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TYPHOID FEVER: PATHOGENESIS AND IMMUNOLOGIC CONTROL (Second of Two Parts)*

R. B. HORNICK, M.D., S. E. GREISMAN, M.D., T. E. WOODWARD, M.D., H. L. DUPONT, M.D., A. T. DAWKINS, M.D., AND M. J. SNYDER, PH.D.

Contribution of Bacterial Endotoxin

Endotoxin is obviously an important component of any gram-negative rod such as the typhoid bacillus. Vi antigen appears important in enhancing human virulence of typhoid strains. It is not as simple to define the importance of endotoxin since this substance has many biologic and pharmacologic activities. Attempts were made in the course of these investigations to gather information on the role of purified endotoxin in the pathogenesis of typhoid fever by quantitating the response to endotoxin's pyrogenic activity in volunteers with and without typhoid fever.

When 0.25 μg of a purified endotoxin from *S. typhosa* was administered intravenously to healthy volunteers, chills, fever, headache, myalgia, anorexia, nausea, thrombocytopenia and leukopenia developed. These manifestations were strikingly similar to those observed in patients with typhoid fever. Repeated and increasingly larger daily doses of the endotoxin given intravenously led to increasing resistance to the pyrogenic and subjective toxic effects. Healthy subjects could thus be rendered solidly tolerant to intravenous doses of the endotoxin as large as 2.5 μg. In spite of such remarkable resistance to injections of endotoxin given intravenously, these volunteers readily became clinically ill after oral challenge with viable typhoid bacilli; their illness was no less severe, and the incubation period no longer, than in controls. In such subjects high specific anti-"O" antibody titers developed during induction of tolerance, which provided evidence that these antibodies did not confer antityphoidal immunity. Moreover, since the mechanisms responsible for the tolerance to the intravenously injected exogenous endotoxin remained functional during typhoid illness (as pointed out below), endogenous release of *circulating* endotoxin did not appear capable of accounting for the *sustained* pyrexia and toxemia of typhoid fever. This basic conclusion was initially in doubt, since tolerance seemed to disappear during illness – that is, the volunteers rendered tolerant before oral challenge with typhoid bacilli were found to react vigorously to intravenously injected endotoxin during typhoid illness.[26] However, further studies demonstrated that in control subjects, an even more marked hyperreactivity to the intravenously injected endotoxin developed – that is, responsiveness to circulating endotoxin was "reset" during typhoid fever in both control and tolerant volunteers. Yet tolerance to endotoxin – the difference in responsiveness between control and treated volunteers – persisted although the clinical illness remained unaffected. In addition, daily single intravenous injections of typhoid endotoxin, as well as continuous intravenous infusions, in other volunteers during overt typhoid fever evoked progressively diminishing pyrogenic and toxic responses. This proved the ready ability of man to acquire tolerance to circulating endotoxin during acute typhoidal illness. Yet in these tolerant subjects, the febrile and toxic courses of typhoid fever, again, were not mitigated, providing additional evidence against the role of endotoxemia in the pathogenesis of sustained symptoms and signs of this illness.

Further evidence against the importance of endotoxemia was provided by the responses of four volunteers addicted to the use of narcotics given intravenously for many years. These men appeared to have acquired a substantial level of endotoxin tolerance since, in contrast to other volunteers who served as infected controls, they all failed to hyperreact to intravenous infusions of purified typhoid endotoxin during typhoid fever. Nevertheless, the febrile and clinical course of their typhoid illness also remained indistinguishable from that observed in the control volunteers capable of hyper-reacting to endotoxin.[27]

The "resetting" of the responsiveness of man to circulating bacterial endotoxin during typhoid fever is remarkable. Volunteers with typhoid fever respond with appreciably more rapid and more intense febrile and subjective symptoms to all doses of endotoxin injected intravenously. As the dose was increased, reactions became progressively exaggerated – that is, the shape of the dose-response relation became steeper during illness. Mechanisms responsible for this hyper-reactivity to circulating endotoxin are unknown, although certain major possibilities have been excluded. The hyper-reactivity to *S. typhosa* endotoxin extended equally to heterologous endotoxin preparations (pseudomonas endo-

*From the Division of Infectious Diseases, University of Maryland School of Medicine (address reprint requests to Dr. Hornick at the Division of Infectious Diseases, University of Maryland School of Medicine, 29 S. Greene St., Baltimore, Md. 21201).

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toxin). Hence, the reaction was not related to hypersensitivity to the "O"-specific terminal polysaccharide side chains of the endotoxin. Delayed dermal inflammatory responses became intensified to *S. typhosa* endotoxin during typhoid fever. This may account, in part, for the intense local inflammatory responses that were previously observed in patients with typhoid fever given vaccine during their acute illness. Enhancement of dermal inflammatory responses to pseudomonas endotoxin did not occur in volunteers during typhoid fever in spite of their systemic hyper-reactivity to this endotoxin when given intravenously. These dermal responses suggest that the systemic hyper-reactivity to endotoxin was not the result of acquired delayed hypersensitivity.

The "resetting" of reactivity to circulating endotoxin during typhoid fever was *not* attributable to fever per se; hyper-reactivity progressed in stepwise fashion during the afebrile incubation period and subsided (in a similar manner) during the afebrile convalescent phase. Moreover, no systemic hyper-reactivity to endotoxin was observed in control subjects with sandfly fever, a mild viral infection.[26] These findings, and the fact that inflammation induced in rabbits with chemical irritants did not enhance pyrogenic responsiveness to bacterial endotoxins, presumably excluded nonspecific effects of inflammation per se.[27] Systemic hyper-reactivity to circulating endotoxin may be based upon increased reactivity of man to intermediate substances released from target tissues by the toxin such as endogenous pyrogens. On the other hand, enhanced release of such substances from target tissues may be responsible. Further studies are required.

Although *circulating* endotoxin did not appear to account for the sustained fever and toxemia of typhoid fever, the endotoxin component of *S. typhosa* could contribute importantly to the pathogenesis of typhoid fever in two ways, the first being that, on the basis of the systemic hyper-reactive responses in infected volunteers, release of even minute amounts of endotoxin into the circulation could evoke abrupt exacerbations of the febrile and toxic course of typhoid illness. Such toxic "spikes," however, would be superimposed upon more basic mechanisms responsible for the sustained fever. Their frequency and intensity would be limited by the ability of the host to acquire tolerance to the increments in circulating endotoxin. Secondly, whereas tolerance to the systemic effects of circulating endotoxin occurs readily during typhoid fever, such tolerance does not indicate a generalized tissue refractoriness to endotoxin. The skin is one local tissue site studied in man. Systemic tolerance induced by repetitive intravenous administration of endotoxin during typhoid fever not only failed to reduce the local dermal inflammatory response elicited by the endotoxin but actually enhanced it. Such dissociation of resistance to the systemic and local toxic effects of endotoxin during tolerance also occurred in healthy subjects in whom dermal inflammatory effects were increased.[28] These findings are consistent with observations in rabbits that tolerance to the fever-evoking activity of intravenously injected endotoxin results from refractoriness of hepatic Kupffer cells, with minimal or no refractoriness of other tissues.[29,30] Therefore, in extrahepatic lesions, release of *S. typhosa* endotoxin could contribute to the local inflammatory process, without being subjected to inhibition by tolerance evoked to the systemic effects of circulating endotoxin. Endotoxin influences the local inflammatory response by chemotactic activity, effects on metabolism of phagocytic cells and endogenous pyrogen-releasing activity. The histologic and clinical responses to *S. typhosa* could in part be attributed to these activities.

Endotoxin is a potent inflammatory-inducing agent, and is markedly chemotactic for polymorphonuclear leukocytes.[31] Part, if not all, of this chemotactic activity is mediated via the complement system.[32] Nanogram quantities of *S. typhosa* endotoxin cause gross dermal inflammatory responses during typhoid fever.[27] Although dense accumulations of polymorphonuclear leukocytes can accumulate in typhoid lesions (noted in typhoidal meningitis, osteomyelitis and pneumonia), the classic lesions are characterized by a predominance of mononuclear cells and a paucity of polymorphonuclear leukocytes.[33,34] Biopsies of the small intestinal mucosa obtained as early as the third day after oral challenge of volunteers with *S. typhosa* (before clinical illness is evident) show focal granulomatous inflammatory lesions with mononuclear histiocytes predominantly and a few polymorphonuclear leukocytes.[16] In 1898 F. B. Mallory[34] noted: "Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis. . . ." We are studying the dermal inflammatory response during typhoid fever induced by purified *S. typhosa* endotoxin. This will be compared with the mononuclear response in "rose spots" and other classic typhoid lesions.[34,35] Our preliminary data reveal histologic similarity.

The role of *S. typhosa* endotoxin on the metabolic activity of mononuclear macrophages, cells that typically dominate the histologic lesions of typhoid fever, is unknown. Large doses of endotoxin (20 to 50 μg) stimulate respiratory, phagocytic and bactericidal activities of mouse peritoneal macrophages as well as their lysosomal content.[36,37] It is unknown whether endotoxin released from intracellular or extracellular typhoid bacilli similarly stimulate human macrophages. Yet Mallory[34] suggested this in 1898:

> . . . the typhoid bacillus produces a mild diffusible toxine, partly within the intestinal tract, partly within the blood and organs of the body. This toxine produced proliferation of endothelial cells which acquire for a certain length of time malignant properties. The new formed cells are epithelioid in character, have irregular, lightly staining, eccentrically situated nuclei, abundant, sharply defined,

acidophilic protoplasm, *and are characterized by marked phagocytic properties.*

Stimulated macrophages appear responsible for the striking microbial resistance to *S. typhimurium* in mice.[38] They may participate similarly in human typhoid fever since neither resistance nor recovery appears to be related to humoral antibodies (as discussed below). Delayed hypersensitivity reactions stimulate the macrophage population,[39] as does the endotoxin molecule. The contribution of each mechanism to macrophage response in typhoid fever requires elucidation.

Endogenous pyrogen elaborated from host tissues after microbial or toxin stimuli or hypersensitivity causes fever in animals.[40] The pyrogen circulates and acts upon the thermoregulatory centers in the hypothalamus.[41] Endotoxin stimulates the release of endogenous pyrogen from leukocytes, spleen cells, hepatic Kupffer cells and macrophages of peritoneum and lung of rabbits[29,42-45]; it is formed by polymorphonuclear cells and monocytes of humans.[46-48] Since human blood polymorphonuclear leukocytes and monocytes are capable of releasing endogenous pyrogen, and since the macrophage is derived from blood monocytes,[49] the inflammatory-derived human macrophage and polymorphonuclear leukocyte can presumably synthesize endogenous pyrogen. On the basis of experimental models,[46] typhoid bacilli and its endotoxin constitute two major stimuli for endogenous pyrogen formation by the macrophages and the few polymorphonuclear leukocytes in the local typhoidal inflammatory lesions. Kupffer cells, on the other hand, appear to be important target cells for mediating the febrile response to circulating endotoxin. They readily become refractory to circulating endotoxin, and tolerance results.[29,30] In contrast, extrahepatic tissues capable of releasing endogenous pyrogen appear less likely to become refractory; tolerant animals continue to release endogenous pyrogen from leukocytes and lung macrophages after endotoxin stimulation.[29,50] Thus, tolerance can be induced to circulating endotoxin during typhoid fever, but fever can continue unabated because endogenous pyrogen synthesis and release continue from stimulated macrophages and polymorphonuclear leukocytes in the local typhoidal inflammatory lesions. This concept best explains why fever and toxemia remain unabated in volunteers with typhoid fever made tolerant to the systemic effects of intravenous infusions of the typhoid endotoxin.

Phagocytic Activity of the Reticuloendothelial System

The phagocytic capacity of the human reticuloendothelial system increased appreciably during typhoid fever.[51] This was shown by measurement of the clearance of radioactively tagged aggregated human serum albumin particles from the blood. These particles were proved to be cleared from the bloodstream by reticuloendothelial cells in the liver and the spleen.[28] Serial determinations of their clearance before, during and after induced disease demonstrated increased activity of the reticuloendothelial cells during acute disease. Similarly, tolerance induced in humans by daily intravenous injections of endotoxin resulted in heightened ability of the reticuloendothelial system to clear the blood of circulating endotoxin. This heightened activity persisted during typhoid fever.[27] It is unknown whether such an increased phagocytic property during typhoid fever was the result of recruitment of newly formed reticuloendothelial cells, of increased efficiency of the existing reticuloendothelial cells or of increased opsonins. Bacterial endotoxins increased the phagocytic capacity of the reticuloendothelial system in animals. Yet circulating endotoxin did not appear to be the major factor responsible for increased reticuloendothelial phagocytic activity during typhoid fever. Certain findings support this statement. Similar increases in phagocytic capacity for aggregated human serum albumin particles occur during pneumococcal pneumonia in man.[51] Endotoxemia is not a part of this infection. Moreover, daily intravenous administration of fever-producing doses of purified endotoxin to healthy men is not associated with detectable increases in reticuloendothelial phagocytic activity for the aggregated albumin particles.[28] The enhanced reticuloendothelial system phagocytic activity in typhoid fever may represent a nonspecific response to bacterial infections, reflecting an adaptive "scavenger" function in response to cellular debris.[51]

It is noteworthy that the increased phagocytic activity of the reticuloendothelial system for albumin particles during typhoid fever was transient, lasting only several days after the disease was brought under control with chloramphenicol.[51] If such a generalized increase in phagocytic activity of the reticuloendothelial system contributes to host resistance during typhoid fever, the rapid decline in phagocytic capability precludes the practical use of agents that act by nonspecifically intensifying phagocytosis by the reticuloendothelial system.

Vascular Hyper-reactivity to Catecholamines

Another striking physiologic alteration during typhoid fever was exaggerated reactivity of the vascular system to catecholamines. There were remarkable augmented pressor responses to intravenous infusions of norepinephrine, enhanced vasomotor responses of the nailfold capillary bed and gross dermal hemorrhagic lesions after intradermal injections of epinephrine and norepinephrine. Pathologically, the dermal responses to catecholamines were characterized by extravasated red blood cells, fibrinoid arteriolar necrosis without thrombus formation and minimal inflammatory cellular responses. No such altered vascular reactivity to catecholamines occurred in volunteers with tularemia or the virus of sandfly fever.[52] The mechanism underlying this vas-

cular hyper-reactivity to catecholamines in typhoid fever was believed initially to be related to endotoxemia since minute amounts evoked similar responses in the rabbit.[53] This hypothesis appears inaccurate. Administration of endotoxin to normal subjects sufficient to cause febrile and subjective reactions as severe as those during typhoid fever failed to induce vascular hyper-reactivity to catecholamines. Intradermal injections of mixtures of large doses of typhoid endotoxin (5 μg) together with epinephrine failed to elicit hemorrhagic lesions in normal persons. Hyper-reactivity to catecholamines persisted for one to three weeks into the febrile convalescent phase of typhoid fever. Such responses in rabbits[53] persisted for less than eight hours after endotoxin injections. Vascular hyper-reactivity did not occur in tularemia, a gram-negative bacterial infection similar clinically to typhoid fever.[52]

The intestinal mucosa of man and animals possesses high serotonin concentrations.[54,55] Intestinal ischemia potentiates the vascular catecholamine-sensitizing properties of portal blood of rabbits apparently by releasing serotonin from the bowel.[56] A variety of inflammatory lesions of the intestine cause vascular hyper-reactivity to catecholamines in man.[57] Therefore, release of serotonin from the inflamed intestinal mucosa during typhoid fever, rather than endotoxemia, could account for the vascular hyper-reactivity to the catecholamines. The clinical importance of the vascular hyper-reactivity to catecholamines during typhoid fever is unknown. It may represent a contributing mechanism to the pathogenesis of the focal necrotic lesions and arteritis[58] that occur in such typhoid.

Acquired and Induced Immunity in Typhoid Fever

The discussion dealing with endotoxin alluded to the lack of correlation of "O" antibodies and subsequent development of typhoid fever, and to the possible importance of local cellular defenses. The volunteer studies indicated no correlation between base-line O, H and Vi antibodies and resistance to illness or relapse. Such serum antibodies in patients with naturally acquired typhoid fever did not correlate with tendency to relapse or resistance to reinfection.

Relapses are common in typhoid patients, occurring in about 8 to 10 per cent of patients in the preantibiotic era and in 15 to 20 per cent of those treated with chloramphenicol. These data imply that antibiotic therapy interferes either with the development of "protective antibodies" or cellular immunity or both. The mechanisms of action of chloramphenicol would support this thesis since protein synthesis is inhibited through the blockade of messenger ribonucleic acid. Indeed, studies have been reported indicating impairment of antibody development in man by chloramphenicol. If a protective antibody is inhibited, the nature of such inhibited antibody in antibiotic-treated patients is unknown. As stated, there was no correlation between humoral O, H or Vi antibodies and tendency to relapse. Relapses occurred at the peak of circulating antibody titers, and about 15 days after cessation of antibiotic therapy. Recrudescence appeared to occur after persisting typhoid bacilli had multiplied sufficiently to cause overt disease. Relapses were usually mild and sometimes self-limiting, implying that the host was better able to eliminate the offending organism, presumably through stimulated macrophages or other cell-associated inhibitory mechanisms.

Reinfection with typhoid fever is rare. In 1953 Marmion et al.[59] reported two epidemics of typhoid fever occurring within five months in a British Air Force camp in Egypt. Fifty-four men in whom typhoid fever had developed in the first epidemic were re-exposed in the second. The subsequent attack rate in this group was 20 per cent, as compared to an overall rate of 34 per cent. These data suggested that natural disease conferred moderate immunity. However, a portion of this incomplete immunity might be explained by different phage types involved in the two epidemics, and a larger dose was probably ingested in the second exposure. In the present study, volunteers were selected because of previous induced disease and rechallenged with identical doses of the same phage-type organisms, two to 12 months later. Their illness rate was now about 25 per cent. In this group of initially susceptible volunteers, substantial immunity appears to have been acquired. No correlation between resistance and O, H and Vi antibody levels was demonstrated.

Vaccine Field-Trial Results

Killed parenterally administered vaccines are known to boost bactericidal activity of serum through the production of O, H and Vi antibodies. This reaction requires the presence of complement. Cellular hypersensitivity may occur, but its role as a defense mechanism in human typhoid illness needs elucidation. The purpose of the volunteer studies has been to determine whether typhoid vaccines can prevent or ameliorate the clinical illness. The evidence presented thus far has deliberately attempted to minimize the importance of known vaccine effects in defense against typhoid. Immunoprophylaxis has been difficult to document. The most convincing fields trials designed to evaluate typhoid vaccines were initiated by the World Health Organization in 1955 and continued for 12 years.[60-62] These trials were well controlled and included the testing of three vaccines. Two test vaccines, K and L, were prepared from the standard Ty2V strain at the Walter Reed Army Institute of Research. K vaccine was monovalent and contained acetone-inactivated and dried typhoid bacilli that had retained Vi antigen. Vaccine L consisted of the conventional heat-killed, formalin-preserved typhoid bacilli. This treatment destroyed most of the Vi antigenicity. The

control vaccine was tetanus toxoid. Studies were conducted in Yugoslavia, Poland and Guyana with the use of identical vaccines. Similar studies were conducted in Russia.[63] Children and adults were participants in the evaluation and were recruited as volunteers. Table 3 outlines results of some of these trials. The best results were obtained in children. K vaccine appeared to be superior to L in inducing protection against typhoid fever. After seven years in Guyana there were 146, 16 and 49 confirmed cases of typhoid fever in children given two doses of the placebo, vaccines K and L groups respectively.[61] An unexpected result of this trial was the finding that only one (vaccine K) and four (vaccine L) cases of typhoid fever occurred in a total of 6690 children given only one dose of vaccine. Twenty-two cases occurred in the control group of 3515. Ashcroft[9] suggests that in Guyana and other endemic areas, repeated ingestion of subinfective doses of typhoid or related organisms may result in an immunized population and that vaccine will enhance the protection. These results confirm that typhoid vaccine lowers the prevalence of disease in susceptible children in endemic areas. The means by which this is accomplished is unknown. The results in adults were somewhat confusing. Different rates of protection were obtained in various countries. In all countries, however, the vaccine-induced immunity was less in adults than in children. Perhaps, adults had an increased opportunity to ingest larger doses of organisms. As in children, vaccine L appeared to be less effective than K in inducing protection.

Vaccines K and L were appraised in Pristina, Yugoslavia, an area of high typhoid endemicity resulting from ingestion of heavily contaminated water (Table 4).[62] Persons not volunteering for the typhoid-vaccine study showed six times the attack rate of the tetanus-toxoid control group. These data suggest that highly motivated persons who volunteer for studies are concerned with all preventive measures important in avoiding typhoid fever. They would therefore be less likely to ingest large numbers of enteric pathogens.

Vaccine Evaluation in Volunteers

Evaluation of the same vaccines was conducted in volunteers.[64,65] In this experimental situation, control of the infectious dose and strain involved and knowledge of humoral antibodies allowed for quantitative and qualitative analyses. In addition to the K and L vaccines, Vi antigen provided by Drs. Maurice Landy and Joseph Lowenthal was employed. Vaccines K and L were given in three 0.5-ml doses, the first two at weekly intervals and the third one month after the second dose. Single 50-μg doses of Vi antigen were given subcutaneously.

No protection was afforded to volunteers challenged with an ID_{50} dose (10,000,000) of *S. typhosa* or higher (Table 5). Illness occurred in vaccinated and unvaccinated subjects in spite of high antibody titers before challenge. Indeed, some of those vaccinated became ill sooner than their nonimmunized controls. When clinically apparent typhoid infections developed after ingestion of either low or high infectious doses, the severity of the illnesses was similar. These findings simulate *S. typhimurium* infections in mice[66]; regardless of the dose of infection and prior state of immunity, histologic changes and mortality were identical once bacteremia occurred.

At the ID_{25} (100,000) challenge dose, protection was demonstrated between vaccinees and controls. Only about 9 per cent of the subjects given vaccines K and L became ill, in contrast to 27 per cent of the nonvaccinated controls. The currently available vaccines thus showed effectiveness of 67 per cent against an infecting dose of 100,000 bacilli. Attempts to correlate prechallenge levels of agglutinins to O and H antigens, of Vi hemagglutinins and serumcidal activity with subsequent clinical course were made; no direct relation was found. Im-

Table 3. Results of WHO Field Trials with Vaccines K and L — Two Doses.*

Country	Period Covered (Yr)	Age Range (Yr)	Composition of Group	Vaccine Group						Effectiveness (%)†	
				K		L		Control		K	L
				cases of typhoid	*persons in trial*	*cases of typhoid*	*persons in trial*	*cases of typhoid*	*persons in trial*		
Yugoslavia	1960-1963	2-50	Mainly schoolchildren	16	5,028	37	5,068	75	5,039	79	51
Guyana	1960-1964	5-15	Schoolchildren	6	24,046	26	23,431	99	24,241	94	73
Poland	1961-1963	5-14	Schoolchildren	4	81,534			31	83,734	87	
USSR	1962-1963		Schoolchildren & young adults			13	36,112	50	36,999		73
1 dose of either vaccine K or L:											
Guyana	1960-1964	5-15	Schoolchildren	0	3,319	3	3,371	14	3,515	100	78
Poland		5-14		0	9,136			3	10,067	100	–

*Modified from data of Cvjetanović & Uemura.[60]

†Effectiveness $= \frac{100\,(b-a)}{b}$; a indicates incidence in vaccinated group, & b incidence rate in controls.

Table 4. Typhoid Fever among the Volunteers and Nonparticipants in a Controlled Field Trial in Pristina, Yugoslavia.*

Type of Vaccine	Cases of Typhoid	No. of Persons	Rate/1000	Effectiveness (%)
K	13	3,346	3.9	70
L	20	3,386	5.9	53
Control	43	3,340	12.9	0
Total volunteers	76	10,072	7.5	
Nonparticipants	777	9,500	81.8	

*Modified from data of Yugoslavia Typhoid Commission.

munoglobulin analyses were carried out on a number of the volunteers' serums. No grouping of titers of IgG and IgM and subsequent response to virulent organisms was apparent. Thus, analysis of humoral antibodies failed to show any demonstrable effect in protecting volunteers from illness.

Seventeen volunteers given a single dose of purified Vi vaccine showed definitely less protection than those given vaccines K and L. Only a single injection was given, and perhaps increases in concentration or frequency of administration could improve the results.

These studies showed a correlation with the WHO field trials and suggested that the infecting dose of typhoid bacilli in nature approximates 100,000 organisms, which might result from a waterborne exposure. Vaccine-induced resistance would falter when contaminated foods, which on prolonged incubation would contain huge numbers of organisms per gram, were ingested.

Little is known of the effect of parenterally administered vaccine in stimulating specific local antibody in the intestine. It is in this organ that the initial contact of the pathogenic organisms and host occurs. Vaccine-stimulated resistance most probably affects the progress of infection only after the primary defenses of the intestinal wall have been broached. For this reason it was decided to attempt to stimulate intestinal barriers. Several potential oral vaccines have been available for evaluation. Killed bacteria in the form of keratinized tablets are uniformly employed as vaccines in various European countries. Two types were evaluated for reactivity and immunizing potential: typhoral (Behringwerke), which is made in Germany and consists of 3×10^9 *S. typhosa* and equal numbers of paratyphoid A and B organisms, was prepared by heat and acetone inactivation. A second oral vaccine, Taboral (Swiss Serum Vaccine Institute), is a monovalent preparation consisting of 100×10^9 acetone-killed *S. typhosa* (strain Ty2) per tablet.

Fourfold rises of serum agglutinins did not consistently develop in volunteers given prescribed doses of either Typhoral or Taboral (Typhoral, nine tablets in three days, and Taboral, six tablets per person over three days). When 12 tablets of Taboral were given to 29 volunteers (twice the recommended dose), rises of somatic (O) agglutinins, flagellar (H) agglutinins and Vi antibodies occurred in 20, 25 and 50 per cent, respectively. There were no intestinal or systemic reactions to the vaccine.

Volunteers who ingested 12 tablets of Taboral were given approximately 100,000 virulent *S. typhosa* of the test strain. Clinically detectable typhoid fever developed in 38 per cent of the vaccinated and 54 per cent of the controls. Positive stool isolation of *S. typhosa* was noted in 33 per cent of those vaccinated and 63 per cent of the control volunteers. The number of vaccinated subjects was small, a total of 21. Yet the results suggested a diminution of multiplication of typhoid bacilli in the intestinal tract after oral vaccination with large doses of killed organisms. There was no correlation between serum agglutinin titers and clinical response to infection in the vaccine group.

Six tablets of Taboral gave no protection when such vaccinated subjects were given 100,000 pathogenic typhoid bacilli. Attack rates approximating 40 per cent were noted in these volunteers and nonvaccinated controls.

These killed vaccine preparations did not appear to induce a level of immunity comparable to that after vaccine given parenterally. However, illnesses in control volunteers in these oral vaccine trials were more frequent than observed in earlier studies. Perhaps a lesser challenge would have

Table 5. Occurrence of Typhoid Fever in Vaccinated Volunteers after Graded Challenges with *S. Typhosa*.*

Vaccine	10^9 Organisms		10^7 Organisms		10^5 Organisms	
	No. challenged	No. with disease	No. challenged	No. with disease	No. challenged	No. with disease
K	3	2 (67%)	28	12 (43%)	43	4 (9%)†
L	4	3 (75%)	24	13 (54%)	45	3 (7%)†
Vi	7	6 (86%)	14	10 (71%)	17	3 (18%)
None	4	4 (100%)	30	15 (50%)	104	28 (24%)

**S. typhi* Quailes strain.

†Chi-square test: K versus control, $p<0.05$; L versus control, $p<0.02$; all vaccinees, including Vi, $p<0.01$.

demonstrated a more marked beneficial effect. The finding that smaller numbers of volunteers showed positive stool cultures in the large-dose Taboral group suggested that local immunity may have developed consequent to the massive antigenic stimulus.

The above investigations, as well as information obtained from evaluations of living shigella vaccines, suggested that repeated doses of an attenuated strain of *S. typhosa* might lead to effective resistance at the portal of entry. Reitman[67] selected, by antibiotic stress, a streptomycin-dependent mutant strain, S-27, of *S. typhosa* that was an effective vaccine in mice. Large doses (10^9) of this strain were ingested by volunteers simultaneously with streptomycin given orally. Multiplication was confirmed by repeated isolation of S-27 strain from stools (with the use of streptomycin-containing medium) for the seven days during which streptomycin was given. Other volunteers given similar numbers of organisms without streptomycin had no fecal cultures positive for strain S-27. This strain contains Vi antigen, but the one-step mutation creating streptomycin dependency led to complete avirulence. No evidence was found that intestinal penetration occurred. Blood cultures and serologic studies were negative. Challenge of these vaccinated subjects with an ID_{50} dose of the virulent Quailes strain resulted in an attack rate comparable to that of the controls. Subsequent studies have employed multiple (four to six) huge doses (10^{11}) of S-27 administered concurrently with streptomycin and sodium bicarbonate. No reactions to this combination have been noted, further attesting to the lack of human virulence of S-27. Evidence of humoral antibody stimulation was confirmed in some of the men. Fourfold rises in H, O and Vi antibodies were found in 42, 6 and 9 per cent, respectively.

Preliminary challenge studies conducted in 30 of these orally immunized volunteers yielded evidence of protection from disease. In addition, a striking difference was noted in the number of positive stool cultures obtained in the vaccinated versus the control populations. There was a significant reduction in the former, most resistant subjects having a single or at most two positive cultures during a six-week follow-up period. Most controls had repeatedly positive stool cultures. Thus, it appears that this attenuated strain can enhance local immunity of the intestinal tract and effectively prevent typhoid bacilli from entering a site in which to multiply. Perhaps we have acclimatized the unsophisticated intestinal tract to typhoid fever through the use of multiple exposures to an attenuated strain. Additional studies are needed to confirm this finding. It is conceivable that similar approaches could be used to prevent other bacterial enteric diseases. Indeed, the shigella studies alluded to previously[16] have already progressed to a level of achievement that seems practical for the prevention of dysentery.

Measurement of local antibodies in the gut of patients with typhoid fever has so far been unsuccessful. Although IgA can be found, it has not been possible to prove its antityphoid activity. Analysis of cellular immunity of the intestinal lining appears to be inaccessible to determination in man at present. This area of research appears to be most important to ascertain the role of this primary line of defense in enteric infections.

The conditions attendant to volunteer studies as outlined in the Declaration of Helsinki were adhered to in these investigations. Initial peer review and approval for these studies was given unanimously by members of the Armed Forces Epidemiological Board. Subsequently, annual reviews were performed by the various commissions of the Board. Intramural approval was achieved by peer-group review. Since the Public Health Service requirement in 1966 for the establishment of volunteer committees, protocols have been approved by this committee at the University of Maryland School of Medicine.

Volunteers in these studies were informed inmates at the Maryland House of Correction. Each was asked to participate after having the nature of the study explained. No coercion was used, and each man was free to withdraw at any time. The willingness of these men to volunteer for subsequent studies and their repeated requests to enter these and similar infectious disease studies during the past 10 years attests to the acceptability of the program. This excellent co-operation is greatly appreciated and has been publicly acknowledged. The officials of the Maryland Prison System have been extremely helpful in the conduct of the study. Many fellows, nurses, technicians and secretaries have given invaluable assistance, and their aid is gratefully acknowledged.

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MEDICAL INTELLIGENCE

HYPERNATREMIA COMPLICATING PARTIAL URINARY-TRACT OBSTRUCTION*

LEWIS LANDSBERG, M.D.

IT is well known that the ability of the kidneys to concentrate urine is impaired by obstruction of the urinary tract.[1-3] Nephrogenic diabetes insipidus[4-7] has been shown to occur both with partial urinary-tract obstruction and in the course of postobstructive diuresis after relief of complete urinary-tract obstruction. That severe hypernatremia and hyperosmolar dehydration may occur in certain patients with partial urinary-tract obstruction, however, is not generally appreciated. Thus, a recent review of hypernatremia[8] and two standard textbook chapters[9,10] on obstructive uropathy do not describe this phenomenon. The course of two illustrative patients seen over the past five years with severe hypernatremia secondary to partial obstruction of the lower urinary tract is detailed below.

*From the Department of Internal Medicine, Yale University School of Medicine and the Yale-New Haven Hospital (address reprint requests to Dr. Landsberg at the Department of Internal Medicine, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. 06510).

CASE REPORTS

CASE 1. M.F. (Y.N.H.H. 60-48-13), a 58-year-old man, was admitted to the hospital with a 1-week history of lethargy, confusion and ataxia. For 3 months before admission there had been progressive urinary frequency, nocturia and incontinence. He had been anorectic and had lost 11 kg in weight. On physical examination the pulse was 95, the blood pressure 150/95, and the temperature 98.6°F. He was drowsy but arousable and oriented. The skin and mucous membranes were dry. The chest was clear, and the heart was normal. The bladder was palpable at the umbilicus. There was tenderness at the costovertebral angles. The prostate was slightly enlarged. There were no focal neurologic signs. Admission laboratory data are shown in Table 1. The striking findings were azotemia (blood urea nitrogen of 110 mg/100 ml) and marked hypernatremia (serum sodium of 183 mEq per liter).

A urethral catheter was inserted, and the bladder was slowly decompressed. He excreted 11 liters of urine over the 1st 48 hours while receiving 20 liters intravenously, at least half of which was 5 per cent dextrose in water. By the 3d