hand. The crude yield in the reduction was 62%, but

extensive recrystallization was required in order to obtain one of the racemic 2-benzamido-3-methoxy-1-hexanols in a state of purity, free from the other racemate.

The application of this method of reduction to the pure D,L-threo- and D,L-erythro-α-benzamido-β-methoxy-n-caproic acids and to their optically active forms remains to be accomplished. As a consequence, the efficacy of the lithium aluminum hydride reduction remains to be determined.

Experimental

2-Hexenoic Acid (25, 26).

To 403.9 g. (3.89 moles) of dry malonic acid in 410 ml. of anhydrous pyridine was added 237.6 g. (3.30 moles) of freshly distilled butyraldehyde. The reaction was allowed to proceed at room temperature for twenty-four hours, at 45° for an additional twenty-four hours, and finally at 60° for three hours. The reaction mixture was chilled, acidified with 6 N sulfuric acid, the non-aqueous phase separated, and the aqueous phase extracted with four 200 ml. portions of ether. The non-aqueous phase and ethereal extracts were combined and dried over anhydrous sodium sulfate. The dry ether solution was filtered, the solvent evaporated, the residue allowed to crystallize at 0°, and the crystals (white needles) collected to give 251.3 g. (67%) of crude 2-hexenoic acid, m.p. 30-32°.*

α-Bromo-β-methoxy-n-caproic Acid. (26).

To a solution of 660 g. (2.07 moles) of mercuric acetate in 3 l. of methanol was added 220 g. (1.93 moles) of crude 2-hexenoic acid. The solution was allowed to stand at room temperature for twenty-four hours. The precipitate which had formed was collected by suction filtration and air-dried to give 631 - 656 g. of addition product as a white powder. The addition compound was

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* This and subsequent melting points are the corrected values. All melting points were obtained in an electrically heated copper block. Melting point determinations were conducted at a heating rate of 1°/min.

dissolved in 1940 ml. of water containing 348 g. (2.92 moles) of potassium bromide. To this solution at 0°, over a period of forty-five minutes there was added, with stirring in direct sunlight, 308 g. (1.93 moles) of bromine dissolved in a solution of 348 g. of potassium bromide in 560 ml. of water. The mixture was allowed to stand at 0° for an additional twenty minutes after which sodium bisulfite was added as required to discharge the color of the remaining bromine. The reaction mixture was rendered basic by the addition of sodium bicarbonate and was extracted with 300 ml. of ether to remove a lachrymatory substance which was present. After neutralization, the aqueous phase was acidified by the addition of 324 ml. of 48% hydrobromic acid and was then extracted with five 300 ml. portions of ether. The combined ethereal extracts were dried over anhydrous sodium sulfate after which the solvent was removed to give 345 - 360 g. (79-83%) of crude α-bromo-β-methoxy-n-caproic acid as an orange oil.

α-Amino-β-methoxy-n-caproic Acid.

A solution of 667 g. (2.96 moles) of crude α-bromo-β-methoxy-n-caproic acid in 6.7 l. of concentrated ammonium hydroxide was heated at 85° for twenty-two hours*.

* For this purpose 375 ml. "citrate" bottles fitted with pressure stoppers were found quite satisfactory. Danger from shattering was minimized by wrapping each bottle first with a layer of electrician's tape, then in a cloth towel. The incubation was carried out in a Fischer "Isotemp" oven.

After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to a volume of two liters. The solution was filtered to remove the small quantity of insoluble material present and the filtrate was evaporated to dryness. Water (1 l.) was added and the solution reconcentrated under reduced pressure. The gummy residue was triturated with 1.5 l. of acetone and the mixture allowed to stand for two days. The solid was collected by suction filtration, washed with acetone, and dried to give 414 g. of crude α-amino-β-methoxy-n-caproic acid. The mother liquor and washings were combined and evaporated to dryness at reduced pressure. The residue was allowed to stand under acetone (1 l.) for an additional two days, when further crystallization occurred, yielding 136 g. of crude amino acid.

A small portion (2.8 g.) of the crude amino acid was dissolved in 10 ml. of hot water. To the hot filtered solution an equal volume of ethanol was added and the solution was allowed to cool and stand until crystallization was complete. After a second recrystallization from 5 ml. of hot water, adding three volumes of ethanol to the hot solution, the crystals were collected and dried to give 0.2 g. of α-amino-β-methoxy-n-caproic acid, white plates, decomposition point 224° (the sample was introduced into the melting point apparatus at 215°).

Analysis. Calculated for $C_7H_{15}O_3N$ (161.2):

α-amino N, 8.69; α-amino acid carboxyl C, 7.45. Found: α-amino N, 8.88, 8.89; α-amino acid carboxyl C, 7.61, 7.62.

α-Benzamido-β-hydroxy-n-caproic Acid and α-Benzamido-β-methoxy-n-caproic Acid.

A solution containing 256 g. of crude α-amino-β-methoxy-n-caproic acid (the ammonolysis product from 1.38 moles of bromo acid) and 110.4 g. (2.76 moles) of sodium hydroxide in one liter of water was distilled at reduced pressure from a water bath until the removal of ammonia was complete. The solution was cooled and maintained at a temperature of 0-10° for a period of one hour, during which time 194 g. (1.38 moles) of benzoyl chloride was added in a dropwise fashion with efficient mechanical stirring. During the addition, a solution of 68.8 g. (1.72 moles) of sodium hydroxide in 344 ml. of water was introduced at a rate sufficient to prevent the pH of the reaction mixture from falling below 8.

Stirring and cooling were continued for one hour longer. The reaction mixture was then slowly acidified to pH 1-2 by the dropwise addition of concentrated hydrochloric acid with mechanical stirring. A light yellow oil separated which crystallized on standing at 4° for three days. The crude acid was collected, dried, and dissolved in 400 ml. of hot diisopropyl ether. An insoluble residue remained which was recrystallized from 1,4-dioxane to give 7.2 g. of D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid, white

rectangular plates, m.p. 177.5-178.3° (with slow decomposition when held at the melting temperature).

Analysis. Calculated for $C_{13}H_{17}O_4N$ (251.3): C, 62.13; H, 6.82; N, 5.58. Found: C, 62.31; H, 6.82; N, 5.69.

The isopropyl ether solution was concentrated by removing 150 ml. of solvent by distillation at reduced pressure. On cooling the solution and allowing it to stand at 4° crystallization occurred slowly. After three days the solid was collected, air-dried, and recrystallized three times, once from chloroform, once from 1,4-dioxane, and once from isopropyl ether, to give 14.6 g. (4.0% based on the bromo acid) of D,L-threo-α-benzamido-β-methoxy-n-caproic acid, square plates from isopropyl ether, m.p. 120-121°.

Analysis. Calculated for $C_{14}H_{19}O_4N$ (265.3): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.35; H, 7.27; N, 5.25.

The mother liquors from the dioxane, chloroform, and isopropyl ether recrystallizations were combined and evaporated to dryness *in vacuo*. The residue was extracted with five 600 ml. portions of hot petroleum ether (b.p. 60-70°). The petroleum ether-insoluble material was dissolved in the minimum quantity of hot isopropyl ether and the solution was cooled and allowed to stand at 4°. A slow deposition of crystals occurred over a period of ten

days. The solid was collected by filtration, washed with fresh solvent, and again recrystallized from isopropyl ether to give 88.4 g. (24.2% based on the bromo acid) of D,L-erythro-α-benzamido-β-methoxy-n-caproic acid, soft white needles, m.p. 111.6-112°. Concentration of the combined mother liquor and washings produced an additional 9.1 g. (2.5%) of the erythro acid, m.p. 107-109°, and 23.4 g. of a viscous red syrup which failed to crystallize.

Analysis. Calculated for $C_{14}H_{19}O_4N$ (265.3): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.21; H, 7.32; N, 5.15.

4-n-Butylidene-2-phenyl-5(4)-oxazolone.

A suspension of 1.00 g. (3.98 millimoles) of D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid in 10 ml. of acetic anhydride was placed in a 25 ml. flask protected by a drying tube containing soda lime and was heated on the steam bath for twenty minutes. At the end of this period all the solid had dissolved. The solution was cooled and poured into 40 ml. of chilled water with vigorous stirring. The crystalline solid which separated was collected, air-dried, and recrystallized from 80% aqueous ethanol to give 0.36 g. (42%) of 4-n-butylidene-2-phenyl-5(4)-oxazolone, white needles, m.p. 56-57°.

Analysis. Calculated for $C_{13}H_{13}O_2N$ (251.2): N, 6.51. Found: N, 6.56.

Purification of Papain.

One hundred g. of finely ground crude papain (Wallenstein "Hygrade") was stirred with 500 ml. of water for two hours at 4°, after which the mixture was filtered by suction (Whatman No. 1 filter). For a period of four hours a slow stream of hydrogen sulfide was passed through the yellow faintly turbid solution thus obtained, the mixture being cooled in an ice-bath throughout. The solution was then clarified by centrifugation at 2000 r.p.m. for twenty minutes. The supernatant was decanted and, with cooling in an ice-salt-bath and with mechanical stirring, two and one-third volumes of methanol were added over a period of forty-five minutes. The resultant suspension was stirred at 0-5° for one hour longer and then centrifuged at 2000 r.p.m. for twenty minutes. The supernatant was discarded and the precipitate was dissolved in 500 ml. of cold, hydrogen sulfide-saturated water.

The process of treating with hydrogen sulfide, centrifuging, precipitating with methanol, centrifuging, and redissolving was repeated three more times. After the fourth precipitation with methanol a 10% aliquot of the aqueous methanolic suspension was set aside for purposes of assay. The remaining enzyme was collected by centrifugation and was dissolved in 285 ml. of a 0.5 F acetic acid - 0.5 F sodium acetate buffer to give 468 ml. of solution.

The aliquot was centrifuged, the precipitate was dissolved in water, and the solution was lyophilized. From the weight of the residue which remained (2.98 g.), the concentration of enzyme in the buffered solution was found to be 57.4 g./l.. The total yield of purified papain from 100 g. of crude enzyme was 29.8 g. .

L-Erythro-α-benzamido-β-hydroxy-p-n-caprotoluide.

A solution of 4.42 g. (0.0176 moles) of D,L-erythro-α-benzamido-β-hydroxy-n-caproic acid, 2.14 g. (0.02 moles) of p-toluidine, and 2.00 g. of L(-)-cysteine hydrochloride in 430 ml. of a 0.5 F acetic acid - 0.5 F sodium acetate buffer was prepared. The pH was adjusted to 4.62 at 40° by the addition of 17 ml. of 2.02 N sodium hydroxide, a volume (40.5 ml.) of enzyme solution containing 2.3 g. of purified papain was added, and the mixture was incubated at 40° for five and one-half days. The precipitate which formed during this period was collected, washed with fresh buffer solution, then with water, and dried to give 3.15 g. of crude L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide.

To the filtrate was added 0.50 g. of L(-)-cysteine hydrochloride, 0.63 g. of p-toluidine, and 3.0 ml. of glacial acetic acid. The solution (pH 4.61) was incubated for two days longer, when an additional 0.09 g. of crude toluide was obtained. The crude toluide was recrystallized twice from a mixture of acetonitrile and 1,4-

dioxane (1:1, v/v) to give 2.05 g. (68.5%) of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide, fine white needles, m.p. 224-225°.

Analysis. Calculated for $C_{20}H_{24}O_3N_2$ (340.4): C, 70.56; H, 7.11; N, 8.23. Found: C, 70.57; H, 7.22; N, 8.25.

Specific Rotation. $[\alpha]_D^{27} = \frac{-0.03 \times 2}{1 \times 0.0684} = -27.2°$ (in pyridine).

D-Erythro-α-benzamido-β-hydroxy-n-caproic Acid.

The filtrate remaining after the removal of the second crop of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide was acidified to pH 1-2 with concentrated hydrochloric acid and the solution was allowed to stand for twenty-four hours at 4°. The crystalline solid (long white needles) which separated was collected, washed with water, and dried. This first crop weighed 1.28 g. and had a specific rotation of -33.5° (C = 6.80 w/v % in absolute ethanol at 25° C.).

The filtrate was extracted with chloroform in a continuous liquid-liquid extractor for thirty hours. The chloroform extract was evaporated to dryness, the residue redissolved in chloroform, and the resulting solution was extracted with 5% sodium carbonate solution. After separation, the carbonate phase was acidified to pH 1-2 with concentrated hydrochloric acid and the crystalline precipitate was collected, washed with water, and dried to give a second

crop of D-erythro-α-benzamido-β-hydroxy-n-caproic acid amounting to 9.62 g.

The combined first and second crops were recrystallized from a 1:1 (v/v) mixture of acetonitrile and 1,4-dioxane to give 1.69 g. (76.5%) of D-erythro-α-benzamido-β-hydroxy-n-caproic acid, obtained as clusters of soft white needles, m.p. 164.5-165.0°, $[\alpha]_D^{23.5} = -34.1^\circ$ (C = 4.32 w/v % in absolute ethanol). On further recrystallization the specific rotation remained unchanged.

Analysis. Calculated for $C_{13}H_{17}O_4N$ (251.3): C, 62.13; H, 6.82; N, 5.58. Found: C, 62.03; H, 6.79; N, 5.61.

Specific Rotation. $[\alpha]_D^{23.5} = \frac{-1.47 \times 2}{1 \times 0.0863} = -34.1^\circ$ (in absolute ethanol).

D-Threo-α-benzamido-β-methoxy-p-n-caprotoluide.

To 9.94 g. (0.0375 moles) of D,L-threo-α-benzamido-β-methoxy-n-caproic acid and 450 ml. of 0.5 F acetic acid - 0.5 F sodium acetate buffer was added 4.30 g. (0.04 moles) of p-toluidine and 2.00 g. of L(-)-cysteine hydrochloride. The mixture was warmed to effect solution and the pH was adjusted to 4.62 at 35° by the addition of 10 ml. of 2.02 N sodium hydroxide. Thirty-seven ml. (equivalent to 2.1 g. of papain) of enzyme solution containing purified papain in a concentration of 57.4 g./l. was added and the solution was incubated at 40°. After three and two-thirds days incubation the precipitated toluide (6.33 g.) was collected,

washed with fresh buffer and with water, and dried.

To the filtrate (pH 4.68 at 25°) was added 0.50 g. of L(-)-cysteine hydrochloride, 1.00 g. (0.0093 moles) of p-toluidine, and 3.42 g. of glacial acetic acid, and the pH was adjusted to 4.60 at 28° by the addition of 4 ml. of 2.02 N sodium hydroxide. The solution was incubated at 40° for four days longer, when a small second crop (0.27 g.) was obtained. This was collected and washed as before.

The crude toluide was recrystallized from toluene and the crystals (white needles) collected, washed with fresh toluene, and dried *in vacuo* giving 5.52 g. (83.0 %) of D-threo-α-benzamido-β-methoxy-p-n-caprotoluide, m.p. 176-177°, $[\alpha]_D^{25} = +17.3°$ (in pyridine). The product showed no change in specific rotation following a second recrystallization from toluene.

Analysis. Calculated for $C_{21}H_{26}O_3N_2$ (354.4): C, 71.16; H, 7.39; N, 7.90. Found: C, 71.20; H, 7.34; N, 7.85.

Specific Rotation. $[\alpha]_D^{25} = \frac{1.69 \times 2}{1 \times 0.1958} = +17.3°$ (in pyridine).

L-Threo-α-benzamido-β-methoxy-n-caproic Acid.

The filtrate remaining after the removal of the second crop of D-threo-α-benzamido-β-methoxy-p-n-caprotoluide was acidified to pH 1-2 by the dropwise addition of concentrated hydrochloric acid, with stirring. The precipitated acid was collected, washed with water, and dried to give

4.25 g. of crude L-threo-α-benzamido-β-methoxy-n-caproic acid, m.p. 149-150°.

The filtrate was evaporated to dryness in vacuo on a steam bath and the residue was extracted with 200 ml. of hot ethanol. The ethanol solution was evaporated to dryness, the residue was taken up in chloroform, and the chloroform solution was extracted with 5% aqueous sodium bicarbonate. The bicarbonate phase was separated and acidified to pH 1-2 with concentrated hydrochloric acid, when an oil separated. The oil was extracted into chloroform and the chloroform solution was dried over anhydrous sodium sulfate. On evaporation of the solvent a syrupy residue remained which readily crystallized on shaking with 5 ml. of isopropyl ether. The crystalline material was collected, washed with isopropyl ether, and dried to give an additional 0.33 g. of crude L-threo-α-benzamido-β-methoxy-n-caproic acid.

The product was recrystallized from isopropyl ether and the crystals (transparent cubes) were collected, washed with fresh isopropyl ether, and dried in vacuo at 55° to give 3.60 g. (72.4%) of L-threo-α-benzamido-β-methoxy-n-caproic acid, m.p. 151-152°, $[\alpha]_D^{25} = -51.4°$ (C = 5.06 w/v % in absolute ethanol). The specific rotation was unchanged by further recrystallization.

Analysis. Calculated for $C_{14}H_{19}O_4N$ (265.3): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.28; H, 7.32; N, 5.33.

Specific Rotation. $[\alpha]_D^{25} = \frac{-2.60 \times 5}{1 \times 0.2529} = -51.4^\circ$ (in absolute ethanol).

L-Erythro-α-benzamido-β-methoxy-p-n-caprotoluide.

A mixture of 5.00 g (0.0181 moles) of D,L-erythro-α-benzamido-β-methoxy-n-caproic acid, 2.15 g. (0.020 moles) of p-toluidine, 1.00 g. of L(-)-cysteine hydrochloride, and 180 ml. of 0.5 F acetic acid - 0.5 F sodium acetate buffer was warmed to 40° to effect solution and the pH was adjusted to 4.6 by the introduction of 20 ml. of 1 N sodium hydroxide. An enzyme solution prepared by extracting 7.0 g. of pulverized crude papain (Wallenstein "Hygrade") with 45 ml. of water at 4° for four hours was added. The reaction mixture was incubated at 40° for four and one-half days. The precipitate was collected, washed with fresh buffer and with water, and dried to give 3.11 g. of crude L-erythro-α-benzamido-β-methoxy-p-n-caprotoluide. The filtrate was incubated for two more days, when an additional 0.25 g. of crude toluide was obtained.

The crude product was recrystallized from acetonitrile, and the crystals (white needles) were collected, washed with fresh solvent, and dried in vacuo at 55°. The yield was 1.68 g. (50.3%) of L-erythro-α-benzamido-β-methoxy-p-n-caprotoluide, m.p. 211-212°. The specific rotation was unaltered by a further recrystallization from absolute ethanol.

Analysis. Calculated for $C_{21}H_{26}O_3N_2$ (354.4): C, 71.16; H, 7.39; N, 7.90. Found: C, 71.25; H, 7.44; N, 7.81.

Specific rotation. $[\alpha]_D^{25} = \frac{-0.78 \times 2}{1 \times 0.1115} = -14.0^\circ$ (in pyridine).

D-Erythro-α-benzamido-β-methoxy-n-caproic Acid.

The filtrate remaining after the removal of L-erythro-α-benzamido-β-methoxy-p-n-caprotoluide was concentrated to dryness at reduced pressure on a steam bath. Water (100 ml.) was added and the mixture filtered. The filtrate was acidified to pH 1-2 by the dropwise addition of concentrated hydrochloric acid. The product separated as an oil which crystallized readily (white needles). The crude product was collected, washed with water, and dried. Two recrystallizations, one from isopropyl ether and one from a mixture of equal volumes of petroleum ether (b.p. 60-70°) and chloroform, gave 1.30 g. (52.0 %) of D-erythro-α-benzamido-β-methoxy-n-caproic acid, needles from isopropyl ether and from petroleum ether-chloroform, m.p. 124-125°. The specific rotation was unchanged by a further recrystallization from petroleum ether-chloroform (1:1, v/v).

Analysis. Calculated for $C_{14}H_{19}O_4N$ (265.3): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.22; H, 7.31; N, 5.22.

Specific rotation. $[\alpha]_D^{25} = \frac{-0.48 \times 1}{1 \times 0.0342} = -14.0^\circ$ (in absolute ethanol).

L-Erythro-α-amino-β-hydroxy-n-caproic Acid.

A. From L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide.

A suspension of 926.9 mg. (2.72 millimoles) of L-erythro-α-benzamido-β-hydroxy-p-n-caprotoluide in 65 ml. of 20% hydrochloric acid was refluxed for eleven hours. The resulting solution was concentrated to dryness at reduced pressure, water was added, and the mixture was again evaporated to dryness. The residue was extracted with 20 ml. of water and the solution filtered. The aqueous solution was treated with an excess of freshly precipitated silver carbonate, the insoluble silver salts were separated by filtration, and the filtrate was saturated with hydrogen sulfide. After removal of the precipitated silver sulfide, the halogen-free solution was evaporated to dryness in vacuo from a water bath, yielding 383.5 mg. (95.8 %) of L-erythro-α-amino-β-hydroxy-n-caproic acid. On recrystallization from 70 % aqueous ethanol the product was obtained in the form of clusters of soft white needles, m.p. 203-205° (decomposition; the sample was introduced into the melting point apparatus at 195°), $[\alpha]_D^{22} = -2.0$ (C = 5.87 w/v % in water). After a second recrystallization from aqueous ethanol the specific rotation remained unchanged.

Analysis. Calculated for $C_6H_{13}O_3N$ (147.2): C, 48.96; H, 8.90; N, 9.52. Found: C, 48.83; H, 8.91; N, 9.47.

Specific rotation. $[\alpha]_D^{22} = \frac{-0.12 \times 2}{1 \times 0.1174} = -2.0^\circ$ (in water). $[\alpha]_D^{25} = \frac{0.67 \times 2}{1 \times 0.0494} = +27.1^\circ$ (in 6.07 N hydrochloric acid).

B. From L-Erythro-α-benzamido-β-methoxy-p-n-caprotoluide.

A suspension of 380.4 mg. (1.07 millimoles) of L-erythro-α-benzamido-β-methoxy-p-n-caprotoluide in 5 ml. of 48% hydrobromic was heated to boiling, when the toluide dissolved readily. After refluxing for three hours, the solution was evaporated in vacuo to dryness, 10 ml. of water was added, and the mixture was re-concentrated to dryness.

Water (10 ml.) was added, the insoluble solid was separated by filtration, and the filtrate was treated with freshly precipitated silver carbonate, in excess. After filtration, the halogen-free solution was saturated with hydrogen sulfide, again filtered, and finally evaporated in vacuo to a volume of 10 ml. The solution was clarified by filtration and evaporated to dryness at room temperature and atmospheric pressure by passing a stream of dry air over the solution.

The crystalline residue was dried in vacuo over phosphorus pentoxide to give 148.4 mg. (94.0%) of L-erythro-α-amino-β-hydroxy-n-caproic acid. The product was

recrystallized from 70% aqueous ethanol, from which it was obtained as soft white needles, m.p. 203-204° (dec.) with discoloration observed at 199° (the sample was introduced in the melting point apparatus at 195°). The specific rotation was unchanged within the limits of experimental error by this recrystallization.

Analysis. Calculated for $C_6H_{13}O_3N$ (147.2): C, 48.96; H, 8.90; N, 9.52. Found: C, 48.90; H, 8.79; N, 9.40.

Specific rotation. $[\alpha]_D^{22} = \frac{-0.07 \times 2}{1 \times 0.0677} = -2.1$ (in water).

D-Erythro-α-amino-β-hydroxy-n-caproic Acid.

A suspension of 493.6 mg. (1.96 millimoles) of D-erythro-α-benzamido-β-hydroxy-n-caproic acid in 15 ml. of 20% hydrochloric acid was heated to the boiling point, when a homogeneous solution was obtained. The reaction mixture was refluxed for six hours, cooled, and evaporated in vacuo to dryness. To the crystalline residue was added 10 ml. of water and the resulting suspension was filtered. The filtrate was treated with an excess of freshly precipitated silver carbonate. The solution remaining after separation of insoluble silver salts was saturated with hydrogen sulfide, filtered, decolorized with Norite, and again filtered. The resulting clear, colorless, halogen-free solution was evaporated to dryness at reduced pressure from a water bath to give 229.8 mg.

(79.7 %) of D-erythro-α-amino-β-hydroxy-n-caproic acid, needles, $[\alpha]_D^{26} = +2.0^\circ$ (C = 8.81 w/v % in water).

On recrystallization from 70% aqueous ethanol, the amino acid was obtained as clusters of soft white needles which, after being washed with fresh solvent, were collected and dried in vacuo at room temperature over phosphorus pentoxide. The observed m.p. was 198-202° (decomposition; the sample was inserted in the melting point apparatus at 191° ; a light brown color could be observed in the sample by 196.5°). The specific rotation in water remained unchanged as a result of this recrystallization.

Analysis. Calculated for $C_6H_{13}O_3N$ (147.2): C, 48.96; H, 8.90; N, 9.52. Found: C, 48.89; H, 8.84; N, 9.46.

Specific rotation. $[\alpha]_D^{26} = \frac{0.18 \times 2}{1 \times 0.1762} = +2.0^\circ$ (in water). $[\alpha]_D^{25} = \frac{-0.72 \times 2}{1 \times 0.0526} = -27.4^\circ$ (in 6.07 N hydrochloric acid).

D-Threo-α-amino-β-hydroxy-n-caproic Acid.

A mixture of 2.10 g. (5.93 millimoles) of D-threo-α-benzamido-β-methoxy-p-n-caprotoluide and 20 ml. of 48% hydrobromic acid was heated to effect solution of the toluide. The solution was refluxed for two and one-half hours, concentrated to dryness by distillation from a water bath at reduced pressure, and, after addition of 10 ml. of water, reconcentrated to dryness.

The residue was extracted with 10 ml. of water and the solution filtered. The filtrate was treated successively with an excess of freshly precipitated silver carbonate, hydrogen sulfide, and Norite. The resulting clear, colorless, halogen-free solution was evaporated to dryness at reduced pressure from a water bath and the residue dried at room temperature over phosphorus pentoxide to give 734.1 mg. (84.2 %) of D-threo-α-amino-β-hydroxy-n-caproic acid with a specific rotation, $[\alpha]_D^{25} = +4.6^\circ$ (C = 3.46 w/v % in water). The product was recrystallized from 70% aqueous ethanol, from which it was obtained in the form of soft white needles. The crystals were collected, washed with fresh solvent, and dried at room temperature over phosphorus pentoxide. The observed melting range was 184–188° (dec.), with sintering from 180° (the sample was introduced at 174°). The specific rotation was unchanged, within the limits of experimental error, by the recrystallization.

Analysis. Calculated for $C_6H_{13}O_3N$ (147.2): C, 48.96; H, 8.90; N, 9.52. Found: C, 48.86; H, 8.80; N, 9.45.

Specific rotation. $[\alpha]_D^{25} = \frac{0.16 \times 2}{1 \times 0.0691} = +4.6^\circ$ (in water). $[\alpha]_D^{25} = \frac{0.45 \times 1}{1 \times 0.0242} = +18.6^\circ$ (in 6.07 N hydrochloric acid).

L-Threo-α-amino-β-hydroxy-n-caproic Acid.

A solution of 1.46 g. (5.49 millimoles) of L-threo-

α-benzamido-β-methoxy-n-caproic acid in 15 ml. of 48% hydrobromic acid was refluxed for three and one-quarter hours. The reaction mixture was evaporated to dryness in vacuo on a water bath, water (15 ml.) was added, and the mixture was reconcentrated to dryness. The residue was extracted with 15 ml. of water and the solution filtered. The filtrate was treated successively, in the usual fashion, first with an excess of freshly precipitated silver carbonate, then with hydrogen sulfide. The filtrate obtained after removal of the precipitated silver sulfide was evaporated to dryness at reduced pressure from a water bath and the resulting residue was dried in vacuo at room temperature over phosphorus pentoxide. There was obtained 768.0 mg. (95.0 %) of L-threo-α-amino-β-hydroxy-n-caproic acid with a specific rotation, $[\alpha]_D^{25} = -4.6^\circ$ (C = 3.5 w/v % in water). On recrystallization from 70% aqueous ethanol, the substance was obtained in the form of white needles, m.p. 185-188° (dec.) with gradual discoloration observed from 179° (the sample was introduced at 174°). The specific rotation in water was unchanged by this recrystallization.

Analysis. Calculated for $C_6H_{13}O_3N$ (147.2): C, 48.96; H, 8.90; N, 9.52. Found: C, 48.89; H, 8.87; N, 9.42.

Specific rotation. $[\alpha]_D^{25} = \frac{-0.16 \times 2}{1 \times 0.0699} = -4.6^\circ$ (in water). $[\alpha]_D^{25} = \frac{-0.42 \times 2}{1 \times 0.0455} = -18.5^\circ$ (in 6.03 N hydrochloric acid).

Oxidative Degradation of L-Threo-α-amino-β-hydroxy-n-caproic Acid. (15).

A solution of 414 mg. (1.82 millimoles) of Chloramine-T in 3 ml. of water was heated to 85° and added with efficient mechanical stirring to a solution of 206.6 mg. (1.40 millimoles) of L-threo-α-amino-β-hydroxy-n-caproic acid in 4 ml. of water, the temperature of the latter being approximately 25°. Almost immediately (within ten seconds) the formation of a copious white precipitate was observed. After stirring for sixteen minutes, the reaction mixture was cooled in an ice bath until precipitation of the toluenesulfonamide was complete. The mixture was filtered, the filter cake thoroughly washed with water, and the washings combined with the original filtrate.

The solution was acidified to pH 1-2 by the addition of 0.2 ml. of 6 N hydrochloric acid and again filtered to remove the small precipitate which had formed. A solution of bromine water containing 0.22 g. (1.4 millimoles) of bromine was added and the mixture was allowed to stand at room temperature in a stoppered vessel for eighteen hours. The solution, which still contained a slight excess of bromine, was concentrated at reduced pressure to a volume of 20 ml. and was then extracted with

seven 20 ml. portions of diethyl ether. The combined ethereal extracts were dried over anhydrous sodium sulfate, the drying agent was separated by filtration, and the dried ether solution was evaporated *in vacuo* at room temperature.

The residue was dissolved in 6 ml. of water and the solution so obtained was clarified by filtration and neutralized to a phenolphthalein endpoint by the dropwise addition of 0.518 N barium hydroxide solution (1.75 ml. were required). Following concentration under reduced pressure to a volume of 2 ml., four volumes of ethanol were added to the hot aqueous solution. Barium α-hydroxy-valerate separated as white square and rectangular plates. The crystals were collected, washed with a small quantity of 80% aqueous ethanol, and dissolved in 1 ml. of hot water. The solution was filtered while hot and 4 ml. of absolute ethanol added. The solution was cooled and allowed to stand until crystallization was complete.

The product was collected, washed with 80% aqueous ethanol, and dried in vacuo (100 μ) at 110° over phosphorus pentoxide for fifteen hours. The anhydrous barium salt had a specific rotation, $[\alpha]_D^{20} = -11.0°$ (C = 3.18 w/v % in water). The salt was again recrystallized from 1 ml. of water and 4 ml. of ethanol and dried at 100 microns pressure and 110° over phosphorus pentoxide to give 132.8 mg. (51.1%) of anhydrous barium α-hydroxy-

valerate. The specific rotation was unchanged by this second recrystallization.

Analysis. Calculated for $BaC_{10}H_{18}O_6$ (371.6): Ba, 36.96; C, 32.32; H, 4.88. Found: Ba, 36.85; C, 32.31; H, 5.00.

Specific rotation. $[\alpha]_D^{20} = \frac{-0.35 \times 1}{1 \times 0.0318} = -11.0^\circ$ (in water). $[\alpha]_D^{20} = \frac{-0.11 \times 1}{1 \times 0.0098} = -11^\circ$ (in water).

To 0.0125 g. (0.0337 millimoles) of the anhydrous barium salt was added 0.134 ml. of 1.008 N hydrochloric acid (0.135 milliequivalents) and sufficient water to bring the total volume to 1.00 ml. This corresponds to a solution of 0.0081 g. of α-hydroxyvaleric acid. The specific rotation of this solution, computed for the free hydroxy acid in the presence of an equivalent amount of barium chloride, was, $[\alpha]_D^{20} = \frac{-0.02 \times 1}{1 \times 0.0081} = -2.5^\circ$ (in 0.067 N hydrochloric acid).

2-Benzamido-3-methoxy-1-hexanol.

A 500 ml., three necked, round-bottomed flask was fitted with an efficient, mercury-sealed, mechanical stirrer, a dropping funnel, and a reflux condenser. A sintered-glass funnel (size 15 M) was placed directly beneath the condenser in such a fashion that the condensate would return to the reaction vessel through the funnel (refer to fig. 1). After drying the apparatus rigorously and replacing the atmosphere of air with one of dry nitrogen, 4.00 g. (15.1 millimoles) of α-benzamido-β-

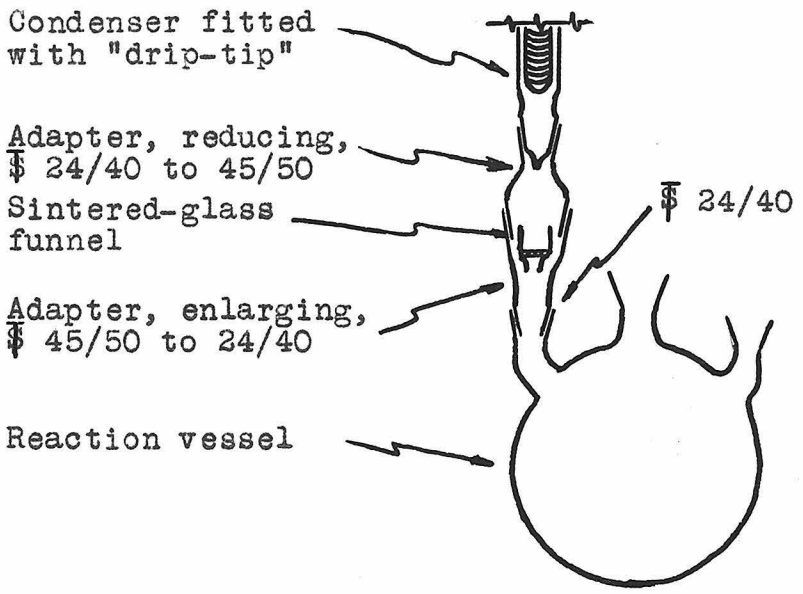

Figure 1.

Sketch of Extractor Permitting Continuous Return of the Extracting Liquid to the Reaction Vessel.

methoxy-n-caproic acid (a mixture of the D,L-threo and D,L-erythro acids) was placed in the sintered-glass funnel. A solution of 25 millimoles of lithium aluminum hydride (27) in 250 ml. of anhydrous diethyl ether was introduced into the reaction vessel through the dropping funnel and the solution was heated to boiling. Refluxing was continued until all the benzamido acid had been extracted into the reaction vessel by the passage of the condensate (thirty-seven hours were required). The reaction mixture was cooled strongly, the excess lithium aluminum hydride was decomposed by the cautious addition of water, and the mixture was then neutralized by the careful addition of 6 N

hydrochloric acid. The ether phase was separated, washed with water and 5% sodium bicarbonate, and dried over anhydrous sodium sulfate.

On separation of the drying agent and evaporation of the ether, there was obtained 2.35 g. (62 %) of an orange oil which crystallized shortly. Two recrystallizations from diisopropyl ether followed by one from water gave 0.97 g. (29.6 %) of 2-benzamido-3-methoxy-1-hexanol, needles from isopropyl ether and from water, m.p. 106-107°.

Analysis. Calculated for $C_{14}H_{21}O_3N$ (251.3): C, 66.90; H, 8.43; N, 5.58. Found: C, 66.97; H, 8.27; N, 5.58.

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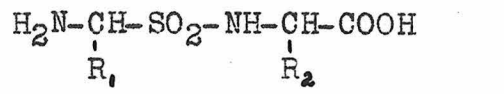
II. EXPERIMENTS ON THE PREPARATION OF α-AMINOALKANESULFONAMIDES

Introduction

The availability of α-aminoalkanesulfonyl derivatives of amino acids (I), which may be considered



(I)

analogs of dipeptides, would make possible the preparation of substrates of considerable value in relation to the characterization of such enzymes as trypsin, chymotrypsin, and papain in terms of the kinetics and specificities of their reactions. In order to develop methods for the preparation of these α-aminoalkanesulfonyl derivatives of α-amino acids, it was proposed to study the preparation of a simpler compound, aminomethanesulfonamide. Were the synthesis of aminomethanesulfonamide to be accomplished, its acidic and basic dissociation constants would then be determined in order to provide information relative to the inductive effect of a substituent α-aminoalkanesulfonyl group.

Indeed, aminomethanesulfonamide is of interest intrinsically. While a number of α-aminoalkanesulfonic acids are known (1), no example of the preparation of an α-aminoalkanesulfonamide is to be found. Further, in spite

of the attention directed to the chemistry of sulfonamides as a result of the importance of aminoarylsulfonamides such as sulfanilamide, little is known concerning the physical properties of the aliphatic sulfonamides. So far as the author is aware, the only sulfonamide of the aliphatic series the dissociation constant of which has been determined is methanesulfonamide (2).

In this section are described exploratory experiments relating to attempts to synthesize aminomethanesulfonamide and α-aminoethanesulfonamide. While these experiments are limited in scope, they are nevertheless recorded here so that they may serve as a point of reference for further endeavor.

Discussion

The most direct method for preparation of aminomethanesulfonamide seemed to be via an N-acyl derivative of aminomethanesulfonic acid, which could, presumably, be converted to its amide over the corresponding sulfonyl chloride. The α-aminoalkanesulfonic acids are easily prepared by treatment of the appropriate aldehyde-bisulfite addition compound with ammonia (3-8). While these acids are rather unstable with respect to decomposition into ammonia, sulfur dioxide, and the original aldehyde, acylation of the amino group stabilizes the molecule to a considerable degree (4).

The sodium salt of benzamidomethanesulfonic acid

was available through the courtesy of Dr. Carl Niemann. When this compound was treated with phosphorus pentachloride (at 80° and at 35°) there was achieved no success in obtaining the desired benzamidomethanesulfonyl chloride, nor could there be found any evidence for its presence in the crude reaction product.

It may well be that if the amino group of aminomethanesulfonic acid were to be substituted by two acyl radicals, the resulting compound would be sufficiently stable to permit of a successful conversion to the sulfonyl chloride. Sodium phthalimidomethanesulfonate would be well suited for this purpose. In this investigation, phthalimidomethyl bromide was prepared by the methods described by Chavane (9) and by Gabriel (10) with a view to producing sodium phthalimidomethanesulfonate by treatment of the bromo compound with sodium sulfite. The latter reaction remains to be accomplished, however.

An attempt was made to prepare α-aminoethanesulfonamide by replacement of the halogen of α-chloroethanesulfonamide by an amino group through the agency of sodamide. The activity, in metathetical reactions, of a halogen atom substituted in the alpha position to a sulfonamide moiety, like that of one alpha to a sulfone group (11, 12), is greatly diminished in comparison to the activity of the halogen atom of an alkyl halide (13). On the other hand, in hot aqueous, alkaline solution chloromethanesulfonamide

(13) and α-chloroethanesulfonamide (8) are surprisingly labile, being hydrolyzed to chloride ion, sulfite ion, and formaldehyde or acetaldehyde, respectively, with extreme ease. It was hoped that by working at the temperature of liquid ammonia the replacement of the halogen of α-chloroethanesulfonamide might be achieved without interference from the base-catalyzed decomposition.

Trithioacetaldehyde was prepared by reaction of hydrogen sulfide and acetaldehyde in ethanol saturated with hydrogen chloride. Oxidation of trithioacetaldehyde with chlorine produced α-chloroethanesulfonyl chloride, which was converted to α-chloroethanesulfonamide by treatment with ammonia in anhydrous diethyl ether.

We were unable to obtain α-aminoethanesulfonamide by reaction of α-chloroethanesulfonamide with sodamide in liquid ammonia. Investigation of this reaction was terminated when it was determined that large quantities of inorganic sulfite were present in the crude reaction product.

Experimental

Reaction of Sodium Benzamidomethanesulfonate with Phosphorus Pentachloride.

A suspension of 71.2 g. (0.30 moles) of sodium benzamidomethanesulfonate in 200 ml. of benzene was placed in a 500 ml. three-necked flask fitted with a mercury-sealed mechanical stirrer, a distilling receiver *, and a reflux condenser. The mixture was refluxed until the condensate no longer contained water, the latter being drawn off via the distilling receiver as it separated from the refluxing solvent.

After cooling the reaction mixture to room temperature, 62.5 g. (0.30 moles) of phosphorus pentachloride was added and refluxing, with stirring, was continued for five hours. The resulting suspension was filtered while hot (giving filtrate A and filter cake B). The filtrate, A, on cooling to room temperature deposited crystals (needles) which were collected and recrystallized from benzene to give 8.7 g. of crystalline solid, m.p. 197-207°. This material was tested qualitatively for the presence of chlorine, nitrogen, and sulfur by methods outlined by Bennett, Gould, Swift, and Niemann (14). These tests afford-

* The distilling receiver used was of the type designated No. 3622 in Catalog No. LP28 (1947), p. 43, Corning Glass Works, Corning, New York.

ed positive results for nitrogen, negative for chlorine and sulfur.*

The mother liquor from filtrate A was evaporated at 55° under reduced pressure (50 mm.). The viscous red liquid which remained was tested qualitatively for the presence of sulfur, with negative results.

The benzene-insoluble fraction (B) from the reaction mixture (34.8 g.) was washed with two 100 ml. portions of ice water and dried in vacuo to give 12.3 g. The results of qualitative tests for the presence in this material of nitrogen, chlorine, and sulfur were positive for nitrogen, negative for chlorine and sulfur.

An attempt to derive benzamidomethanesulfonyl chloride by reaction of sodium benzamidomethanesulfonate with phosphorus pentachloride in the presence of anhydrous diethyl ether (reflux temperature for twelve hours) was similarly without success.

Phthalimidomethanol (9, 16).

A mixture of 100.0 g. (0.68 moles) of phthalimide and 225 g. of a 10% aqueous formaldehyde solution (0.75 moles of formaldehyde) was placed in an autoclave and heated,

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* The solid was tested by fusion with soda-lime according to the procedure described by Cheronis and Entrikin (15). A positive test for an amide group was obtained. The substance failed to yield a hydroxamic acid on treatment with alcoholic hydroxylamine. When the substance was saponified by refluxing with 20% sodium hydroxide (6 hr.) and the resulting solution was acidified, a water-insoluble acid was obtained which did not melt when heated to 320°, but decomposed gradually.

with continuous shaking, for five hours at 100°. The reaction mixture was removed from the autoclave while still warm and allowed to cool. On cooling, phthalimidomethanol crystallized in the form of white needles. The crystals were collected by suction filtration and thoroughly washed with water. After drying in vacuo at room temperature over concentrated sulfuric acid, the product was recrystallized from toluene to give 104.5 g. (86.8%) of phthalimidomethanol (white needles), m.p. 146.5 - 148.5°.

For analysis a sample was recrystallized a second time from toluene, after which the compound exhibited a m.p. of 147.5-148.5°. Reported (9), 143-144°.

Analysis. Calculated for $C_9H_7O_3N$ (177.2): C, 61.01; H, 3.99; N, 7.91. Found: C, 61.05; H, 4.13; N, 7.92.

Phthalimidomethyl Bromide (9, 10).

To 20.0 g. (0.11 moles) of phthalimidomethanol was added the solution resulting from the addition of 20 ml. of concentrated sulfuric acid (sp. gr. 1.835) to 40 ml. of 48% hydrobromic acid. The reaction mixture was heated to 50°, when the majority of the phthalimidomethanol entered solution. Within five minutes a solid precipitated to such an extent that the reaction mixture set to a thick mass. By the addition of 20 ml. of 48% hydrobromic acid and 5 ml. of concentrated sulfuric acid the consistency of the mixture was reduced to a slurry. Heating was continued for two hours

at 45-60° with mechanical stirring, after which the suspension was cooled to 4° until crystallization was complete.

The solid was collected by suction filtration through a sintered glass filter, washed, first with 45 ml. of cold 10% ammonium hydroxide, then with a like volume of water, and dried in vacuo over concentrated sulfuric acid in a desiccator from which light was excluded. The crude product was recrystallized from benzene to give 22.1 g. (78.4%) of phthalimidomethyl bromide, m.p. 149-152°, as equant and columnar crystals. A second recrystallization from benzene raised the melting point to 150-152°. Reported (9), 150-151°.

Analysis. Calculated for $C_9H_6O_2NBr$ (240.1): C, 45.04; H, 2.51; N, 5.84. Found: C, 45.00; H, 2.67; N, 5.97.

Trithioacetaldehyde (17).

In a three-necked round-bottomed flask fitted with a sealed, mechanical, Hershberg stirrer (18) was placed 600 ml. of absolute ethanol saturated with dry hydrogen chloride. Acetaldehyde, prepared by depolymerization of paraldehyde, was condensed directly into the ethanol solution until a total of 180.0 g. (4.1 moles) of acetaldehyde had been added.

After replacing the air in the reaction vessel by an atmosphere of hydrogen sulfide, the reaction mixture was

cooled in an ice bath. With continuous cooling and stirring, hydrogen sulfide was passed in at a rate equal to its rate of absorption by the solution. After circa fifteen minutes solid trithioacetaldehyde commenced to separate from solution; after one-half hour had elapsed the reaction mixture was of the consistency of a thick paste. Absorption of hydrogen sulfide was now quite slow. Hydrogen sulfide was passed into the mixture with continued cooling and stirring for an additional thirty minutes.

The white solid was collected by suction filtration and washed with 125 ml. of fresh absolute ethanol. The crude product was recrystallized from 80% ethanol and the crystals collected, washed with fresh solvent, and dried to give 187.2 g. (76.1%) of trithioacetaldehyde, m.p. 78.5-90°. Since both α- and β-trithioacetaldehyde are reported to serve equally well for the preparation of α-chloroethanesulfonyl chloride (19), no effort was made to separate the product into its isomeric constituents, but it was used directly for oxidation to α-chloroethanesulfonyl chloride.

By concentration of the mother liquors an additional 27.8 g. (11.3%) of trithioacetaldehyde, m.p. 70-83°, was obtained, bringing the total yield to 87.4% of the theoretical amount based on the acetaldehyde used.

α-Chloroethanesulfonyl Chloride (8).

A suspension of 45.0 g. (0.25 moles) of trithioacetaldehyde in 900 ml. of water was placed in a 2-liter flask fitted with an efficient mechanical stirrer. The reaction mixture was cooled in an ice bath and, with vigorous stirring, a stream of chlorine was passed into the suspension until 177.3 g. (2.5 moles) of chlorine had been absorbed (thirty-six minutes were required).

The reaction mixture now consisted of two liquid phases: a yellow oil, the lower phase, and an acidic aqueous solution, the upper phase. Ether (150 ml.) was added to dissolve the oil and the layers were separated. The aqueous phase was extracted with two 150 ml. portions of ether and the extracts were combined. The ethereal solution was washed, first with 250 ml. of 1 F sodium thiosulfate, then with a like volume of water, after which it was dried over anhydrous magnesium sulfate.

The dried ether solution was evaporated and the residual oil distilled from a modified Claisen flask (20). The fraction distilling from 64.0-65.0° at 9 mm. was collected to give 58.0 g. (47.4%) of α-chloroethanesulfonyl chloride as a pale pink liquid. Reported boiling range (8): 48-53° at 3 mm.; 70° at 13 mm.

α-Chloroethanesulfonamide (8).

A solution of 58.0 g. (0.36 moles) of α-chloroethane-

sulfonyl chloride in 175 ml. of anhydrous diethyl ether was placed in a 300 ml. flask fitted with an inlet tube and a condenser through which ice water was circulating. Over a period of one hour a stream of ammonia was passed through the solution.

The ammonium chloride which had formed was separated by filtration and the filter cake was washed with two 75 ml. portions of fresh ether. The washings were combined with the initial filtrate and the solvent evaporated. The oily residue which remained crystallized slowly on standing at room temperature. After two recrystallizations from benzene there was obtained 25.0 g. (48.4%) of α-chloroethanesulfonamide, soft white plates, m.p. 64-65°. Reported (8): 65-66°.

By concentration of the mother liquors a second crop amounting to 5.0 g. (9.7%), m.p. 62-63° was obtained.

Reaction of α-Chloroethanesulfonamide With Sodamide

A solution of 0.3 moles of sodamide in 250 ml. of liquid ammonia was prepared from 7.0 g. of sodium according to the procedure of Vaughn, Vogt, and Nieuwland (21). The sodamide solution was placed in a 500 ml. round-bottomed flask equipped with a sealed mechanical stirrer and a dry-ice condenser protected with a drying tube containing soda-lime. With strong cooling, by means of a dry-ice - acetone bath, a solution of 14.4 g. (0.1 moles) of α-chloroethane-

sulfonamide in 100 ml. of liquid ammonia was added.

The cooling bath was removed and the reaction mixture allowed to reflux for three hours. At the end of this period the ammonia was allowed to evaporate. After decomposing the remaining sodamide by the cautious addition of ethanol, the residue was taken up in water, the solution was filtered, and the filtrate was brought to neutrality by the addition of concentrated hydrochloric acid. The neutral solution was evaporated to dryness at reduced pressure from a 65° water bath. The greenish-white solid residue weighed 26.1 grams.

A portion of the residue (1 g.) was dissolved in 5 ml. of water. When 1 ml. of this solution was acidified, a gas was evolved which, on the basis of its odor, was tentatively identified as sulfur dioxide. The experiment was discontinued after confirming this identification by the following tests:

(1) The solution readily reduced bromine.

(2) The addition of barium ion to a second 1 ml. portion of the solution immediately produced a voluminous precipitate which dissolved on acidification.

(3) The evolved gas was tested for reducing properties by suspending a drop of iodine in starch-iodide solution over circa 1 mg. of the precipitated barium salt to which four drops of 60% perchloric acid had been added. (The technique resembles the "hanging drop" method described

by Bennett, Gould, Swift, and Niemann (14) for qualitative detection of arsenic and sulfur.) The iodine- starch-iodide drop was rapidly decolorized.

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III. THE INFLUENCE OF NERVE IMPULSE SEQUENCE ON THE CONTRACTIONS OF DIFFERENT CRUSTACEAN MUSCLES

by

C. A. G. Wiersma and R. T. Adams

Introduction

Blaschko, Cattell, and Kahn (1) have described the effect of single intercalated stimuli on the sustained contraction obtained by a continuous low frequency stimulation of the whole nerve in different decapod nerve-muscle preparations. From their description and illustrations it follows that the response may take one of two forms: in one case the only effect is a sudden twitch followed quickly by relaxation to about the base level of the sustained contraction; in the other case relaxation is greatly delayed and, hence, the muscle remains shortened to a considerable degree and to a much greater extent than would have been observed without the intercalated stimulus. Katz (2) has termed this a "trigger" effect, a name which seems rather appropriate.

Wiersma and van Harreveld (3) have shown that at least in the closer of Cancer anthonyi Rathburn the effect can be obtained when the isolated fast fiber of the closer muscle is stimulated, but not by stimulation of the isolated slow fiber. During the fast contraction they found a sustained enhancement in shortening similar to that reported

by Blaschko et al (loc. cit.) for other crabs.

The present investigation was undertaken to obtain more comparative data on the effect of intercalated impulses, and to study the relation between this phenomenon and that of stimulus-spacing, an effect which will be described below.

Methods

In all cases the experiments have been carried out on preparations in which either single motor fibers had been prepared or in which a bundle, known to contain only a single motor fiber, could be stimulated. The latter method was resorted to only for studying the opener contraction of the crayfish. In all preparations special care was taken to exclude inhibitory fibers (for methods of preparation of single fibers see, e.g., ref. 4).

In the experiments with intercalated single shocks, the nerve fiber was stimulated sometimes faradically by means of an induction coil, but more often with electronically obtained square wave pulses (0.3 msec.) of a lower frequency. Through the same pair of micromanipulated platinum electrodes, through which the basic stimuli were given, an extra shock, obtained with a second induction coil, was administered at random moments during the stimulation. With appropriate signal recorders the stimulation times were recorded, together with the isotonic contraction, on a

kymograph.

In the other series of experiments the same preparations were used, but a different method of stimulation was employed. This consisted of applying a series of double shocks in which the interval between the shocks could be varied at will at any desired fundamental frequency of the double shocks. Isotonic contractions were here recorded for stimulation periods of three or five seconds duration. In most cases, it was first determined what contraction was obtained with equal spacing of impulses, after which the stimuli were delivered in pairs and the contraction again observed. To illustrate, if the double shock frequency were 10 d.s./sec., the distance between the two impulses was made 50 msec., and thus a shock was delivered exactly every 1/20 second. After registration of this contraction for 5 seconds, a short rest was given and the contraction with a short interval between the two impulses, e.g. 3 msec., was registered. The number of shocks was still 20 per second, but now they came in pairs. Again the preparation was stimulated for exactly 5 seconds. It was then necessary, after a further short rest period, to repeat the stimulation in the first manner before any conclusion as to similarity or difference between the contractions could be drawn. In practice, in most cases, other intervals could also be investigated, and often the procedure could be repeated with another fundamental

frequency.

The animals used included the crayfish, Cambarus clarkii Girard, the rock lobster, Panulirus interruptus Randall, the shore crab, Pachygrapsus crassipes Randall, and the edible crab, Cancer antennarius Stimpson. In the first instance, van Harreveld's solution, with the other forms sea water, was used to bathe the nerve.

Results

Since it was found that there was a rather close relation between the results obtained in different muscles with the two methods described above, these will be presented together for the different groups. In the cases in which a muscle was investigated by only one method, the findings will not be described unless the system in question presented features which were not so pronouncedly present in other systems. The order in which we shall present the results will be: first, the effects on slow systems; second, the effects on fast systems; third, on opener and stretcher systems; and, fourth, on systems with quadruple motor innervation.

Slow Closer, Bender, and Extensor Systems.

For most species these systems may be considered together. On the whole the response is small in both types of experiments.

In some systems intercalated shocks did not produce

any visible increase in the mechanical contraction in any of the preparations. Since, in order to have an effect, the random shock must fall outside the refractory period of the previous stimulus and, moreover, should itself not suppress the next impulse in a similar way, an effect can at best be expected from only a part of such arbitrarily superimposed stimuli. Therefore, it is necessary, during any one contraction, to repeatedly attempt the intercalation of an extra shock in order to determine if it have any influence. Repetition of these trials is the more necessary since, it was observed, in cases in which the extra shock does lead to an increase in the contraction, this effect may be more pronounced at the start than during the later stages of the contraction.

Intercalated stimuli produced no visible effect at all, notwithstanding repeated trials, with two of the slow systems studied, viz., the slow closers of Pachygrapsus and Cambarus. Reproductions of the contractions of these systems are shown in figures 9 and 10, and in figure 15, respectively; any enhancement of the contractions due to the intercalated stimuli is completely absent. The other slow systems investigated were the slow closers of Cancer (figs. 21 and 22) and Panulirus (fig. 27), the slow benders of Cambarus (fig. 17) and Cancer (fig. 25), and the slow extensor of Panulirus (fig. 32). With these muscles very small increases were, at least sometimes, observed. In no

instance were these large enough to be called a "trigger" effect, or to permit a definitive statement as to whether or not they contributed significantly to the development of the contraction. In some cases, however, it seemed likely that a material contribution resulted when an active stimulus fell during the quickly rising first phase of the contraction.

Data of a more quantitative nature are obtained with the second method used. Taking into account the fact that slow systems give smooth tetani at any effective frequency of stimulation, even though it be low, and that the response to intercalated shocks is small or lacking, it was predicted that in these systems the spacing of the impulses would have little or no effect. This prediction was borne out by the results, as may be seen by reference to Table I. In this and subsequent tables, there has been listed for each case the maximum and minimum intervals between the double shocks which were used. The difference between the contractions obtained with each of these intervals has been expressed as a percentage, the contraction resulting with the longer stimulus-spacing being considered as unity.

A majority of the preparations showed no difference in behavior on changing the stimulation patterns. Figure 1 shows a record typical of those obtained by the shock-spacing method with these systems. When shock-spacing did have an influence, the effect was quite small (in direct correlation

TABLE I

Effect of Stimulus-Spacing for Slow Closer, Bender, and Extensor Systems.

Preparation	No.	Frequency of double shocks (d.s./second)	Maximum interval (msec.)	Minimum interval (msec.)	Percentage difference
Slow closer, Pachygrapsus	1	20	18	2	0
	2	20	18	2	+10
	3	20	18	2	0
	4	10, 20	18	2	0
	5	20, 30	18	1.4	0
	6	15, 20	18	5	0
	7	10, 20	18	2	0
	8	10, 20	18	5	0
Slow closer, Panulirus	1	20	18	1.8	+15
Slow closer, Cambarus	1	10	50	4	-10
	2	10	50	3.5	+25
	3	5	90	5	0
	4	10	50	2	-15
	5	10	50	5	0
Slow bender, Pachygrapsus	1	20, 30	18	2	0
	2	20	18	2	+5
	3	30	16	5	+30
Slow bender, Panulirus	1	20	18	4	0
	2	10, 20	50	5	0
Slow bender, Cambarus	1	15	25	5	+20
	2	20	18	4	+10
Slow extensor, Panulirus	1	20	18	4	+10
	2	5	90	5	+10
	3	10	50	5	0

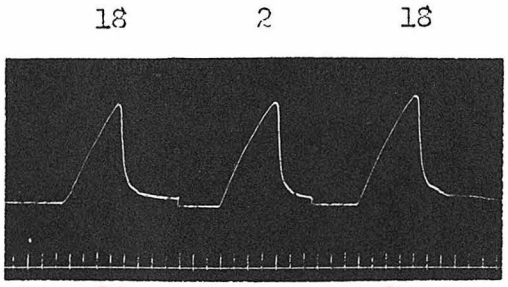
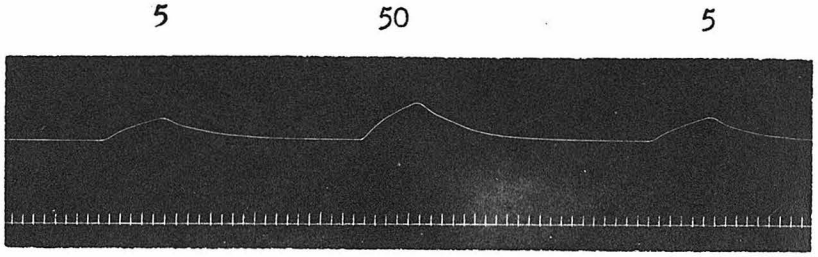
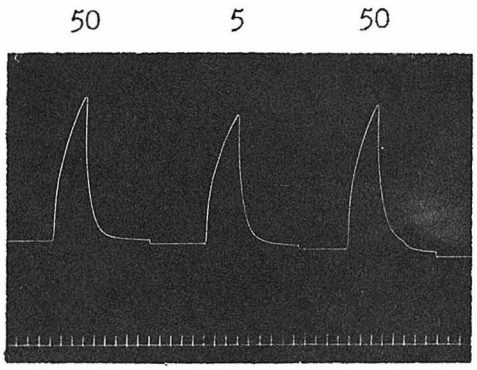


Figure 1. Slow Closer of Pachygrapsus. Stimulation with 20 d.s./sec. Figures above contractions give interval in msec. between the shocks in each pair. Duration of stimulation 3 seconds. No influence of spacing. Time in seconds.



A.



B.

Figure 2. Slow Closer of Cambarus. Two preparations giving negative spacing effect. Duration of each stimulation 3 seconds. Stimulation with 5 d.s./sec. (A) and 10 d.s./sec. (B). Shorter intervals give smaller contractions.

with the intercalated shock experiments). These effects were generally positive; that is to say, the muscle contracted more strongly when the pair of shocks were separated by a short interval than when they were further apart.

The slow closer of the crayfish is noteworthy in view of the negative effects observed in part of the preparations. These negative values indicate that the contraction with widely spaced shocks was actually larger than that with closely following shocks. Figure 2 shows reproductions of the records obtained with two preparations of the slow closer of Cambarus which gave negative responses, one preparation (A) being stimulated with a basic frequency of 5 d.s./sec., while the other (B) was studied at a basic frequency of 10 d.s./sec. While this negative effect has been encountered in slow systems only in the slow closer of Cambarus, it was observed also in three fast systems, as will be shown later.

Fast Closer, Bender, and Extensor Systems.

In general, intercalated shocks are effective in these systems. However, the effect on the ensuing contraction is variable, which is well illustrated by the two contractions of the fast closer of Cancer antennarius reproduced in figure 23. In A there is complete relaxation after each extra twitch; in B (the same preparation at a later stage) there is hardly any relaxation and, hence, the contraction remains considerably larger after each effective

intercalated stimulus. As may be noticed in the figure, some intercalated shocks have more effect than others, which is due to the timing of the extra stimulus. Due to shortage of material no experiments with shock-spacing are available for this case.

The fast extensor of Panulirus showed in all three of the intercalated shock experiments a noticeable increase in the contraction as a result of the extra stimuli (fig. 33). This system responded very strongly (in a positive fashion) to decreases in stimulus-spacing (see Table II). Contractions of one of the examples of this system are shown in figure 3.

It is of interest to note that one of our preparations (designated no. 5 in table II) when fresh did not give a contraction with wide spacing (90 msec.) at a frequency of 5 d.s./sec., whereas almost smooth tetani were obtained with close spacing; thus, the effect in this case was infinite. The unexpected aspect in this instance is that such contractions are tetani rather than a series of unconnected single twitches.

Table II summarizes the influence of shock-spacing on the contractions of the fast systems studied. It is apparent that the fast closer and bender of Pachygrapsus and the fast bender of Cambarus all showed a greater order of positive activity than did the corresponding slow systems, less pronounced in the last instance than in the first two cases.

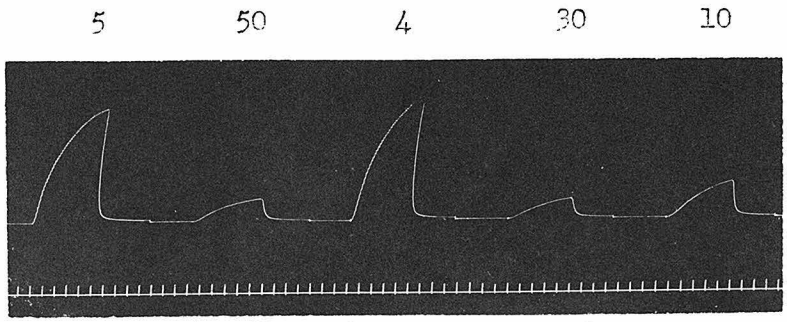


Figure 3. Fast Extensor of Panulirus. Stimulation with 10 d.s./sec. Pronounced positive effect of spacing. Duration of each test 3 seconds. Note the completeness of all tetani.

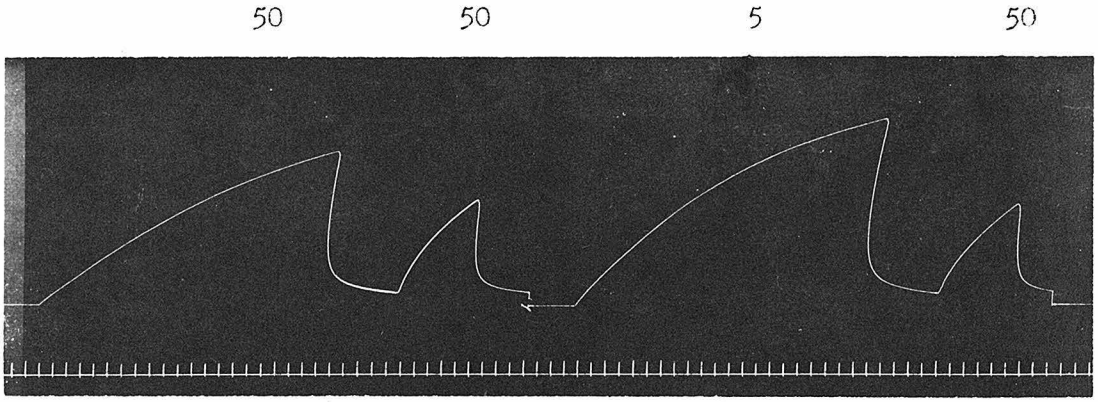


Figure 4. Fast Extensor of Panulirus. Absence of difference in facilitation caused by two long-lasting contractions though height of contraction varies with spacing. Frequency of stimulation 10 d.s./sec. First facilitating contraction of 20 sec. duration with 50 msec. spacing. After 5 sec. rest, test contraction of 5 sec. duration with same spacing. After a long rest, second facilitating contraction, again of 20 sec. duration, 5 msec. spacing. Notwithstanding pronounced difference in height of the two facilitating contractions, the second test contraction (5 sec. duration with 50 msec. spacing, registered after 5 sec. rest as before) is not greater than the first.

TABLE II

Effect of Stimulus-Spacing for Fast Closer, Bender and Extensor Systems.

*(C indicates smooth tetanus; c indicates nearly smooth tetanus, but with vestiges of tops remaining; i indicates incomplete tetanus.)

Preparation	No.	Frequency of double shocks (d.s./second)	Maximum interval (msec.)	Minimum interval (msec.)	Percentage difference*
Fast closer, Pachygrapsus	1	10, 20	18	2	i +100(10); C +70(20)
	2	10	18	2	c +120
	3	15, 20	18	2	i +500(15); c +130(20)
	4	15	18	2	C +110
	5	10, 20	18	2	i +200(10); C +60(20)
	6	10, 15, 20	18	2	i +150(10); i +350(15); C +400(20)
	7	20	20	5	C +100
Fast bender, Pachygrapsus	1	10	18	2	C +170
	2	15	30	4	C +300
	3	15	18	5	i +130
	4	10	18	2	i +40
	5	15	18	2.4	C +300
	6	15	18	5	C +140
Fast closer, Panulirus	1	10	50	5	c -20; later +500
	2	10	50	5	c +40; later +350
	3	10, 15	50	3	C first inf.; later +6000; C +700(15)
	4	10	50	5	c +15
Fast bender, Panulirus	1	20	18	3	C +100
	2	20	18	5	C +30
	3	10, 15	50	5	C -35(10); C -25(15)
Fast extensor, Panulirus	1	10	50	4	C +4000
	2	10, 15	50	4	C +450(10); C +200(15)

Table II cont.

Preparation	No.	Frequency of double shocks (d.s./second)	Maximum interval (msec.)	Minimum interval (msec.)	Percentage difference
Fast extensor, Panulirus	3	5	90	5	c +5000
	4	5	90	5	c +75
	5	5	90	5	c inf., later less
	6	10	50	5	C+35; later+70
Fast closer, Cambarus	1	5	90	4	i -5; later c+250
	2	5	90	5	i -55; later c +15
	3	5	90	5	i -50; later c +25
	4	5	90	5	i -45; later i 0
	5	5	90	5	i -60; later i -75
Fast bender, Cambarus	1	10	50	5	C +45
	2	10	50	3	C +35

With intercalated shocks strong trigger effects were obtained with the fast closer (fig. 11) and fast bender (fig. 12) of Pachygrapsus. Of two preparations of the fast bender of Cambarus (figs. 18 and 19), one gave a medium effect whereas in the other very little increase could be seen at all.*

The data from the intercalated shock and the

* One preparation of the fast bender of Cancer was studied, the record for which is shown in fig. 24. No examples of the effect of shock-spacing on this system are available.

stimulus-spacing experiments check rather well with each other and tend to show that there may be an inherent difference between various preparations which determines the strength of both effects.

The remaining fast systems, the fast closer and fast bender of Panulirus and the fast closer of Cambarus, require separate treatment since, in these as in the slow closer of Cambarus, negative effects as well as positive ones were encountered.

Only one example of the influence of intercalated shocks on the fast closer of Panulirus is at hand (fig. 28). A small effect without a permanent increase in shortening was observed. Toward shock-spacing this system reacted in general in a positive fashion, sometimes quite strongly. With one preparation (no. 1, table II), however, the response was first negative, becoming positive as the preparation grew older.

The fast bender of Panulirus likewise showed both negative and positive responses to decreases in shock-spacing. The effect of intercalated stimuli was moderate in this system (fig. 29).

In the fast closer of Cambarus the response in the shock-spacing experiments was always negative with the fresh preparation. As the preparation grew older, however, the effect changed in the positive direction in four of the five examples available (fig. 5). With respect to intercalated

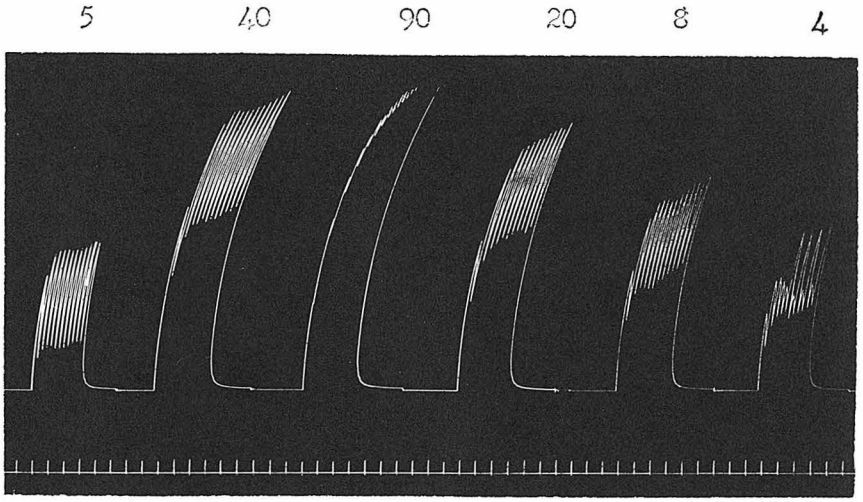
shocks, there was in this system a strong trigger effect, but returns to the baseline were rapid (fig. 16). This is, of course, a special preparation since it is the only one used in which single impulses, at least in fresh preparations, will give very strong contractions.

Openers and Stretchers.

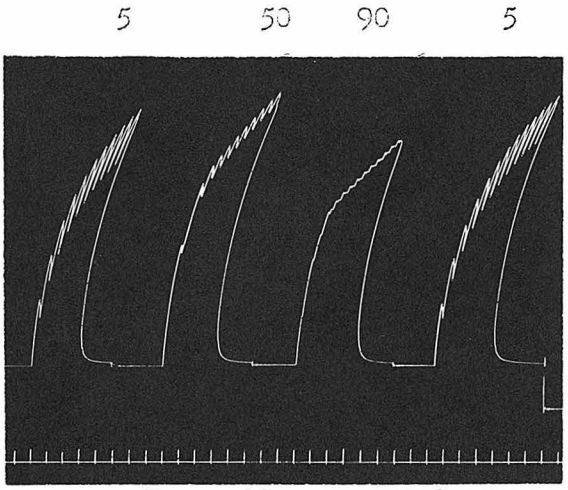
In all species these muscles receive only a single motor axon. It has not yet been possible to determine whether the contractions of these muscles are to be considered slow or fast. With respect to facilitation and inhibition, most of the systems which have been investigated seem to be comparable to slow systems. However, in at least one case, the opener of Eupagurus, summation of two impulses gives results which are more like those of a fast system (5).

The present investigation has shown that, on the whole, slow systems are influenced to a much smaller extent either by intercalated shocks or by changes in shock-spacing than are the corresponding fast systems. It is, therefore, of considerable interest to see what results this group of muscles presents.

A decided difference from one species to another was observed. In the opener of Pachygrapsus, intercalated shocks had no effect (fig. 8). Similarly, changes in stimulus-spacing had an effect only in one of the three preparations examined, and then only to an extent of 35% (cf. Table



A.



B.

Figure 5. Fast Closer of Cambarus. Contractions of 5 sec. duration with stimulating frequency of 5 d.s./sec. Reversal of spacing effect after repetition of stimulation. A shows negative effect (-55%); B, same preparation shortly afterwards, shows positive effect (+15%). Note the more complete fusion in B.

TABLE III

Effect of Stimulus-Spacing for Opener and Stretcher Systems.

Preparation	No.	Frequency of double shocks (d.s./second)	Maximum interval (msec.)	Minimum interval (msec.)	Percentage difference
Opener, Pachygrapsus	1	20	18	2	0
	2	15	18	5	+35
	3	10	18	2	0
Stretcher, Pachygrapsus	1	15	18	2	0
	2	10, 15	18	2	+40(10); +40 (15)
	3	10, 15, 20	18	2	0(10); +5(15, 20)
Opener, Panulirus	1	10	50	3	+160
	2	5, 10	90	5	0(5);+5(10)
Stretcher, Panulirus	1	20	18	5	+40
Opener, Cambarus	1	20	13	4	0
	2	10	50	3	+200
	3	15, 20	18	5	+30(20); +90(15)
	4	15	30	5	+100
	5	10	50	4	+90
	6	10	50	4	+200

III).

On the other hand, the opener of Cambarus showed a temporary trigger reaction with intercalated shocks (fig. 13) in two of the three preparations studied, while the third showed none (fig. 14). With stimulus-spacing, most preparations showed a positive response, in two cases as great as +200% (fig. 6).

The opener of Panulirus (fig. 26) responded in both

types of experiment to a degree intermediate between the response of Pachygrapsus and that of Cambarus.* Whereas in Pachygrapsus the opener behaves as though it were a typical slow system, in Cambarus the effect approaches that of certain fast systems.

Main Flexor of Panulirus.

Some experiments have been performed with both methods on the four contraction types of the main flexor of Panulirus. Table IV summarizes the effects obtained with

TABLE IV

Effect of Stimulus-Spacing on Panulirus Main Flexor Systems.

Preparation	No.	Frequency of double shocks (d.s./second)	Maximum interval (msec.)	Minimum interval (msec.)	Percentage difference**
Fastest	1	10, 15	30	5	C inf.(10); C +500(15)
	2	10	40	3	C +3000
2nd Fast	1	10	50	4	C +40
3rd Fast	1	10, 15	50	4	C 0(10); C +100(15)
	2	15, 20	30	5	C +70(15); C +20(20)
Slow	1	30	16	5	C +35
	2	30	16	5	C +30

** C indicates complete tetanus.

* Fig. 20 shows the record for the preparation of the opener system of Cancer which was examined by the intercalated shock method. No data are at hand for the behavior of this system under the influence of stimulus-spacing.

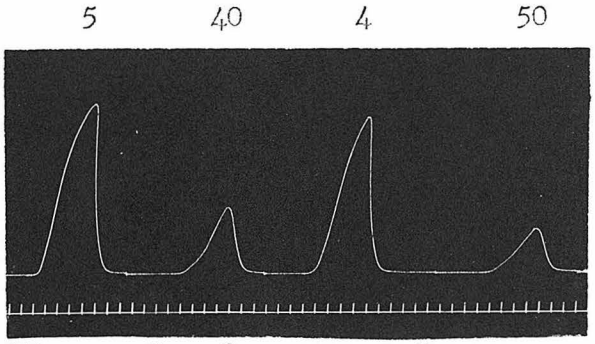


Figure 6. Opener of Cambarus. Preparation with positive spacing effect. Contractions of 5 sec. duration. Stimulating frequency 10 d.s./sec.

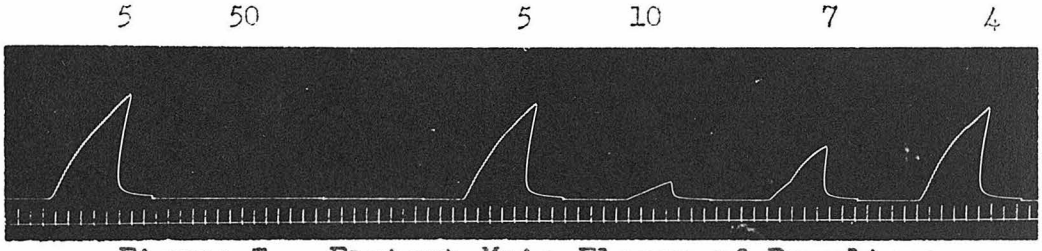


Figure 7. Fastest Main Flexor of Panulirus. Preparation stimulated with 10 d.s./sec. Pronounced positive effect of spacing (infinite).

the stimulus-spacing method.

It was observed that the slower systems were influenced to only a relatively small extent by either type of stimulation (fig. 30). The fastest system was remarkable. A very quick relaxation following the twitch due to an intercalated shock was found (fig. 31), while a very pronounced positive effect was obtained on decreasing the stimulus-spacing (fig. 7.). The second fast responded in the same way, but to a lesser degree.

Effect of Spacing on Subsequent Facilitation.

It was of interest to determine whether the amount of facilitation resulting from a closely-spaced strong contraction differed noticeably from that obtained during a widely-spaced weaker contraction. In systems in which there was a large spacing effect, the following method was applied to elucidate this point. First, a close-spaced contraction was elicited for from 5 to 15 seconds (the latter when it was desired to obtain maximal facilitation). Soon after relaxation (e.g., 5 seconds after a 15 second contraction) a 3 second contraction was elicited with equal spacing of the stimuli. Such a contraction is somewhat larger than a similar one which has not been preceded by a long contraction. After a rest period to allow the facilitation to decay, a 15 second wide-spaced contraction was produced, followed (after a 5 second interval as before) by a 3 second test contraction (with equal spacing).

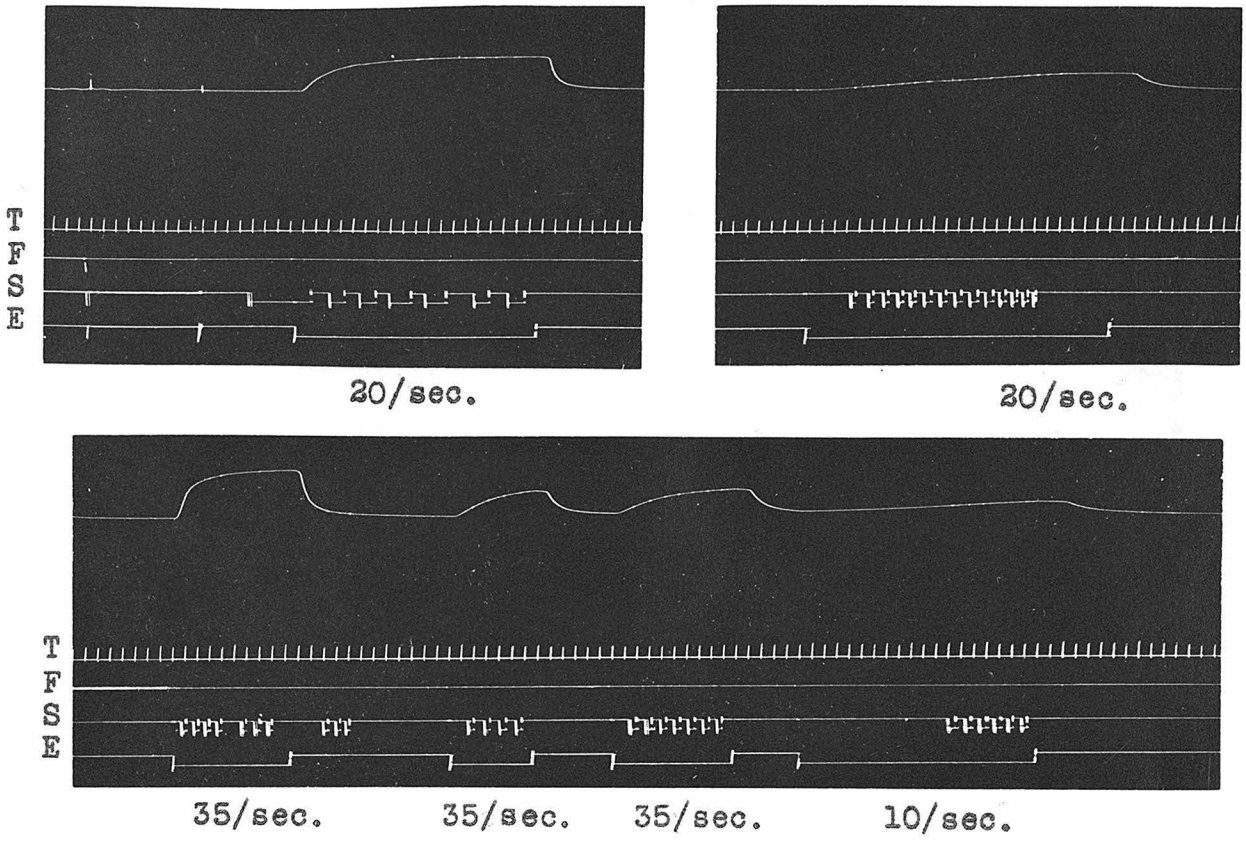


Figure 8. Opener of Pachygrapsus.
Preparation stimulated with electronically obtained square wave pulses (E) at frequencies indicated. Intercalated shocks (S) were without effect. Time in seconds (T).

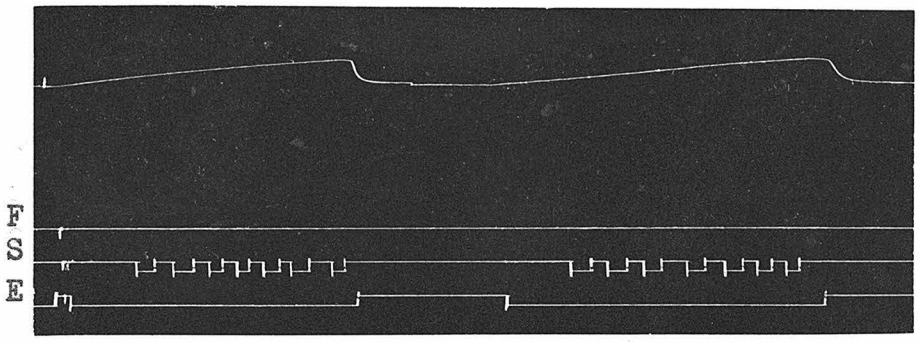


Figure 9. Slow Closer of Pachygrapsus.
Intercalated shocks (S) were without effect. Frequency of electronic stimulation (E), 20 per second.

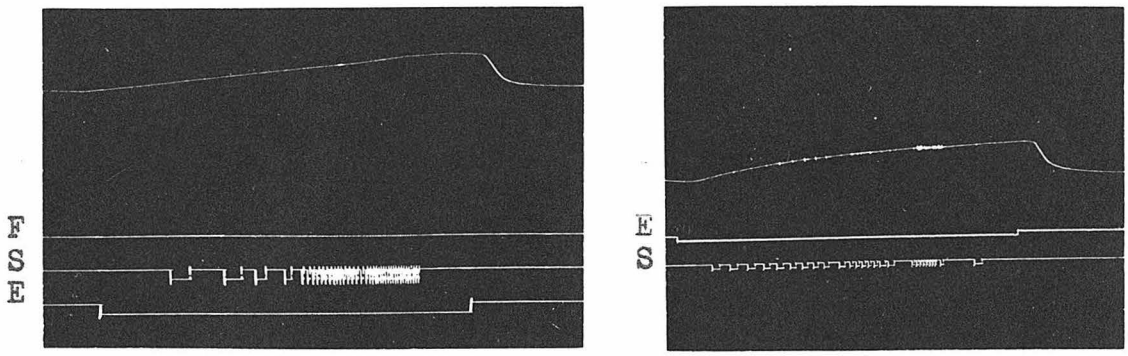


Figure 10. Slow Closer of Pachygrapsus.
Two preparations of the slow closer of Pachygrapsus. The frequency of electronic stimulation (E) was 20 pulses per second in A, 30/second in B. Extra single shocks (S) had no effect.

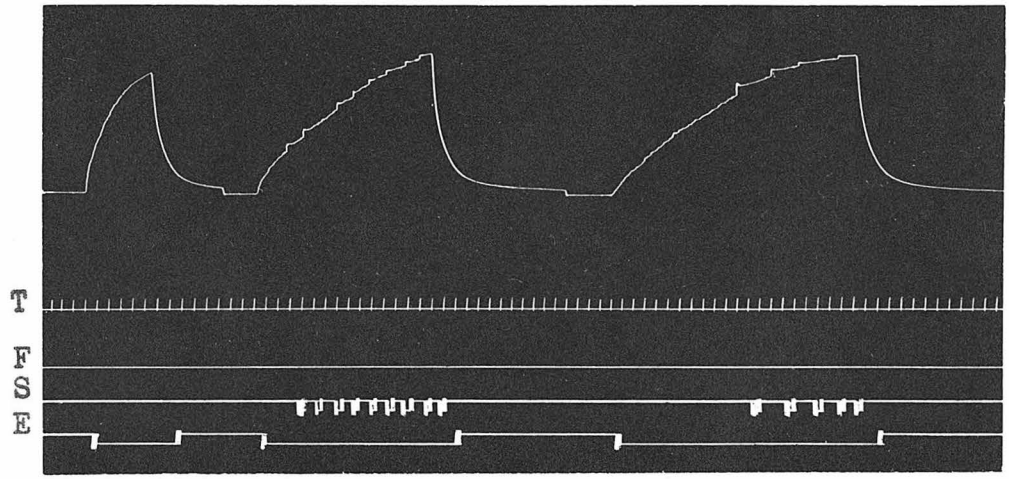
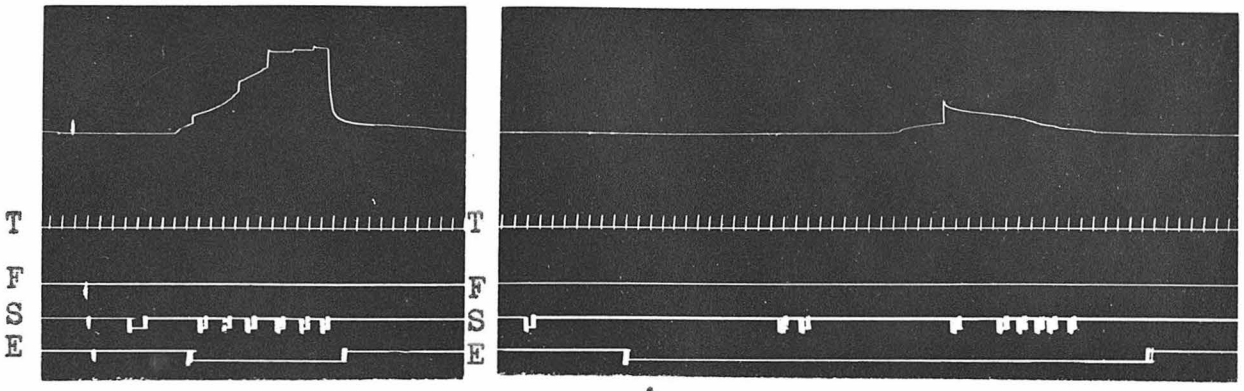
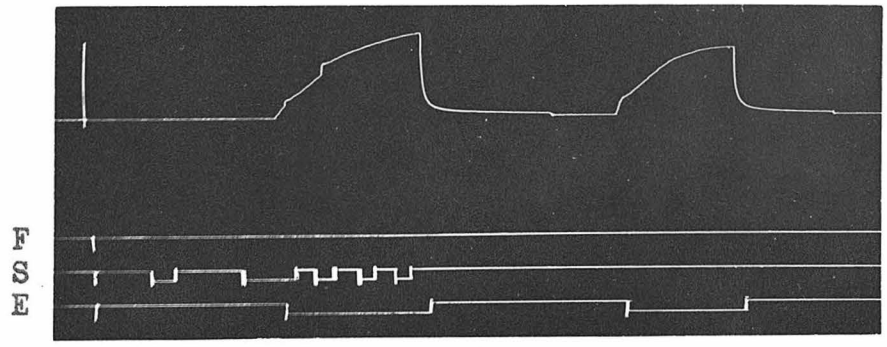


Figure 11. Fast Closer of Pachygrapsus.
The effect of intercalated stimuli (S) on the contraction produced with electronically obtained square-wave pulses of a frequency of 35 per second. Time in seconds (T).



A.



B.

Figure 12. Fast Bender of Pachygrapsus.
Records of contractions of two preparations of the fast bender of Pachygrapsus showing the trigger effect obtained with intercalated shocks (S). Frequency of basic stimulation was 35 per second (E).

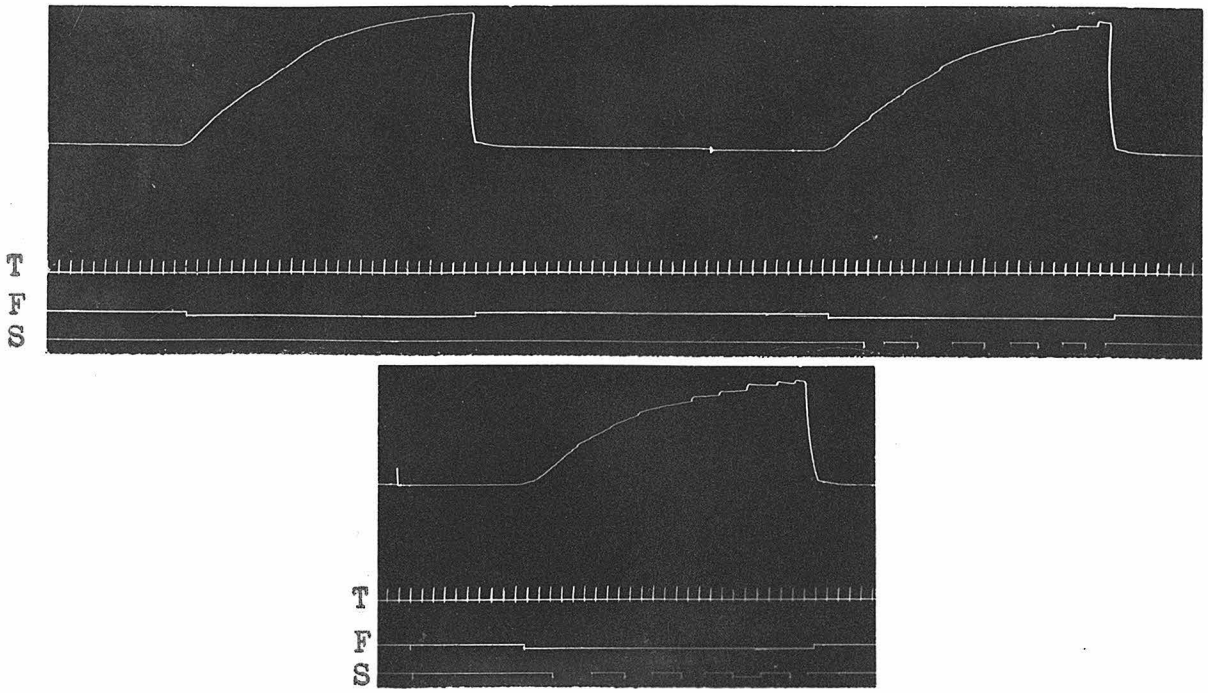


Figure 13. Opener of Cambarus.
The effect of single shocks (S) intercalated during faradic stimulation (F) of the opener nerve bundle. Time (T) in seconds.

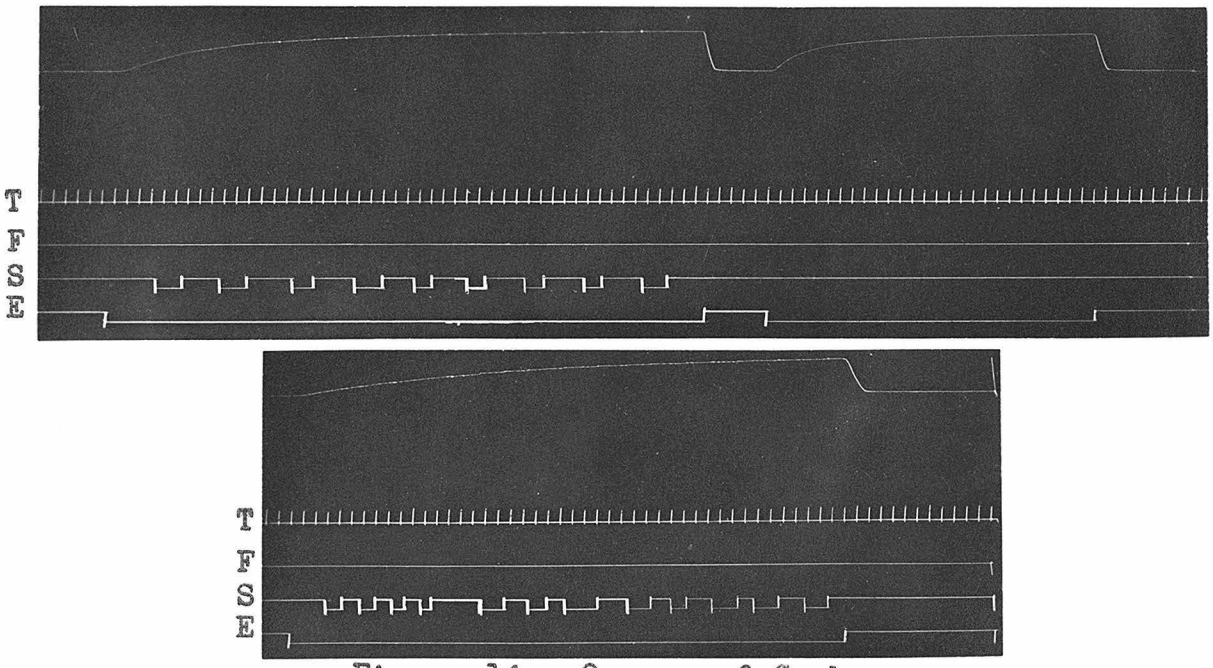


Figure 14. Opener of Cambarus.
Another preparation of the opener of Cambarus, stimulated with electronically obtained square-wave pulses (E) of a frequency of 30 per second. Extra single shocks (S) had no effect. Time (T) in seconds.

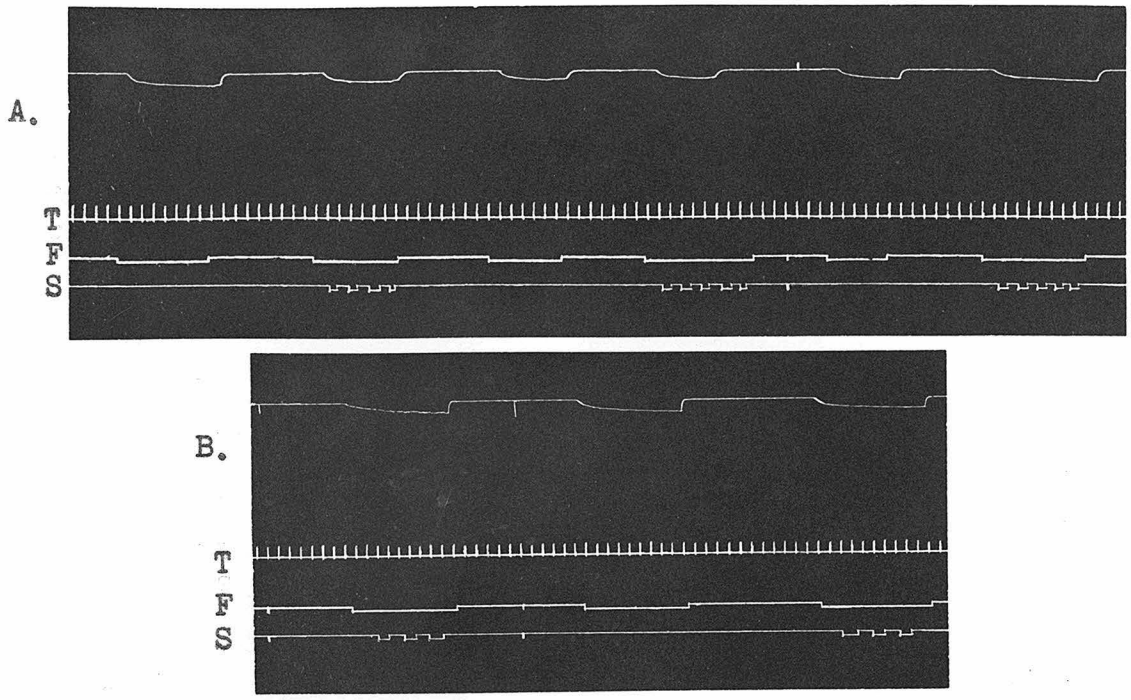


Figure 15. Slow Closer of Cambarus.
Absence of any visible effect due to single shocks (S) applied during faradic stimulation (F). Time in seconds (T). A and B are different preparations.

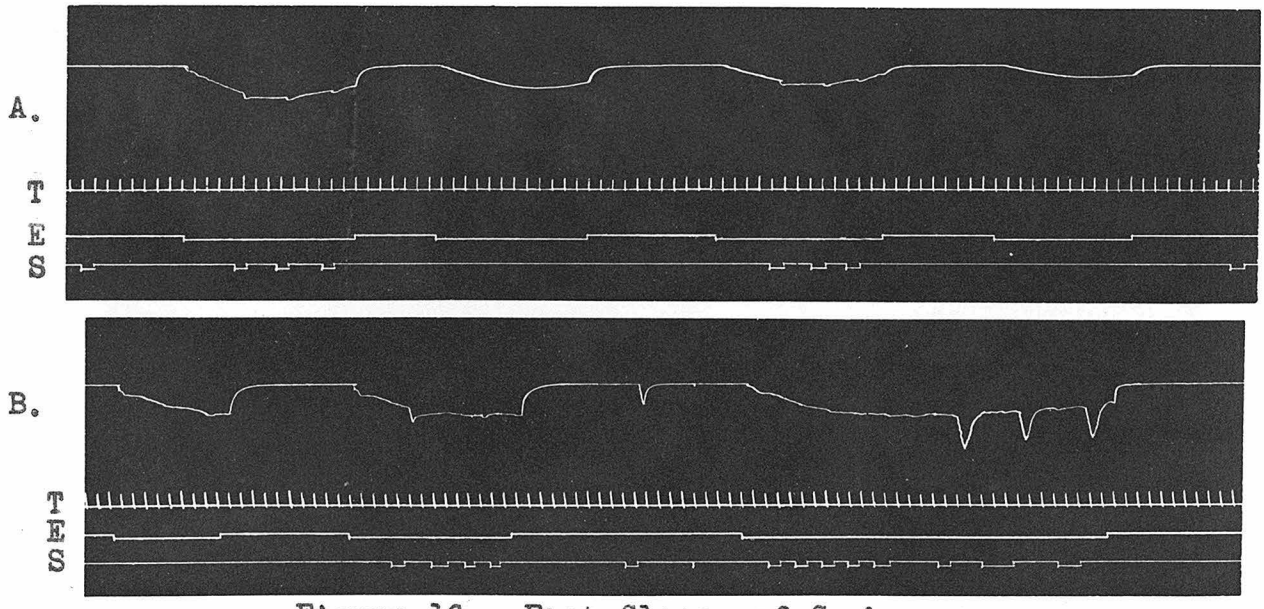


Figure 16. Fast Closer of Cambarus.
Records from two preparations of the fast closer of Cambarus showing the effect of intercalating extra stimuli (S) during the contractions resulting from electronic stimulation (E). Frequency of stimulation 20 pulses per second (A) and 10 per second (B). Time in seconds (T).

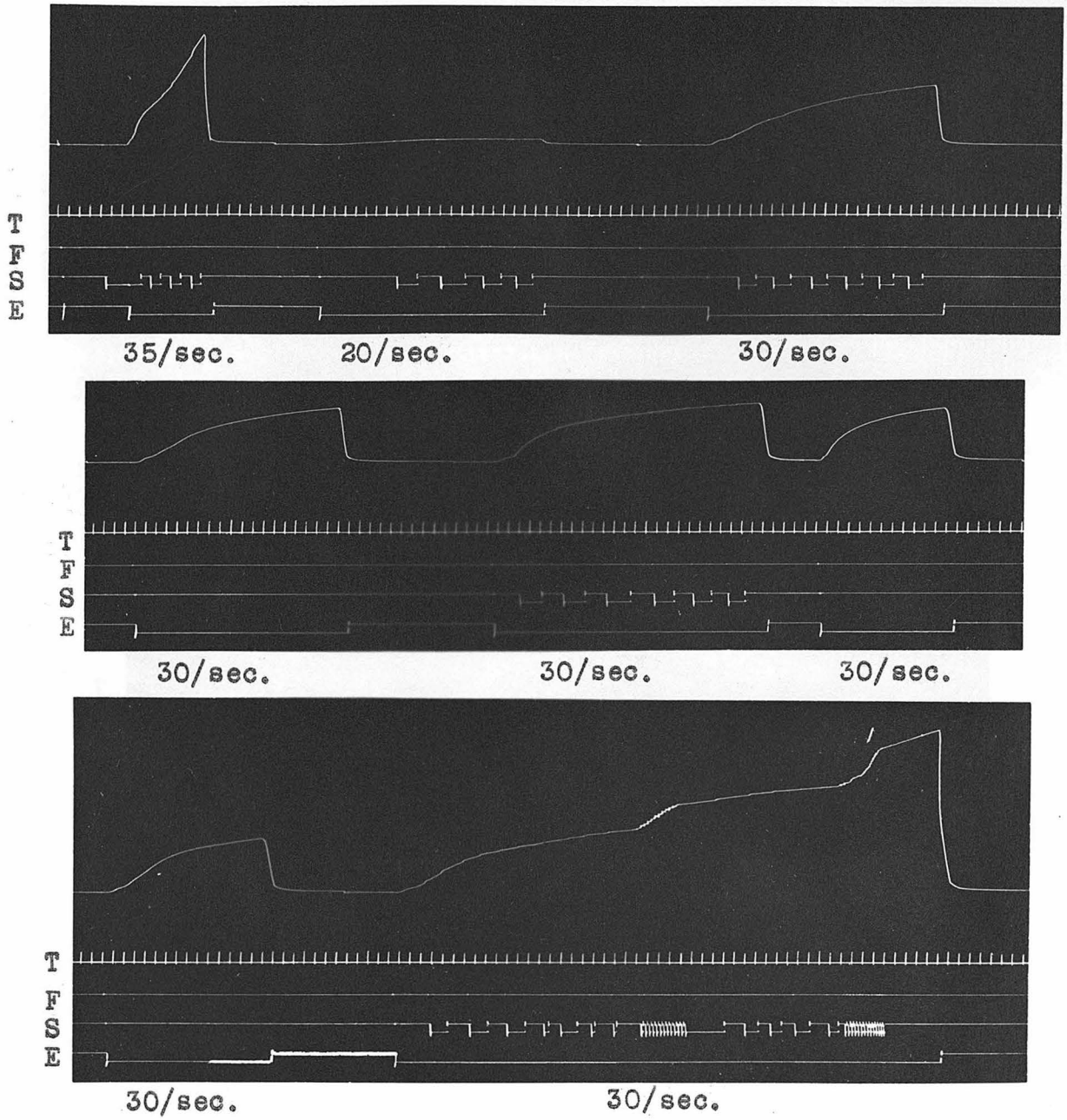


Figure 17. Slow Bender of Cambarus.
The effect of intercalated stimuli (S) on the contraction of the slow bender of Cambarus. Electronic stimulation (E) at the frequencies indicated. Time in seconds (T).

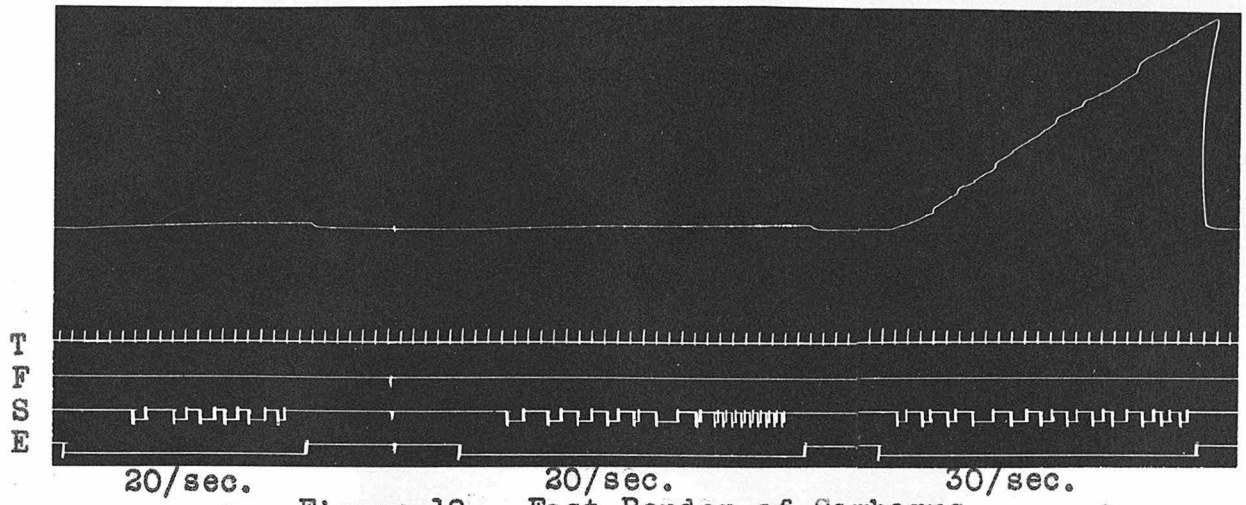


Figure 18. Fast Bender of Cambarus.
The effect of single shocks (S) on the contraction of the fast bender. Electronic stimulation (E). Time in seconds (T).

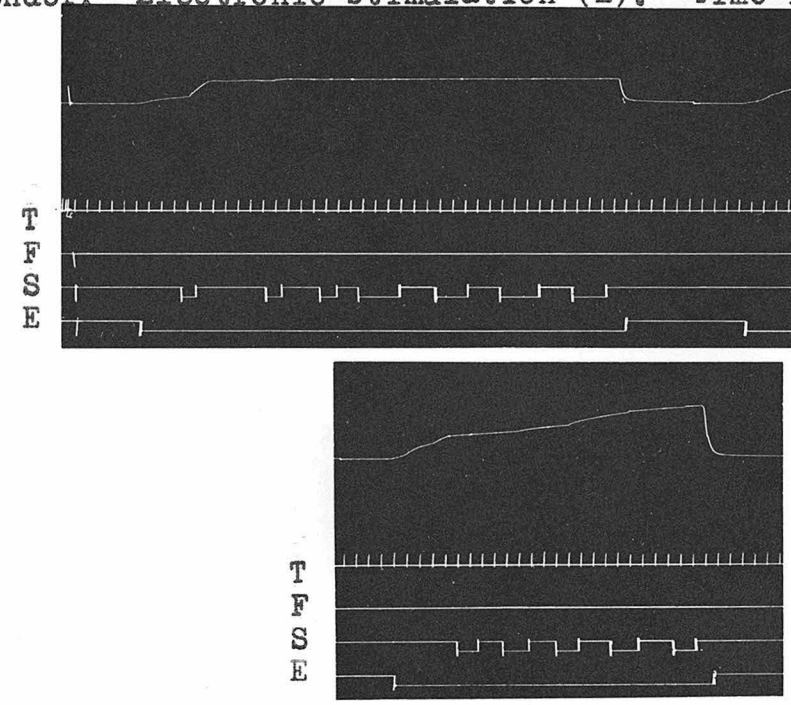


Figure 19. Fast Bender of Cambarus.
Registration of the contraction elicited with square-wave pulses of frequency 20 per second (E). Single shocks (S) have little effect. Time in seconds (T).

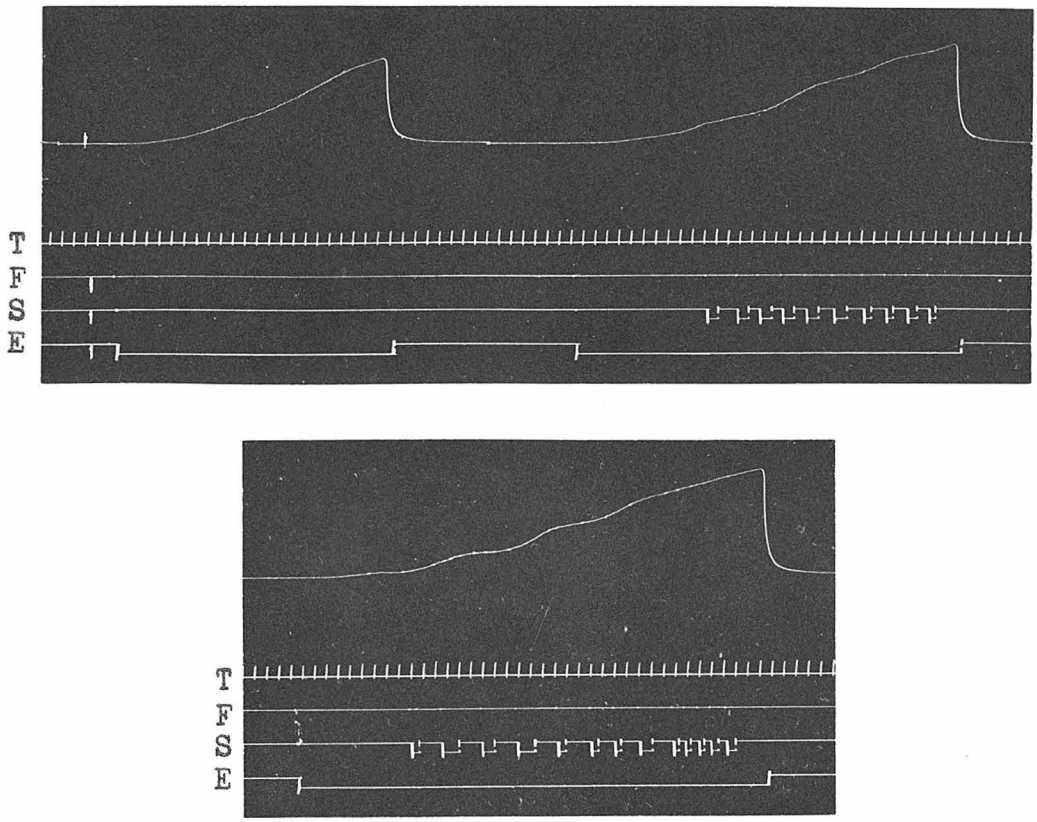


Figure 20. Opener of Cancer.
Single shocks (S) applied during electronic stimulation (E) at a frequency of 30 per second produced only a slight effect. Time in seconds (T).

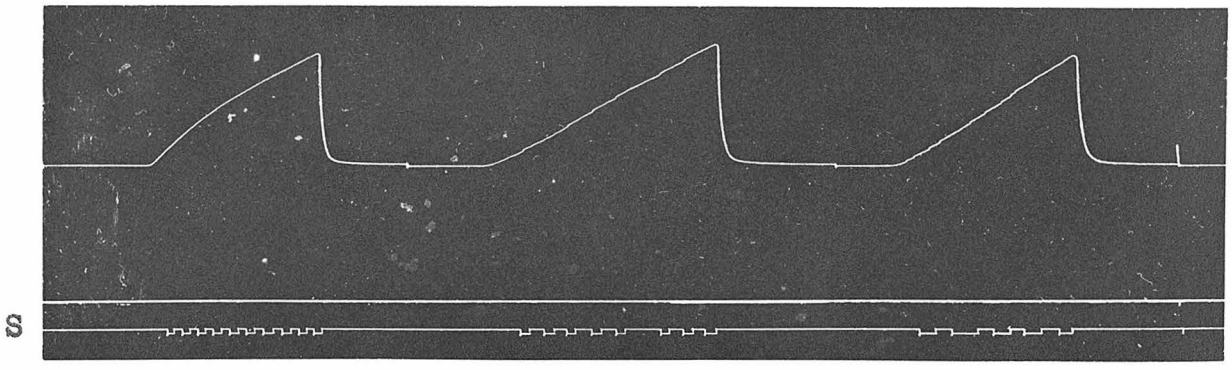


Figure 21. Slow Closer of Cancer.
Record of contractions of the slow closer of Cancer obtained with intercalated shocks (S) during faradic stimulation.

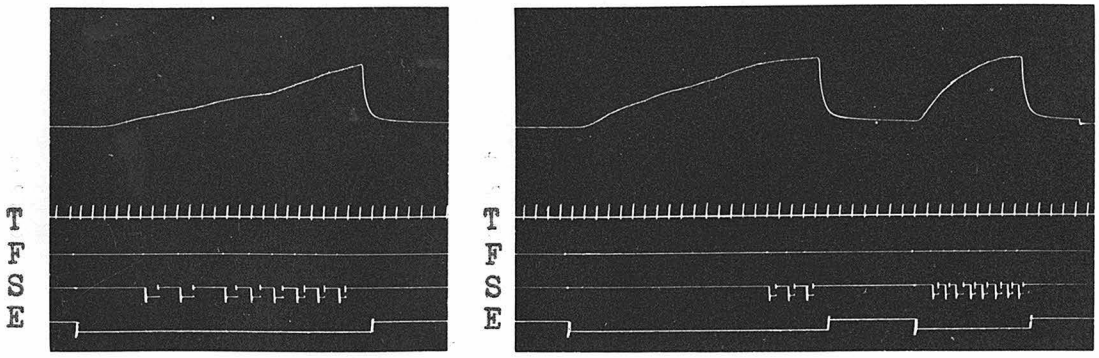


Figure 22. Slow Closer of Cancer.
Contractions of the slow closer of Cancer obtained with square-wave pulses of a frequency of 30 per second (E) and intercalated shocks (S). Time in seconds (T).

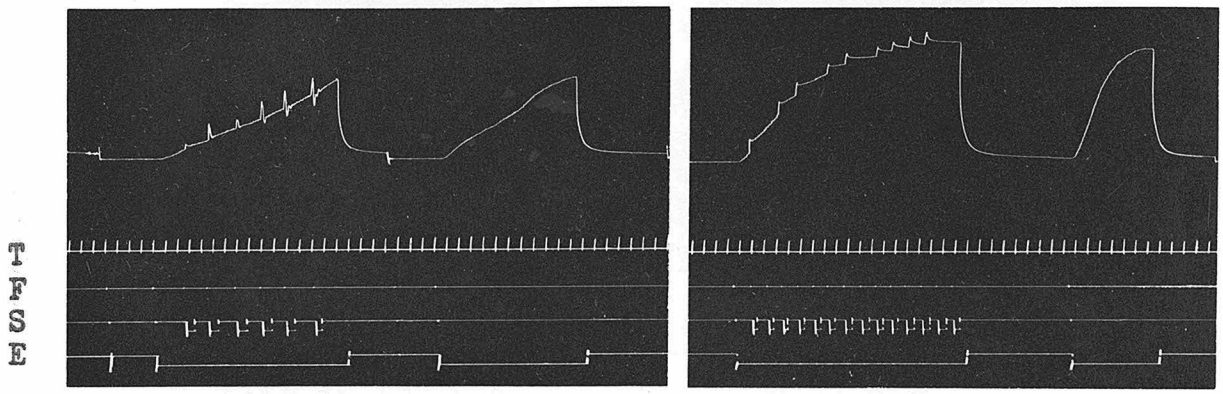
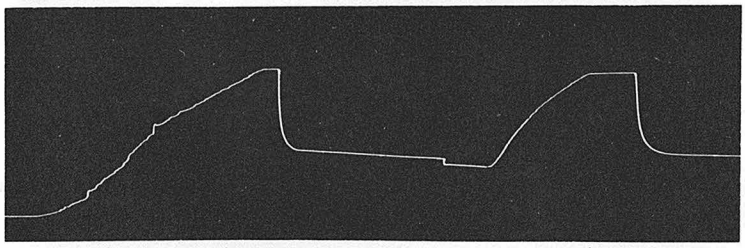


Figure 23. Fast Closer of Cancer.
Contractions of the fast closer of Cancer showing the effect of imposing extra single shocks (S) during stimulation at a basic frequency of 30 per second. In A, the fresh preparation, relaxation after each extra twitch is rapid. In B, the same preparation at a later stage, relaxation is slow.



(a) (b)

Figure 24. Fast Bender of Cancer.
(a) Faradic stimulation plus intercalated shocks. (b) Faradic stimulation alone.

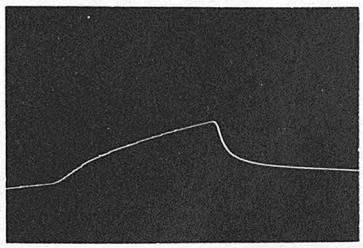


Figure 25. Slow Bender of Cancer.
Faradic stimulation with intercalated stimuli.

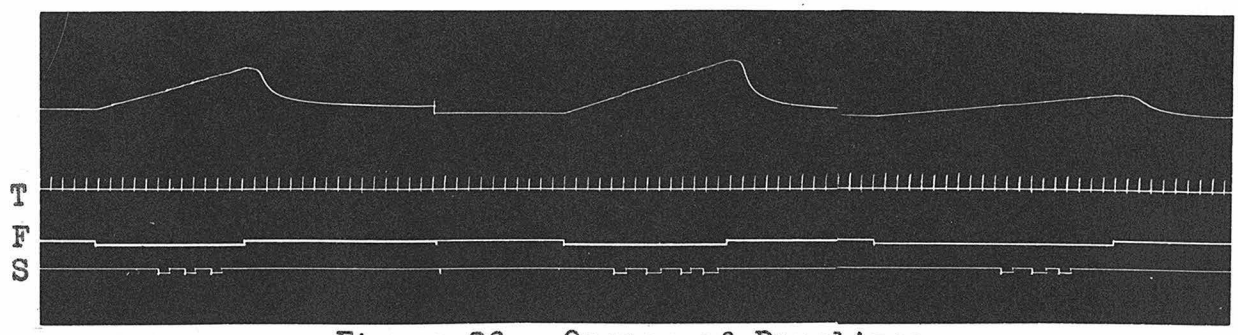


Figure 26. Opener of Panulirus.
Intercalation of extra stimuli (S) during faradic stimulation (F). Time in seconds (T). Extra shocks had very slight effect.

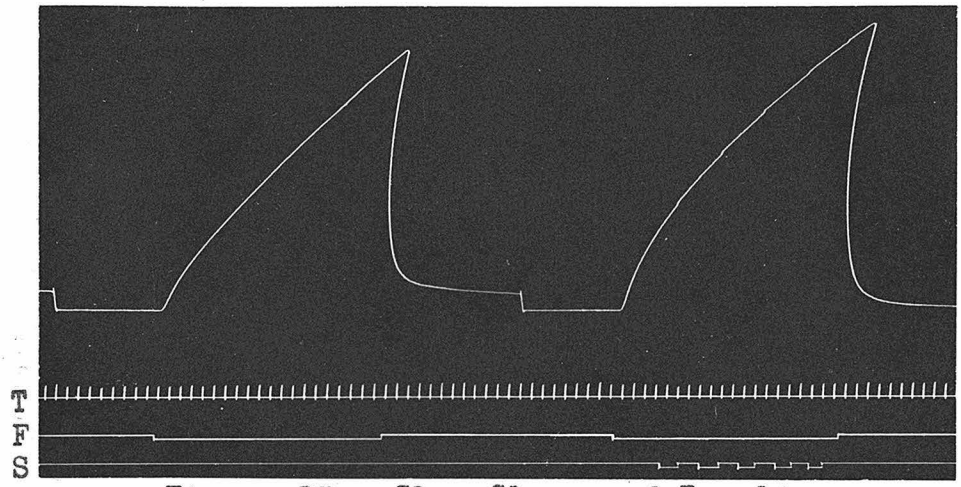


Figure 27. Slow Closer of Panulirus.
Effect of intercalating single shocks (S) during a contraction produced with faradic stimulation (F) Time in seconds (T).

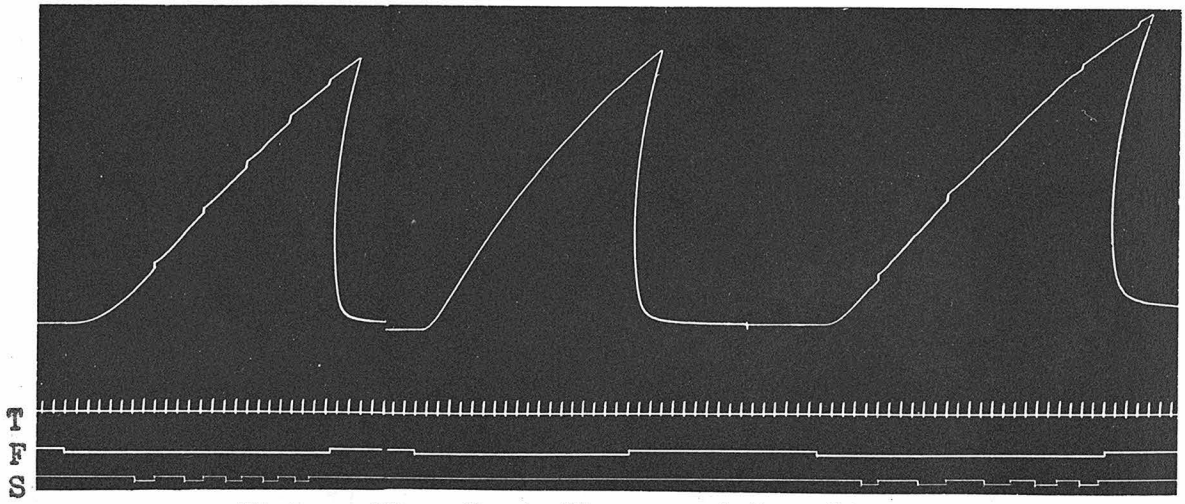


Figure 28. Fast Closer of Panulirus.
The effect of intercalated shocks (S) during faradic stimulation (F) of the fast closer. Time in seconds (T).

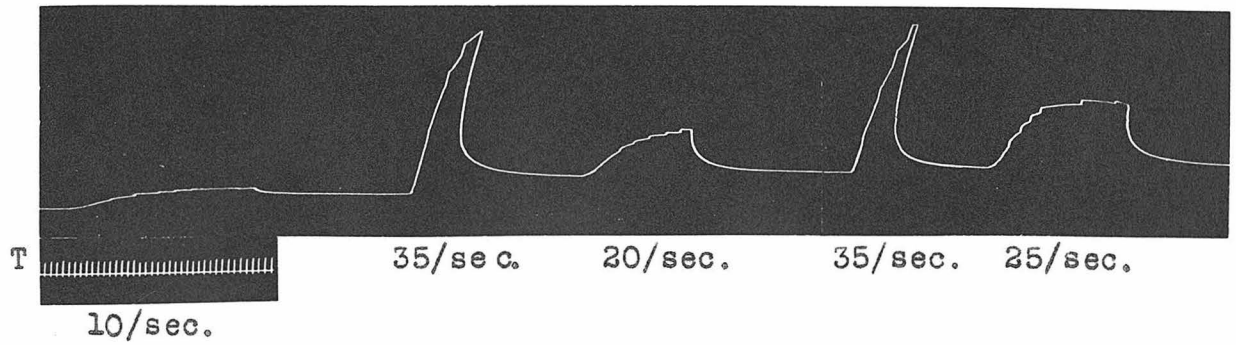


Figure 29. Fast Bender of Panulirus.
The effect of intercalated shocks on the contraction of the fast bender of Panulirus. Basic frequencies of electronically obtained square-wave pulses as indicated. Extra single shocks applied during all contractions shown. Time in seconds (T).

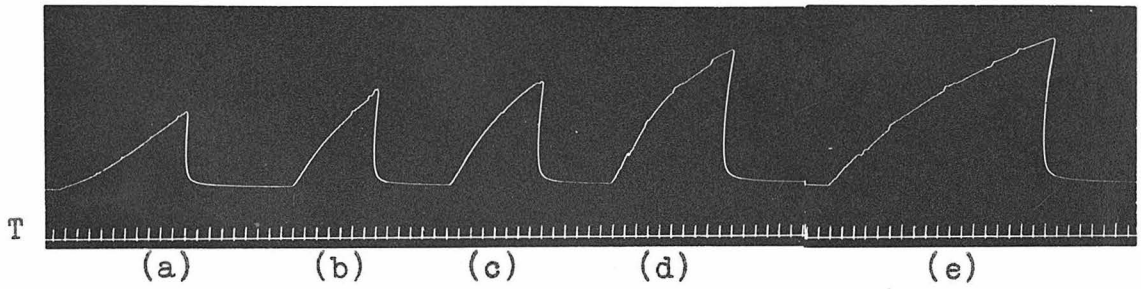


Figure 30. Second Fastest Main Flexor of Panulirus.
The effect of intercalating single stimuli during faradic stimulation of the second fastest main flexor. Single shocks were imposed during contractions (a), (b), (d), and (e). Time in seconds (T).

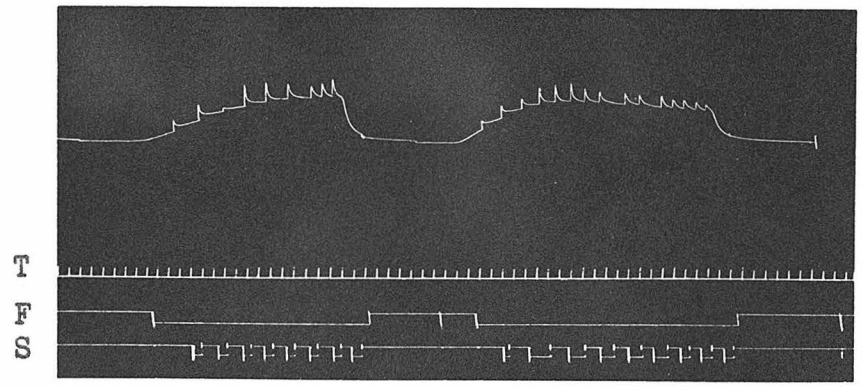


Figure 31. Fastest Main Flexor of Panulirus.
Showing the effect of intercalating single shocks (S) during faradic stimulation (F). Relaxation following the extra twitch is quite rapid. Time in seconds (T).

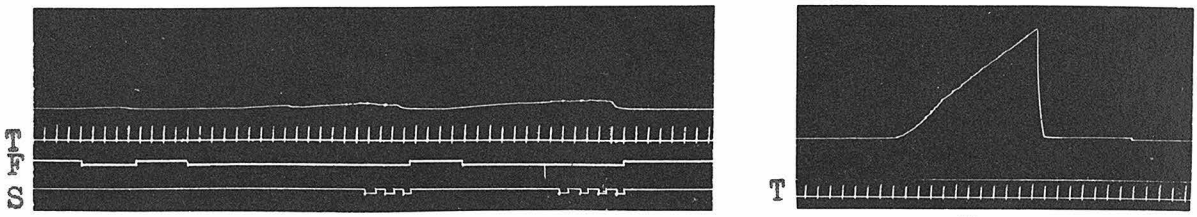


Figure 32. Slow Extensor of Panulirus.
Two preparations of the slow extensor of Panulirus. Preparation A was stimulated faradically (F) and with intercalated shocks (S). Preparation B was stimulated with square-wave pulses at a frequency of 30 per second and extra stimuli were imposed during the contraction. Time in seconds (T).

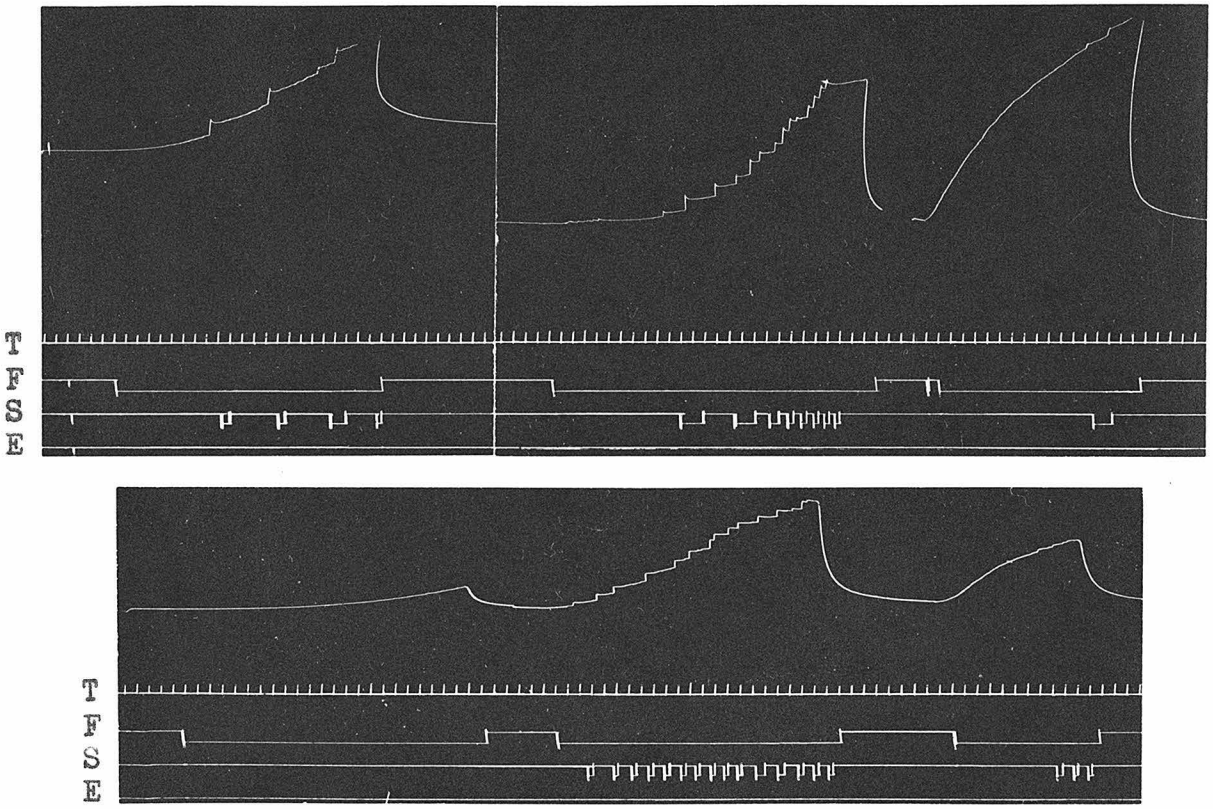


Figure 33. Fast Extensor of Panulirus.
Record of contractions of the fast extensor of Panulirus showing the effect of single shocks (S) during faradic stimulation (F). Time in seconds (T).

Systems with which this method was used include: fast extensor, Panulirus, fastest main flexor, Panulirus, fast bender, Cambarus, and opener, Cambarus. If the wide-spaced shocks gave less facilitation, the test contraction following the wide-spaced contraction would be smaller than that following the close-spaced contraction. This proved not to be the case. In all the preparations investigated the differences found were small and within the limits of accuracy of the method. Figure 4 illustrates a typical record obtained with this method.

Discussion

From the above experiments it will be seen that only a limited number of systems can give a pronounced "trigger" reaction. There is, however, a more or less continuous "spectrum" ranging from systems totally lacking such a reaction to systems which invariably give a strong effect. Even though any given system varies from one preparation to another and at different states of fatigue of the same preparation, certain broad distinctions can be drawn. In slow systems the effect is either completely absent or, if present, is small. In fast systems, on the other hand, it is generally present, and is often large.

The after result of the trigger response is less predictable in these preparations. From all indications it is much more susceptible to variation with the physical state of the preparation than the trigger effect itself.

This is not unexpected, since the nature of the after-effect will depend to a considerable degree on the speed of relaxation, which in all muscles is known to be dependent on the previous history of the muscle.

It seems questionable whether the trigger phenomenon as such is of much biological value in muscles with multiple motor innervation. In such muscles it is quite likely that the slow fiber fires at low frequency during postural reflexes. Fast movements will be executed by sudden high frequency bursts in the fast fiber. In order to obtain a real trigger action, it would be necessary that the fast fiber be firing first at a low frequency, which seems doubtful, but against which there is no experimental evidence. It may be pointed out that in those opener (and stretcher) systems which do show a trigger effect, this mechanism may be of considerable functional importance, since in these muscles the innervation is by a single motor axon.

The conditions which prevail during stimulations with double shocks are almost certainly far removed from any occurring under normal circumstances. These experiments serve, however, to show that synaptic structures may be profoundly influenced by impulse spacing. This is of general interest because it seems possible that impulse patterns do play a role in transmission in central nervous systems. For example, through the agency of pattern a fiber

might be used to convey, in addition to information regarding strength and location of peripheral sensations, a difference in modality (e.g., color in vision). Such possibilities have recently been mentioned by Granit (6) and by Adrian (7). In order that real benefit from the presence of pattern sensitivity can be obtained, it will be necessary that both pattern-sensitive and pattern-unsensitive elements be connected to the nerve fiber in which the impulses are transmitted. Otherwise, if only the first were present, the information of non-pattern impulses in the fiber would become lost. There are some reasons to believe that in the pattern-sensitive opener systems this possibility is realized. Here low frequency equally-spaced impulses will give a smooth tetanus, and close-spaced double impulses a much larger one. It seems possible that the different nerve endings on the single muscle fiber have different properties, some being pattern-sensitive and others not. In this way the single nerve fiber innervating the opener would combine the properties of a fast and a slow fiber to a considerable extent. Even if this be not the case, it serves as a good illustration of the possibilities offered by the combination of both kinds of excitability in one system.

The question arises as to what constitutes the difference between pattern-sensitive and pattern-unsensitive transmission mechanisms. Before going further, it may be

well to point out that there is between these mechanisms a difference only in degree: if the pattern be coarse enough it will be transmitted in any case. The factor which gives rise to the difference must be sought in differences in facilitation. In slow systems, unsensitive to pattern, it is obvious from, among others, the experiments with intercalated shocks that the amount of facilitation caused by a single nerve impulse is never large. Furthermore, relaxation is always slow, which follows from the fact that the tetani of these systems are smooth, even at low frequencies of stimulation. On the other hand, facilitation is long lasting and builds up to a considerable degree. With slow systems then, it is the total number of impulses during a given short time interval which is the factor determining the strength of the ensuing contraction. The actual pattern of the impulses during this period is relatively unimportant.

Pronounced pattern sensitivity requires that a single nerve impulse cause considerable facilitation which must decay rather quickly. It is clear, from the variable results obtained with fast systems, that a considerable increase in facilitation as caused by an intercalated impulse, though a prerequisite, is in itself not sufficient to guarantee a positive response from spacing. In order to obtain this, it is necessary that at the time of arrival of each odd-numbered impulse the amount of facilitation re-

maining be larger with close-spacing than with wide-spacing. Negative effects will be observed when the reverse is the case, which would occur when the facilitation after the even-numbered impulses decays so quickly that its increase with close spacing is over-compensated by its decrease during the longer intervals. Though these factors are sufficient to explain the negative effects found in the incomplete tetani of the fast closer of Cambarus, they may be wanting for the explanation of negative effects encountered in smooth tetani of the slow closer of Cambarus and the fast closer and bender of Panulirus.

References

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2. B. Katz, Biol. Rev., 24, 1 (1949). Neuromuscular transmission in invertebrates.

3. C. A. G. Wiersma and A. van Harreveld, J. Exp. Biol., 15, 18 (1938). A comparative study of the double motor innervation in marine crustaceans.

4. C. A. G. Wiersma, J. Comp. Neurol., 74, 63 (1941). The inhibitory nerve supply of the leg muscles of different decapod crustaceans.

5. C. A. G. Wiersma and A. van Harreveld, Arch. neerl. Physiol., 19, 426 (1934). On the nerve-muscle system of the hermit crab. I. The contraction of the abductor and the adductor of the claw.

6. R. Granit, "Sensory mechanisms of the retina." London, Oxford Univ. Press, 1947.

7. E. D. Adrian, "The Physical Background of Perception." Oxford Clarendon Press, 1947.

Propositions Submitted by Robert T. Adams

Ph. D. Oral Examination, May 12, 1950, 1:00 P.M., Crellin Conference Room.

Committee: Professors Niemann (Chairman), Bates, Buchman, Lucas, Wiersma, and Wulf.

1. In order to establish the configurational relationships about carbon atoms two and three of sphingosine, I propose the following degradation:

$$C_{13}H_{27}-CH=CH-\underset{AcO}{CH}-\underset{NHAc}{CH}-CH_2OAc \xrightarrow{KMnO_4} HOOC-\underset{AcO}{CH}-\underset{NHAc}{CH}-CH_2OAc$$

$\xrightarrow{2\ \text{steps}}$ D- or L- Erythronic or Threonic Acid.

2. The observation of Levene and Compton* that the saponification of 5-tosyl-2,3-isopropylidene-L-rhamnofuranose with sodium methoxide yields methyl 2,3-isopropylidene-6-desoxy-D-allofuranoside may be reconciled with other detosylation reactions of the sugars by a mechanism involving formation of the aldehydo sugar followed by successive formation and scission of a 4,5-anhydro ring.

* Levene and Compton, J. Biol. Chem., 116, 169 (1936).

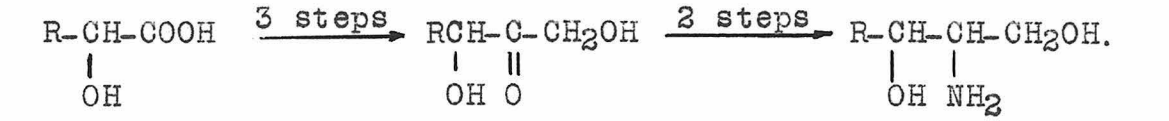
3. The reaction of hydrazine with permanganate in acid solution consumes in the limiting case 1.45 equivalents of permanganate per mole of hydrazine destroyed*. The course of this reaction may be explained with the aid of the assumption that the rate of oxidation of hydrazine by manganate is very fast compared with the rate of decomposition of manganate.

* Cuy, Rosenberg, and Bray, J. Am. Chem. Soc., 46, 1810 (1924).

4. Application of the mass spectrometer should permit resolution of the possible conflict between the conclusions of Bawn and Milstead* and of Rosenblum** concerning the fate of methylene radicals in the presence of molecular hydrogen. Telluroformaldehyde may be a convenient source of methylene radicals for such investigations.

* Bawn and Milstead, Trans. Faraday Soc., 35, 889 (1939).
** Rosenblum, J. Am. Chem. Soc., 63, 3322 (1941).

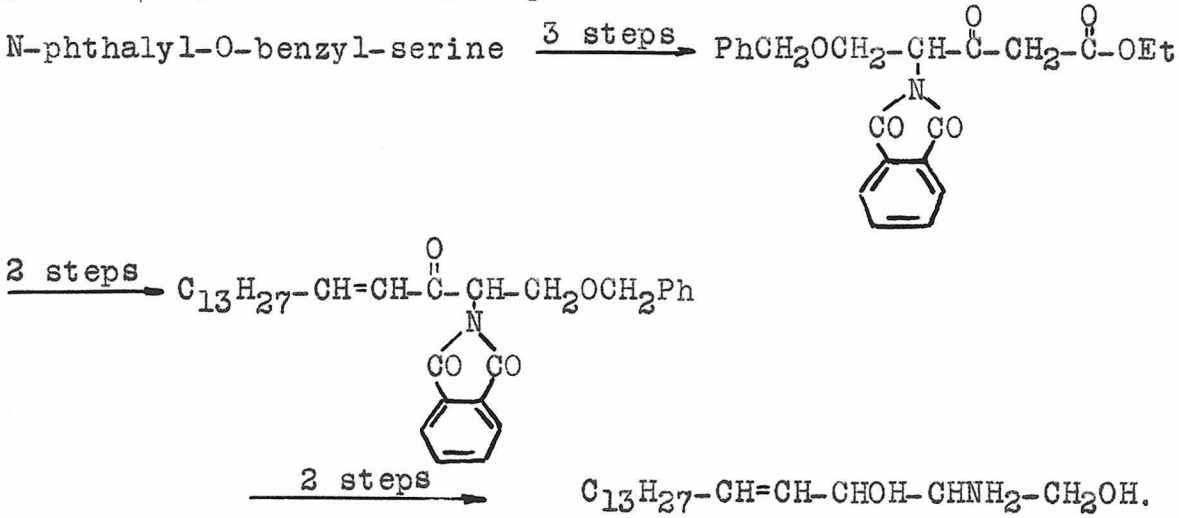
5. The following reactions constitute a general synthesis of 1,3-dihydroxy-2-amino-n-alkanes.



6. It is proposed that the lack of success attending efforts to reduce α-benzamido-β-methoxy-n-caproic acid by "reductive desulfurization" of its thiol ester* may be attributed to formation of an azlactone during the attempted preparation of the acid chloride of the amino acid.

* This Thesis.

7. The following reaction scheme outlines a possible synthesis of sphingosine.



8. Salicylaldehyde forms a stable compound with ammonia whereas p-hydroxybenzaldehyde does not.* This difference may be explained on the basis of hydrogen bonding.

* Delepine and Rivals, Compt. rend., 129, 520 (1899).

9. I propose that ethylene glycol possesses considerable utility as an agent for use in codistillations.

10. (A) A laboratory course in "Advanced Organic Preparations" for first year graduate students of organic chemistry would be of substantial value to the student and to the organic department.

(B) I propose that there would be merit in requiring each candidate for a higher degree to be instructed in the art of public speaking.