MEDICAL PROGRESS

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

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TYPHOID fever is a disease unique to man. No other animal species acquires an illness simulating typhoid fever after oral ingestion of the organism. This lack of a suitable experimental animal has hindered the acquisition of knowledge on pathogenesis and control. This presentation will summarize the experience gained in the study of healthy volunteers infected with typhoid bacilli. The purpose of these ongoing investigations has been to develop better methods of prevention and control of disease. The primary objective has been the quantitative evaluation of experimental and conventional vaccines. Such studies have permitted a careful analysis of pathogenesis.

Typhoid vaccine is one of the oldest bacterial vaccines employed in man. In 1896 Wright[1] injected killed typhoid bacilli into two Indian medical officers. Subsequently, one of these volunteers was inoculated with viable *Salmonella typhosa*. The vaccine was assumed to be protective since illness did not occur. This experiment was carried out 16 years after Eberth[2] had described the bacterial rods in histologic sections of mesenteric lymph nodes and spleen, and 11 years after the causative organism was isolated from stool specimens by Pfeiffer.[3] Wright's studies were based upon the concept that protective antibodies could be induced in man by typhoid bacilli. Pfeiffer and Kolle[4] had shown that serum of patients who had recovered from typhoid fever protected guinea pigs against lethal doses of typhoid bacilli. These "protective bodies" were demonstrated in man 11 days after killed bacteria were inoculated. In 1896 Gruber and Durham[5] reported that serum from immunized guinea pigs when mixed with bacteria caused them to "stick together in large balls" and lose their motility. These substances were called agglutinins. In the same year, Widal[6] added serum from patients with typhoid fever to broth cultures of *Salmonella typhosa (S. typhi)* using serial dilutions, and reported gross flocculation after 24 hours' incubation. The stage was finally set for attempted control by vaccination. Wright's initial study began an intensive investigation lasting for nearly 60 years.

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SUSCEPTIBILITY TO TYPHOID FEVER

Susceptibility to typhoid fever may be conditioned by the nature of the environment in which man lives. In many areas of the world today (for instance, Chile and Egypt), the highest incidence of typhoid fever is found in children. In these locales, it may be assumed that the first few exposures to typhoid organisms usually result in infection, and in a few persons disease develops. Adults, on the other hand, having had frequent encounters with these micro-organisms (mild, self-limiting infections), have developed substantial resistance. Other countries present less opportunity to acquire repeated subclinical infections and have populations susceptible at all ages. Thus, in the United States, scattered epidemics follow food contamination by a carrier, and all exposed are at risk. This hypothesis has been promulgated by several authorities.[7-9]

In the studies reported below, the population studied presumably represents a highly susceptible group, not comparable to adults residing in other areas of the world where typhoid is more common. The volunteers have an unsophisticated intestinal tract in terms of experience with typhoid bacilli. Their reactions to various numbers and strains of typhoid organisms would be representative of those of persons residing in countries with a low incidence of typhoid fever. However, the results obtained in these men in terms of susceptibility to infection and levels of induced immunity may have added meaning because of this lack of previously acquired resistance to typhoid. Vaccine trials conducted in endemic areas measure the additive effect of vaccine immunoprophylaxis upon the background level of acquired resistance. Under these circumstances, such studies might be expected to provide various results, depending on the extent of this background immunity.

The administration of known numbers of a well characterized bacterial strain to persons with a known vaccine history allows for precise determinations of acquired resistance. Unfortunately, in nature, the virulence and number of ingested organisms are unknown, and in a specific person, the state of resistance at the time of exposure cannot be determined. Volunteers were selected only after they were identified as healthy and thus could be expected to have the general nonspecific attributes that aid resistance to disease. It is in such healthy, informed volunteers that information regarding path-

ogenesis and resistance to typhoid fever has been gathered.

Nature of Induced Disease

Clinical Description

A pathogenic strain of *S. typhosa* (Quailes strain), obtained from a carrier, was used to induce infection; the exact number of viable organisms required to infect, and their antigenic content, mouse pathogenicity and time of infection were known. This strain, which possesses appreciable amounts of the Vi (envelope) antigen, was propagated on solid and liquid mediums and harvested approximately six hours after 37°C incubation. It was suspended in 30 ml of milk and administered by gargling and swallowing. The characteristics of this strain have been well studied, and its virulence in relation to other classic typhoid strains has been defined. This information is presented in a subsequent section.

The symptoms and signs associated with the illness resulting in volunteers were identical to those observed in patients with naturally acquired typhoid fever. Fever was the earliest indication of disease, rising over a period of two to three days in a stepwise fashion. Headache and abdominal pain occurred shortly thereafter. Tenderness to palpation in the lower abdominal quadrants, with associated sensation of displacing, under the palpating fingers, loops of bowel filled with air and fluid, was a pathognomonic physical sign. Subsequently, anorexia, myalgia and fatigue occurred. Chills and sweats were uncommon; the latter appeared only after antibiotic therapy was started. Herpes-simplex infection was not observed. Chloramphenicol treatment always aborted illness. Figure 1 presents the graphic record of a characteristic typhoid infection in a volunteer; seven days after oral challenge bacteremia was documented. Two days later, malaise, headache, anorexia and fever developed. In several days, the temperature reached 103.5°F, and toxemia characteristic of typhoid was present. Headache and other toxic signs abated in 24 hours, and defervescence was complete approximately 3.5 days after chloramphenicol treatment was begun. Typhoid bacilli were noted in the feces on the first two days after pathogen ingestion. Subsequent stool specimens failed to reveal typhoid bacilli. The highest typhoid "O" titer was noted on the 15th and 17th days.

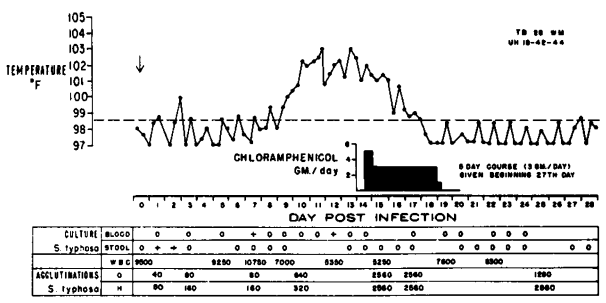

Figure 1. Summary of the Clinical Course of Induced Typhoid Fever in a Volunteer.

Therapy consisted of two courses of chloramphenicol, the first given for five days; after a week without specific treatment, chloramphenicol was administered for an additional five-day period.

Once illness occurred, the clinical courses were comparable regardless of the dose of the infectious inoculum. The median incubation period varied inversely with the size of inoculum. However, within each group incubation periods varied greatly (Table 1).

Table 1. Relation of Dosage of *S. typhosa* — Quailes Strain — to Disease.

No. of Viable *S. Typhosa*	Total Volunteers Challenged	No. with Disease	Incubation Periods—Days	
			Median	Range
10^9	42	40 (95%)	5	3-32
10^8	9	8 (89%)		
10^7	32	16 (50%)	7.5	4-56
10^5	116	32 (28%)	9	6-33
10^3	14	0 (—)		

Clinical manifestations were arrested by chloramphenicol administration; usually, the volunteers were afebrile by the third to the fifth day of therapy. A standard protocol was established to estimate vaccine effect. Thus, antibiotic treatment was begun when the temperature reached 103°F or higher, by mouth, and persisted at that level for 24 to 36 hours. The majority of patients in whom disease developed reacted in this rather uniform fashion. A minority were treated when their temperature remained under 103°F for longer than five days and their clinical condition warranted therapy. During the course of these studies, 250 men received antibiotic treatment (215 with chloramphenicol) for typhoid fever. Two cases of moderate hemolytic anemia occurred before chloramphenicol was given. In several volunteers temporary episodes of confusion developed. There have been no permanent typhoid carriers.

Dose-Response Data

The development of typhoid in man after various doses of bacilli was determined to establish a meaningful challenge dose for immunized volunteers. Attack rates for disease were determined when the illness consisted of toxic symptoms and signs such as headache, malaise, anorexia and a temperature of 103°F or higher by mouth for at least 24 to 36 hours. At this point specific treatment was initiated. The infectious dose (ID) of *S. typhosa* (Quailes strain) for healthy volunteers is shown in Table 1. The ID_{50} is about 10^7 (10,000,000) bacilli as compared with a rate of approximately 95 per cent in volunteers ingesting 10^9 organisms. The most commonly

employed dose, 100,000 *S. typhosa*, caused disease in 28 per cent of the volunteers, whereas the smallest dose employed, 1000 cells, failed to induce disease in any of the 14 volunteers. Little information is available to compare these results with numbers of organisms causing naturally acquired disease. Most data have been acquired after the incubation period was passed and the first few cases of disease have occurred. The number of organisms actually ingested in contaminated water or food is conjectural. If the incubation periods are well documented, as in the Zermatt epidemic of 1963,[10] it is possible to compare attack rates in the naturally acquired cases with our present volunteer data and thereby to estimate the dose of organisms involved. Indeed, in Zermatt, it appears that certain groups of tourists (that is, those with well documented incubation periods) probably ingested less than 100,000 typhoid bacilli. This inference seems justified, since the virulence of one strain isolated from patients in the epidemic is comparable to that of the Quailes strain (as discussed below).

Characterization of the Intestinal Phase of Infection

Bacteriologic studies revealed that bacilli multiplied rapidly in the intestinal tract. Stools yielded *S. typhosa* within 24 hours after ingestion and were often positive during the incubation period. Continued culturing of fecal specimens in the first days of illness was rarely rewarding. During the second week, usually when the patient had recovered, cultures of the stools again revealed typhoid bacilli. Their appearance was not directly affected by chloramphenicol administration.

In volunteers given 10^5 to 10^7 organisms, the presence of *S. typhi* in the stools for the first five days after challenge did not necessarily mean that they would become ill. However, such evidence of early intraintestinal multiplication did increase the likelihood of subsequent disease. Thus, in the first few days of interaction between host and bacteria, the initial evidence of bacterial multiplication in the gut is not a definite indication of failing host defense. Many volunteers, especially in the groups given the lower infectious dose, were able to contain the infection in the intestinal tract, and overt disease did not materialize. In fact, volunteers in whom disease failed to develop (for example, those exposed to the ID_{50} dose) frequently shed typhoid bacilli in the stools for several weeks. Moreover, the pattern of excretion remained similar to that of the subjects who became ill. In both groups during the second week after challenge, the frequency of positive stool cultures reached a peak, continued at that level for about three more weeks and then rapidly disappeared, with few positive stools six weeks after challenge. Since chloramphenicol was employed in the overtly ill group, it was apparent that this antibiotic did not shorten the period of multiplication or residence of typhoid bacilli in the gut.

On the other hand, lack of evidence of bacterial multiplication in the gut did not exclude occurrence of illness. Volunteers did acquire typhoid fever without evidence that organisms were shed during the incubation period. In these men, it seems likely that rapid penetration by appreciable numbers of ingested typhoid bacilli into the intracellular environment without detectable intraluminal multiplication was sufficient to cause subsequent disease. Sprinz[11] has contrasted the events caused by various enteric pathogens that may lead to an enteric infection. Each of the major agents, such as *Vibrio cholerae*, shigella and salmonella, is unique in its manner of producing enteritis. Cholera vibrios appear to cause diarrhea by altering the permeability of the epithelium by means of a toxin. No penetration by the organisms occurs. Pathogenic shigella, to cause disease, must invade and multiply in the lining cells. This process results in necrosis and intense inflammation and finally small abscess formation. Salmonellae are readily transported through the intestinal epithelial lining and do not cause as gross injury as shigellae. There is little evidence of multiplication in the epithelial cells. Gerichter[12] demonstrated remarkable dissemination of typhoid bacilli in mice after oral challenge. In these animals, typhoid bacilli were isolated from the blood in 20 seconds and from the spleen and liver shortly thereafter. The rapidity and site of penetration of the intestinal epithelial lining in man is unknown. In volunteers it was possible to rule out the pharynx as a noteworthy portal of entry by having participants expectorate the gargled suspension (10^9); in no volunteer was disease initiated. When chimpanzees were challenged orally,[13] salmonellae readily penetrated the epithelium of the gut without causing appreciable inflammation. It was at the next level of defense, the mesenteric lymph nodes, that an obstruction to further spread occurred. Chimpanzees[14] have also been inoculated intravenously and directly into mesenteric lymph nodes. Some animals showed fecal excretion, but typhoid bacilli were consistently identified in lymph tissue such as the tonsils and mesenteric lymph nodes several days after infection was initiated. The mesenteric lymph nodes apparently serve to halt spread temporarily, but as a consequence, allow for additional multiplication of typhoid bacilli.

The human stomach, with its marked acidity, is generally regarded as a nonspecific host defense for degradation of swallowed bacteria. Attempts to isolate *S. typhosa* from the gastric secretions of volunteers were successful for as long as 30 minutes after ingestion. This organ may have a substantial role in defense against enteric infections since the number of organisms is reduced through its inhibitory effect. Small doses of sodium bicarbonate given to volunteers before ingestion of shigellae have markedly enhanced the rates of illness and stool isolates.[15] Gastritis is a consequence of shigella infection in

monkeys and in man, suggesting that these organisms can penetrate the gastric mucosa. However, such a condition is rare in typhoid fever. On the other hand, specimens of jejunum obtained by biopsy after infection with *S. typhosa*[16] have shown inflammatory changes. These findings imply that salmonellae entered the mucosa at these sites. These biopsy specimens were obtained late in the incubation period, before demonstrable bacteremia. Conceivably, typhoid bacilli were limited to the draining lymph nodes at that time.

Additional information pertaining to the rapidity with which typhoid bacilli enter cells or find other areas in which to survive hostile environmental factors can be inferred from results of studies designed to test the efficacy of antibiotics to prevent typhoid fever. Four volunteers were given an ID_{95} dose of typhoid bacilli; 24 hours later each was begun on chloramphenicol therapy in doses of 1.0 g three times a day. Two of the men continued this regimen for seven days, and the others for 28 days. None became ill while on therapy. Blood cultures were taken during the late stages of the expected incubation period and periodically thereafter. Despite administration of this effective drug, positive blood cultures were obtained in one of the four, and all showed significant increases of antibody titers while receiving the antibiotic treatment; simultaneously, control subjects were ill and demonstrated similar laboratory findings. In one of the two volunteers treated for seven days chemoprophylactically disease subsequently developed about nine days after the drug was stopped. The men maintained on antibiotic for 28 days failed to have symptoms or signs of illness but did show antibody responses. These data suggest that infecting organisms found an intracellular habitat within 24 hours and therefore were not eliminated by the antibiotic. Tissue-culture studies performed by Showacre et al.[17] demonstrated intracellular persistence of typhoid bacilli despite the presence of antibiotic in the menstruum – that is, "antibiotic indifference"; when the antibiotic was washed out (after 21 days) the organisms multiplied.

Typhoid bacilli are susceptible in vitro to many antibiotics. Several drugs (such as polymyxin and colistin) are markedly bactericidal; yet none cure patients with typhoid fever.[18] Antibiotic control requires a drug like chloramphenicol that perhaps inhibits the growth of the pathogen by effectively altering both cellular and bacterial metabolism through interference with nucleic acid synthesis. The process is slow; patients require 3½ to five days to become afebrile and eradication is incomplete. Stool cultures remain positive for several weeks to months after therapy, and relapses are common (15 to 20 per cent). The unique response of *S. typhosa* to antibiotics in vivo – that is, only to chloramphenicol (somewhat less to ampicillin)[19] – attests to the effective adaptive capabilities of this organism in man. A means of aborting these capabilities would provide an excellent method of control.

Japanese and American investigators[20,21] showed that streptomycin inhibits growth of anaerobes of the genera bacteroides and lactobacillus, each partially responsible for maintaining an acid pH in the intestine of mice through their production of short-chain fatty acids. In the acid medium, salmonellae were inhibited. Thus, in the mouse antibiotic given orally lowered the dose needed to induce disease from 10^6 to less than 10 *S. enteritidis* organisms.

Oral prechallenge of volunteers with streptomycin (no effect on the typhoid bacilli) may have similarly influenced intestinal defense mechanisms. Whereas clinically detectable typhoid fever did not occur in 14 volunteers given 1000 viable typhoid bacilli, one of four treated volunteers became clinically ill. Concentrations of short-chain fatty acids change during some enteric diseases, but their role in protecting the large intestine from colonization has not yet been defined.

Typhoid Carriers

A striking example of intestinal resistance to infection with *S. typhosa* is the typhoid carrier. These people serve as the reservoir of typhoid fever. Women exceed men as carriers by a ratio of 3:1. There may be as many as 10^{11} virulent typhoid organisms per gram of feces. These organisms reside in scarred foci of the biliary tree, migrate through the bile ducts and over the vast surface area of intestinal epithelium, and yet they do not cause typhoid fever in the carrier. Frequently, antibody levels are not elevated, and in a few carriers tested, tolerance to endotoxin is not apparent. Thus, these virulent organisms are excluded from the host presumably by local humoral or cellular immune mechanisms. An improved understanding of this remarkable example of microbial persistence and the associated local defenses may provide leads to better control measures against enteric infections. One interesting and as yet unconfirmed observation was recently reported by Chernokhvostova et al.[22] They noted that typhoid carriers were deficient in IgM and did not respond to Vi antigen administration with any IgM-type antibodies. This information suggests that carriers are selected because of their deficiency of IgM, which is very active in promoting bactericidal activity in serum. The obvious anatomic alterations in the biliary tract are very important in the production of the carrier state. A deficiency of IgM and impaired drainage or scarring of the biliary tree could produce an environment conducive to prolonged bacterial persistence.

Characterization of Virulence Factors in *S. typhosa*

In addition to the numbers of organisms ingested, the antigenic makeup is another determinant in the virulence potential of a particular strain. Sorting the

pathogenic role of each antigen in causing disease delineates the factors that need to be inhibited to prevent disease. This information could lead to better vaccines. The data cited above for the infectious dose of S. *typhosa* for man were obtained with one strain that contained Vi antigen. It caused typhoid fever and was isolated from a carrier. Other strains with differing antigenic makeup were studied for their human pathogenicity (Table 2). The Zermatt strain obtained from Dr. Vischer, of Basel, Switzerland, is one of the strains isolated during the epidemic occurring in Zermatt in 1963. This strain contains Vi antigen and is highly virulent for mice by intraperitoneal injection. Vaccines have been derived from the classic Ty2V strain, isolated by Felix from a patient in a small Russian village in 1915.[23] A strain that lacked Vi antigen was derived from Ty2V cultures and called Ty2W.[23] Absence of Vi antigen renders this strain less pathogenic for mice. Strain 0-901, which was obtained by Felix in the same locale as Ty2V,[23] lacks both Vi and H antigens. Mouse virulence is of the same order for the 0-901 as Ty2W – both significantly less than Ty2V. These strains not only differed in their antigenic makeup but had an additional variable of being long established laboratory cultures, far removed from viable human tissue.

Table 2. Virulence of Certain Strains of Typhoid Bacilli for Man (Effect of Vi Antigen on Virulence, with a Challenge Dose of Approximately 1×10^7 Organisms).

Strain	LD_{50} Mouse Virulence*	Human Virulence		
		Disease†	Infection‡	No infection§
Quailes	2.8×10^7	16 of 30	12 of 30	2 of 30
Zermatt	3.0×10^4	6 of 11	4 of 11	1 of 11
Ty2V	3.0×10^6	2 of 6	3 of 6	1 of 6
Totals (Vi strains)		24 of 47 (51%)¶	19 of 47 (40%)	4 of 47 (9%)
0-901	3.11×10^8	6 of 20	6 of 20	8 of 20
Ty2W	1.9×10^8	4 of 19	10 of 19	5 of 19
Totals (non-Vi strains)		10 of 39 (26%)¶	16 of 39 (41%)	13 of 39 (33%)

*Mouse virulence evaluated by intraperitoneal inoculation of organisms in saline; Zermatt-strain virulence determined with organisms in gastric mucin.

†Temperature of 103°F or higher for >36 hr & treatment with antibiotic.

‡Low-grade fever or significant serological response, or positive blood culture or excretion of *S. typhosa* in stools for >5 days & no specific therapy.

§No clinical, cultural or serologic evidence.

¶Chi-square test difference between incidence of disease caused by Vi & non-Vi strains significant ($p < 0.05$).

After a dose of 10,000,000 organisms, disease rates were significantly higher in volunteers who ingested Vi-containing strains than with non-Vi strains: 51 versus 26 per cent (p less than 0.05). Vi antigen thus appeared to be an important determinant of human virulence for typhoid bacilli. This was thought to be a nontoxic antigen. Its virulence-enhancing effect in animals was related to its role as an envelope antigen that protects "O" antigen from "O" antiserum. In this manner it interfered with bactericidal activity in serum.[23] Also, Vi antigen may be a deterrent to phagocytosis. Similar roles in man may be inferred from the data in these studies.

In most volunteers infected with Vi-containing strains positive blood cultures developed at the onset of fever and persisted until therapy was initiated. Attempts were made in certain groups of volunteers (mainly in those receiving large inocula) to determine the presence of asymptomatic bacteremia. In two of 64 patients this condition was documented.[24] In one volunteer, bacteremia occurred for 10 days; there was no overt clinical disease and no fever. He was tested for pyrogenic tolerance to endotoxin and did manifest the expected febrile response after intravenous endotoxin. The tissue source for these bacteria – that is, Peyer's patches, mesenteric lymph nodes, liver and so forth – can only be surmised; however, the lesions had to be self-limiting. Reasons for the apyrexia are not known. Recent unpublished observations[25] have amply demonstrated prolonged asymptomatic bacteremia in Egyptian patients with *Schistosoma hematobium* infestation of the genitourinary system and typhoid infection. These patients have severe anemias that serve as a clue to the double infection. Clearing the host of schistosoma usually eliminates the typhoid-carrier state. The salmonellae appeared to be limited to the genitourinary tract in the scarred areas created by the schistosomal disease. These patients did not excrete typhoid bacilli in the stools but only in the urine. It is unknown whether such patients were tolerant to endotoxin. It is apparent that typhoid bacilli can circulate in the blood without causing any apparent host reaction.

At the other end of the bacteremic spectrum were the volunteers infected with non-Vi-containing strains of typhoid bacilli. Attempts to isolate typhoid bacilli were successful in 40 per cent from the blood or feces in contrast to over 80 per cent of volunteers infected with fully virulent strains (Vi antigen containing). The clinical illness of these volunteers infected with non-Vi strains was typical of enteric fever; host ability to phagocytize such strains probably accounted for fewer positive cultures. Serologic data, on the other hand, were of

more help in confirming these cases. Conceivably, persons infected naturally with similar, less virulent strains may be seen with obscure fever and negative blood and fecal cultures.

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MEDICAL INTELLIGENCE

CURRENT CONCEPTS

Warfarin Therapy (First of Two Parts)*

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THE coumarin anticoagulants are widely used in the management of thromboembolic vascular disease. Of the coumarin agents, sodium warfarin is the most frequently employed in the United States. Recent studies have advanced our understanding of the pharmacology of warfarin and allow a reassessment of current practice in the initiation and maintenance of warfarin therapy.

Warfarin is a water-soluble derivative of coumaric acid. When given by mouth, it is absorbed completely from the small intestine. It is transported in the blood, loosely bound to albumin. Its distribution in the body and in body fluids, therefore, is essentially the "albumin space." However, warfarin will cross the placenta, and it does appear in milk. Warfarin is degraded in the liver by enzymes located on the endoplasmic reticulum. The degradation products of warfarin are also bound to albumin and are excreted in the urine. No undegraded warfarin is excreted in the urine. Although the half-time of disappearance from the blood of an orally administered dose of the drug is a result of a balance between the rate of absorption and the rate of degradation, it is primarily a function of the rate of degradation. The rate of disappearance of warfarin remains constant, but there is wide variation in its rate of disappearance from the blood in different persons. Thus, in a series of normal subjects given a standard oral dose of warfarin, the average half-time of disappearance of the drug was 44 hours, but the range of values extended from 15 to 56 hours.[1]

The therapeutic effect of warfarin is a function of its ability to inhibit the action of vitamin K, a fat-soluble vitamin. There are two sources of vitamin K: the diet and the intestinal bacterial flora. There is disagreement over the relative importance of each of these sources, and the available data do not allow an unambiguous statement that the bacterial flora is the sole or even the most important source of vita-

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