a rise in arterial but also a rise in venous pressure. In five experiments the venous pressure rise in three normal persons was 8–83 mm. water simultaneously with rises of 13–54 mm. mercury in systolic pressure. There was also a considerable decrease of blood flow in the hand, which fell from an average of 27 cc. to 3 cc. per minute per 100 cc. of tissue during a pressor response of 43–67 mm. systolic in two normal persons. The cardiac output showed in three determinations a very slight decrease of 0.2 liter per minute, while the blood pressure rose 42–56 mm. systolic and 36–42 mm. diastolic.

Summary

A method is described by which renin, a pressor protein of the kidney, can be prepared in good yields and with a high degree of purity. The effect of human renin and hog renin in human beings, rabbits and dogs has been studied. Repeated injections of large amounts of pig renin did not produce permanent hypertension in rabbits.

Specificity Studies on Enzymes Hydrolyzing Esters of Substituted Amino and Nitrogen Heterocyclic Alcohols[1]

BY DAVID GLICK

Earlier work on the specificity of cholinesterase[2] led to certain generalizations, among which was one that the presence of nitrogen groups, such as amino, alkyl-substituted amino, or heterocyclic structures in the acid component of an ester renders the compound refractory to hydrolytic enzyme action. Evidence given in the present communication extends this rule to include esters of mono- and dialkylamino alcohols as well as those of piperidyl alcohols, even though the latter are attacked by an enzyme which, from data to follow, is probably not the same as cholinesterase.

There is no term in the literature for the class of esterases that act on the nitrogen-alcohol esters. Therefore, it is proposed that the term "azolesterase" be applied to this group to distinguish it from other types of esterase. Among the "azolesterases" are cholinesterase, morphinesterase,[3] and certain tropinesterases such as atropinesterase, cocainesterase, and tropacocainesterase. From differences in occurrence, evidence has been presented that the three tropinesterases may be distinct enzymes.[4]

Differentiation of enzyme entities among the azolesterases has been suggested, within certain limitations, by the use of various sera as enzyme sources.[4] Thus, horse serum contains the usual lipase and esterase and, in addition, it is a rich source of cholinesterase; but it is devoid of all of the tropinesterases studied except tropacocainesterase. There are rabbit sera with and without atropinesterase; both types contain cholinesterase, cocainesterase, and tropacocainesterase. Horse serum and both kinds of rabbit serum were employed in the present study.

Most of the substrates used in this investigation are local anesthetics or antispasmotics; their enzymatic hydrolysis might be a significant factor in determining the duration, and possibly the intensity, of the action of these drugs *in vivo*. The following groups of substrates were used: esters of β-diethylaminoethanol, certain alkyl-substituted aminoalkyl benzoates, dialkylamino-alkyl 2-furoates, and piperidylalkanol esters.

Experimental

The manometric method employing the Warburg apparatus was used for the enzyme measurements in the same manner as in preceding studies.[2,4] In the present case, enzyme activity has been expressed in terms of cmm. of carbon dioxide liberated in two hours at 30° with 1% substrate and 2.5% serum in a total volume of 4 ml. One % substrate was chosen since this concentration is sufficiently high to ensure maximum hydrolytic velocities. Manometer readings were taken every fifteen minutes, and activity was determined by the slope of the linear portion of the activity-time curve.

In a number of cases it was necessary to bring the substrate solutions to the desired pH of 7.4 by addition of alkali. The weighed substrate was dissolved in a small volume of bicarbonate Ringer solution, either N or 0.1 N sodium hydroxide (depending on the amount of base required) was added a drop at a time until the solution was neutralized to brom thymol blue used as an outside indi-

(1) Aided by a grant from the Sidney C. Keller Research Fund.
(2) D. Glick, *J. Biol. Chem.*, **125**, 729 (1938); **130**, 527 (1939); **137**, 357 (1941).
(3) C. I. Wright, *J. Pharmacol. Exp. Therap.*, **71**, 164 (1941).
(4) D. Glick and S. Glaubach, *J. Gen. Physiol.* **25**, 197 (1941).

cator, and then it was made up to the required volume with bicarbonate Ringer solution. Practically all of the compounds are insoluble in water as the free bases and soluble as the salts. However, many were insoluble at pH 7.4, and hence were used in suspension; these cases have been indicated in the tables.

β-Diethylaminoethyl Benzoate Hydrochloride (no. 1).— Cold benzoyl chloride was dropped into diethylaminoethanol in a flask kept cold with an ice-salt mixture until an excess of acyl chloride had been added. After the initial reaction subsided, the mixture was heated on a steam-bath under a reflux condenser for three hours, and allowed to stand at room temperature overnight. The material was dissolved in absolute alcohol and precipitated with absolute ether. The solid was redissolved and reprecipitated twice more and finally dried *in vacuo* over phosphorus pentoxide. The final product consisted of colorless needles containing 13.6% Cl; calcd. 13.7.

β-Diethylaminoethyl *p*-Chlorobenzoate Hydrochloride (no. 2).—Prepared with *p*-chlorobenzoyl chloride in the same manner as compound no. 1. The reaction mixture was merely warmed on the steam-bath before letting stand overnight. The product, which was brownish at first, required six precipitations to yield the colorless compound containing 11.8% Cl as chloride, and 23.9% total Cl; calcd. 12.1 and 24.2%, respectively.

β-Diethylaminoethyl phenylacetate hydrochloride (no. 11) was prepared as above with phenylacetyl chloride. The reaction mixture was heated on the steam-bath for three hours before letting stand overnight. Ether precipitation from alcohol yielded a yellow oil; the four subsequent precipitations were made from acetone solution, and the product which came down was still an oil, but it crystallized on stirring and cooling. The final product, though colorless, took on a yellow tint during drying. *Anal.* Found: Cl, 13.0, calcd. 13.0.

β-Dimethylaminoethylbenzoate hydrochloride (no. 19) was prepared from the acyl chloride and substituted amino alcohol as above. The reaction mixture was heated on a steam-bath for three hours, and the product reprecipitated four times from alcohol by ether; final colorless needles contained 15.3% Cl; calcd. 15.4.

The remaining compounds were obtained from various investigators[5] with the exception of no. 18 which was described in the first paper in this group.[3]

Discussion

Rabbit serum (I) (containing atropinesterase) could hydrolyze any of the esters tested that were acted upon by either horse serum or rabbit serum (II) (devoid of atropinesterase), Tables I–IV. This was also true for the tropine esters

(5) Compound no. 16 was supplied by Dr. L. A. Pirk of Hoffman-La Roche, Inc. (It is commonly prepared as the phosphate salt, but could not be used in this form because of the buffer action of phosphate; hence it was converted to the free base, from which the chloride was prepared); no. 41 by Dr. W. E. Cass of New York University; 4, 5, 17, 29 by Dr. W. A. Lott of the Squibb Institute for Medical Research; 12-15 by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc.; 34-38 by Dr. L. A. Walter of the Maltbie Chemical Co.; 24, 25, 30-33 by Dr. E. S. Cook of the Institutum Divi Thomae; 6-10, 23, 26, 40 by Dr. H. R. Hulpieu of the Indiana University School of Medicine; 20, 21 by Dr. S. D. Goldberg of the Novocol Chemical Mfg. Co.; and 43 by Dr. R. T. Major of Merck and Co.

previously investigated.[4] The action of rabbit serum (I) on compound 6 was not studied because of the accidental loss of the substrate.

The only known azolesterases in horse serum are cholinesterase and tropacocainesterase. Hence if a substrate is not attacked by horse serum, but is by rabbit serum (I), then it should be tested with rabbit serum (II) to determine whether the tropinesterase contained in (I), but not in (II), is required for the enzymatic scission. This has been done in the present work, and it may be seen that compounds 4, 6, 7, 8, 22, 23, 29, 34, 37–40 were hydrolyzed by serum (II). Therefore, these compounds that are unaffected by horse serum, but are hydrolyzed by both types of rabbit sera, must be split by an enzyme or enzymes that are probably none of the known azolesterases with the possible exception of cocainesterase[4] which exists in both serum (I) and (II).

The effect upon hydrolysis of varying the structure of the acid component of esters of β-diethylaminoethanol is shown in Table I. The insolubility of many of these substances, as well as that of certain esters given in the other tables, is probably a factor in reducing their splitting, but certain compounds, such as nos. 2, 11, 12 undergo considerable scission in spite of this.

From the data obtained by the action of horse serum on these substrates, further information concerning the specificity of cholinesterase is available; for undoubtedly the enzyme hydrolyzing the choline esters is the same as that operating on these compounds. Not only is the aminobenzoate (no. 3) impervious to the enzyme action, as would be expected of any ester of an amino acid, but the alkoxy and thioalkyl benzoates are also unaffected. The action on the cyclohexylacetate is less than that on the phenylacetate, while the introduction of two rings regardless of the degree of their saturation (nos. 13–15), abolishes all enzymatic effect. The introduction of a hydroxymethyl group into the phenylacetate (no. 16) also blocks enzyme action.

The hydrolysis of the thioalkyl benzoates (nos. 7–10) varies with the size of the alkyl group: increasing from thiomethyl to thioethyl, decreasing to thiopropyl, and becoming even less with thiobutyl.

The result of variations of the alkyl substituents attached to the nitrogen in the alcohol may be seen from compounds 1, 18, 19, 24, 25 (Tables

I, II). Apparently horse serum acts equally well on the trimethyl- and diethylamino esters, less

Compound	Substrate	Non-enzymatic hydrolysis	Enzymatic hydrolysis[a] Horse serum	Rabbit serum (I)	Rabbit serum (II)
1	Benzoate	120	136	252	
2	p-Chlorobenzoate[b]	43	80	692	100
3	p-Aminobenzoate (Novocaine, Procaine)	10	0	0	
4	p-Ethoxybenzoate	32	0	46	28
5	p-n-Propoxybenzoate[b]	20	0	0	
6	p-Thioethylbenzoate[b]	8	0	...	36
7	m-Thiomethylbenzoate[b]	0	0	392	51
8	m-Thioethylbenzoate[b]	6	0	680	58
9	m-Thio-n-propylbenzoate[b]	0	0	156	3
10	m-Thio-n-butylbenzoate[b]	0	0	38	0
11	Phenylacetate[b]	640	64	192	
12	Cyclohexylacetate[b]	58	19	180	
13	Diphenylacetate[b] (Trasentin)	0	0	0	
14	Phenylcyclohexylacetate[b]	7	0	0	
15	Dicyclohexylacetate[b,c]	0	0	0	
16	Hydroxymethylphenylacetate (Syntropan)	28	0	6	
17	α-Ethyl cinnamate[b]	30	0	0	

[a] Cmm. carbon dioxide liberated in two hours at 30° with 1% substrate and 2.5% serum, 4 ml. total volume. [b] Insoluble, suspension used. [c] Sulfate, instead of chloride salt.

vigorously on the dimethyl- compound, and not at all on the dibutyl esters. The ratio of activities of horse and rabbit sera (I) on substrates nos. 1, 18, 19, varies widely. Inclusion of the nitrogen of the alcohol component in a piperidyl ring completely prevents horse serum action, but allows scission by rabbit sera, Table IV.

The diethylaminoalkyl 2-furoates, Table III, are split by all three sera, indicating that cholinesterase may act upon them, but the dibutyl compounds 32, 33 are more irregular. The insolubility of no. 32 is doubtless a factor in the negligible hydrolysis of this substance, and no. 33 is peculiar in that only rabbit serum (II) appreciably affects it.

The piperidylalkanol esters, Table IV, are hydrolyzed by an azolesterase probably different from cholinesterase, atropinesterase, or tropacocainesterase, as already mentioned. Compounds 41–43 were included for comparison. Little can be said concerning the hydrolysis of the vitamin B₆ triacetate (no. 43) since no attempt was made to determine which ester linkages were hydrolyzed.

TABLE II

HYDROLYSIS OF CERTAIN OTHER ALKYL-SUBSTITUTED AMINOALCOHOL ESTERS

Compound	Substrate	Non-enzymatic hydrolysis	Enzymatic hydrolysis Horse serum	Rabbit serum (I)	Rabbit serum (II)
18	$(CH_3)_3N(Cl)CH_2CH_2OCOC_6H_5$	17	133	84	
19	$(CH_3)_2NH(Cl)CH_2CH_2OCOC_6H_5$	102	56	480	
20	$C_2H_5NH_2(Cl)CH_2CH_2OCOC_6H_4NH_2\text{-}p$	30	0	0	
21	$n\text{-}C_3H_7NH_2(Cl)CH_2CH_2OCOC_6H_4NH_2\text{-}p$	8	0	0	
22	Apothesine	11	0	16	60
23	$(C_2H_5)_2NH(Cl)CH_2CH_2CH_2OCOC_6H_4SCH_3\text{-}m$	15	0	146	14
24	$n\text{-}(C_4H_9)_2NH(Br)CH_2CH_2CH_2OCOC_6H_5{}^a$	0	0	0	0
25	$n\text{-}(C_4H_9)_2NH(Br)CH_2CH_2OCOC_6H_5{}^a$	0	0	0	0
26	$n\text{-}(C_4H_9)_2NH(Cl)CH_2CH_2OCOC_6H_4SC_2H_5\text{-}o^a$	0	0	0	0
27	Stovaine	2	0	0	
28	Alypin	8	0	0	
29	$(CH_3)_2NH(Cl)CHCH_2OCOC_6H_5{}^b$ $\quad\mid$ $CH(OH)C_6H_5$	13	0	33	15

[a] Insoluble, suspension used. [b] Saturated solution used, almost 1%.

TABLE III

HYDROLYSIS OF DIALKYLAMINOALKYL 2-FUROATES

Compound	Substrate (alcohol component)	Non-enzymatic hydrolysis	Enzymatic hydrolysis Horse serum	Rabbit serum (I)	Rabbit serum (II)
30	β-Diethylaminoethyl·HCl	302	48	258	32
31	γ-Diethylaminopropyl·HCl	35	26	114	30
32	β-Di-n-butylaminoethyl·HBr[a]	0	2	3	5
33	γ-Di-n-butylaminopropyl·HBr	9	12	260	4

[a] Insoluble, suspension used.

Summary

The term "azolesterase" is proposed for the group of esterases acting on nitrogen-alcohol esters.

Measurements have been made of enzymatic and non-enzymatic hydrolysis of esters of β-diethylaminoethanol and certain alkylsubstituted aminoalkyl benzoates, dialkylaminoalkyl 2-furoates, and piperidylalkanol esters. Horse serum and rabbit sera with and without atropinesterase were used as sources of enzyme.

TABLE IV

HYDROLYSIS OF PIPERIDYLALKANOL ESTERS

Compound	Substrate	Non-enzymatic hydrolysis	Enzymatic hydrolysis		
			Horse serum	Rabbit serum (I)	Rabbit serum (II)
34	β-(2-Piperidyl)-ethyl benzoate·HCl	13	0	120	29
35	β-(2-Piperidyl)-ethyl p-aminobenzoate·HCl[a]	0	0	0	0
36	β-(2-Piperidyl)-ethyl o-aminobenzoate·HCl	3	0	0	0
37	β-(2-Piperidyl)-ethyl p-ethoxybenzoate·HCl	10	0	20	15
38	β-(2-Piperidyl)-ethyl cinnamate·HCl[a]	17	0	12	9
39	Metycaine	10	0	22	20
40	β-(N-Piperidyl)-ethyl m-thiobenzoate·HCl[a]	4	0	460	18
41	2-(N-Methylpiperidyl)-methyl acetate methiodide	52	352	52	50
42	Atropine sulfate	4	0	50	0
43	Vitamin B$_6$ triacetate	170	96	182	

[a] Insoluble, suspension used.

Further confirmation of the rule, that the presence of certain nitrogen groups in the acid component of an ester prevents hydrolytic enzyme action, was obtained.

Evidence was presented for the possible existence of an enzyme or enzymes distinct from any of the known azolesterases, with the exception perhaps of cocainesterase, that can hydrolyze certain esters of β-diethylaminoethanol and related compounds, as well as piperidylalkanol esters.

NEWARK, N. J.   RECEIVED SEPTEMBER 19, 1941

Studies in Chemotherapy. IV. Sulfanilamidopyrimidines[1]

BY RICHARD O. ROBLIN, JR., PHILIP S. WINNEK AND JACKSON P. ENGLISH

In a previous paper[2] of this series, sulfadiazine and several other sulfanilamidopyrimidines were described. The successful application of sulfadiazine as a chemotherapeutic agent[3] made it seem desirable to carry out a more extensive investigation of the various possible pyrimidine derivatives. The present report describes the preparation and properties of 5-sulfanilamidopyrimidine and a number of alkyl, alkoxy, amino and halogen substituted compounds. The results are recorded in Table I. For comparison the derivatives of this series which have been reported previously are included in the table.

Since 5-aminopyrimidine and several of the other aminopyrimidines required were unknown, it was first necessary to devise a synthesis of these intermediates. The sodium salt of 2-hydroxy-5-

nitropyrimidine[4] provided the starting point in the synthesis of 5-aminopyrimidine and several derivatives as outlined below.
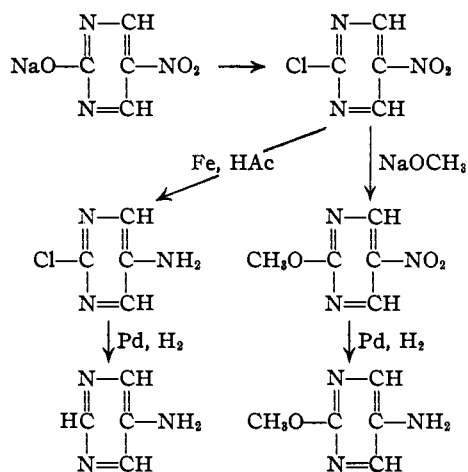
An attempt to prepare 5-aminopyrimidine more directly by the condensation of sodium nitromalondialdehyde and formamidine followed by reduction was unsuccessful. No 5-nitropyrimidine could be isolated from the condensation reaction. A one step catalytic reduction of 2-

(1) Presented in part before the Divisions of Medicinal and Organic Chemistry, Atlantic City meeting of the American Chemical Society, September 10, 1941.

(2) Roblin, Williams, Winnek and English, THIS JOURNAL, 62, 2002 (1940).

(3) Feinstone, Williams, Wolff, Huntington and Crossley, Bull. Johns Hopkins Hospital, 68, 427 (1940); Plummer and Ensworth, Proc. Soc. Exptl. Biol. Med., 45, 734 (1940); Long, Can. Med. Assoc. J., 44, 217 (1941); Flippin, Rose, Schwartz, Dorn and Doak, Am. J. Med. Sci., 201, 585 (1941); Finland, Strauss and Peterson, J. Am. Med. Assoc., 116, 2641 (1941).

(4) Hale and Brill, THIS JOURNAL, 34, 82 (1912).