Went on tomatoes (*4*) and those of Dorland and Went on chili peppers (*5*) as far as vegetative growth is concerned. Thus, the peppers showed greatest vegetative growth, while still small, at 27°-day: 30°-night. Young tomato plants also showed maximum stem elongation at the highest pair of temperatures tested (27°-day: 27°-night).

The gynophore of the peanut has no close parallel in tomatoes or peppers, being most conveniently considered a stage intercalated between flower development and fruit development. Correspondingly, the evidence concerning a temperature optimum for growth of the gynophore finds no parallel in published results on flower or fruit development in tomatoes and pepper.

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The Antimicrobial Principle of *Clematis Dioscoreifolia*

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In the course of a survey designed to test green plants of this region for antimicrobial activity, it was noted that freshly prepared aqueous extracts of *Clematis dioscoreifolia* showed unusually strong activity against gram-positive and gram-negative bacteria and thus resembled a number of other species of Ranunculaceae tested previously (*1*). The activity was maintained for more than two months of storage in a refrigerator but diminished quickly at room temperature. Extracts of plant material from dried leaves were inactive.

Extraction of the aqueous solution with a variety of organic solvents, of which ethyl acetate appeared to be the best, caused the activity to move into the organic layer. In an attempt to isolate the active principle, the aqueous solution resulting from the extraction of approximately 1 kg of freshly picked plant was extracted with ethyl acetate. Removal of the organic solvent at reduced pressure, followed by several recrystallizations of the residual gum from a mixture of ethyl acetate and ligroin, yielded 204 mg of shiny white plates (mp, 151° C), which were shown to be identical with anemonin (*2, 3*) by analysis, color reactions, and mixed melting point.[1]

The distribution of anemonin in a number of Ranunculaceae has been discussed recently (*4*). Since the solutions used in our work possessed the extremely irritating properties of protoanemonin commented upon by other workers (*2, 3*), there can be little doubt that protoanemonin is responsible for the antimicrobial activity of *Clematis dioscoreifolia,* but dimerized to the inactive anemonin under the conditions employed for its isolation.

[1] We wish to thank Beatrice C. Seegal and S. Raymond for an authentic sample of anemonin.

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The Chemical Kinetics of Procaine and Chloroprocaine Hydrolysis

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Kisch (*1*) in 1943 reported his studies on procaine esterase. Since that time a number of microanalytical methods have been recommended for the determination of procaine and *p*-aminobenzoic acid in biological fluids. A reliable and simple method was described by Ting *et al.* (*2*). The authors of this paper have shown that Ting's method is also applicable to 2-chloroprocaine[1] and 2-chloro-4-aminobenzoic acid (*3*). Ting's method was utilized in the study of the chemical kinetics of the alkaline and enzymatic hydrolysis of procaine and chloroprocaine reported in this paper.

Bullock (*4*) demonstrated the instability of alkaline-buffered procaine solutions and measured the rate of decomposition of procaine at various pH's and temperatures. Although no actual mention is made in his paper of the kinetics of the reaction, the data presented seem to indicate that the alkaline hydrolysis of procaine is a first or second order reaction.

To study the alkaline hydrolysis, solutions containing around 4×10^{-4} moles/l of procaine or chloroprocaine and 7×10^{-4} moles/l NaOH were incubated at

TABLE 1

THE RELATIONSHIP BETWEEN TIME AND THE QUANTITY OF PROCAINE AND CHLOROPROCAINE HYDROLYZED IN ALKALINE MEDIA

Time (min)	Quantity procaine hydrolyzed (moles/l × 10^5)			Quantity chloroprocaine hydrolyzed (moles/l × 10^5)		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
30	5.2	8.0	7.9	12.0	13.2	9.4
60	10.3	12.8	13.1	21.2	21.8	16.4
90	16.4	17.4	17.5	27.2	27.5	22.0
120	20.1	21.8	21.8	30.6	30.3	25.2
150	24.1	25.3	24.6	32.1	32.2	27.8
180	27.2	28.5	27.2	33.5	33.7	29.7
210	—	31.0	29.5	—	34.3	30.7
240	—	32.8	32.8	—	34.6	32.0

[1] The chloroprocaine and the 2-chloro-4-aminobenzoic acid were supplied through the courtesy of L. Reiner, of Wallace and Tiernan Products, Belleville, N. J.

37° C. Samples were withdrawn at known time intervals into excess acid, thus stopping the hydrolysis. The procaine and *p*-aminobenzoic acid or chloroprocaine and chloro-aminobenzoic acid content of the samples was determined. Table 1 shows the results of three experiments.

The data of Table 1 indicate that the amount of procaine and chloroprocaine hydrolyzed increased logarithmically. Furthermore, since the initial ester and hydroxide concentrations were of the same order of magnitude, the reaction should be of the second order, expressed by the differential equation

$$\frac{dx}{dt} = K(a-x)(b-x),$$

where dx is the amount of ester or hydroxide reacted during the time dt, a is the initial amount of the ester, b the initial amount of the hydroxide, and K the reaction rate constant. The integration and rearrangement of the above equation leads to

$$t = \left[\frac{2.303}{K(a-b)} \log \frac{b}{a}\right] + \frac{2.303}{K(a-b)} \log \frac{a-x}{b-x}.$$

Since a and b are constants, t plotted against log $[(a-x)/(b-x)]$ should give a straight line the slope of which is equal to $2.303/K(a-b)$. The curves obtained in one of the procaine and one of the chloroprocaine hydrolysis experiments are shown in Fig. 1. It can be seen that the points fall close to a straight line, indicating that the reaction is of second order.

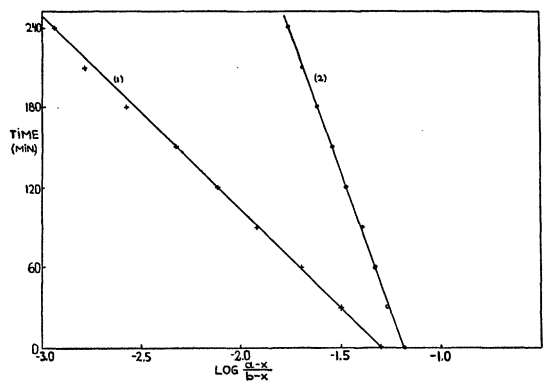


FIG. 1. Reaction rate curves of the alkaline hydrolysis of procaine (2) and chloroprocaine (1).

The average reaction rate constant of procaine was found to be 0.784 l/min × mole, that of chloroprocaine 2.29 l/min × mole, which means that chloroprocaine in alkaline media is hydrolyzed about three times faster than procaine.

The enzymatic hydrolysis of procaine and chloroprocaine was studied by incubating human plasma samples containing 3.7×10^{-4} moles/l of procaine or 3.3×10^{-4} moles/l of chloroprocaine at 37° C. At various time intervals samples were withdrawn into 3.75% trichloracetic acid, the hydrolysis stopped, and the procaine and *p*-aminobenzoic acid, or chloroprocaine and chloro-aminobenzoic acid, content determined. The results expressed as the quantity of procaine hydrolyzed in a given time interval are given in Table 2; similar data for chloroprocaine are presented in Table 3.

TABLE 2

RELATIONSHIP BETWEEN TIME AND THE QUANTITY OF PROCAINE HYDROLYZED IN PLASMA

Time (min)	Quantity procaine hydrolyzed (moles/l × 10^5)	
	Plasma 1	Plasma 2
2	3.5	—
3	5.7	3.9
5	9.9	10.7
8	16.3	16.8
10	19.3	25.2
12	25.7	—
13	—	31.2
14	28.2	—
15	32.6	36.6

TABLE 3

RELATIONSHIP BETWEEN TIME AND THE QUANTITY OF CHLOROPROCAINE HYDROLYZED IN PLASMA

Time (min)	Quantity chloroprocaine hydrolyzed (moles/l × 10^5)	
	Plasma 1	Plasma 2
1	8.5	10.4
2	18.3	20.7
3	28.5	31.0

The relationship between time and the quantity of procaine or chloroprocaine hydrolyzed (Fig. 2) indicates that there is a linear relationship between time and the quantities hydrolyzed. The independence of the reaction rate from the initial concentration is also evident from Fig. 3. The points on this graph were obtained by hydrolyzing 3 plasma samples containing 73.2, 36.6 and 18.3×10^{-5} moles/l of procaine, respectively, at 37° C, and withdrawing samples at the indicated time intervals. The amount of nonhydrolyzed procaine was plotted against time. The 3 lines drawn through the obtained points are parallel, indicating

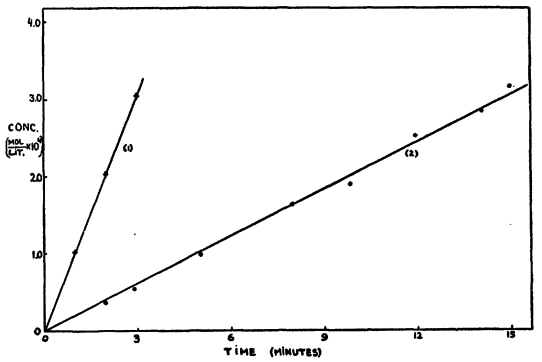


FIG. 2. Reaction rate curves of the enzymatic hydrolysis of procaine (2) and chloroprocaine (1).

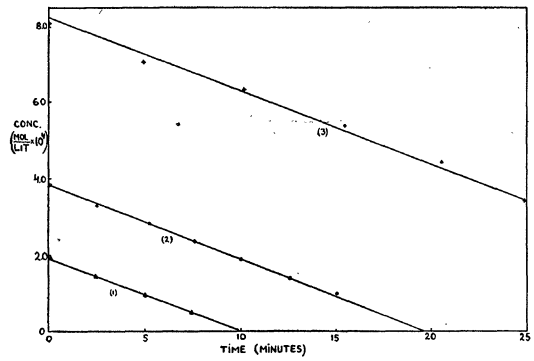


FIG. 3. Relationship between time and the quantity of non-hydrolyzed procaine in samples of various concentrations.

that the reaction rates were equal in all samples. This means that the enzymatic hydrolysis of procaine and chloroprocaine is a zeroth order reaction. The reaction rate constants obtained for three different plasma samples are given in Table 4. As can be seen from this table, chloroprocaine is hydrolyzed about four times faster than procaine.

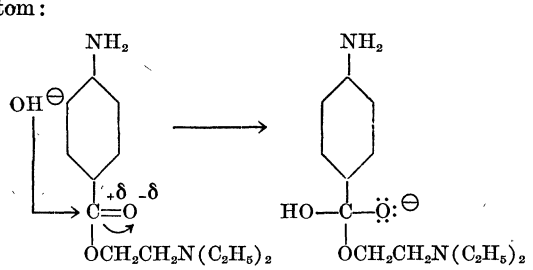
TABLE 4

THE REACTION RATE CONSTANTS (K) OF THE ENZYMATIC HYDROLYSIS OF PROCAINE AND CHLOROPROCAINE AT 37° C

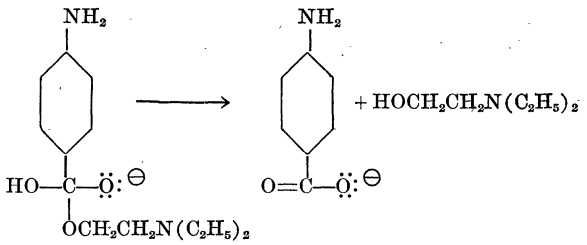
	K in moles/l × min		
	Exp 1	Exp 2	Exp 3
Procaine	2.58×10^{-5}	2.41×10^{-5}	2.45×10^{-5}
Chloroprocaine	1.03×10^{-4}	0.92×10^{-4}	1.04×10^{-4}
$\frac{K_{\text{chloroprocaine}}}{K_{\text{procaine}}}$	3.99	3.83	4.24

Although the mechanism of the alkaline and the enzymatic hydrolysis is different, it is of interest that the ratio of the reaction rate constants of chloroprocaine and procaine hydrolysis in both cases is not only of the same order of magnitude, but actually very close.

On the basis of purely theoretical considerations it was not definitely predictable what effect the substitution of a chlorine atom in the benzene ring would have on the speed of hydrolysis of the procaine molecule. The positive center at which the hydroxyl ion attack should be expected is the carbonyl carbon atom:



The decomposition of this complex will then proceed as follows:



Since the rate-determining step in the above reaction chain is the attachment of the hydroxyl ion to the carbonyl carbon atom, any electrophilic substituents in the benzene ring, which withdraw electrons from the carbonyl carbon atom, will facilitate this reaction and speed up the hydrolysis. The substitution of chlorine conceivably can have one of two effects: It can either exert its electron-attracting permanent polarization effect, or its electron-releasing resonance effect can be predominant. Our experimental findings indicate that when chlorine is substituted in the procaine molecule, the first of these two possibilities is prevalent.

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The Oncological Aspect of the "Immunity" of *Colchicum* to Colchicine

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In the course of his early colchicine work, the senior author came to the conclusion (*1*), as other workers had (*2, 3*), that *Colchicum autumnale* was totally resistant to the toxic, stathmokinetic, and oncogenic actions of colchicine.

Since then, this immunity has been seriously questioned by Cornman (*4*), who elicited typical colchicine mitoses in the roots of *Colchicum* by using higher concentrations of the drug than previous investigators. Levan and Steinegger (*5*) have, however, argued that the striking results obtained by the American author were not due to the alkaloid, but to the chloroform of crystallization in the drug.

These findings have revived old problems and have raised new ones that seem worth investigating—this so much the more so as in the references at our disposal we have found information only on the responses of the roots, whereas previous experiments of Havas[2] have furnished ample indications of the divergent oncogenic and growth effects of extracts of the various organs of *Colchicum*. If different organs of

[1] CNRS and IUBS—Unesco fellow for 1950–51. (Died June 9, 1951.)

[2] Observations and photographic records in press.