Ecological Studies of Intestinal Bacteria. Relation between the Specificity of Fecal ABO Blood Group Antigen—Degrading Enzymes from Enteric Bacteria and the ABO Blood Group of the Human Host

LANSING C. HOSKINS

*From the Gastroenterology Service, Veterans Administration Hospital and the
Department of Medicine, Case Western Reserve University School of Medicine,
Cleveland, Ohio 44106*

ABSTRACT This report presents evidence for enteric bacterial adaptation to genetically controlled environmental factors in the individual human host. Human feces contains bacterial enzymes that degrade water-soluble A, B, and H antigens, and both the presence and the specificity of ABH blood group antigens in human gut mucous secretions are genetically determined for each individual. In this study, partially purified fecal blood group antigen—degrading enzymes from 31 subjects of known blood group and secretor status were obtained and their relative specificity for A, B, and H antigen was measured.

Patterns of enzyme specificity were found that were related to the ABO blood type of each subject. This was most evident for enzyme preparations of blood group A, B, and AB secretors: enzyme preparations from group A secretors rapidly degraded A antigen but degraded B antigen only slightly during the same time interval; enzyme preparations from group B secretors degraded B antigen rapidly and A antigen only slightly, and enzyme preparations from group AB secretors degraded both A and B antigens.

Bacterial adaptation of blood group A antigen—degrading enzyme activity was demonstrated in an *in vitro* anaerobic culture of fecal bacteria from a blood group B secretor.

This work was presented in part at the National Meeting of the American Federation for Clinical Research, Atlantic City, New Jersey, on April 30, 1967.

Received for publication 19 August 1968 and in revised form 6 December 1968.

These findings are compatible with enteric bacterial adaptation of degradative enzymes to the genetically determined blood group antigens in their host's gut mucous secretions.

INTRODUCTION

37 yr ago Schiff and his coworkers discovered enzymes in human feces and saliva that degraded water-soluble blood group substances (1–5). The bacterial origin of fecal blood group—degrading (BGD) enzymes was disputed and not established (2, 6, 7) despite evidence that unidentified oral bacteria produced BGD enzymes in saliva (6, 8, 9). The significance of fecal BGD enzyme activity to the host and to the resident enteric flora was not pursued.

I have reinvestigated fecal BGD enzymes to explore their significance in the degradation of gastrointestinal mucins and in the host—enteric flora ecosystem (10, 11). Evidence was obtained that BGD enzymes are synthesized by intestinal bacteria and not by gastrointestinal tissues (11). Since the capacity to secrete water-soluble ABH antigens in gastrointestinal mucous secretions and the blood group specificity of the antigens secreted are genetically determined characteristics of each human host, it seemed possible that intestinal microorganisms might adapt to their host environment by synthesizing BGD enzymes which preferentially degraded the specific blood group antigens secreted by their host. The present study was performed to test this possibility. Fecal BGD enzyme preparations from individuals of known

blood group and secretor status were tested for their relative ability to degrade water-soluble A, B, and H blood group antigens.

METHODS

Subjects. Stools were obtained from 21 healthy biomedical colleagues and from 10 patients hospitalized on the Orthopedic Service for elective operative procedures. None had historical, physical, or laboratory evidence of active gastrointestinal disease, acute disease involving other organ systems, or had recently ingested antibiotics. All subjects were eating their usual diet or a standard hospital diet at the time of collection. Their ages ranged from 24 to 68 yr. Seven were females. Neither age, race, nor sex of these adults appeared to influence the results.

Each subject's blood type was determined by the slide method using commercial human immune anti-A and anti-B typing sera. For determining blood group secretor status fresh saliva from each subject was heated at 100°C for 10 min and centrifuged. The titer of A, B, or H antigen in the supernatant fluid was determined by the hemagglutination inhibition technique described below. The saliva of secretors contained their phenotype antigen at a titer of 1:64 or more; saliva of nonsecretors contained their phenotype antigen at a titer of 1:4 or less.

Preparation of crude fecal blood group-degrading enzymes. Stools were obtained from each subject by defecation into containers lined with polyethylene bags and were refrigerated and processed within 24 hr. All steps were performed at 4°C. Each stool was weighed and homogenized for 4 min with enough chilled 0.15 M NaCl to make a 20% w/v suspension. To remove particulate matter the suspension was centrifuged at 700 g for 20 min and the supernatant fluid centrifuged at 20,000 g for 60 min, yielding a clear, brown supernatant fraction.

Fecal BGD enzyme activity in the stool supernatant fraction of the first five subjects (subjects 2, 3, 7, 22, and 27) was partially purified by passing the supernatant fraction through Sephadex G25 followed by chromatography on DEAE Sephadex A50. Although yielding active enzyme preparations, this procedure was unsuitable for processing large volumes of stool supernatant fraction. Accordingly, a different procedure was employed with the stool supernatant fractions from all other subjects. This procedure utilized acetone precipitation followed by adsorption and elution from calcium phosphate gel. An equal volume of acetone was added to the supernatant fraction at the rate of 2 ml/min at 0°C. The precipitate, recovered by centrifugation at 700 g for 15 min, was dissolved in 20–30 ml of 0.01 M sodium acetate-acetic acid buffer, pH 5.2, and was dialyzed against at least four 2-liter volumes of the same buffer. A suspension of calcium phosphate gel, prepared by the method of Keilin and Hartree (12), was then added to the dialyzed fraction in the amount of 35 mg (dry weight) of gel for every 100 mg of protein. The suspension was stirred for 30 min and centrifuged. The gel was washed three times with 10–15 ml of the acetate buffer, and the washings were discarded. BGD enzyme activity was eluted by stirring the gel for 15 min in 15 ml of 0.75 M ammonium sulfate in 0.1 M Tris(hydroxymethyl)aminomethane buffer, pH 7.8, centrifuging the suspension, and retaining the supernatant fraction. Elution was repeated twice more with buffered 0.75 M ammonium sulfate. The three supernatant fractions were pooled, dialyzed against at least four 2-liter volumes of distilled water, and were then lyophilized.

TABLE I
Partial Purification of Blood Group B Antigen-Degrading Enzymes from the Stool of a Blood Group B Secretor

Fraction	Total protein mg	BGD enzyme activity	
		Specific activity units*/mg protein	Total units
1. 20,000g supernatant fraction of stool homogenate	860	5.6	4800
2. 50% v/v acetone precipitate	478	14	6700
3. Ca ₃ (PO ₄) ₂ gel eluate	113	30	3400

* 1 unit of enzymatic activity causes a 50% decrease in B antigen substrate titer ($\Delta n=1$) during incubation for 30 min at 37°C, pH=6.4.

The lyophilized preparations were stored over Drierite in a desiccator at room temperature and were assayed within 2 wk. BGD enzyme activity in these preparations appears to be stable. Essentially identical degradation rates were found in two lyophilized preparations when these were re-assayed after storage for 16 and 23 months respectively.

Blood group antigen substrates. Saliva from one blood group A secretor, saliva from one blood group B secretor, and gastric juice from two group O secretors were employed as A, B, and H antigen substrates. The salivas were heated at 100°C for 10 min and centrifuged at 700 g for 10 min. The supernatant fluid was dialyzed against four 2-liter volumes of distilled water at 4°C and was lyophilized. The gastric juice was centrifuged, dialyzed, and lyophilized in the same manner.

Measurements of blood group-degrading enzyme activity. BGD enzyme activity was measured by the decrease in the titer of antigen substrate during incubation with enzyme preparation. For the assays the lyophilized antigen substrates and lyophilized enzyme preparations were dissolved separately in 0.02 M phosphate buffer, pH 6.4, containing 0.01 M MgCl₂. The final concentration of A or B antigen substrate in each incubation mixture was 0.5 mg/ml and that of H antigen substrate was 1 mg/ml. The final enzyme concentration in each incubation mixture, previously selected by preliminary testing to produce significant degradation of the subject's blood group phenotype antigen after the first hour of incubation, varied from 0.06 mg/ml to 12.5 mg/ml and for most enzyme preparations was 0.5 mg/ml. The enzyme solution was added to separate tubes containing A, B, or H antigen substrate, and the mixtures were incubated at 37°C for 6 hr. Aliquots were removed from each tube immediately after mixing and at various times thereafter including 1, 2, and 6 hr. BGD enzyme activity was stopped by placing each aliquot in a boiling water bath for 10 min, and the titer of antigen substrate remaining in each aliquot was then determined. The titer of antigen incubated without enzyme does not change under these conditions. Specificity of BGD activity in each preparation was evaluated by comparing the rates of degradation of A, B, and H antigen during incubation.

Hemagglutination inhibition measurements of antigen titers. The blood group antigen titer in each aliquot of the incubation mixtures was determined by the macroscopic he-

FIGURE 1 Decrease of H antigen titer during incubation with fecal blood group-degrading enzyme. Subject 2; 37°C; pH = 6.4; lyophilized enzyme concentration = 0.5 mg/ml.

magglutination inhibition procedure of Kabat (5). Twenty hemagglutinating units of human immune anti-A and anti-B sera (National Bioserums, Inc., Brooklyn, N. Y.) and anti-H (*Ulex europaeus*) were employed in the procedure. Hemagglutination inhibition end points were checked by recentrifuging and redetermining hemagglutination in the relevant tubes. To eliminate bias each tube was identified after it was examined for hemagglutination. The antigen titer in the aliquot was defined as the highest dilution capable of completely inhibiting hemagglutination. This rarely varied by more than ± 1 serial dilution in replicate tests.

Antigen titers are expressed as the value of the exponent, n , of serial 1:2ⁿ dilutions of each aliquot at the hemagglu-

tination inhibition end point. BGD enzyme activity is expressed as the decrease in antigen titer, Δn , during the period of incubation.

Bacterial adaptation of BGD enzyme activity in vitro. To determine if bacterial adaptation of BGD enzyme activity could be demonstrated in vitro, 1 g fresh feces from a blood group B secretor (subject 7, Table II) was suspended in 10 ml of thioglycollate broth (Difco Laboratories, Inc., Detroit, Mich.), and 1 ml of this suspension was inoculated into each of two culture flasks containing 200 ml of thioglycollate broth and 1 mg/ml D-glucose. One of the flasks also contained 0.2 mg/ml of hog gastric mucin (HGM) (Nutritional Biochemicals Corporation, Cleveland, Ohio) which possessed

FIGURE 2 Linear relationship between the rate of blood group B antigen degradation and blood group antigen-degrading enzyme concentration in the incubation mixture. Subject 26; incubation time = 30 min; 37°C, pH = 6.4.

FIGURE 3 Fecal BGD enzyme activity from a group A secretor showing rapid degradation of A and H antigen and much slower degradation of B antigen. Subject 2.

a high titer of blood group A antigen but no B antigen. The titer of A antigen in this medium was 1:16,000 whereas no A antigen was detectable in the medium that did not contain HGM. Neither medium contained detectable B antigen. After incubation at 37°C for 4 days bacteria were removed by centrifugation and filtration of the supernatant culture fluids through a Seitz filter. The cell-free culture fluids were dialyzed against four 4-liter volumes of distilled water and the nondialyzable portions were then lyophilized. BGD enzyme activity for A, B, and H antigens was assayed in each of the lyophilized culture media in the same manner as the fecal BGD enzyme preparations. The concentration of lyophilized culture media in the assay incubation mixtures was 2.5 mg/ml.

Protein concentration. Protein concentration during preparation of the fecal BGD enzymes was measured in appropriately diluted aliquots from the optical density at 280 mμ and 260 mμ by the method of Warburg and Christian (13). For the measurement of enzyme specific activity (Table I), protein concentration was measured by the method of Lowry, Rosebrough, Farr, and Randall (14) using 4× crystallized human albumin (Nutritional Biochemicals Corporation) as reference standard.

RESULTS

Partial purification and some properties of BGD enzyme activity. The specific activity and recovery of crude blood group B-degrading enzyme obtained from the stool of a blood group B secretor is shown in Table I. Enzyme activity in the calcium phosphate gel eluate in this and other preparations increased 5- to 15-fold over that in the stool homogenate. The increase in total enzyme units recovered in the acetone precipitate was usually obtained in other preparations and may represent removal of enzyme inhibitors.

TABLE II
Degradation of A, B, and H Antigens by Partially Purified Blood Group-Degrading Enzymes from the Stools of Blood Group A, B, AB, and O Secretors

Blood group	Subject	Decrease in A, B, and H antigen titer, Δn.					
		After 1 hr			After 6 hr		
		A	B	H	A	B	H
A	1	3	0	3	≥11*	2	≥10
	2	9	0	5	≥13	8	≥8
	3	3	1	2	≥10	4	2
	4	4	0	2	≥10	0	8
	5	2	1	3	≥11	2	≥9
	6	4	1	5	≥12	2	≥8
B	7	0	9	2	0	≥14	≥9
	8	0	7	0	2	≥10	3
	9	0	7	0	1	≥10	0
	10	0	≥9	7	8	≥9	≥9
	11	0	≥10	3	6	≥10	7
AB	12	≥10	9	1	≥10	≥10	3
	13	2	7	0	7	≥10	3
	14	5	4	6	11	8	≥7
O	15	0	0	2	0	0	8
	16	0	0	3	0	0	6
	17	0	0	2	0	2	6
	18	0	3	2	1	≥10	6
	19	0	2	4	0	5	≥10
	20	0	3	2	0	≥10	≥7
	21	0	3	5	1	≥10	≥7

* The prefix ≥ before a value for Δn indicates that no detectable antigen remained at this time.

FIGURE 4 Fecal BGD enzyme activity from a group B secretor showing rapid degradation of B antigen, slower degradation of H, and no degradation of A antigen. Subject 7.

During the early phase of incubation with most BGD enzyme preparations, the exponential value, n , of the antigen titer decreased at a constant rate (Fig. 1). Under these incubation conditions, the rate of antigen degradation, Δn per unit time, may be used as a measure of enzyme activity and is proportional to enzyme concentration in the incubation mixture (Fig. 2).

Degradation of A, B, and H antigens by fecal BGD enzymes of ABO secretors. The relative specificity of

fecal BGD enzymes of secretors for the subject's blood group phenotype antigen is illustrated in Figs. 3–7, and the extent of antigen degradation after 1 and 6 hr incubation is summarized for all secretors in Table II. Fecal BGD enzyme preparations from group A secretors rapidly degraded A antigen and degraded B antigen very slowly (Fig. 3). In contrast, fecal BGD enzyme preparations from group B secretors rapidly degraded B antigen and degraded A antigen very slowly (Fig. 4).

FIGURE 5 Fecal BGD enzyme activity from a group AB secretor showing rapid degradation of A and B antigen and much slower degradation of H antigen. Subject 12.

FIGURE 6 Fecal BGD enzyme activity from a group O secretor illustrating relative specificity for H antigen only. Subject 15.

Fecal BGD enzyme preparations from the three group AB secretors degraded both A and B antigen (Fig. 5). Fecal BGD enzyme preparations from seven group O secretors showed two patterns of antigen degradation. Preparations from three of these subjects (subjects 15–17) rapidly degraded H antigen but produced little degradation of either A or B antigen (Fig. 6), and preparations from the remaining group O subjects rapidly degraded B antigen in addition to H antigen (Fig. 7, Table II). No preparations from group O secretors appreciably degraded A antigen during 6 hr of incubation.

H antigen was degraded at variable rates by BGD enzyme preparations from all but one subject (subject

9). This is consistent with observations (15) that H antigen is present in variable titer in the secretions of blood group A, B, and AB secretors as well as group O secretors.

Degradation of A, B, and H antigens by fecal BGD enzymes of ABO nonsecretors. Although the ABH blood group antigens are either absent or else present in trace amounts in the mucous secretions of nonsecretors, fecal BGD enzymes from the 10 nonsecretors did degrade A, B, and H antigens (Table III). Fecal BGD enzymes from six of these (subjects 22–24, 26–28) showed a pattern of relative specificity for the subject's blood group phenotype antigen similar to that ob-

FIGURE 7 Fecal BGD enzyme activity from a group O secretor illustrating degradation of B antigen as well as H antigen. Subject 18.

FIGURE 8 Fecal BGD enzyme activity from a group A nonsecretor showing lack of specificity towards the subject's phenotype antigen. Subject 25.

served among the secretors. The enzyme preparation from the one group O nonsecretor in this category (subject 28) degraded both B and H antigens similar to preparations from four of the group O secretors. Fecal BGD enzymes from the remaining nonsecretors degraded A, B, and H antigens at comparable rates (Fig. 8, Table III).

Production of BGD enzymes in anaerobic cultures, and bacterial adaptation of A antigen-degrading enzymes in an in vitro culture of feces from a blood group B se-

cretor. When a mixed culture of human fecal bacteria is incubated anaerobically, BGD enzyme activity can be consistently demonstrated in the cell-free culture medium after 24 hr of incubation. Furthermore, the BGD enzymes in the culture medium show specificity for the host's blood group phenotype antigen similar to that found in his stools. This is illustrated in Fig. 9 where the BGD enzymes in the control culture containing thio-glycollate broth and glucose inoculated with feces from a blood group B secretor degrade B antigen more rapidly than A or H antigen. BGD enzymes in the cultures are clearly produced during bacterial growth and are not introduced with the fecal inoculum: no BGD enzyme activity is detectable immediately after inoculation, the specific activity of BGD enzyme increases during bacterial growth (11), and the capacity to produce BGD enzymes can generally be demonstrated in subcultures made at 24 hr.

When compared with the BGD enzyme activities produced in the control culture, there was a definite increase in A antigen-degrading activity in the culture containing A antigen (Fig. 9). There was essentially no difference in the rate of B or H antigen degradation, indicating that the effect of A antigen was the preferential adaptation of A-degrading enzyme activity. This relatively specific in vitro adaptation of A-degrading enzyme by fecal bacteria grown in the presence of HGM A antigen was confirmed in other experiments. However, the specificity of enzyme adaptation depended upon the concentration of HGM A antigen in the medium. At concentrations less than 0.2 mg/ml, the HGM failed to stimulate production of A-degrading activity. At a concentration of 0.2 mg/ml only A-degrading activity was

TABLE III
Degradation of A, B, and H Antigens by Partially Purified
Blood Group-Degrading Enzymes from the Stools of
Blood Group A, B, and O Nonsecretors

Blood group	Subject	Decrease in A, B, and H antigen titer, Δn ,					
		After 1 hr			After 6 hr		
		A	B	H	A	B	H
A	22	2	0	0	6	0	0
	23	8	1	1	$\geq 10^*$	≥ 10	≥ 9
	24	5	1	4	≥ 11	≥ 10	≥ 8
	25	4	4	1	≥ 11	≥ 10	≥ 9
B	26	1	≥ 9	2	7	≥ 9	≥ 9
	27	0	8	4	2	≥ 10	6
O	28	0	2	4	1	≥ 12	7
	29	≥ 10	≥ 9	4	≥ 10	≥ 9	≥ 8
	30	0	1	1	8	≥ 9	≥ 8
	31	3	7	2	8	≥ 10	6

* The prefix $\geq$ before a value for Δn indicates that no detectable antigen remained at this time.

FIGURE 9 Degradation of A, B, and H antigens by BGD enzymes in a 4-day old anaerobic culture of feces from a blood group B secretor. Open circles, thioglycollate broth with 1 mg/ml D-glucose. Closed circles, same medium with 0.2 mg/ml hog gastric mucin (A antigen titer = 1:16,000).

increased, whereas in cultures containing 10 mg/ml of HGM the A-, B-, and H-degrading enzyme activities were all increased over their activities in control cultures.

DISCUSSION

The present data indicate that the specificity of fecal BGD enzymes among blood group secretors varies in a generally predictable manner that is related to the ABO blood group phenotype of each individual. Fecal BGD enzymes of secretors showed a specificity for the individual's blood group phenotype antigen together with variable degrees of activity toward H antigen. Since the gut mucous secretions of secretors contain high titers of the host's blood group phenotype antigen together with variable amounts of H antigen (5, 16-20), fecal BGD enzyme specificity appears to be directly related to the blood group antigens in the host's gut mucous secretions. This relationship is most definite among the group A and group B secretors where enzyme specificity is clearly directed toward the phenotype antigen, A or B, secreted by each in his epithelial mucous secretions. Degradation of both A and B antigens by the fecal enzymes of group AB secretors also conforms to this re-

lationship as does the relative specificity for H antigen observed in preparations from three of the seven group O secretors. The remaining group O secretors, however, deviated from this relationship by excreting BGD enzymes that also degraded B antigen.

Schiff and Akune (1), Schiff and Weiler (2), and Stimpfl (4) stated that the strength of A and B antigen-degrading activity in crude stool filtrates was unrelated to the individual's blood group. In the present study, enzyme assays of crude stool supernatant fractions from several secretors revealed the same pattern of preferential degradation of phenotype antigen that was exhibited by the partially purified enzyme preparations. The reasons for the discrepancy between this and the earlier studies are unclear. However, it appears that the earlier workers used assay conditions that were unsuitable for detecting differences in the initial rates of antigen degradation which provide the basis for the enzyme specificity reported here.

Although the majority of nonsecretors secrete Lewis^x (Le^x) antigen in place of A, B, or H antigen in their gut mucous secretions, the fecal BGD enzymes of nonsecretors did degrade A, B, and H antigens by one of

two patterns. In one pattern the host's blood group phenotype antigen was preferentially degraded; in the other the three antigens were degraded at comparable rates. Interpretation of these findings is hampered by the complex relationships between Le^a and the ABH antigens, both within the oligosaccharide side chains of blood group substances where the Le^a antigenic determinant may coexist with the A, B, or H antigenic determinants (16), and in the gut lumen of the nonsecretor where A, B, or H phenotype antigen on cell membranes is continuously introduced via shed intestinal epithelium (21).

It appears likely that the relative specificity of fecal BGD enzymes from ABO secretors for their blood group phenotype antigen and H antigen represents bacterial adaptation of blood group-degrading enzymes to the antigens in their host's gut mucous secretions. This conclusion is strengthened by the *in vitro* fecal culture experiment which demonstrated enhanced A antigen-degrading activity in the culture medium containing A antigen. Howe, MacLennan, Mandl, and Kabat (22) demonstrated a similar adaptation of A-degrading activity by a strain of *Clostridium tertium* when grown in a medium containing blood group substance.

The water-soluble blood group substances that are substrates for BGD enzymes are glycoproteins closely related in composition and structure (16, 23, 24). A, B, H, and Le^a antigen specificities are determined by saccharides at the nonreducing end of oligosaccharide side chains covalently linked to a polypeptide core via serine and threonine residues. A antigen specificity is conferred by α -N-acetyl-D-galactosaminoyl terminal glycosides, B specificity by α -D-galactosyl terminal glycosides, and H and Le^a specificities by unsubstituted α -L-fucopyranosyl glycosides. The action of BGD enzymes obtained from several microorganisms is known (25-30). With the exception of the deacetylase from *Clostridium tertium* described by Marcus, Kabat, and Schiffman which hydrolyzes the acetyl group from the terminal α -N-acetyl-D-galactosaminoyl antigenic determinant of A substance (29), these are glycosidases which cleave the antigenic determinant saccharides from oligosaccharide side chains of blood group substances.

Blood group-degrading enzyme activity is found in saliva (6, 8, 9) and in the contents of the terminal ileum and colon (20). Although strains of several species of microorganisms, notably those of the genus *Clostridium*, produce BGD enzymes (25-30), it is not known whether these or other species are responsible for BGD enzymes in the majority of human stools.

There is evidence that intestinal bacteria degrade gastrointestinal mucins and utilize the constituents of mucus, especially the carbohydrate moieties (10, 31). BGD enzymes may play a role in this process by cleaving the

antigenic determinant sugars from oligosaccharide components of mucous glycoproteins.

The present observations indicate that among the numerous factors determining the ecology of enteric flora and human host, one is the genetically determined blood group and secretor status of each host. If the observed patterns of BGD enzyme specificity are indicative of a normal ecological relationship, then studies of fecal BGD enzyme specificity may offer one approach to detecting disturbed host-enteric flora ecology in subjects with diarrheal and inflammatory intestinal diseases.

ACKNOWLEDGMENTS

I thank Miss M. Jo Ann Woods and Miss Lillian Hejduk for their technical assistance, and Doctors Don M. Carlson, C. W. Shuster, and Leslie T. Webster, Jr. of the Departments of Biochemistry, Microbiology, and Pharmacology for their advice and encouragement.

This work was supported by a research grant from the Veterans Administration.

REFERENCES

- Schiff, F., and M. Akune. 1931. Blutgruppen und physiologie. *Muenchen. Med. Wochenschr.* **78**: 657.
- Schiff, F., and G. Weiler. 1931. Fermente und Blutgruppen. I. *Biochem. Z.* **235**: 454.
- Schiff, F., and G. Weiler. 1931. Fermente und Blutgruppen. II. *Biochem. Z.* **239**: 489.
- Stimpfl, A. 1932. Zur kenntnis der Blutgruppenfermente. *Z. Immunitaetsforsch Allergie Klin. Immunol.* **76**: 159.
- Kabat, E. A. 1956. Blood Group Substances, Their Chemistry and Immunochemistry. Academic Press Inc., New York.
- Witebsky, E., and T. Satoh. 1933. Zur Frage des Blutgruppenferments und der Ausscheidung von Blutgruppen-substanz. *Klin. Wochenschr.* **12**: 948.
- Schiff, F. 1935. Über den Abbau gruppenspezifischer Substanzen durch *Clostridium welchii*. *Klin. Wochenschr.* **14**: 750.
- Sievers, O. 1935. Beitrage zur Kenntnis der Wirkung des sogenannten "Blutgruppenferments." *Z. Immunitaetsforsch Allergie Klin. Immunol.* **85**: 163.
- Hartmann, G., and J. Hartmann. 1941. Destruction of group antigens by enzymes. *Acta Pathol. Microbiol. Scand.* **18**: 367.
- Hoskins, L. C., and N. Zamcheck. 1968. Bacterial degradation of gastrointestinal mucins. I. Comparison of mucus constituents in the stools of germ-free and conventional rats. *Gastroenterology.* **45**: 210.
- Hoskins, L. C. 1968. Bacterial degradation of gastrointestinal mucins. II. Bacterial origin of fecal ABH(O) blood group-destroying enzymes. *Gastroenterology.* **54**: 218.
- Dixon, M., and E. C. Webb. 1964. Enzymes. Academic Press Inc., New York. 2nd edition. 42.
- Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. In *Methods in Enzymology*. S. P. Colowick and N. O. Kaplan, editors. Academic Press Inc., New York. **3**: 451.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.

15. Morgan, W. T. J., and W. M. Watkins. 1948. The detection of a product of the blood group O gene and the relationship of the so-called O-substance to the agglutinogens A and B. *Brit. J. Exp. Pathol.* **29**: 159.
16. Watkins, W. M. 1966. Blood group substances. *Science*. **152**: 172.
17. Glynn, L. E., and E. J. Holborow. 1959. Distribution of blood group substances in human tissues. *Brit. Med. Bull.* **15**: 150.
18. Szulman, A. E. 1960. The histological distribution of bloodgroup substances A and B in man. *J. Exp. Med.* **111**: 785.
19. Szulman, A. E. 1962. The histological distribution of the blood group substance in man as disclosed by immunofluorescence. II. The H antigen and its relation to A and B substances. *J. Exp. Med.* **115**: 977.
20. Witebsky, E., and E. Neter. 1935. Distribution of blood group properties and blood group property destroying factors in the intestinal tract of man. *J. Exp. Med.* **62**: 589.
21. Cowan, W. K. 1962. Blood groups and disease. ABH antigens on human duodenal cells. *Brit. Med. J.* **2**: 964.
22. Howe, C., J. D. MacLennan, I. Mandl, and E. A. Kabat. 1957. Enzymes of *Clostridium tertium*. Effects on blood group and virus receptor substances. *J. Bacteriol.* **74**: 365.
23. Lloyd, K. O., E. A. Kabat, and E. Licerio. 1968. Immunochemical studies on blood groups. XXXVIII. Structures and activities of oligosaccharides produced by alkaline degradation of blood-group Lewis^a substance. Proposed structure of the carbohydrate chains of human blood group A,B,H,Le^a and Le^b substances. *Biochemistry*. **7**: 2976.
24. Carlson, D. M. 1968. Structures and immunochemical properties of oligosaccharides isolated from pig submaxillary mucins. *J. Biol. Chem.* **243**: 616.
25. Stack, M. V., and W. T. J. Morgan. 1949. The preparation and properties of enzymes from *Cl. welchii* (Type B) filtrates which destroy blood group substances. *Brit. J. Exp. Pathol.* **30**: 470.
26. Gyorgy, P., C. S. Rose, and G. F. Springer. 1954. Enzymatic inactivation of bifidus factor and blood group substances. *J. Lab. Clin. Med.* **43**: 532.
27. Naylor, I., and H. Baer. 1959. Studies of an enzyme produced by *Bacillus fulminans* that inactivates blood group O substance. *J. Bacteriol.* **77**: 771.
28. Yamamoto, S., K. Fujisawa, and S. Iseki. 1962. Chemical effects of purified A- and B-decomposing enzymes on blood group A and B substances. *Gunma J. Med. Sci.* **11**: 248.
29. Marcus, D. M., E. A. Kabat, and G. Schiffman. 1963. Immunochemical studies on blood groups. XXXI. Destruction of blood group A activity by an enzyme from *Clostridium tertium* which deacetylates N-acetylgalactosamine in intact blood group substances. *Biochemistry*. **3**: 437.
30. Watkins, W. M. 1959. Enzymes from *Trichomonas foetus*. The action of cell-free extracts on blood group substances and low-molecular weight glycosides. *Biochem. J.* **71**: 261.
31. Lindstedt, G., S. Lindstedt, and B. E. Gustafsson. 1965. Mucus in intestinal contents of germfree rats. *J. Exp. Med.* **121**: 201.