mycin, the gramicidins A–D, and tyrocidine (Lardy *et al.*, 1964). The wide variety of agents that affect mitochondrial swelling and ion movements should be of value in testing the hypothesis of Mitchell (1961) and Mitchell and Moyle (1965) that proton gradients may supply energy for synthesis of ATP.

Acknowledgment

We are grateful to Professor V. Prelog for supplying the principal antibiotics used in this work and especially for preparing a quantity of pure nonactin when none was available in his laboratory.

References

Chappell, J. B., and Crofts, A. R. (1965), *Biochem. J. 95*, 393.

Connelly, J. L., and Lardy, H. A. (1964), *Biochemistry 3*, 19.

Gomez-Puyou, A., Tuena, M., Campillo-Serrano, C., and Diaz-Maqueo, J. (1965), *Arch. Biochem. Biophys. 109*, 221.

Graven, S. N., Lardy, H. A., Johnson, D., and Rutter, A. (1966), *Biochemistry 5*, 1729 (preceding paper).

Lardy, H. A., Connelly, J. L., and Johnson, D. (1964), *Biochemistry 3*, 1961.

Lardy, H. A., and Graven, S. N. (1965), *Federation Proc. 24*, 424.

Lardy, H. A., and Wellman, H. (1952), *J. Biol. Chem. 195*, 215.

Lehninger, A. L. (1961), *Biochim. Biophys. Acta 48*, 324.

Mitchell, P. (1961), *Nature 191*, 144.

Mitchell, P., and Moyle, J. (1965), *Nature 208*, 147, 1205.

Moore, C., and Pressman, B. C. (1964), *Biochem. Biophys. Res. Commun. 15*, 562.

Neubert, D., and Lehninger, A. L. (1962), *Biochim. Biophys. Acta 62*, 556.

Pressman, B. C. (1965a), *Federation Proc. 24*, 425.

Pressman, B. C. (1965b), *Proc. Natl. Acad. Sci. U. S. 53*, 1076.

Rasmussen, H., Fischer, J., and Arnaud, C. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 1198.

Sallis, J. D., and DeLuca, H. (1964), *J. Biol. Chem. 239*, 4303.

The Action on Soluble Blood Group A Substances of an α-*N*-Acetylgalactosaminidase from *Helix pomatia**

Hans Tuppy and Walter L. Staudenbauer

ABSTRACT: An α-*N*-acetylgalactosaminidase obtained from the hepatopancreas of *Helix pomatia* and purified 300-fold was allowed to act on soluble blood group A substances from human ovarian cyst fluid and hog gastric mucosa. This treatment resulted in an almost complete loss of serological A specificity and an 8- to 16-fold enhancement of blood group H activity as measured by hemagglutination inhibition tests. *N*-Acetylgalactosamine was found to be released. This indicates that the enzyme destroys the serological A specificity by removing from the antigenic sites terminal *N*-acetylgalactosamine residues thereby exposing structures similar to those present in H substances. Quantitative determinations suggest an average number of 120 antigenic sites per molecule of human blood group A substance, each of them being composed of two *N*-acetylgalactosamine, one *N*-acetylglucosamine, two galactose, and two fucose residues.

There is ample evidence suggesting that the soluble blood group substances obtained from human and other sources consist of a protein backbone to which numerous oligosaccharide units are attached (Kabat, 1956; Morgan, 1959). The latter are considered to carry the group specificity. Enzymes are known which destroy the serological activity of blood group substances by degrading the carbohydrate portions of the mucopolysaccharide molecule with the liberation of reducing sugars.

Enzyme preparations from the hepatopancreas of the snail *Helix pomatia* were found by Freudenberg and Eichel (1935) to inactivate blood group A substance and to release from it *N*-acetylhexosamine and galactose. The presence of A-decomposing enzymes was also demonstrated in liver extracts from the snail *Buscyon* (Howe and Kabat, 1953), and in various microorganisms (Iseki and Masaki, 1953; György *et al.*, 1954; Watkins, 1959; Yamamoto *et al.*, 1962). All these enzyme preparations, however, contained mixtures of

* From the Institut für Biochemie, Universität Wien, Vienna, Austria. *Received January 6, 1966.*

TABLE I: Purification of α-*N*-Acetylgalactosaminidase.

Procedure	Enzyme Act. (mU)	Enzyme Recovery (%)	Total Protein (mg)	Sp Act. (mU/mg)
(1) Acetone powder ext	71,000	100.0	12,200	5.8
(2) pH 4.5 supernatant	70,600	99.5	5,630	13.5
(3) First acetone pptn	71,600	101.0	2,470	29
(4) Ammonium sulfate (36–50%)	73,500	103.5	454	162
(5) Second acetone pptn	63,000	89.0	236	266
(6) Sephadex G-100	22,200	31.3	26	850
(7) ECTEOLA-cellulose	19,500	27.5	14	1,380
(8) CM-cellulose	19,100	27.0	10.8	1,750

glycosidases which caused further breakdown of the group substances after their loss of serological specificity.

Recently, Harrap and Watkins (1964) were able to define the molecular changes which occurred in human A substance when it was exposed to an A-decomposing enzyme from *Trichomonas foetus*. In the course of the incubation of the blood group substance with the enzyme, which resulted in an extensive loss of A specificity and in an eightfold rise of the H inhibition titer, 17% of the total *N*-acetylgalactosamine, but only 3.3% of the total fucose and small amounts of galactose, were found to be released. The results suggested that the glycosidase responsible for the destruction of A specificity removed from A substance terminal *N*-acetylgalactosamine residues, thereby exposing structures closely related to those present in H substances. The present study describes the action of an α-*N*-acetylgalactosaminidase (α-2-acetamido-2-deoxy-D-galactoside acetamidodeoxygalactohydrolase) partially purified from the hepatopancreas of *H. pomatia*, on soluble A substances of human and porcine origin.

Materials and Methods

Blood Group Substances. Water-soluble human A substance (HO-A) was prepared from a pseudomucinous ovarian cyst fluid by precipitation with ethanol, extraction of the precipitate with 90% phenol, and dissolution of phenol-insoluble material in water (Annison and Morgan, 1952). Blood group A and H substances from hog gastric mucosa (HGM-A and HGM-H) were obtained by the procedure of Bendich *et al.* (1946).

Immunochemical Methods. Human anti-A was obtained from Oesterreichisches Institut fuer Haemoderivate, Vienna. An anti-H reagent was prepared from the seeds of *Ulex europaeus* (Boyd and Shapleigh, 1954). The anti-A serum and the plant agglutinin solution were diluted to contain eight completely hemagglutinating doses.

Hemagglutination inhibition tests were performed on a Salk plate as follows: 0.1 ml of anti-serum (or agglutinin solution) was added to 0.1 ml of serial dilutions of blood group substances. The mixtures were left 30 min at 37° and 0.1 ml of a 1% suspension of the appropriate erythrocytes was then added. The degree of agglutination was determined after 1 hr at 37°.

Enzyme Preparation (see Table I). For assays of enzymatic activity α-phenyl-*N*-acetylgalactosaminide, which had been prepared by a modification of the procedure of Roseman and Dorfman (1951), was used as the substrate. One enzyme unit was defined as that amount of enzyme which released from the substrate, at pH 5.4 and 37°, 1 μmole of phenol/min. The phenol released by the enzyme was determined with the Folin–Ciocalteu reagent as described by Roseman and Dorfman (1951).

Pooled *H. pomatia* hepatopancreas was homogenized in acetone at −20° in a Waring Blendor. The powder was collected by centrifugation and dried. Unless otherwise indicated, all further operations were carried out at +4° and centrifugations were performed for 30 min at 23,000*g* in a MSE refrigerated centrifuge. Acetone powder (30 g) was extracted with 250 ml of 0.01 M sodium acetate buffer at pH 5.4. The extract was clarified by centrifugation and the pH of the supernatant was adjusted to 4.5 with 2 N acetic acid. The precipitated protein was removed by centrifugation and the pH of the supernatant was readjusted to 5.4 with 2 N NaOH. To 250 ml of enzyme solution 320 ml of acetone was added at −10° and the precipitate was collected by centrifugation and dissolved in 300 ml of 0.2 M sodium acetate, pH 5.4. Ammonium sulfate was added to 36% of saturation and the precipitate discarded after centrifugation. The concentration of ammonium sulfate was then increased to 50% of saturation and the resulting precipitate was dissolved in 500 ml of 0.2 M sodium acetate, pH 5.4. 375 ml of acetone was added at −10°, and the precipitate was discarded. The protein fraction which precipitated when 250 ml of acetone were added to the supernatant was dissolved in 10 ml of 0.2 M sodium acetate, pH 6.0. This solution was applied to a column of Sephadex G-100 (3.5 × 50 cm), equilibrated with 0.025 M ammonium acetate containing 0.005 M $CaCl_2$, pH 6.0, and the same buffer was used for elution of the enzyme. The peak of enzyme activity appeared immediately after the void volume. The respective fractions were combined and dialyzed *vs.* ammonium ace-

TABLE II: Time Course of the Degradation of Hog Blood Group A Substance by Snail α-*N*-Acetylgalactosaminidase as Followed by Hemagglutination Inhibition Tests.[a]

	Time of Incubation with Enzyme (hr)	Serial Dilutions of HGM-A												
		1:2[4]	1:2[5]	1:2[6]	1:2[7]	1:2[8]	1:2[9]	1:2[10]	1:2[11]	1:2[12]	1:2[13]	1:2[14]	1:2[15]	1:2[16]
Blood Group A Act.	0	−	−	−	−	−	−	−	−	−	−	+	+	+
	3	−	−	−	−	−	−	−	+	+	+	+	+	+
	6	−	−	−	−	+	+	+	+	+	+	+	+	+
	10	−	+	+	+	+	+	+	+	+	+	+	+	+
Blood Group H Act.	0	−	+	+	+	+	+	+	+	+	+	+	+	+
	3	−	−	−	−	−	+	+	+	+	+	+	+	+
	6	−	−	−	−	−	+	+	+	+	+	+	+	+
	10	−	−	−	−	−	−	+	+	+	+	+	+	+

[a] No agglutination (−). Weak agglutination (+ or ++). Strong agglutination (+++ or ++++).

tate–$CaCl_2$ (0.005:0.01 M), pH 5.4. Subsequently the enzyme preparation was further fractionated on columns (2 × 5 cm) of Cellex-ECTEOLA and Cellex-CM using ammonium acetate–$CaCl_2$ (0.005:0.01 M), pH 5.4, for equilibration and elution. On either column, the enzyme was not appreciably retained.

The enzyme is most active in the range between pH 3 and 4, and virtually inactive >pH 6. It is stable at −20° for several months and can be thawed and refrozen without appreciable loss of activity.

Enzyme Treatment of Blood Group A Substances. Blood group substance (20 mg) was dissolved in 2 ml of dialyzed enzyme solution containing approximately 2.5 mg of protein (five enzyme units) and, after adjustment of the pH to 4.3 with diluted acetic acid, was incubated at 37° in the presence of toluene. Aliquots of the incubation mixture were removed in 3-hr intervals and assayed for hemagglutination inhibition. Finally, the reaction was terminated by heating the incubation mixture in a boiling water bath for 5 min, and the protein precipitate was separated by centrifugation and washed twice with distilled water. The supernatant and the washings were combined and dialyzed at 4° for 48 hr *vs.* several changes of distilled water. Both the diffusible and the nondiffusible material were concentrated under reduced pressure at 50°.

Paper and Thin Layer Chromatography. Ascending paper chromatography was performed on Schleicher Schuell 2043a paper impregnated with 0.025 M sodium tetraborate (Ohkuma and Shinohara, 1964) using 1-butanol–pyridine–water (6:4:3). In this system the sugar components of the blood group substances are clearly separated and have the following R_F values: *N*-acetylglucosamine 0.32, *N*-acetylgalactosamine 0.17, fucose 0.13, and galactose 0.08. Reducing sugars were detected with *p*-anisidine phthalate or silver nitrate, and *N*-acetylhexosamines with the Elson–Morgan spray reagent (Partridge, 1948).

For thin layer chromatography, cellulose layers and the solvent system ethyl acetate–pyridine–water (2:1:2) were used (Schweiger, 1962). Reducing sugars were detected with *p*-anisidine phthalate.

Quantitative Determinations of Sugar Components. Blood group substances, before and after enzyme treatment, were hydrolyzed in 2 N HCl at 100° for 2 hr. In the hydrolysates total hexosamine was determined by a modification of the method of Reissig *et al.* (1955). Galactosamine was estimated in the presence of glucosamine according to the procedure of Pogell and Koenig (1958). The amount of glucosamine was calculated from total hexosamine and the separately determined galactosamine, the difference in color yields of the two amino sugars in the Elson–Morgan reaction being taken into account. Galactose and fucose were estimated by the cysteine–H_2SO_4 reaction according to Dische's method (Dische, 1955). Reducing sugar was assayed by the method of Hagedorn and Jensen (1923). All determinations were carried out in duplicate.

Results

Treatment of Group A Substances with Snail α-N-

Acetylgalactosaminidase. Table II indicates that after 3 hr of incubation of hog gastric mucosal A substance with the snail enzyme preparation, the A activity had already decreased by 87.5%. Within the same period the H activity had risen eightfold. After a 10-hr period of incubation A activity was almost completely destroyed (99.8%), whereas the H activity had remained on its raised level. As shown in Table III both human and hog group A substances were degraded to the same extent. The H activity of the degradation products as assayed with *Ulex* anti-H was of the same order as that of an H substance from hog gastric mucosa (HGM-H), which showed practically no A activity.

TABLE III: The Serological Properties of Blood Group Substances before and after Treatment with α-*N*-Acetylgalactosaminidase.

Substance	Treatment	Min Amount of Substance (μg/ml) Giving Complete Inhibn of Hemagglutination: A Erythrocytes + Human Anti-A	O Erythrocytes + *Ulex* Anti-H
HO-A	Untreated	0.2	500
	Enzyme treated	62.5	15.6
HGM-A	Untreated	0.1	62.5
	Enzyme treated	62.5	7.8
HGM-H	Untreated	500	7.8

Chromatographic Study of the Diffusible Material Released in the Enzymatic Reaction. Thin layer chromatography showed one single reducing sugar spot which had the mobility of an *N*-acetylhexosamine. The substance was identified by chromatography on borate-impregnated paper where it gave a single purple spot with the Elson–Morgan spray reagent corresponding to *N*-acetylgalactosamine (R_F 0.17). None of the other sugars occurring in blood group substances was detectable in the dialyzable portion of the incubation mixture, which indicated that the enzyme preparation was sufficiently pure to split off terminal *N*-acetylgalactosamine residues without further breakdown of the blood group substances.

Quantitative Analysis of the Native and Enzyme-Treated Group Substances. The molar ratios of the sugar components before and after enzyme treatment are given in Table IV. They have been related to a galactose value of 2.00. After enzyme treatment the relative amounts of fucose, galactose, and glucosamine were essentially unchanged, whereas there was a significant decrease in galactosamine.

In Table V the sugar composition of the undegraded group substances and the amounts of terminal galactosamine are listed in per cent by weight. The number of antigenic determinants per molecule of blood group substance has been calculated assuming a molecular weight of 300,000.

Discussion

The purified A-decomposing enzyme of *H. pomatia* used in the present investigation has been found to bring about, within a few hours, an almost complete loss of the A activity of blood group A substances. The enzyme destroys the serological specificity at a much higher rate than the crude enzyme preparation previously obtained from the liver of the snail *Buscyon*, which required 5–7 weeks for full inactivation (Howe and Kabat, 1953). The reaction rate is similar to that described for the decomposition of blood group A substances by an enzyme of *T. foetus* (Harrap and Watkins, 1964).

The enzyme isolated from the hepatopancreas of *H. pomatia* is active on α-phenyl-*N*-acetylgalactosaminide, but not on the corresponding α-glucosaminide, and has thus proved to be a specific α-*N*-acetylgalactosaminidase.

The isolation, from hydrolysates of A substances, of oligosaccharides containing *N*-acetyl-α-galactosamine at the nonreducing end and the finding that these oligosaccharides were able to inhibit the agglutination of A erythrocytes by anti-A sera (Côté and Morgan,

TABLE IV: Molar Ratio of Sugar Components in Blood Group A Substances before and after Treatment with α-*N*-Acetylgalactosaminidase.

Substance	Treatment	Molar Ratio Fuc:Gal:GlcN:GalN	GalN Released in % of Total GalN
HO-A	Untreated	2.24:2.00:1.06:2.69	
	Enzyme treated	2.09:2.00:0.94:1.44	46.5
HGM-A	Untreated	0.75:2.00:1.21:2.55	
	Enzyme treated	0.75:2.00:1.12:2.07	18.8

TABLE V: Chemical Characterization of Blood Group Substances and Estimation of the Number of Antigenic Determinants.

Substance	Reducing Sugar (Calcd as Glucose)	Fuc	Gal	GlcN	GalN (Total)	GalN Terminal	No. of Determinant Groups/Mol of Blood Group Substance
	mg/100 mg of Blood Group Substance						
HO-A	42.5	12.0	11.7	6.2	15.7	7.3[a]	122
HGM-A	53.5	6.7	19.4	11.6	24.5	4.6[b]	77
HGM-H	39.0	3.5	13.2	12.4	14.5		

[a] 15.7 × 46.5/100. [b] 24.5 × 18.8/100.

1956; Schiffman *et al.*, 1962) had previously indicated that terminal *N*-acetyl-α-galactosamine residues were responsible for the serological specificity of blood group A substances. The destruction of A activity by an established α-*N*-acetylgalactosaminidase provides additional evidence for this fact.

From human ovarian A substance nearly half of the total galactosamine was released by the snail enzyme (Table IV). On the supposition that each *N*-acetylgalactosamine molecule released corresponded to one antigenic determinant, the number of antigenic determinants per molecule of human ovarian A substance, assuming its molecular weight to be 300,000, could be calculated as approximately 120. This is in good agreement with values based on results of the enzymatic deacetylation of terminal *N*-acetylgalactosamine residues in intact A substances (Marcus *et al.*, 1964). A similar estimate was made by Zarnitz and Kabat (1960) for the number of antigenic determinants in group B substance, from the amount of galactose liberated by treatment with coffee bean α-galactosidase.

The carbohydrate portion of human ovarian A substance contains fucose, galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine in a molar ratio of approximately 2:2:1:2 (Table IV). If all the oligosaccharide units from which the terminal *N*-acetylgalactosamine residues are enzymatically removed are structurally identical, each of them will contain two fucose, two galactose, one *N*-acetylglucosamine, and two *N*-acetylgalactosamine residues. The results are compatible with a simple sequence of *N*-acetylhexosamine and galactose units, as proposed by Rege *et al.* (1963)

α-GalNAc-(1–3)-β-Gal-(1→3 or 4)-
β-GlcNAc-(1–3)-β-Gal-(1–3)-GalNAc-

to which two α-L-fucose residues are laterally attached.

The destruction of A specificity by the snail enzyme was accompanied by a significant enhancement of H activity. Similar observations have been made by Harrap and Watkins (1964) using the *T. foetus* enzyme and by Marcus *et al.* (1964), who removed the terminal *N*-acetylgalactosamine residues by enzymatic de-*N*-acetylation followed by deamination with nitrous acid. The development of H activity in the course of enzymatic degradation of B substance has also been reported (Iseki and Ikeda, 1956; Watkins *et al.*, 1962). These findings suggest that the terminal *N*-acetylgalactosamine residues are attached to a basic structure which shows H specificity and is probably common to all ABO antigens. In group A substances H activity is obviously blocked by the terminal *N*-acetylgalactosamine residues.

The porcine A substance studied in this work differed from the human A substance in containing only about half as much fucose. A similar difference has been previously reported by Baer *et al.* (1948).

It also appears that in the hog gastric mucosal A substance some of the oligosaccharide units are incomplete and without terminal *N*-acetylgalactosamine residue. This is indicated by a lower number of A-antigenic determinants (Table V) and a higher H activity of the hog substance (Table III) as compared with human ovarian A substance. These shorter chains may represent intermediate stages in a stepwise biosynthesis of the oligosaccharide units in accordance with the scheme formulated by Watkins and Morgan (1959).

References

Annison, E. F., and Morgan, W. T. J. (1952), *Biochem. J. 52*, 247.

Baer, H., Dische, Z., and Kabat, E. A. (1948), *J. Exptl. Med. 88*, 59.

Bendich, A., Kabat, E. A., and Bezer, A. E. (1946), *J. Exptl. Med. 83*, 485.

Boyd, W. C., and Shapleigh, E. (1954), *J. Lab. Clin. Med. 44*, 235.

Côté, R. H., and Morgan, W. T. J. (1956), *Nature 178*, 1171.

Dische, Z. (1955), *Methods Biochem. Analy. 2*, 313.

Freudenberg, K., and Eichel, H. (1935), *Ann. 518*, 97.

György, P., Rose, C. S., and Springer, G. F. (1954), *J. Lab. Clin. Med. 43*, 543.

Hagedorn, H. C., and Jensen, B. N. (1923), *Biochem. Z. 135*, 46.
Harrap, G. J., and Watkins, W. M. (1964), *Biochem. J. 93*, 9P.
Howe, C., and Kabat, E. A. (1953), *J. Am. Chem. Soc. 75*, 5542.
Iseki, S., and Ikeda, T. (1956), *Proc. Japan Acad. 32*, 201.
Iseki, S., and Masaki, S. (1953), *Proc. Japan Acad. 29*, 460.
Kabat, E. A. (1956), Blood Group Substances, New York, N. Y., Academic.
Marcus, D. M., Kabat, E. A., and Schiffman, G. (1964), *Biochemistry 3*, 437.
Morgan, W. T. J. (1959), *Naturwissenschaften 46*, 181.
Ohkuma, S., and Shinohara, T. (1964), *Nature 202*, 593.
Partridge, S. M. (1948), *Biochem. J. 42*, 238.
Pogell, B. M., and Koenig, D. F. (1958), *Nature 183*, 127.
Rege, V. P., Painter, T. J., Watkins, W. M., and Morgan, W. T. J. (1963), *Nature 200*, 532.
Reissig, J. L., Strominger, J. L., and Leloir, L. F. (1955), *J. Biol. Chem. 217*, 959.
Roseman, S., and Dorfman, J. (1951), *J. Biol. Chem. 191*, 607.
Schiffman, G., Kabat, E. A., and Leskowitz, S. (1962), *J. Am. Chem. Soc. 84*, 73.
Schweiger, A. (1962), *J. Chromatog. 9*, 374.
Watkins, W. M. (1959), *Biochem. J. 71*, 261.
Watkins, W. M., and Morgan, W. T. J. (1959), *Vox Sanguinis 4*, 97.
Watkins, W. M., Zarnitz, M. L., and Kabat, E. A. (1962), *Nature 195*, 1204.
Yamamoto, S., Fujisawa, K., and Iseki, S. (1962), *Gunma J. Med. Sci. 9*, 248.
Zarnitz, M. L., and Kabat, E. A. (1960), *J. Am. Chem. Soc. 82*, 3953.

Labeling of Amine-Acceptor Cross-Linking Sites of Fibrin by Transpeptidation*

L. Lorand and H. H. Ong

ABSTRACT: The transpeptidating enzyme of blood plasma which cross-links fibrin can also be used to introduce specific tracers into fibrin itself. Three known cross-linking inhibitors (glycine ethyl ester, hydroxylamine, and hydrazine) were shown to become incorporated to the extent of 1 mole/100,000 g of bovine fibrin. The sedimentation behavior of the modified protein indicates that glycine ethyl ester prevented cross-linking of fibrin by blocking mostly monomeric units.

The incorporation of amine substrates into fibrin can be taken as evidence for the existence of a fibrinyl–enzyme intermediate in the transpeptidation reaction with the cross-linking enzyme. Of the protein derivatives prepared by the enzymatic transpeptidation, fibrin hydroxamate seems to be best suited for further identification of the acceptor carbonyl groups in the polymerizing centers of fibrin. Since the number of acceptor sites in fibrin seems to equal that of the peptide bonds broken by thrombin in fibrinogen, it is suggested that the uncovering of these sites occurs as a result of the fibrinogen–fibrin transition (*i.e.*, through the release of the fibrinopeptide fragments). This is supported by the finding that, in the presence of the cross-linking enzyme, fibrinogen incorporates glycine ethyl ester at a much slower rate than fibrin.

The physiological cross-linking of fibrin is thought to occur (Lorand *et al.*, 1962; Lorand and Jacobsen, 1964; Lorand, 1965) by a transpeptidating mechanism in which donor amino groups of one fibrin (F) molecule react with acceptor carbonyl functions (COY) of another (F'). Dimerization between two of the protein molecules could thus be written as

$$H_2NFCOY + H_2NF'COY \longrightarrow H_2NFCONHF'COY + HY$$

Extended in both directions, this reaction represents the last event in normal blood clotting, and is catalyzed by FSF*[1] (Lorand and Konishi, 1964a; Konishi and Lorand, 1966). It also illustrates the ultimate bio-

* From the Biochemistry Division, Department of Chemistry, Northwestern University, Evanston, Illinois. *Received February 4, 1966.*

[1] Abbreviations used: FSF*, thrombin-activated fibrin stabilizing factor; GEE, glycine ethyl ester; TAME, N^{α}-*p*-tosyl-L-arginine methyl ester.