contents of one flask were used in one kinetic determination. The concentration of I was about 0.00049 mole, that of II about 0.0004 mole and of III 0.00024 mole, the largest amount that could be dissolved. The concentrations did not affect the rate constants within that range, because the use of lower concentrations for the first two compounds (0.00038 for I and 0.00027 for II) did not alter them. Rate constants were calculated from the integrated form of the first-order rate equation; the errors reported in Table I are average deviations. Data for one determination are listed in Table II. With an initial concentration of 0.0002736 of bromo compound the rate constant was 2.81×10^{-5} sec.$^{-1}$.

TABLE II

THE REACTION BETWEEN 3-NITRO-4-BROMOBIPHENYL WITH PIPERIDINE IN DIOXANE AT 25°

Time, sec.	Moles $\times 10^4$ of bromide	$(a - x) \times 10^4$	$k \times 10^5$, sec.$^{-1}$
3600	4.042	3.629	(2.99)
7200	4.020	3.267	2.88
9000	4.017	3.099	2.88
12600	4.017	2.799	2.87
15720	4.009	2.538	2.91
18060	4.042	2.412	2.86
21660	4.060	2.182	2.87
25020	4.070	2.022	2.80
31440	4.048	1.644	2.87
31980	4.035	1.631	2.83
50100	4.042	0.946	2.90

Results qualitatively similar to those reported in Table I were obtained in preliminary experiments conducted in boiling benzene (40 ml.) to which 4 ml. of piperidine had been added.

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Absence of Wall Effects in a Typical α-Chymotrypsin Catalyzed Hydrolysis[1]

BY RICHARD A. BERNHARD AND CARL NIEMANN[2]

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In the past[3,4] it has been tacitly assumed that α-chymotrypsin catalyzed hydrolyses, which are conducted under the conditions ordinarily used in *in vitro* studies of the mode of action of this enzyme, proceed entirely in solution and that wall effects, arising from the interaction of the reactants with the walls of the container, are unimportant. However, it appears that no one has ever determined whether or not the above assumption is a valid one. Therefore, we have, in this investigation, examined a representative α-chymotrypsin catalyzed hydrolysis with respect to possible wall effects arising from the nature and surface area of the container.

The initial velocities were determined by the method of Jennings and Niemann from both zero- and first-order plots,[5] for the α-chymotrypsin catalyzed hydrolysis of acetyl-L-tyrosinhydroxamide,[4,6,7] in aqueous solution at 25° and *p*H 7.6 and 0.3 *M* in the THAM[8] component of a THAM–HCl buffer, under conditions where the enzyme concentration was maintained at 0.0266 mg. protein-nitrogen/ml., *i.e.*, *ca.* 0.76×10^{-5} *M*,[9] the initial specific substrate concentration at 10×10^{-3} *M*, and where only the nature or the surface area of the container was varied. It will be seen from the data presented in Table I, for experiments 1 to 3, inclusive, that there is no significant difference in the initial velocities when either Pyrex or Kimble glass or polyethylene containers of equivalent surface area were employed. Furthermore, the addition of powdered Kimble glass to either the Kimble or Pyrex glass containers, or powdered Pyrex glass to the Pyrex container (*cf.* Table I, experiments 4 to 8, inclusive) was without effect even though the increase in surface area was of the order of twenty-fold in the extreme cases.

TABLE I

α-CHYMOTRYPSIN CATALYZED HYDROLYSES OF ACETYL-L-TYROSINHYDROXAMIDE[a]

Expt.	Nature of vessel	Powd. glass added Nature	Powd. glass added Weight[b]	Total area[c]	v_0[d]
1	Pyrex glass	.....	..	22	0.160
2	Kimble glass	.....	..	22	.159
3	Polyethylene	.....	..	22	.156
4	Kimble glass	Kimble[e]	0.30	83	.153
5	Kimble glass	Kimble	1.50	412	.157
6	Kimble glass	Pyrex[f]	0.20	61	.160
7	Pyrex glass	Pyrex	0.27	81	.157
8	Pyrex glass	Pyrex	1.33	406	.157
			Average of all values		0.157

[a] In aqueous solutions at 25° and *p*H 7.62 and 0.3 *M* in the THAM component of a THAM–HCl buffer, [E] = 0.0266 mg. protein–nitrogen/ml., $[S]_0 = 10 \times 10^{-3}$ *M*. [b] In g. [c] In units of cm.^{2} [d] In units of 10^{-3} *M*/min. [e] 150–200 mesh, density 2.5 g./cm.^{3}. [f] 150-200 mesh, density, 2.25 g./cm.^{3}.

Therefore, it may be concluded from the results of this study that, for the case at hand, wall effects are experimentally unimportant under the conditions which are employed generally in *in vitro* studies with α-chymotrypsin and that it is reasonable to assume that in all α-chymotrypsin catalyzed reactions which are studied under these conditions the reaction can be postulated as proceeding in solution in so far as can be determined within the limits of experimental error.

The average of the eight values of v_0 which are given in Table I, *i.e.*, 0.158×10^{-3} mole/min., may be compared with the value of $0.166 \pm 0.028 \times 10^{-3}$ mole/min. calculated on the basis of [E] = 0.0266 mg. protein-nitrogen/ml., $[S]_0 = 10 \times 10^{-3}$ *M*, $K_S = 43 \pm 4 \times 10^{-3}$ *M* and $k_3 = 33 \pm 3 \times 10^{-3}$ *M*/min./mg. protein-nitrogen/ml.[4]. The fact that these two values agree, within the limits of experimental error, can be taken as evidence of the consistency of the value of v_0 reported in this communication with those determined earlier.[4,6,7]

Experimental

Containers.—The Pyrex and Kimble glass containers used in this study were standard 10-ml. glass-stoppered volumetric flasks which had been cleaned with hot water containing a detergent and then thoroughly washed with distilled water. The polyethylene container was a 60-ml. screw cap bottle which had been treated similarly.

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(2) To whom inquiries regarding this article should be sent.

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(8) Tris-(hydroxymethyl)-aminomethane

(9) Based upon a molecular weight of 22,000 and a nitrogen content of 16.0% for monomeric α-chymotrypsin.[4]

Powdered Glass.—Pyrex and Kimble glass tubing was ground in an iron mortar and pestle, the product which passed a 150-mesh screen collected on a 200-mesh screen, digested with concentrated hydrochloric acid for 48 hours at 25–30°, thoroughly washed with distilled water and dried at 145° for 2 hours. In the calculation of surface areas an average diameter of 0.0088 cm. was assumed. The density of Pyrex glass was taken to be 2.25 g./cm.[3] and that of the Kimble glass 2.5 g./cm.[3].

Enzyme and Specific Substrate.—The α-chymotrypsin was an Armour preparation lot No. 10705. The acetyl-L-tyrosinhydroxamide, m.p. *ca.* 140° dec., $[\alpha]^{25}D$ 37.0° (*c* 5% in water), was prepared essentially as described by Hogness and Niemann.[6]

Enzyme Experiments.—All reactions were conducted as described by Hogness and Niemann[6] except that the improved analytical procedure described by Foster, Jennings and Niemann[10] was substituted for the one used earlier.[6] In every case plots of both $([S]_0 - [S]_t)$ *vs.* *t* and $\ln [S]_0/[S]_t$ *vs.* *t* were made and then corrected as described by Jennings and Niemann[6] using a value of $K_S = 43 \times 10^{-3}$ *M*. The values of v_0 given in Table I are the averages of those obtained from the corrected zero and first order plots which in no case differed by more than $\pm 0.005 \times 10^{-3}$ *M*/min.

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Epimerization and Fragmentation of Glucose by Quaternary Ammonium Base Type Anion Exchange Resins[1]

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The interconversion and degradation of mono- and disaccharides through the action of anion exchange resin (quaternary ammonium base type) in its hydroxyl form has been reported recently by several workers.[2-4] By analogy one would expect the mechanism of these reactions to be similar to that of the interaction of saccharides and strong alkali; however, failure to find mannose in the interconversion products has been cited by Rebenfeld and Pacsu[4] as evidence that the ene-diol mechanism for the Lobry du Bruyn transformation is not involved. In the case of the degradation products, five acidic compounds have been detected, but only two have been identified, these being lactic and glycolic acids.[2,3] The mechanism of the formation of these acids has not been investigated thoroughly.

In the present study, mechanism of the interconversion and degradation of glucose through the action of Amberlite IRA-400, a quaternary ammonium base type resin, was probed by the use of radioactive glucose-2-C^{14}. The interconversion products were examined by means of chromatography and radioautography. Lactic and glycolic acids were isolated from the degradation products and their respective isotopic distribution patterns is determined by degradation studies.

Experimental

Isolation of Fractions.—A solution containing 1.1 g. of glucose-2-C^{14} with a total activity of 1.66×10^5 c.p.m. was passed through a column 1.6 cm. diameter and 32 cm. long charged with 39 g. of IRA-400 (hydroxyl form). The absence of radioactivity in the effluent indicated that there was complete retention of the sugar on the column.

After standing 24 hours at room temperature the stoppered column was eluted with 1 *N* $(NH_4)_2CO_3$. After the removal of $(NH_4)_2CO_3$ by partial evaporation, the eluate subsequently was passed through an IR-112 cation exchange column to remove any remaining ammonium ions, and through an IR-4B weakly basic exchange column to remove any acidic constituents. The effluent from the latter thus contained only the sugars and other neutral components and gave a total activity of 1.07×10^5 c.p.m. or 64% of the original activity.

The original column was eluted with 2 *N* H_2SO_4 to recover the acidic products retained on the IRA-400. This was subsequently combined with the 2 *N* H_2SO_4 eluate of the IR-4B column. Organic acids then were recovered from the combined eluates by evaporation and exhaustive ether extraction. The mixed acid fraction so obtained had an activity of 2.48×10^4 c.p.m. or 15% of the original activity.

Identification of Interconversion and Degradation Products.—Paper chromatography of the mixed sugar fraction in the 80% phenol–water system revealed three components when sprayed with the normal carbohydrate reagents such as aniline phthalate and 3,5-dinitrosalicylic acid (R_f 0.33, 0.41 and 0.50). However, when ammoniacal silver nitrate was used, a fourth component of low R_f value (R_f 0.16) also slowly appeared. Radioautographs of these chromatograms showed all four spots to be radioactive. Three of these components (R_f 0.33, 0.41 and 0.50) were identified by co-chromatography as glucose, fructose and mannose, respectively. The fourth component is as yet unidentified.

Paper chromatography of the mixed acid fraction using a pentanol–formic acid–water system and a multiple-spray developing technique[5] revealed six components (R_f 0.08, 0.15, 0.23, 0.35, 0.56 and 0.74). Radioautography, however, indicated only three of these were radioactive (R_f 0.35, 0.56 and 0.74). These were identified, by color reactions with the various spray reagents and co-chromatography with authentic samples, to be: lactic acid (major constituent), glycolic acid and lactyllactic acid. The latter compound presumably results from the autoesterification of lactic acid during concentration of the samples for paper chromatography.[5,6] The other three non-radioactive components have not yet been identified.

Isolation of Acids.—Half of the mixed acid fraction was subjected to silica gel column chromatography according to the procedure of Bulen, Varner and Burell.[7] Three peaks were observed: a large radioactive lactic acid band containing essentially all of the starting activity, a very small radioactive glycolic acid band and a small non-radioactive band. The purity and identity of all fractions were established by paper chromatography. The unknown non-radioactive acid component could not be detected readily on paper chromatograms and it is as yet unidentified.

The glycolic acid content of the combined glycolic acid fractions was assayed colorimetrically to be 0.95 mg. according to the procedure of Newburgh and Burris.[8] After addition of the carrier, the diluted glycolic acid was isolated as its calcium salt. The lactic acid was isolated from the corresponding fractions as the zinc salt without dilution; yield 41.5 mg. Zn lactate trihydrate.

Degradation of Glycolic and Lactic Acids.—The glycolic acid was oxidized with lead tetraacetate according to a modification of the procedure of Schou, *et al.*[9] To a frozen mixture of calcium glycolate in glacial acetic acid was added

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