the resorcinol complex of the benzothiazolium isomer (6.2 g, 53%), mp 202–204°. The uv spectrum of the complex showed only the peak (420 mμ) characteristic of the benzothiazolium isomer.

2-(3-Pyridylvinyl)benzothiazole Dimethiodide (35).—A solution of pyridine-3-carboxaldehyde methiodide[18] (5.0 g, 0.02 mole) and 2,3-dimethylbenzothiazolium iodide (5.8 g, 0.02 mole) in EtOH (50 ml) containing 0.3 ml of piperidine was refluxed for 2 hr. The product separated on standing, mp 226–228°, yield 85%.

Compound **36** was prepared similarly from *p*-anisaldehyde.

Thioflavin Hydroxide (41) and 2-Methoxy-2-(*p*-dimethylaminophenyl)-3,6-dimethylbenzothiazoline (42).—Thioflavin bromide dihydrate (300 g, 0.75 mole), dissolved in MeOH (6 l.), was passed through a column of IRA-400 (OH^- form) (1 l.) previously washed with MeOH. The effluent was evaporated to dryness, and the residual solid, consisting of a mixture of **41** and **42**, was extracted with Et_2O (1 l.). The insoluble hydroxide was removed by filtration, mp 159–160°, yield 100 g (45%). *Anal.* ($C_{17}H_{20}N_2OS$) C, H, N, O, S.

The extract was evaporated, and the residue was recrystallized from EtOH–Et_2O at room temperature to give pure **42**, mp 97–98°, yield 125 g (52%). *Anal.* ($C_{18}H_{22}N_2OS$) C, H, N, O, S.

Compound **42** was also prepared by adding MeONa [from 0.23 g (0.01 g-atom) of Na] to thioflavin bromide (4.0 g, 0.01 mole) in MeOH (50 ml) at room temperature. The product gradually separated, mp 95–97°, yield 2.7 g (86%).

Derivatives of 2-(*p*-Dimethylaminobenzoylamino)-5-methylbenzenethiol (43).—Thioflavin bromide dihydrate (40 g, 0.1 mole) was added to 400 ml of 5% aqueous NaOH and the suspension was boiled until all the solid had dissolved. One-tenth of this solution (0.01 mole) was treated with I_2 (2.5 g) in 15 ml of 5% NaOH and the solution was boiled for 2 hr. The precipitate obtained on cooling was crystallized from MeOH, giving the disulfide **44**, mp 200–202°, yield 2.0 g (67%). *Anal.* ($C_{36}H_{38}N_4O_2S_2$) C, H, N, O, S.

A further portion of the alkaline solution of the thiol was treated with MeI to give the S-methyl derivative **45a**, mp 126–128°, in 90% yield. *Anal.* ($C_{18}H_{22}N_2OS$) C, H, N.

The S-benzyl derivative **45b** was obtained by treatment of **43** with benzyl bromide, mp 122–123° (80%). *Anal.* ($C_{24}H_{26}N_2OS$) C, H, N.

Addition of benzoyl chloride in Me_2CO to the thiol gave the S-benzoyl derivative **45c**, mp 129.5–130.5° (95%). *Anal.* ($C_{24}H_{24}N_2O_2S$) C, H, N.

Similarly, the 3,4-dichlorobenzoyl derivative **45d**, mp 150–152°, was obtained in 32% yield. *Anal.* ($C_{24}H_{22}Cl_2N_2O_2S$) C, H, N.

Acknowledgment.—The authors are indebted to Dr. A. O. Geiszler for invaluable help in the coordination of the research program.

(18) L. Panizzon, *Helv. Chim. Acta*, **24**, 24E (1941).

Catalysis by Poly-L-lysine of Aminolysis of Penicillin by Tris(hydroxymethyl)aminomethane

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The rate of penicillin loss in solutions containing both poly-L-lysine (PLL) and Tris in the pH range 7–9 is much more rapid than with either Tris or PLL alone. The rate is directly proportional to PLL concentration at low concentrations of PLL but becomes independent of Tris at high concentrations of the latter. Evidence is presented to show that the reaction taking place is aminolysis of penicillin by Tris catalyzed by PLL. Based on these results a mechanism is proposed which involves complex formation between PLL and penicillin prior to attack by Tris. This reaction may be a model for aminolysis of penicillin *in vivo*, leading to formation of antigen involved in penicillin allergy.

The principal antigenic determinant in penicillin allergy is the penicilloyl group bound by amide linkage to ε-amino groups of lysine residues on proteins.[1] One pathway by which formation of this hapten–protein conjugate may occur is the direct aminolysis of penicillin by the amino group.[2,3] Investigation of the mechanism of this reaction in a model system, using glycine as the amine, revealed that general base catalysis by a second molecule of glycine anion is required.[4] Further study of the reaction of benzylpenicillin with diamines suggested that intramolecular general base catalysis by one of the amino groups was involved and could increase reaction rate.[5] The present work was initiated to study the reaction of benzylpenicillin with poly-L-lysine (PLL), where it was thought that the large number of amino groups on the same molecule would accelerate the rate and perhaps be a better model for *in vivo* conditions.

As will be seen, however, in the presence of Tris buffer the reaction which takes place is aminolysis of the penicillin by Tris and this reaction is markedly catalyzed by PLL.

(1) A. L. DeWeck and G. Blum, *Int. Arch. Allergy*, **21**, 221 (1965).
(2) F. R. Batchelor, J. M. Dewdney, and D. Gazzard, *Nature*, **206**, 362 (1965).
(3) C. H. Schneider and A. L. DeWeck, *ibid.*, **206**, 57 (1965).
(4) M. A. Schwartz and G. M. Wu, *J. Pharm. Sci.*, **55**, 550 (1966).
(5) M. A. Schwartz, *ibid.*, **57**, 1200 (1968).

Results and Discussion

In the presence of 0.167 *M* Tris the rate of penicillin loss from solution at pH 8.8 is directly proportional to the concentration of PLL as shown in Figure 1. The small intercept represents reaction rate due to 0.167 *M* Tris alone at this pH. In Figure 2 is shown the dependence of reaction rate upon Tris concentration at several pH values when PLL concentration was kept constant at 9.0×10^{-5} *M* (0.04 *M* monomer). There is a saturation effect of the Tris and these curves can be fit by an equation of the form

$$k_{obsd} = \frac{a + b(T)}{c + (T)} \quad (1)$$

where (T) represents Tris concentration and *a*, *b*, and *c* are constants. Plots of the reciprocal of k_{obsd} *vs.* 1/(T) were nonlinear as would be expected from eq 1.

The pH dependence of reaction rate at both high Tris concentration and in absence of Tris is given in Figure 3.

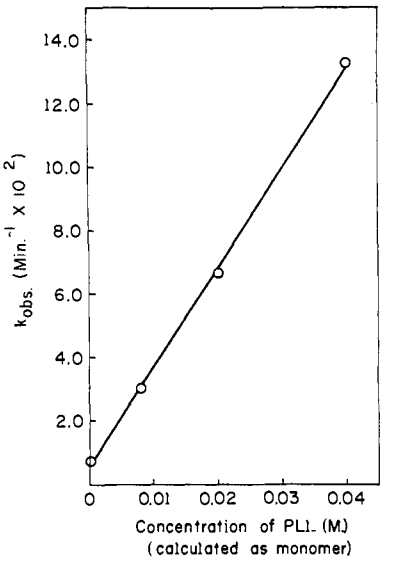


Figure 1.—Dependence of observed rate on PLL concentration. 0.04 *M* calculated as monomer is equivalent to 9.0×10^{-5} *M* polymer.

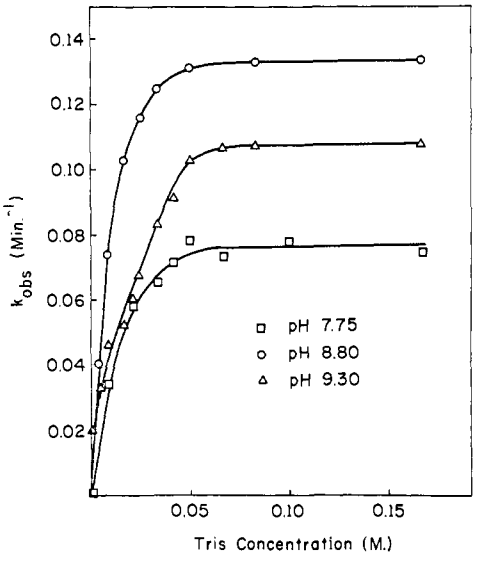


Figure 2.—Dependence of observed rate upon Tris concentration.

The dashed curve was obtained by substracting the rate constants observed without Tris from those determined with Tris. The maximum occurring at about pH 8.6 is indicative of participation in the reaction of both the positively charged PLL and the free base form of Tris. In the absence of Tris the reaction with PLL is very slow below pH 9. It is known that in highly alkaline solution penicillin reacts with PLL to form a multipenicilloated PLL which is used as a diagnostic agent in penicillin allergy.[6]

In order to get some idea of the nature of the reaction products, samples of reaction solutions taken after seven half-lives were dialyzed exhaustively and assayed for the penicilloyl group by the penamaldate method.[7] The results, presented in Table I, show that while most of the penicillin reacted with PLL when no Tris was present, only a small percentage reacted with PLL in the presence of Tris.

The fact that Tris was consumed in the reaction was

(6) B. B. Levine, *J. Med. Chem.*, **7**, 675 (1964).
(7) B. B. Levine, *ibid.*, **5**, 1025 (1962).

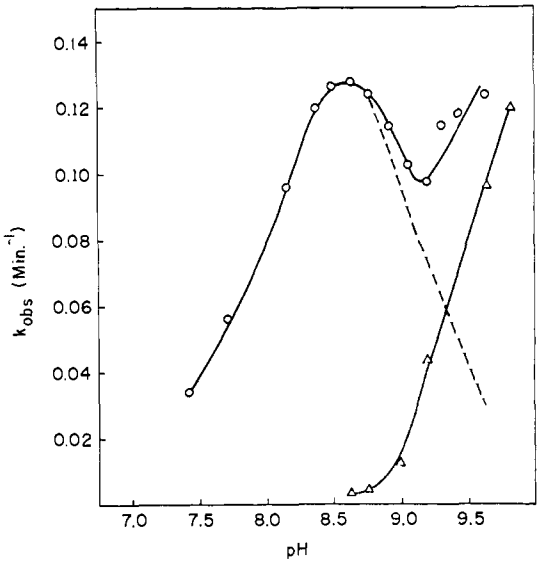


Figure 3.—Dependence of observed rate upon pH: ○ in presence of 0.167 *M* Tris, △ without Tris. All solutions contained 9.0×10^{-5} *M* PLL. The dashed line was obtained from the difference between the two curves.

TABLE I
PRODUCT ASSAYS[a]

Tris concn, *M*	mg of PLL reed[b]	% of orig penicillin as penicilloamide
0.167	45.1	19.7
0	42.7	64.5

[a] From runs at pH 8.8. [b] From 50 mg in sample. Determined by optical rotation.

proven by the observation, shown in Figure 4, of second-order kinetics when the initial concentration of both penicillin and Tris was 0.02 *M*. Titration of the solution after completion of reaction showed that over 80% of the Tris amino group was consumed.

Experiments were also carried out in which the polymer was replaced by the monomers ϵ-aminocaproic acid (EACA) and propylamine. As can be seen by the results in Table II neither of these substances had a large effect on reaction rate indicating that general acid catalysis by the individual charged amino groups on the polymer is not the principal mechanism. Further evidence that the catalytic action of PLL depends more on its polymer character came from experiments with PLL of molecular weight 38,000. The reaction rates with this material were much faster than with the 95,000 molecular weight PLL when calculated on the basis of concentration of amino group, but almost the same based on *polymer* concentration.

TABLE II
REACTION RATES AT pH 8.8

Tris, *M*	EACA, *M*	Propylamine, *M*	PLL, *M*	$k_{obsd.}$ min^{-1}
0.167	0.04	...	...	0.0078
0.167	...	0.04	...	0.010
0.167	...	...	...	0.0070
0.167	...	...	9.0×10^{-5}	0.129
...	...	...	9.0×10^{-5}	0.0060

One possible mechanism which was considered was that PLL may form complexes with Tris which are very reactive with penicillin. This was eliminated when no

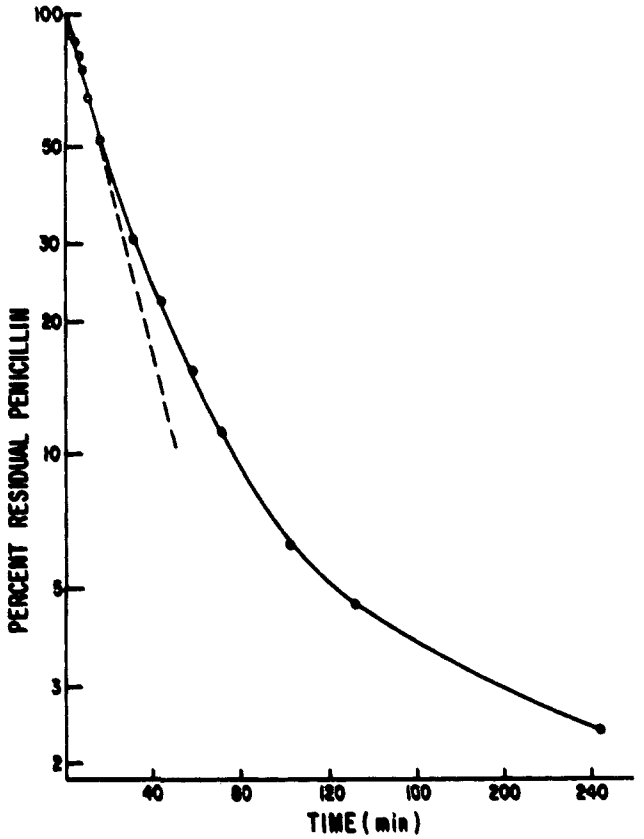
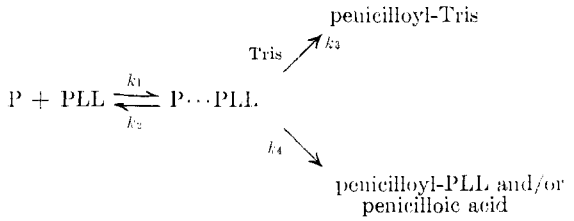


Figure 4.—Semilog plot of penicillin loss from solution equimolar in penicillin and Tris (0.02 *M*) plus 9.0 × 10^{-6} *M* PLL. Dashed line shows expected first-order plot of Tris is not consumed.

such complexes could be detected from dialysis studies. A second and more likely possibility is shown in the following scheme. Here it is assumed that penicillin is



attracted to the oppositely charged polymer and "activated" for attack by Tris or by a free amino group on the polymer or water to form the designated product. Taking a steady state in the intermediate the following equation is obtained

$$k_{obsd} = \frac{k_1k_4(\mathrm{PLL})_t + k_1k_3(\mathrm{PLL})_t(\mathrm{T})}{k_2 + k_4 + k_3(\mathrm{T})} \qquad (2)$$

where $(\mathrm{PLL})_t$ represents the stoichiometric concentration of polymer. This equation is of the same form as eq 1 showing the correspondence between the observed kinetics and this mechanism.

While further studies are needed (and are in progress) to elucidate the nature of the intermediate and determine how PLL exerts its catalytic effect, it is extremely interesting to note that a polymeric material can so markedly catalyze a reaction which is involved in penicillin antigen formation. A similar catalytic mechanism may operate *in vivo* and may be one route to rapid formation of antigen.

A caution in the use of Tris buffers in studying reaction kinetics should be mentioned here. Other workers noted enhanced rates in the presence of Tris. Shah and Connors, for example, recently reported that Tris increased the rate of chymotrypsin-catalyzed hydrolysis of a carbonate ester.[8] Thus, whenever Tris is utilized care should be taken to assess its effect, if any, on the system under study.

Experimental Section

Materials.—Potassium benzylpenicillin was kindly supplied by Bristol Laboratories. Poly-L-lysine hydrobromide was purchased from Pilot Laboratories, Watertown, Mass. The molecular weights were determined by the manufacturer. All other materials were reagent grade.

Kinetic Studies. With PLL.—The polymer was dissolved in 5 ml of Tris buffer containing sufficient KCl to make the ionic strength 0.5 *M*. At zero time 1.0 ml of penicillin solution was added and samples withdrawn at appropriate intervals were assayed for residual penicillin by the method of Brandriss, *et al.*[9] When Tris was absent or the buffer capacity too low, pH was maintained constant by a Radiometer TTT-1 pH-Stat using 1.0 *M* NaOH as titrant. The volume of titrant added during the course of a run was negligible.

With PLL Absent.—The reaction solution was prepared exactly as above but after adding the penicillin, 5 ml of reactant mixture was transferred to a 1-dm thermostated polarimeter cell and the change in optical rotation was followed on a Perkin-Elmer Model 141 polarimeter at 313 mμ with a Sargent SR recorder. Guggenheim plots were used to determine the apparent first-order rate constants.

All rate measurements were made at 35° in thermostated glass cells and, with the exception of the case where both penicillin and Tris concentrations were 0.02 *M*, the initial penicillin concentration was 1.0 × 10^{-4} *M* so that pseudo-first-order kinetics would be observed.

Assay for Penicilloyl-PLL.—The solution to be assayed was dialyzed in the cold for 2 days against a large volume of water containing Amberlite CG-400 anion exchanger. The recovery of PLL was determined by measuring optical rotation and the penicilloyl content by the penamaldate method.[7]

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(8) A. A. Shah and K. A. Connors, *J. Pharm. Sci.*, **57**, 282 (1968).

(9) M. W. Brandriss, E. L. Denny, M. A. Huber, and H. G. Steinman, *Antimicrobial Agents Chemotherapy*, 626 (1962).