Plasma Kallikrein Activation and Inhibition during Typhoid Fever

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ABSTRACT As an ancillary part of a typhoid fever vaccine study, 10 healthy adult male volunteers (nonimmunized controls) were serially bled 6 days before to 30 days after ingesting 10^5 *Salmonella typhi* organisms. Five persons developed typhoid fever 6–10 days after challenge, while five remained well. During the febrile illness, significant changes ($P < 0.05$) in the following hematological parameters were measured: a rise in α_1-antitrypsin antigen concentration and high molecular weight kininogen clotting activity; a progressive decrease of platelet count (to 60% of the predisease state), functional prekallikrein (55%) and kallikrein inhibitor (47%) with a nadir reached on day 5 of the fever and a subsequent overshoot during convalescence. Despite the drop in functional prekallikrein and kallikrein inhibitor, there was no change in factor XII clotting activity or antigenic concentrations of prekallikrein and the kallikrein inhibitors, C1 esterase inhibitor (C$\bar{1}$-INH) and α_2-macroglobulin. Plasma from febrile patients subjected to immunoelectrophoresis and crossed immunoelectrophoresis contained a new complex displaying antigenic characteristics of both prekallikrein and C$\bar{1}$-INH; the α_2-macroglobulin, antithrombin III, and α_1-antitrypsin immunoprecipitates were unchanged. Plasma drawn from infected-well subjects showed no significant change in these components of the kinin generating system. The finding of a reduction in functional prekallikrein and kallikrein inhibitor (C$\bar{1}$-INH) and the formation of a kallikrein C$\bar{1}$-INH complex is consistent with prekallikrein activation in typhoid fever. The correlation of these changes with the drop in platelet count suggests that a common mechanism may be responsible.

INTRODUCTION

The pathogenesis of the hematologic changes, including the thrombocytopenia and the leukopenia seen during typhoid fever, is not fully understood. Although endotoxin has been postulated to play a role (1), circulating endotoxin has been searched for but not detected during the febrile illness (2). Disseminated intravascular coagulation occurs in some patients with typhoid fever (3) and may be initiated by activation of Hageman factor as has been documented in other types of sepsis (4, 5). Plasma kinins are potent mediators of the increased vascular permeability characteristic of the inflammatory response (6). In addition, the coagulation (7) and fibrinolytic (7, 8) systems are functionally interrelated with the plasma kinin forming system (9).

The development of the coagulation, enzymatic, and immunochemical assays (10) for the bradykinin generating system, which includes prekallikrein, kininogen, and kallikrein inhibitors (11), has recently helped to clarify the dynamics of the plasma kinin system by permitting simultaneous and accurate measurements of its major components. We report here functional and immunochemical assays of six plasma proteins involved in the kinin forming system measured sequentially during the development and resolution of typhoid fever. Three are participants in the reactions leading to kinin liberation including factor XII (Hageman factor), prekallikrein, and high molecu-

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lar weight (HMW) kininogen.[1] The other three are plasma protease inhibitors which may limit the extent of kinin formation; namely, α_1-antitrypsin, α_2-macroglobulin, and C1 esterase inhibitor (C$\bar{1}$-INH). The results suggest that the activation of prekallikrein to kallikrein as well as its inhibition through complex formation with C$\bar{1}$-INH) occurs concomitantly with typhoid fever.

METHODS

Clinical groups and plan of study. As an ancillary part of a study done in 1974 to evaluate the efficacy of an experimental typhoid vaccine, 20 healthy adult male volunteers served as the nonimmunized control group (12). Volunteer subjects were inmates of the Maryland House of Correction, Jessup, Md. No coercion whatsoever was exacted in the selection of volunteers. All participants were fully informed of the experimental objectives, procedures, and potential discomforts and risks. Oral and written consent were obtained from each volunteer, and subjects understood that they could withdraw from the study at any time. Investigations were performed in accordance with the Declaration of Helsinki and the new Federal Regulations for Research in Humans promulgated May 1974. The study protocol was reviewed and approved by the Human Experimentation Committee of the University of Maryland Medical School. All men had histories, physical examinations, complete blood counts, platelet counts, blood urea nitrogen, glucose, serum glutamic-oxalacetic transaminase, electrocardiograms, and cholecystograms performed before the study. Men who were food handlers were excluded.

All men were bled once, 6 days before and, sequentially, for 30 days after ingesting an infectious dose (ID_{50}) of 1×10^5 Quailes strain of *Salmonella typhi* in a glass of milk (13). After challenge, men were seen daily and questioned as to the occurrence of fever, chills, abdominal pain, constipation, diarrhea, epistaxis, or vomiting. Daily temperatures were taken, and stool or rectal swabs were collected daily. All men with a temperature in excess of 100°F were admitted to the research ward with a presumptive diagnosis of typhoid fever. Each had a daily stool collection for a period extending 1 mo beyond his hospitalization. Blood cultures were taken every 8 h after admission before therapy, every 6 h for 2 days after the start of therapy, and then daily for an additional 2 days. Sick individuals were treated with chloramphenicol, 1 g orally every 8 h for 12 days. Individuals who did not develop fever were bled on the same schedule and served as controls. Plasma samples, frozen immediately at −50°C, were available for this study on five volunteers with fever and five individuals who did not contract the disease (controls). All determinations were performed on coded samples. The volunteers who developed typhoid fever did so 6–10 days after the day of ingestion and within 3 days after the onset of positive stool cultures. Three typhoid patients and three controls developed O-, H-, or Vi-agglutinating antibody titers to *S. typhi*, and the titer rose four-fold or more within 6 wk of ingestion.

All data were expressed as percent of the base-line level obtained before ingestion. Because the time of onset of fever varied, the values in the individuals with typhoid fever were expressed as the mean±standard error of the mean (SEM) on the days after the onset of fever. Control patients were analyzed by tabulating the mean±SEM starting with the day of challenge. Student's t test was used to determine whether the difference between a particular day and the base-line level was significant ($P < 0.05$).

Laboratory tests. Platelet counts were performed by the method of Bull et al. (14) and expressed as number per microliter using 5 ml whole blood collected in 5-ml tubes containing 9.0 mg disodium ethylenediamine tetraacetate at 20°C.

Blood (4.5 ml) for prekallikrein, factor XII, HMW kininogen, and proteolytic inhibitor assays was added to chilled polypropylene tubes containing 0.5 ml of 3.8% sodium citrate, centrifuged at 900 *g* for 20 min, and the plasma was separated and frozen at −50°C in plastic tubes. Plasma factor XII activity (15) and HMW kininogen activity (16) was determined by a modification of the activated partial thromboplastin time using congenitally deficient plasma (17) and was expressed as a percent of the mean of 20 healthy adult subjects.

The activities of plasma prekallikrein (micromoles per milliliter per hour of tosyl arginine methyl ester hydrolyzed) and kallikrein inhibitors (inhibitor units) were determined by enzymatic assay (11). This assay is based on the observation that citrated human plasma collected without glass contact contains very low levels of arginine esterase activity. The addition of kaolin facilitates the conversion of factor XII (Hageman factor) to activated Hageman factor and its fragments (18), which in turn converts prekallikrein to kallikrein (18, 19). Although other enzymes may be activated by contact, it appears that most of the enzymatic activity of 1 min with L-tosyl arginine methyl ester as a substrate results from the formation of kallikrein. Evidence that kaolin-activated arginine esterase activity primarily reflects kallikrein is provided by identical substrate specificity, similar inhibition profiles (12), release of bradykinin in parallel with arginine esterase activity (19), and exclusion of other enzymes such as plasmin and thrombin (12). Furthermore, an immunochemical assay of prekallikrein in normal plasma correlates well with the arginine esterase assay (20). Although several kallikrein inhibitors exist in plasma (21), 85–100% of the decrease in arginine esterase activity from 1 to 5 min is due to the stoichiometric combination of kallikrein with the C$\bar{1}$-INH. This conclusion is supported by the observation that similar kinetics of inhibition of purified kallikrein are observed upon incubation with plasma (22), and by the minimal loss of esterase activity (<15%) obtained upon activation of the plasma of patients with hereditary angioedema who lack the C$\bar{1}$-INH (11, 22). One inhibitor unit is defined as 50% inhibition of the arginine esterase activity formed at 1 min. To measure the hydrolysis of L-tosyl arginine methyl ester, the methanol formed is oxidized to formaldehyde and coupled to chromotropic acid to give a purple color. Duplicate assays agreed within ±5%.

The antigenic concentrations of prekallikrein, α_1-antitrypsin, α_2-macroglobulin, and C$\bar{1}$-INH were determined by the method of Mancini (23) as modified by Bagdasarian et al. (20). All antisera were obtained from Behring Diagnostics (Somerville, N. J.) except the rabbit antikallikrein antibody which was prepared as previously described (20). On both immunodiffusion and immunoelectrophoresis this antisera gave a single precipitin line against normal plasma but no immunoprecipitate against Fletcher trait plasma. Immunoelectrophoresis was performed by the method of Scheidigger (24), except that agarose (Litex, Denmark) was prepared as a 1% gel in sodium barbital buffer (Harleco, Gibbstown, N. J.) $I = 0.0375$, pH = 8.6. The electrophoresis was performed on glass plates (85 × 95 mm) at 15°C at 250 V on an LKB Multiphor apparatus (LKB Instruments, Inc., Rockville, Md.) until the tracking dye (bromphenol blue) migrated 3–4 cm. Gel and running buffers were the same. Crossed immuno-

[1] *Abbreviations used in this paper:* C$\bar{1}$-INH, C1 esterase inhibitor; HMW kininogen, high molecular weight kininogen.

electrophoresis was performed by a modification of the method of Laurell (25). The buffer used for both dimensions was sodium barbital as described for immunoelectrophoresis. Electrophoresis of the prechallenge and fever plasma in dimension 1 was performed on a single glass plate (85 × 95 mm) at 15°C at 250 V and run until the tracking dye migrated 4–5 cm. The agarose strips containing the plasma samples were sliced and transferred to separate uncoated glass plates (50 × 75 mm) where an antibody-agarose mixture was poured. Electrophoresis in the second dimension was performed at 100 V for 18 h at 15°C on an LKB Multiphor apparatus. The concentration (volume percent) of antiserum in the total volume (4 ml) of agarose solution in the second dimension was 3.8% for kallikrein, 2.5% for C1-INH, 7% for α_1-antitrypsin, 2% for antithrombin III, and 1.6% for α_2-macroglobulin. The volumes of plasma used were 50 μl for kallikrein, 14 μl for C1-INH, 0.7 μl for α_1-antitrypsin, 5 μl for antithrombin III, and 5 μl for α_2-macroglobulin. For immunoelectrophoresis and crossed immunoelectrophoresis, the plates were soaked overnight in 0.1 M sodium phosphate, containing 0.5 M sodium chloride, pH 7.0, dried in an oven, and stained with 0.5% Coomassie Brilliant Blue R250 in 5 parts methanol, 5 parts water, 1 part glacial acetic acid and destained in the same solvent.

RESULTS

Although the time-course of the changes in typhoid fever was variable, a typical course is illustrated in Fig. 1. 10 days after ingestion of *S. typhi*, blood cultures became positive for that organism accompanied by the development of fever. α_1-Antitrypsin, a known acute phase reactant, rose to a peak value on day 7 after fever and then fell to normal during convalescence. HMW kininogen increased to a maximum on day 5–7 and returned to normal levels by 13–14 days after fever. In contrast, a parallel decrease of platelets, prekallikrein, and kallikrein inhibitory activity occurred with a nadir on day 5 after the onset of fever (day 15 after ingestion). Thereafter, all of these components returned to base line by day 9-11 after the fever with development of an

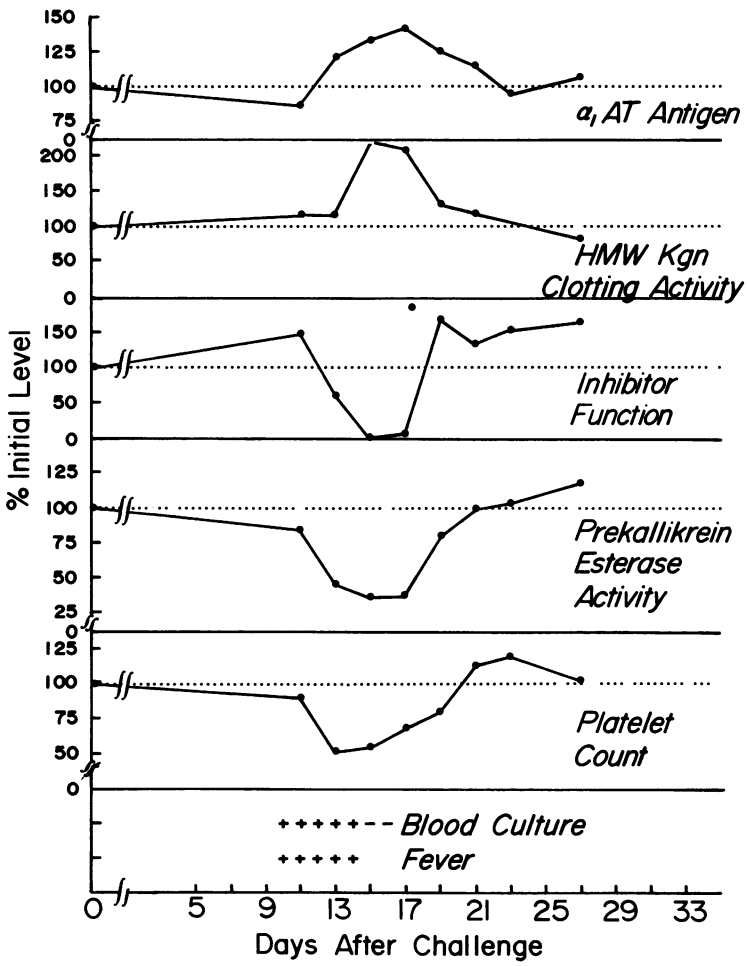

FIGURE 1 Course of one typical volunteer who developed typhoid fever. Each assay is plotted as percent of prechallenge level on the ordinate and days after ingestion of bacteria on the abcissa. α, AT, α_1-antitrypsin; HMW Kgn, HMW kininogen.

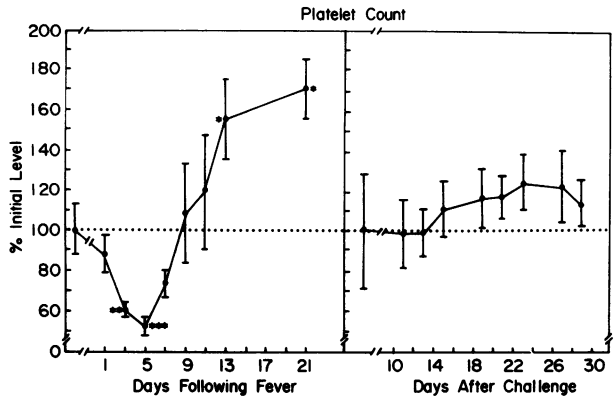

FIGURE 2 Platelet count changes in typhoid fever. In Figs. 2–6, the left side of each graph represents determinations in febrile volunteers expressed as the mean (●)±SE of the mean (I) for each postfebrile day, whereas the right side represents determinations in volunteers who remained afebrile after ingestion of *S. typhi*. *, $P < 0.05$, **, $P < 0.02$, ***, $P < 0.01$ indicate the significance of the difference from prechallenge levels.

overshoot as the fever subsided. No consistent changes occurred in coagulant factor XII or in immunochemical levels of prekallikrein, CĪ-INH, or α_2-macroglobulin (not shown). Similar correlations were observed in the other four patients, and the results of the entire group of patients are summarized in the following paragraphs.

The platelet count decreased to 60% at day 5 ($P < 0.01$) after the onset of fever and showed an overshoot to 160% on days 13–17 (Fig. 2). A similar drop without overshoot was noted in the total white blood count. Although no significant change was noted in the peripheral polymorphonuclear leukocyte or monocyte counts, the total lymphocyte count decreased to 47% on the first day of fever ($P < 0.01$) and returned to base-line level after the fever subsided. In contrast, no such changes were detected in the control patients.

Factor XII (Hageman factor), the protein that initiates the activation sequence of kallikrein system, did not increase significantly in either the patients with typhoid fever (Fig. 3) or in the afebrile individuals. HMW kininogen, which potentiates the activation and function of factor XII, showed a statistically significant ($P < 0.05$) rise, reaching a peak on the day of highest fever (day 5), persisting through day 13, and returning to base-line levels during convalescence. The HMW kininogen levels in the controls rose and remained elevated throughout the period of observation. However, there was wide variation and only on day 19 after challenge was the increase statistically significant.

Functional prekallikrein declined to 55% of the base line levels (Fig. 4) reaching a nadir on the day of maximum fever (day 5, $P < 0.02$) with a return to base-line levels by day 11. In contrast, the control patients showed no significant change, fluctuating between 96 and 126% of base-line levels. However, when prekallikrein was measured immunochemically, the level of kallikrein antigen remained between 90 and 114% in both the typhoid fever patients and the control individuals.

Determination of the functional activity of kallikrein inhibitor, which primarily measures the effect of CĪ-

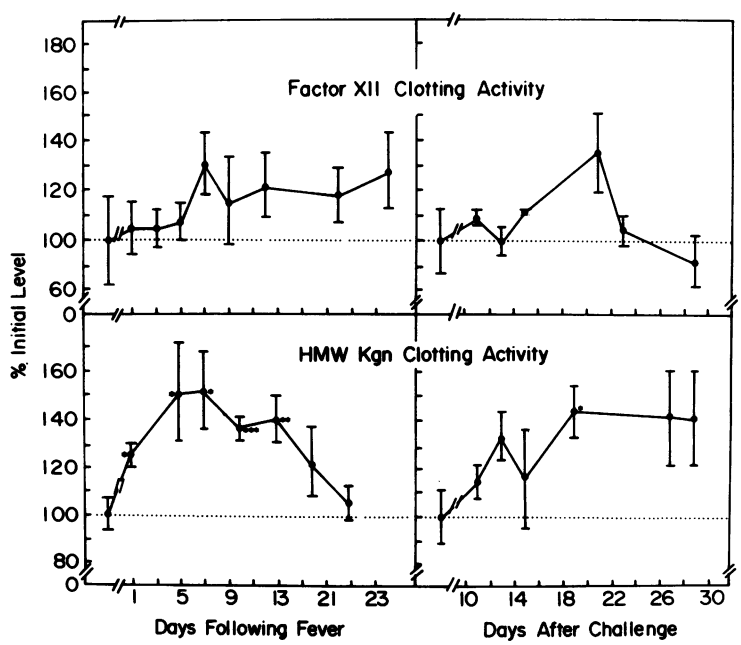

FIGURE 3 Factor XII and HMW kininogen concentrations in typhoid fever. kgn, kininogen.

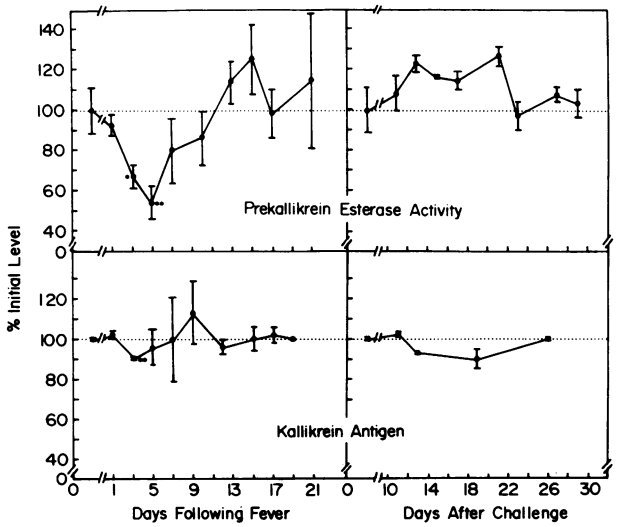

FIGURE 4 Prekallikrein esterase activity and kallikrein antigen concentrations in typhoid fever.

INH, showed a significant decrease to 45–50% of base line on day 5–7 ($P < 0.05$) with a significant overshoot to 146% on day 9 (Fig. 5). The mean of the control individuals showed no significant change. In contrast, CĪ-INH antigen did not increase significantly in the typhoid patients ($P > 0.05$) or the controls.

α_1-Antitrypsin antigen rose in the typhoid fever patients to 145% of base-line values on fever day 7 ($P < 0.02$) with a return to base-line levels during convalescence (Fig. 6), whereas the control patients varied from 90 to 110%. Immunological α_2-macroglobulin showed no change in the afebrile individuals and showed a minimal (10% decline) on day 5 in the typhoid fever patients.

One possible explanation for the decrease in functional kallikrein and its inhibitor with apparently normal immunochemical levels of both proteins is the formation of a nonfunctional circulating complex of kallikrein and CĪ-INH in which both proteins maintain their antigenic identity. Therefore, plasma obtained before ingestion of *S. typhi* and 5–7 days after the onset of fever were compared in all five affected individuals. The results were similar in all five patients, and a representative immunoelectrophoretic pattern is seen (Fig. 7). In the base-line period, plasma tested against antikallikrein exhibited a single immunoprecipitin arc representing prekallikrein (lower well of plate). During typhoid fever a second arc (arrow), in a more anodal position showing a line of partial identity with prekallikrein, is seen (upper well of plate). In the plasma obtained during the base-line period only a single arc is detected by anti-CĪ-INH antisera, whereas during the febrile episode a second arc (arrow) migrating more cathodally and continuous with the CĪ-INH arc is detected. These observations support the

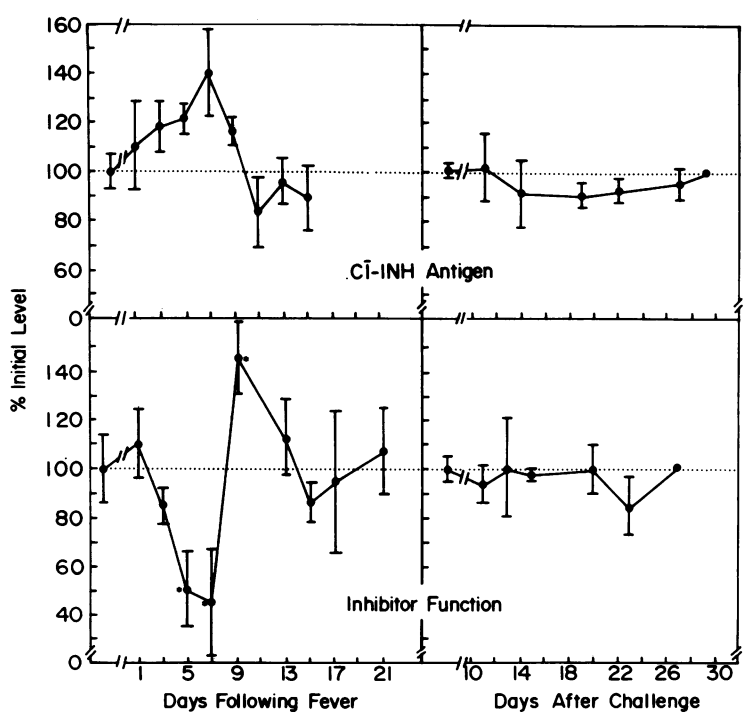

FIGURE 5 Functional kallikrein inhibitor activity and CĪ-INH antigen concentrations in typhoid fever.

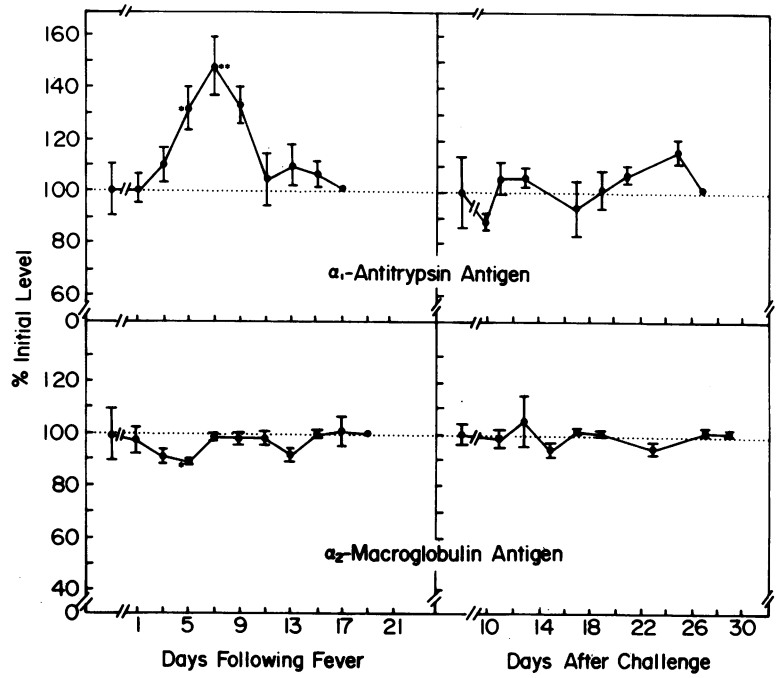

FIGURE 6 α_1-antitrypsin and α_2-macroglobulin antigen concentrations in typhoid fever.

existence of a complex displaying antigenic characteristics of both prekallikrein and CĪ-INH. the CĪ-INH and kallikrein antigen arcs in their normal position probably represent uncomplexed protein because the activity measurements indicate that only a portion of the prekallikrein had been activated in vivo. The relative proportion of the arcs differs from patient to patient and seems to correlate with residual esterase activity (data not shown). In contrast, the precipitin arcs for α_2-macroglobulin, α_1-antitrypsin, and antithrombin III show no difference in their position or appearance in the plasma obtained during typhoid fever compared with base-line plasma samples.

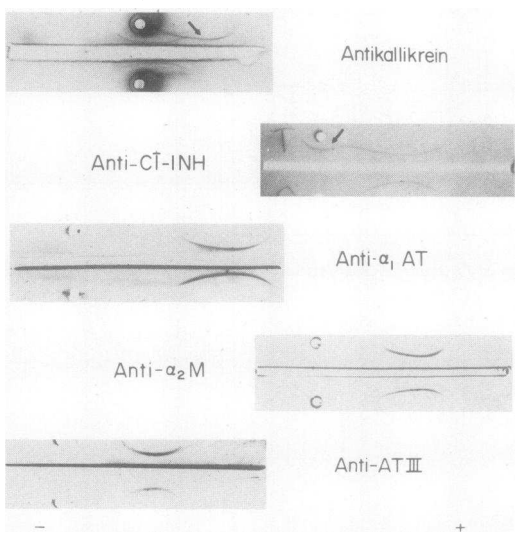

FIGURE 7 Immunoelectrophoresis of plasma of one typical febrile volunteer. The top well of each immunoplate represents patient plasma on day 5 of fever, whereas the bottom half represents the same patient before challenge with *S. typhi*. The arrows indicate new precipitin arcs interpreted as a kallikrein-inhibitor complex (see text).

This kallikrein CĪ-INH complex was also demonstrable by crossed immunoelectrophoresis using antikallikrein antiserum (Fig. 8). In the prechallenge plasma (P), a single kallikrein antigen peak (arrow 1) is seen. During the febrile episode (F), in addition to uncomplexed kallikrein, a new complex (arrow 2) appears continuous with the kallikrein arc but migrating in a more anodal portion expected for a kallikrein-inhibitor complex.

To confirm the identity of the inhibitor with which the kallikrein was complexing, crossed immunoelectrophoresis was performed with antiserum to CĪ-INH, α_1-antitrypsin, α_2-macroglobulin, and antithrombin III (Fig. 9). The superimposition of the prechallenge and fever immunoplates showed a cathodal shift in the F sample only when developed with CĪ-INH but showed no differences with the other kallikrein inhibitors (α_1-antitrypsin, α_2-macroglobulin, or antithrombin III). This change was consistent in all five plasmas (data not shown). The failure of appearance of a trough between free and complexed CĪ-INH may represent the close resemblance between these two forms noted previously (20).

DISCUSSION

In this study we have described changes in the kinin forming system in typhoid fever by serially following the changes in function and/or immunological behavior of six proteins before and after ingestion of *S. typhi*. We have compared the pattern of response in patients who developed the disease and controls who did not, and we have confirmed the correlation of a fall in platelet count with the development of the fever (1). This change had been attributed to the direct effect of endotoxin which is known to aggregate platelets in vitro. However, no circulating endotoxin has been demonstrated (2), and the reasons for the transient thrombocytopenia has not yet been determined.

From in vitro studies of the kinin generating system, one would expect the following sequence (9) if prekallikrein activation occurred in vivo. Precursor factor XII in the presence of HMW kininogen and prekallikrein would be cleaved to yield an active enzyme which remains surface-bound and, in the fluid phase, yields Hageman factor fragments (molecular weight 30,000). These fragments would then serve as prekallikrein activators converting prekallikrein to kallikrein (18, 26). Kallikrein would then combine stoichiometrically with C1̄-INH (27) to form a complex which has neither enzymatic nor inhibitory activity. A complex has been previously produced in human plasma in vitro by addition of kallikrein and detected by immunoelectrophoresis developed with kallikrein antibody (20). A kallikrein-C1̄-INH complex has also been shown in purified systems by electrophoresis in sodium dodecyl sulfate (28). Kallikrein in purified systems also reacts with α_2-macroglobulin, but unlike the complex with C1̄-INH, this resulting enzyme-inhibitor complex retains much of its esterolytic activity though its proteolytic activity is lost (29). In summary, if kallikrein activation occurs in vivo, one might expect a decrease in prekallikrein and kallikrein inhibitor activities. Because the enzyme-inhibitor complex might retain its antigenicity, immunochemical assays of these proteins might not show a similar decrease.

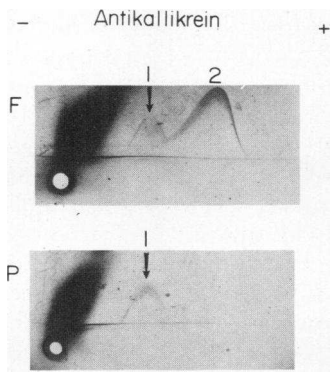

FIGURE 8 Crossed immunoelectrophoresis vs. antikallikrein antiserum. The prechallenge plasma (P) and the plasma on day 5 of fever (F) were run on a single glass plate for dimension 1 (see Methods) and transferred to separate plates for dimension 2. Arrow 1 indicates the position of uncomplexed kallikrein. Arrow 2 indicates the formation of a new precipitin peak.

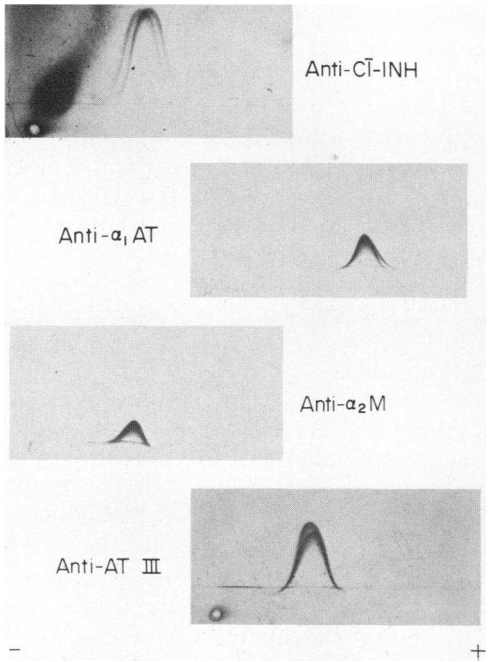

FIGURE 9 Crossed immunoelectrophoresis vs. inhibitor antiserum. The procedure was as in Fig. 8. For photographic purposes, the origins of the plates for each pair of prechallenge and fever plasma from a given patient were aligned. One photograph was made for each antibody (indicated on the plate) used to develop the precipitin peaks.

Such a sequence of events has been demonstrated during the febrile phase of typhoid fever. There is a prompt decrease of both prekallikrein and kallikrein inhibitor functional activity to 50% of normal occurring at the height of fever 5 days after onset which also correlates with the decrease in platelet count. Similar decrease in functional prekallikrein activity was found in children with dengue and other acute febrile illnesses (30). However, kallikrein inhibitory activity remained normal, and the decrease in prekallikrein did not correlate with the onset of shock. In gram-negative sepsis, decreased prekallikrein and kallikrein inhibitory activity (4, 5) correlated with fever, and hypotension and elevated bradykinin levels (5) have been documented. This decrease in the present study cannot be the result of the ingestion of the bacilli per se, because individuals who did not develop the disease after *S. typhi* challenge did not show these changes. Although chloramphenicol can inhibit protein synthesis, the

return of prekallikrein and kallikrein inhibitory activity began long before the cessation of antibiotic therapy at 12 days, suggesting that the therapy was not producing the changes. Mild hepatic dysfunction occurs in typhoid fever but this cannot by itself account for the changes observed. A different pattern of changes has been defined in compensated cirrhosis with a decrease in both prekallikrein activity and antigen (20) with normal kallikrein inhibitory activity (9). Any effect on protein synthesis would involve a decrease of both antigen and functional activity. Instead, in the typhoid fever patients, no change in antigenic prekallikrein or $C\bar{1}$-INH occurred.

Supporting the concept of activation of prekallikrein, a kallikrein-$C\bar{1}$-INH complex was present by immunoelectrophoresis and was detectable by antisera against both kallikrein and $C\bar{1}$-INH inhibitor. Furthermore, crossed immunoelectrophoresis confirmed the existence of such a complex which, in vitro, forms only with kallikrein and not prekallikrein. Inasmuch as the complex was demonstrable only in the plasma of patients after the onset of fever but not in the plasma of patients without fever, its genesis appears to be related to typhoid fever.

Unlike the kallikrein-$C\bar{1}$-INH complex, a plasma kallikrein-α_2-macroglobulin complex might possess residual esterolytic and proteolytic activity like the plasmin-α_2-macroglobulin complex (31). α_2-Macroglobulin antigen did not increase in the febrile episode consistent with the observation that it is not an acute phase reactant (32) and the inhibitor is not elevated in typhoid fever (33). Although a complex was not demonstrated between kallikrein and α_2-macroglobulin by immunoelectrophoresis or crossed immunoelectrophoresis, it is possible that this is the result of a close resemblance between the complex and uncomplexed α_2-macroglobulin. In in vitro experiments, we observed a decrease in the size of the α_2-macroglobulin precipitin arc after the addition of kallikrein (20), and a kallikrein-α_2-macroglobulin complex has been demonstrated on sodium dodecyl sulfate electrophoresis (28).

No complex was demonstrated between kallikrein and α_1-antitrypsin in individuals with typhoid fever. Evidence has been presented that α_1-antitrypsin is not a kallikrein inhibitor (34). Furthermore, plasma lacking α_1-antitrypsin shows no decrease in kallikrein inhibitory activity (35). Moreover, no complex of kallikrein with partially purified α_1-antitrypsin inhibitor was demonstrated in vitro (20). The rise in α_1-antitrypsin during typhoid fever, subsiding during convalescence and not occurring in afebrile patients, is compatible with the observation that this protein is an acute phase reactant and confirms previous results in typhoid fever (33).

Antithrombin III has been demonstrated (36) to be a weak inhibitor of purified plasma kallikrein, and the rate of the reaction is accelerated by heparin. However, in normal plasma, the presence of heparin does not increase the rate of extent of kallikrein inhibition. Only in the absence of $C\bar{1}$-INH does heparin augment the total inhibitory capacity of plasma apparently due to antithrombin III (36). In agreement with these observations, no complex between kallikrein and antithrombin III was demonstrable by immunoelectrophoresis.

Factor XII is capable of cleaving prekallikrein enzymatically, and in the fragmented form, its ability to activate prekallikrein may increase compared with surface-bound active Hageman factor (26). Therefore, only a small number of Hageman factor fragment molecules may be needed to convert significant quantities of prekallikrein to kallikrein, and this may not be detectable with current methods. Although factor XII must be converted to an active form to initiate kinin formation, the coagulant assay measures both precursor and active factor XII. However, in forming Hageman factor fragments (18), factor XII has been shown to lose 95% of its coagulant activity (37). Thus, in more extreme situations, as in septicemic hypotension where prekallikrein decreased to 10% of normal (4), factor XII coagulant activity decreased to 40%. The less extensive changes in typhoid fever may explain the lack of hypotension in the disease. This conclusion is consistent with the moderate decrease in prekallikrein documented in normotensive bacteremia (4).

The pathogenesis of the changes in HMW kininogen are less clear. Although bovine HMW kininogen loses activity after cleavage by kallikrein (38), this does not appear to be the case in human preparations (16). Liberation of all the kinin from human HMW kininogen failed to change its coagulant activity. The variation of this protein in disease is incompletely understood because its vital role in plasma proteolysis has been only recently appreciated (10, 16, 39–41). Saito et al. (42) have reported a decrease in HMW kininogen in liver disease and disseminated intravascular coagulation. The striking rise of HMW kininogen in typhoid fever may indicate that it is an acute phase reactant. Zeitlin et al. (43), for example, have found that plasma kininogen is elevated in acute rheumatic fever and rapidly decreases when the disease is treated with indomethicin or aspirin.

Endotoxin has been suggested as a mediator of the hematologic changes in typhoid fever (1). However, circulating endotoxin was not detected by the sensitive limulus lysate assay in any of 21 typhoid fever patients studied (2). Furthermore, investigations (44) have shown failure of induced tolerance to endotoxin to alter the subsequent course of typhoid fever. Thus, endotoxin activation of Hageman factor, although documented in vitro (45), probably does not occur systemically in typhoid fever. However, endotoxin may contribute to

typhoid illness by inciting inflammatory lesions at the site of bacterial lodgment, a hypothesis supported by the production of inflammatory reactions in the skin at levels of endotoxin below those necessary to produce fever (46). The possibility thus exists that endotoxin activation of the kinin forming system occurs in blood circulating through these areas of inflammation (47) without a rise in circulating endotoxin or associated hypotension (48). The precise mechanism of activation of the kallikrein system remains obscure.

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REFERENCES

1. Greisman, S. E., R. B. Mornick, F. A. Carozza, and T. E. Woodward. 1963. The role of endotoxin during typhoid fever and tolerance in man. I. Acquisition of tolerance to endotoxin. *J. Clin. Invest.* **42:** 1064–1075.
2. Butler, T., W. R. Bell, J. Levin, N. N. Linh, and K. Arnold. 1977. Typhoid fever: studies of blood coagulation, bacteremia and endotoxemia. *Arch. Intern. Med.* In press.
3. Allen, N., Y. Normanbhoy, D. Green, and G. Durea. 1969. Typhoid fever with consumption coagulopathy. J. A. M. A. *(J. Am. Med. Assoc.).* **208:** 689–690.
4. Mason, J. W., U. Kleeberg, P. Dolan, and R. W. Colman. 1970. Plasma kallikrein and Hageman factor in gram-negative bacteremia. *Ann. Intern. Med.* **73:** 545–551.
5. O'Donnell, T. F. Jr., G. H. A. Clowes, R. C. Talamo, and R. W. Colman. 1976. Kinin activation in the blood of patients with sepsis. *Surg. Gynecol. Obst.* **143:** 539–545.
6. Elliott, D. G., E. W. Horton, and G. P. Lewis. 1960. Actions of pure bradykinin. *J. Physiol.* **153:** 473–480.
7. Margolis, J. 1958. Activation of plasma by contact with glass: evidence for a common reaction which releases plasma kinin and initiates coagulation. *J. Physiol.* **144:** 1–22.
8. Niewiarowski, S., and O. Prou-Watelle. 1959. Role de facteur contact (Facteur Hageman) dans la fibrinolyse. *Thromb. Diath. Haemorrh.* **3:** 593–603.
9. Colman, R. W. 1974. Formation of human plasma kinin. *N. Engl. J. Med.* **291:** 509–515.
10. Colman, R. W., and A. Bagdasarian. 1976. Human kallikrein and prekallikrein. *Methods Enzymol.* **45:** 303–324.
11. Colman, R. W., J. W. Mason, and S. Sherry. 1969. The kallikreinogen-kallikrein enzyme system of human plasma. *Ann. Intern. Med.* **71:** 763–773.
12. Gilman, R. H., R. B. Hornick, W. E. Woodward, H. C. Dupont, M. S. Snyder, M. M. Levine, and J. P. Libonati. 1977. Evaluation of an epimeraseless mutant of *Salmonella typhi* as a live oral vaccine. *J. Infect. Dis.* In press.
13. Hornick, R. B., T. E. Woodward, and F. R. McCrumb. 1966. Study of induced typhoid fever in man. I. Evaluation of vaccine effectiveness. *Trans. Assoc. Am. Physicians.* **79:** 361–367.
14. Bull, B. S., M. A. Schneiderman, and G. Brecher. 1965. Platelet counts with the Coulter counter. *Am. J. Clin. Pathol.* **44:** 678–688.
15. Mason, J. W., and R. W. Colman. 1971. The role of Hageman factor in disseminated intravascular coagulation induced by septicemia, neoplasia, or liver disease. *Thromb. Diath. Haemorrh.* **26:** 325–331.
16. Colman, R. W., A. Bagdasarian, R. C. Talamo, C. F. Scott, M. Seavey, J. A. Guimares, J. V. Pierce, and A. P. Kaplan. 1975. Williams trait: human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman dependent pathways. *J. Clin. Invest.* **56:** 1650–1662.
17. Proctor, R. R., and S. I. Rapaport. 1961. The partial thromboplastin time with kaolin: a simple screening test for first-stage plasma clotting factor deficiencies. *Am. J. Clin. Pathol.* **36:** 212–219.
18. Kaplan, A. P., and K. F. Austen. 1970. A prealbumin activator of prekallikrein. *J. Immunol.* **105:** 802–811.
19. Girey, G. J. D., R. C. Talamo, and R. W. Colman. 1972. The kinetics of the release of bradykinin by kallikrein in normal human plasma. *J. Lab. Clin. Med.* **80:** 496–505.
20. Bagdasarian, A., B. Lahiri, R. C. Talamo, P. Wong, and R. W. Colman. 1974. Immunochemical studies of plasma kallikrein. *J. Clin. Invest.* **54:** 1444–1454.
21. Harpel, P. C. 1970. Human plasma alpha-2-macroglobulin: an inhibitor of plasma kallikrein. *J. Exp. Med.* **132:** 329–352.
22. Gigli, I., J. W. Mason, R. W. Colman, and K. F. Austen. 1970. Interaction of plasma kallikrein with Cl inhibitor. *J. Immunol.* **104:** 574–581.
23. Mancini, G., A. O. Carbonaro, and J. F. Hereman. 1965. Immunochemical quantitation of antigen by single radial immunodiffusion. *Immunochemistry.* **2:** 235.
24. Scheidigger, J. J. 1955. Une micro-méthode de immunoelectrophoresè. *Int. Arch. Allergy Appl. Immunol.* **7:** 103–110.
25. Laurell, C. B. 1965. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* **10:** 358.
26. Bagdasarian, A., B. Lahiri, and R. W. Colman. 1973. Origin of the high molecular weight activator of prekallikrein. *J. Biol. Chem.* **248:** 7742–7747.
27. Gigli, I., J. W. Mason, R. W. Colman, and K. F. Austen. 1970. Interaction of plasma kallikrein with the Cl inhibitor. *J. Immunol.* **104:** 574–581.
28. Harpel, P. C., M. W. Mosesson, and N. R. Cooper. 1975. Studies on the structure and function of α_2-macroglobulin and $C\bar{1}$ inactivator. *In* Proteases and Biological Control. E. Reich, D. B. Ripkin, and E. Shaw, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 387–404.
29. Harpel, P. C. 1971. Separation of plasma thromboplastin antecedent from kallikrein by the plasma α_2-macroglobulin-kallikrein inhibitor. *J. Clin. Invest.* **50:** 2084–2090.
30. Edelman, R., S. Nimmannitya, R. W. Colman, R. C. Talamo, and F. H. Top, Jr. 1975. Evaluation of the plasma kinin system in dengue hemorrhagic fever. *J. Lab. Clin. Med.* **86:** 410–421.
31. Harpel, P. C., and M. W. Mosesson. 1973. Degradation of human fibrinogen by plasmin α_2-macroglobulin-enzyme complexes. *J. Clin. Invest.* **52:** 2175–2184.
32. Crockson, R. A., C. J. Payne, A. P. Ratcliff, and J. F. Soothill. 1966. Time sequence of acute phase reactive proteins following surgical trauma. *Clin. Chim. Acta.* **14:** 435–441.

33. Bostian, K. A., B. S. Blackburn, R. W. Wannamaker, Jr., V. G. McGann, W. R. Beisel, and H. L. Dupont. 1976. Sequential changes in the concentration of specific serum proteins during typhoid fever infection in man. *J. Lab. Clin. Med.* **87**: 577–585.
34. Heck, L. W., and A. P. Kaplan. 1974. Substrates of human Hageman factor. I. Isolation and characterization of PTA (factor XI) and its inhibition by α_1-antitrypsin. *J. Exp. Med.* **140**: 1615–1630.
35. Colman, R. W., and B. Mitchell. 1972. Specificity of inhibitors lacking in α_1-antitrypsin deficiency and hereditary angioedema toward human proteolytic enzymes. *Clin. Chim. Acta.* **39**: 5–14.
36. Lahiri, B., A. Bagdasarian, B. Mitchell, R. C. Talamo, R. W. Colman, and R. D. Rosenberg. 1976. Antithrombin-heparin cofactor: an inhibitor of plasma kallikrein. *Arch. Biochem. Biophys.* **175**: 737–747.
37. Kaplan, A. P., and K. F. Austen. 1971. A prealbumin activator of prekallikrein. II. Derivation of activators of prekallikrein from active Hageman factor by digestion with plasmin. *J. Exp. Med.* **133**: 696–712.
38. Matheson, R. T., D. R. Miller, M. J. Lacombe, Y. N. Han, S. Iwanaga, H. Kato, and K. D. Wuepper. 1976. Flaujeac factor deficiency: reconstitution with highly purified bovine high molecular weight kininogen and delineation of a new permeability-enhancing peptide released by plasma kallikrein from bovine high molecular weight kininogen. *J. Clin. Invest.* **58**: 1395–1406.
39. Wuepper, K. D., D. R. Miller, and M. J. Lacombe. 1975. Flaujeac trait: deficiency of human plasma kininogen. *J. Clin. Invest.* **56**: 1663–1672.
40. Saito, H., O. D. Ratnoff, R. Waldman, and P. Abrahon. 1975. Fitzgerald trait: deficiency of a hitherto unrecognized agent, Fitzgerald factor participating in surface-mediated reaction of clotting, fibrinolysis, generation of kinins and the property of diluted plasma enhancing vascular permeability (PF/dil). *J. Clin. Invest.* **55**: 1082–1089.
41. Donaldson, V. H., H. I. Glueck, M. A. Miller, H. Z. Movat, and F. Habat. 1976. Kininogen deficiency in Fitzgerald trait: role of high molecular weight kininogen in clotting and fibrinolysis. *J. Lab. Clin. Med.* **87**: 327–337.
42. Saito, H., G. Goldsmith, and R. Waldmann. 1976. Fitzgerald factor (high molecular weight kininogen) clotting activity in human plasma in health and disease and various animal plasmas. *Blood.* **48**: 941–947.
43. Zeitlin, I. J., J. N. Sharma, P. M. Brooks, and W. C. Dick. 1976. An effect of indomethicin or raised plasma kininogen levels in rheumatoid patients. *In* Chemistry and Biology of the Kallikrein-Kinin System in Health and Disease. J. J. Pizano and K. F. Austen, editors. U. S. Government Printing Office. 483–486.
44. Greisman, S. E., R. B. Hornick, H. N. Wagner Jr., W. E. Woodward, and T. E. Woodward. 1969. The role of endotoxin during typhoid fever and tularemia in man. IV. The integrity of the endotoxin tolerance mechanisms during infection. *J. Clin. Invest.* **48**: 613–629.
45. Morrison, D. C., and C. G. Cochrane. 1974. Direct evidence for Hageman factor (factor XII) activity by bacterial lipopolysacchrides (endotoxins). *J. Exp. Med.* **140**: 797–811.
46. Greisman, S. E., and R. B. Hornick. 1972. Cellular inflammatory responses of man to bacterial endotoxin: a comparison with PPD and other bacterial antigens. *J. Immunol.* **109**: 1210–1222.
47. Hornick, R. B., S. E. Greisman, T. E. Woodward, H. L. DuPont, A. T. Dawkins, and M. J. Snyder. 1970. Typhoid fever: pathogenesis and immunologic control. *N. Engl. J. Med.* **283**: 686–691.
48. Greisman, S. E., R. B. Hornick, F. A. Carozza, and T. E. Woodward. 1964. The role of endotoxin during typhoid fever and tolerance in man. II. Altered cardiovascular responses to catecholamines. *J. Clin. Invest.* **43**: 986–999.