STUDIES ON INFECTION AND IMMUNITY IN EXPERIMENTAL TYPHOID FEVER

I. Typhoid Fever in Chimpanzees Orally Infected with Salmonella typhosa

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Plates 6 and 7

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Few infectious diseases of man have lent themselves to experimental study and reproduction in the laboratory animal with the classic simplicity and clarity that underlay the pioneer experiments of Pasteur and Koch on anthrax, rabies, and tuberculosis. Thus it was perhaps not too difficult for Koch to lay down his famous postulates; but it has not always been easy to follow them. In the case of typhoid fever—which has been the subject of our particular attention—the paucity of successful attempts to reproduce the disease has been striking in view of the 80 years that have passed since Klebs and Eberth first observed the typhoid bacillus. Although many experimental patterns, involving a variety of experimental animals, have been employed in laboratory studies of the pathogenic behavior of *Salmonella typhosa*, few investigators have attempted to reproduce the essential features of infection with this organism as observed in man: infection *via* the alimentary tract, invasion of the blood stream, and a marked, extensive and sustained enteritis.

Fränkel and Simmonds (1), and later Remlinger (2) were apparently the first to claim success in the production of oral infection in laboratory animals with *S. typhosa*. In both rabbits and mice, ingestion of typhoid-contaminated food after 2 or 3 days of fasting was followed by enteritis, severe and frequently fatal septicemia, and engorgement or ulceration of the Peyer's patches (2). Grünbaum (3), by feeding typhoid cultures or infected stools to 4 chimpanzees, produced malaise, diarrhea, fever, a rising or positive Widal reaction, and

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characteristic changes in the Peyer's patches, but did not describe his procedures and results in full detail.

A few years later Metchnikoff and Besredka (4) undertook more extensive studies. They reported experiments in 14 chimpanzees, 3 of which were fed pure cultures of *S. typhosa*, the other 11 receiving mixtures of contaminated feces and cultures or else simply fecal material. Fever, enteritis, enlarged Peyer's patches, positive blood cultures and a rising agglutinin titer were observed in most of their chimpanzees, although not all such findings were checked regularly in every animal. They also produced essentially similar findings in one gibbon. In contrast, out of 10 guinea pigs, 18 young rabbits, 6 adult rabbits, and 51 monkeys of four different species, only two *cynomolgus* monkeys showed any sign of infection following ingestion of typhoid bacilli. Grünbaum had previously obtained similarly negative results with *Macacus rhesus* monkeys. In a subsequent study (5), Metchnikoff and Besredka challenged 10 additional chimpanzees, 7 of which were subjected to procedures calculated to provide some degree of immunity. Six of the ten animals developed fever and typhoid bacteriemia 6 to 8 days following challenge, with supporting findings similar to those observed in the previous experiments.

Except for two reported successes in producing infection with *S. typhosa* by the oral route in mice (6, 7), the matter has rested here since the work of Metchnikoff and Besredka. In reporting their studies on infection by the oral route, Metchnikoff and Besredka concluded that pathogenesis and immunity in typhoid fever could only be studied effectively in experiments wherein the route of infection, the general clinical course and the pathological alterations induced resembled those seen in typhoid fever. They remarked—tartly but correctly—that "Entre la péritonite typhique du cobaye[1] et la fièvre typhoïde de l'homme, il n'y a de commun que le nom du microbe" (4). Nevertheless, experimental infection with *S. typhosa* has, during the past 50 years, been virtually confined to intraperitoneal inoculation of the guinea pig and, in recent years, the mouse. There is no doubt that the many small-animal studies (review in reference 8, for example), including the ingenious variations of intracerebral inoculation (9–11) and of inoculation into the mesenteric lymph nodes (12), have contributed greatly to the characterization of the typhoid bacillus, its antigenic composition, the relation between antigenic components and invasiveness following parenteral inoculation, and the immune response in animals and man. But during this period the investigative emphasis was not on the problems of the portal of entry, the distribution of organisms in the host, the nature of the basic immune response, and other fundamental problems in the experimental pathology of oral infection with *S. typhosa*. Instead, the

[1] The guinea pig was the usual small animal employed in laboratory experiments with *S. typhosa* 50 years ago.

emphasis was on relatively more accessible and more readily isolated problems amenable to conventional microbiological, immunological, and histopathological techniques.

It is therefore no matter of surprise that, despite the 60 years that have passed since typhoid vaccination was first practiced in man, there is still no general agreement on the essential antigenic components that must be present in a typhoid vaccine, on the manner in which the vaccine should be prepared, or on the way in which its acceptability could best be measured. The demonstration of the "Vi" antigen in certain strains of *S. typhosa* (13), and the correlation of the presence of this antigen with high virulence for mice, effective immunization of mice, and perhaps with severity of infection in man (14), have been regarded as indicating that the Vi antigen is of primary importance in human immunization. A corollary conclusion was that the widely used heat-killed phenol-preserved type of typhoid vaccine, which usually appears to possess rather low Vi antigenicity, would be relatively ineffective, and that it should be replaced by an alcohol or acetone-killed vaccine which normally was richer in Vi antigen (15, 16). This conclusion did not, however, appear to be clearly supported by such field observations as were available, there being massive (though statistically uncontrolled) evidence that considerable reduction in the incidence of typhoid fever has been achieved by the use of the phenol-type vaccine (17). On the other hand, recent experiences in the British Armed Forces in the Middle East (18) re-emphasized the complexity of the problem, inasmuch as recovery from typhoid fever itself did not appear to produce significant immunity to the disease as seen in that area.

Meanwhile, typhoid fever has remained a serious public health problem throughout most of the world, causing probably well over a million attacks of illness in man per year. It was apparent that further progress in the understanding and control of the disease would be contingent on a better understanding of its pathogenesis and the factors involved in acquired resistance to the infection. Since chimpanzees appeared to afford the most promising available pathway for such studies, we undertook to reproduce and more sharply delineate the disease in these animals; to define the pathological changes resulting from oral infection with *S. typhosa;* and to observe and characterize the immune response and resistance to infection achieved by various anti-typhoid vaccines, by the major antigenic components of the typhoid bacillus, and by recovery from clinical or subclinical infection with *S. typhosa.* Preliminary reports of these studies have appeared elsewhere (19–25). The present communication provides detailed findings on the establishment and course of typhoid fever in the chimpanzee. Subsequent papers will be concerned with immunity to typhoid fever and a more complete description of the pathological picture as seen in this animal.

Materials and Methods

Experimental Animals.—Young Congo and West African chimpanzees of both sexes, ranging in weight from 10 to 30 pounds, were employed throughout this study, after examination of several stool cultures had shown them to be free of Group D *Salmonellae.*

Challenge Organisms.—The well characterized Ty2 strain of *Salmonella typhosa*, phage type E_1, was selected for the basic experiments because of its high mouse virulence and inagglutinability by typhoid O antiserum. This culture has shown extreme stability in maintaining antigenic composition and mouse virulence for over two decades of laboratory handling (26), and was furnished to us by the late Dr. A. Felix, Lister Institute for Medical Research, several years ago. A second, more recently isolated culture, designated as strain 2593, was selected for comparative studies. This culture had been isolated late in 1955 from a carrier responsible for a case of typhoid fever. It was obtained from the laboratory of Dr. H. J. Shaughnessy of the Illinois State Department of Health, where it was found to be phage type T. Preliminary experiments in our laboratory established the infectivity of this strain for chimpanzees and also demonstrated that it displayed the same degree of O inagglutinability and mouse virulence as Ty2.

Preparation of Challenge.—The first experiment was carried out with a culture obtained from a stock ampule of lyophilized strain Ty2. All subsequent experiments with this strain were carried out with stab cultures derived directly from tissues of infected chimpanzees and maintained at room temperature in the laboratory until use. With strain 2593 a fresh ampule was employed for each experiment. A suspension of the contents of an ampule, or a loopful of inoculum from a stab culture, was streaked on meat extract agar plates. After overnight incubation at 37°C., the plates were examined for the presence of smooth, iridescent colonies, typical of Vi-containing typhoid bacilli (27). Such colonies were streaked on additional meat extract agar plates which were incubated at 37°C. for 12 hours. The growth on each plate was checked microscopically as before and then suspended in 3 ml. of veal infusion broth which was then added to a Kolle flask containing veal infusion agar. Flasks were incubated at 37°C. in an inverted position to avoid excess inoculum on the agar surface. After 4½ to 5½ hours, growth in each flask was harvested with 5 ml. sterile saline by means of a metal rake, and the harvests were combined. Concentration of this harvest pool was determined spectrophotometrically and appropriate dilutions were made for chimpanzee challenge, for determination of mouse LD_{50}, and for serological examination. The estimated number of bacilli in the harvest pool was checked by viable counts, using the usual pour plate procedure.

Mouse Virulence.—Groups of 20 mice were injected intraperitoneally with 5-fold increments of the challenge preparation suspended in saline. Mortality

was observed over a 72 hour period and the LD_{50} of the preparation was determined graphically on probit paper.

Serological Examination of the Challenge Culture.—The presence of Vi antigen and the O inagglutinability of each challenge culture was checked by a slight modification of the method described by Felix and Pitt (13, 26), employing O and Vi antisera obtained from rabbits immunized with *S. typhosa* 0-901 and *Paracolobactrum ballerup* respectively.

Bacteriologic Procedures.—Stool and other specimens except blood were cultured regularly on SS agar and occasionally on MacConkey and Wilson-Blair bismuth sulfite agar. Non-lactose fermenting colonies were transferred to Kligler's iron agar (KIA) slants which were observed subsequently for biochemical changes. Typical or suspected KIA cultures were then examined by the slide method for Vi agglutination and O inagglutinability. From time to time, confirmatory checks of these cultures were made by more extensive biochemical and serological procedures. The general effectiveness of this method in isolating small numbers of *S. typhosa* was confirmed by the examination of chimpanzee stool specimens previously inoculated with typhoid bacilli. It is of interest to note, however, that an occasional chimpanzee stool inhibited the growth of added *S. typhosa* so that these organisms could not be recovered after 30 minutes' incubation at room temperature. Stools from the same chimpanzee were not always inhibitory.

Blood samples were cultured by inoculating 2 to 4 ml. of freshly drawn blood into 40 ml. of trypticase soy broth containing 0.1 per cent agar and 0.1 per cent sodium citrate. Subcultures to SS agar were made as soon as there was evidence of growth, or after 7 days of incubation even when growth was not apparent. Typical or suspected colonies were treated in the same maner as those from stool specimens. Blood cultures were considered negative if growth of *S. typhosa* failed to occur within 7 days.

Serological Examination of Chimpanzee Sera.—Prior to and during the course of experiments, O and H antibody levels in chimpanzee sera were determined by the usual agglutination test procedures employed by the Department of the Army (28), while Vi antibody was measured by the hemagglutination technique described by Landy and Lamb (29).

Challenge Procedures.—By means of a 2 ml. Luer lock syringe and a 4 inch, 22 gauge needle, the desired quantity of challenge culture for each animal was injected into a whole unbroken banana. Immediately afterward, each chimpanzee to be infected was presented with an inoculated banana, and since the animals had been fed sparingly for 24 hours prior to challange, the bananas were entirely consumed within a few minutes, usually 5 minutes or less. One hour later the animals were returned to their regular feeding regimen.

Clinical and Laboratory Observation of Challenged Animals.—For a period prior to challenge, and for the duration of the experiment after challenge,

animals were followed by daily rectal temperatures, daily bacteriological examination of stools, blood cultures either daily or every other day, close daily observation for alterations in appetite, appearance or behavior, and periodic examination of serum for the presence and titer of O, H, and Vi antibodies.

RESULTS

Bacteriological and Parasitological Findings in Stools of Normal Chimpanzees.—In order to determine the prevailing aerobic enteric bacterial flora of normal chimpanzees, stools from all animals in this study, as well as from all other chimpanzees used in this laboratory, were examined bacteriologically prior to infection with typhoid bacilli. One hundred and fifty-eight stool specimens from 67 chimpanzees were found to contain the bacteria listed in Table I.

Stools from most of the animals were also examined for animal parasites,[2] and revealed that infestation, in varying degree, was universal. Table II shows the occurrence of parasites in 108 fecal specimens obtained from 62 chimpanzees. No attempt was made to treat the animals for parasitism prior to infecting them with *S. typhosa.*

Anti-Salmonella Antibodies in Normal Chimpanzees.—102 normal animals were examined for Vi antibody prior to exposure to *S. typhosa.* All but 2 of these were also tested for the presence of O antibody, and 79 were checked for anti-H antibody. The results are tabulated in Table III. O antibodies were demonstrated in one-fourth of presumably normal chimpanzees, although the titers found in such animals were generally quite low; anti-H and anti-Vi antibodies were uncommon. There was no apparent correlation between these pre-infection antibody levels and subsequent response of the animals to oral infection.

Relationship of Viability of Ingested Organisms to Observed Antibody Response.—As the studies progressed, it became clear that a significant proportion (actually 7 out of 37) of the animals exposed to infection by the oral route developed a definite antibody rise with no other detectable signs of infection. Indeed every animal showed a rise in O antibodies within 10 to 14 days following infection, and all but 2 rose at least fourfold. Consequently, it was considered important to determine whether such an antibody response, in the absence of other evidence of infection, was in fact a manifestation of subclinical infection, or whether it was merely a response to oral administration of antigen. Therefore a group of 8 chimpanzees,[3] with no previous known ex-

[2] We are indebted to Mr. Ralph Duxbury, Department of Medical Zoology, Walter Reed Army Institute of Research, for the examination of stools for parasites.

[3] We are greatly indebted to Dr. Albert B. Sabin, of the Children's Hospital Research Foundation, Cincinnati, for making these animals available to us for this purpose, and for his active cooperation in the accomplishment of this phase of the studies described.

TABLE I

Bacteria Isolated from Fecal Specimens of 67 Normal Chimpanzees, 158 Stools Examined

Genus or type of bacteria isolated	No. of chimpanzees from which isolates were made
Coliform types	63
Klebsiella sp.	4
Proteus sp.	39
Pseudomonas sp.	5
Paracolobactrum sp.	11
Beta hemolytic streptococci, Group D	1
Alpha hemolytic streptococci, Group G	1
Achromobacter sp.	1
Aerobacter sp.	4
Shigella sp.	2
Alcaligenes sp.	1

TABLE II

Parasites Found in Fecal Specimens Obtained From 62 Normal Chimpanzees, 108 Stools Examined

Parasite	No. of chimpanzees infested
Troglodytella sp.	16
Strongyloides sp.	55
Endamoeba coli	53
Trichomonas sp.	40
Balantidium sp.	3
Hookworm	35
Trichuris sp.	18
Enterobius sp.	2
Endamoeba histolytica	7
Hymenolepis sp.	1
Giardia sp.	7
Chilomastix sp.	19
Coccidium	1
Endolimax nana	10
Iodamoeba sp.	13
Ascaris sp.	1
Unidentified flagellate	3

posure to *Salmonella* infection, was fed graded amounts of acetone-killed and dried *S. typhosa* strain Ty2, prepared according to the method of Landy (16). This preparation had been shown to be highly antigenic with respect to the three major antigens of this organism when given by the subcutaneous route

(16, 30). One-half billion, 5 billion, and 100 billion killed typhoid bacilli were fed to 3, 3, and 2 animals respectively. No significant change in O, H, or Vi antibody levels was seen at either 12 or 26 days after ingestion of the organisms. It appeared therefore that antibody response to ingested typhoid antigens occurred only as a consequence of proliferation of live bacilli.

General Findings.—With few exceptions, it was found that stool cultures were positive for *S. typhosa* within 24 hours after the bananas had been ingested, thus assuring that each animal was exposed to viable typhoid bacilli throughout its gastrointestinal tract. An incubation period of 4 to 7 days

TABLE III

Typhoid O, H, and Vi Antibody Levels of Normal Chimpanzees

Titer*	Number of chimpanzees showing indicated titers		
	O Antibody	H Antibody	Vi Antibody
Negative	74	73	94
5	2	3	
7.5			4
10	6	1	
15			3
20	10	1	
30			1
40	7	1	
60	1		
Total No. of animals tested	100	79	102
No. Positive	26/100	6/79	8/102

* Reciprocal.

elapsed before fever was noted or blood cultures became positive. With the advent of bacteriemia, temperatures began to rise, reaching maxima of 3 to 5.7°F. above normal. Intermittently positive stool and blood cultures were obtained during the febrile period. Mild lethargy and anorexia were seen in several animals but were by no means the rule. The character of the stools was not significantly different from that of normal animals. No evidence of pulmonary disease was noted, and rose spots were not observed. Symptoms generally subsided within 2 weeks after infection, and specific O and H antibodies were found in the sera of all chimpanzees after the 6th day. Vi antibodies developed in about one-third of the infected animals (See Table IV). In the animals which were sacrificed and autopsied between the 9th and 23rd day after infection, gross findings ranged from characteristic changes, including enlarged Peyer's patches and mesenteric lymph nodes, to absence of

apparent tissue alteration. Fig. 1 illustrates typical gross findings in the ileocecal region. In all animals the bowel was free of hemorrhage or ulceration. Cultures of typhoid bacilli were obtained irregularly from several organs, particularly the spleen and gallbladder.

Significant microscopic findings were confined largely to the gastrointestinal tract, the lymphoid tissue, spleen, liver, and gallbladder. All animals displayed a diffuse enteritis which was present in every section of small bowel examined, and which involved the cecum in two of the animals. The enteritis was characterized by an edema of the mucosa and a mixed infiltrate of lymphocytes, monocytes, plasma cells, and large macrophages exhibiting cytophagocytosis. The lacteals were distended and contained debris-laden macrophages. Mallory's "typhoid cells" were seen. There was no hemorrhage, necrosis, or ulceration of the intestinal epithelium. In addition to the diffuse enteritis, the solitary follicles and Peyer's patches, as well as the lymphoid tissue of the mesenteric nodes and spleen, were involved in two distinct alterations. There was a marked reactive follicular hyperplasia of the lymphoid tissue which was seen to about an equal degree in all animals, and there was, in addition, a typhoid-specific granulomatous proliferation of mononuclear cells with formation of ill-defined nodular masses of these reticulohistiocytic elements seen principally in the mesenteric lymph nodes and spleen (Figs. 2 and 3). This latter feature varied greatly in intensity within the group of chimpanzees. In all animals the gallbladder was involved by a chronic cholecystitis. Occasionally, typhoid nodules, so called typhomas, were noted in the liver.

Analysis of Over-All Results in Terms of Disease Induced.—The results observed in 10 experiments involving 37 unimmunized, previously unexposed chimpanzees are presented in Table V. The infections detected could reasonably be separated into "febrile" and "afebrile". Definition of "febrile" was necessarily arbitrary, especially since rectal temperatures in chimpanzees are readily subject to fluctuations due to various, sometimes undetermined, causes. However, after considerable experience it was felt that the definitions given below were satisfactory when applied to groups of animals, although there were individual chimpanzees which did not fit satisfactorily into either category:

Febrile Infection.—Rectal temperature greater than 1.5°F. above the average prechallenge level of the animal in question for 1 or more days, occurring within 10 days of challenge. (All febrile animals exhibited bacteriemia and showed at least a fourfold increase in 1 or more typhoid antibodies.)

Afebrile Infection.—Rectal temperature of 1.5°F. or less above the average prechallenge level, with or without bacteriemia, plus a *fourfold* or greater antibody rise to 1 or more typhoid antigens.

Table V shows that somewhat more than half of the animals exhibited febrile infection within 10 days of ingesting the infectious organism. This

result, however, is a summation of data influenced by two known and planned variables, *viz.:*

(*a*) *Challenge dose.*—Nineteen out of the 25 animals receiving 3 billion or more strain Ty2 bacilli developed febrile infection. On the other hand, 2 of the

TABLE IV

Peak Antibody Responses of Chimpanzees to Oral Administration of Viable S. typhosa

Titer*	No. of chimpanzees showing indicated titers					
	O Antibody		H Antibody		Vi Antibody	
	I	II	I	II	I	II
Negative	25		28		36	24
5	1					
7.5						4
10	4			1		
15					1	2
20	4	1				
30						3
40	2	2		3		
60	1	2		1		2
80		3				
120		3		1		1
160		6		1		
180						1
240		6		1		
320		6		7		
480		4				
640		3		7		
960				1		
1280		1				
2560				3		
5120				2		

I, pre-challenge antibody levels.
II, peak post-challenge antibody levels.
* Reciprocal.

3 animals given 0.425 billion cells of this strain developed infection without fever. The 3rd animal suffered an intercurrent infection and its response was therefore uninterpretable. Thus, from the limited data available, it appeared that there might well be a threshold dose of strain Ty2, below which febrile infection was unlikely to occur.[4] Nevertheless, the data indicated that afebrile

[4] Subsequent studies by one of us have shown that ingestion of 35 million Ty2 cells could occasionally induce febrile infection.

"inapparent" infections—manifested sometimes only by a specific antibody rise—may well be induced by a wide range of doses.

(*b*) *Challenge Strain.*—With strain 2593, febrile infection appeared to be irregularly induced even with as many as 100 billion organisms, and the impression was gained that this strain requires a larger infecting dose than strain Ty2 in order to produce a febrile response. Because of the scarcity of chimpanzees, a more precise answer to this question was not sought.

TABLE V

Summary of Results of Challenge Experiments

Experiment No.	Challenge dose × 10^9	No. challenged	No. infected	
			Febrile	Afebrile
9	81	2	2	0
10	4.5	2	2	0
13	5	7	5	2 (2)*
14	0.425	3‡	0	2 (1)
15	3	5	4	1 (1)
16	7	4	2	2 (2)
18	3	5	4	1 (1)
19	100§	5	1	4 (0)
21	100§	2	2	0
22	4§	2	0	2 (0)
		37	22	14 (7)

* Figures in parentheses indicate number of afebrile animals without demonstrable bacteriemia.

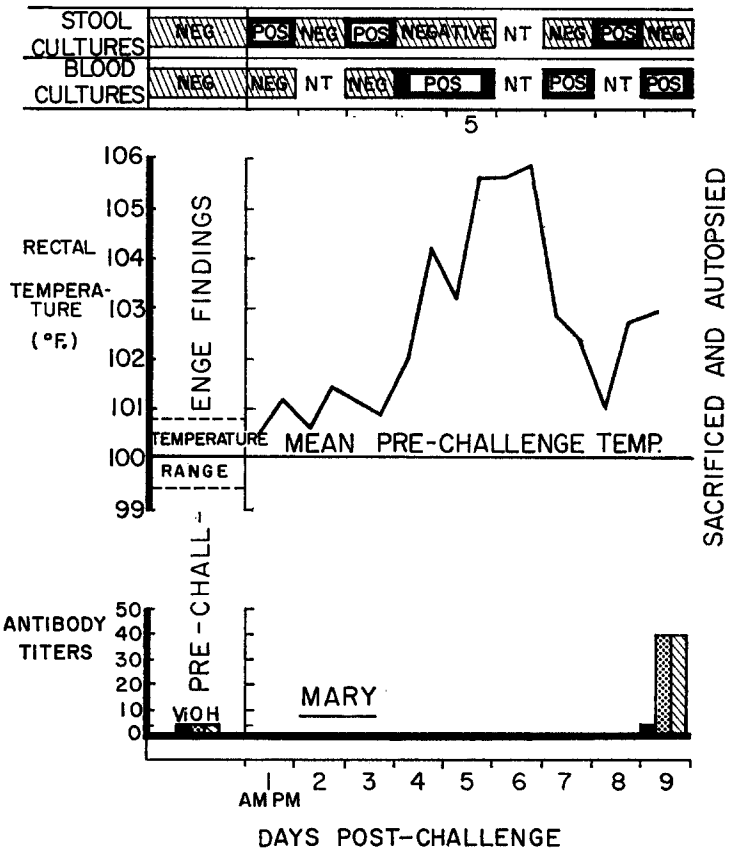
‡ One of these 3 animals developed a febrile intercurrent infection and is therefore excluded from the final tabulation.

§ Challenged with strain 2593 in Experiments 19, 21, and 22; strain Ty2 used for challenge in all other experiments.

The essential findings in three typical clinical infections and 1 closely observed afebrile infection were as follows:—

Chimpanzee Mary (see Text-fig. 1), a 16 pound female, ingested 81 billion *S. typhosa* strain Ty2, derived from a lyophilized stock culture which had been through 3 passages on agar prior to harvesting the challenge inoculum. Stool cultures from Mary were positive on the 2nd day of the experiment and intermittently thereafter. A marked febrile response began on the 4th day postinfection, rising to more than 5°F. above her normal by the 6th day and then rapidly subsiding. Positive blood cultures were obtained on several occasions beginning with the 1st day of fever. Clinically, the animal was markedly lethargic, anorectic, constipated, and dehydrated. On the 9th day she was

sacrificed, and autopsy revealed enlarged mesenteric lymph nodes, enlarged spleen, and markedly hyperplastic, prominent, opaque Peyer's patches. Characteristic histopathological findings are illustrated in Figs. 2 and 3. Neither discoloration, ulceration nor free blood were observed in the bowel. Positive cul-



TEXT-FIG. 1. Chimpanzee Mary: response to oral challenge with 81 × 10^9 *S. typhosa* Ty2. NT, not tested.

tures were obtained from the spleen, liver, bile, and a mesenteric lymph node. Serum secured just prior to sacrifice showed H and O titers of 1:40, while Vi antibody could not be demonstrated.

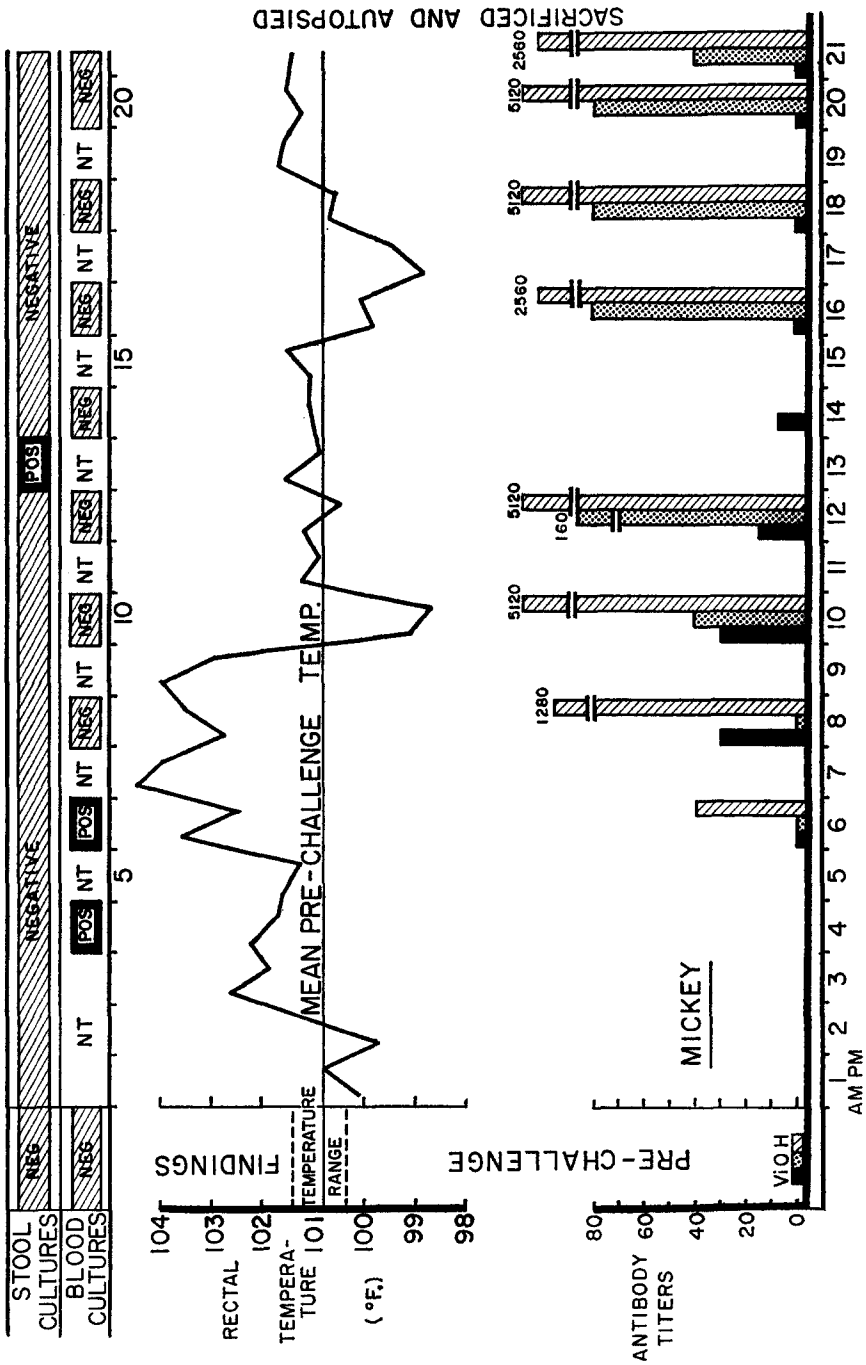
Chimpanzee Mickey (Text-fig. 2), a 25 pound male, ingested 4.5 billion *S. typhosa* from a culture isolated from the spleen of chimpanzee Mary. His stools were consistently negative except for a single isolate during convalescence. His temperature rose sharply on the 6th day after challenge, remained elevated

for 3 more days and then dropped suddenly to normal. Only two blood cultures —just before and with the onset of fever—were positive. H antibodies rose early in the disease, followed shortly by rises in O and Vi antibodies. The Vi response was transitory, and fell to less than 5 by the 16th day postinfection, whereas the H and O titers remained at generally high levels up to the time of sacrifice. Clinically the animal showed a marked loss of appetite for about a week during and immediately after the febrile episode, and its usual aggressiveness was noticeably reduced during this period. On postmortem examination, 21 days after challenge, the only obvious gross pathologic change was the swelling of the Peyer's patch just proximal to the ileocecal valve. The mesenteric lymph nodes were not appreciably increased in size. A unique finding was a retrocecal abscess, from which a pure culture of typhoid bacilli was isolated. The organisms were also isolated from the spleen, but not from blood, bile, or stool.

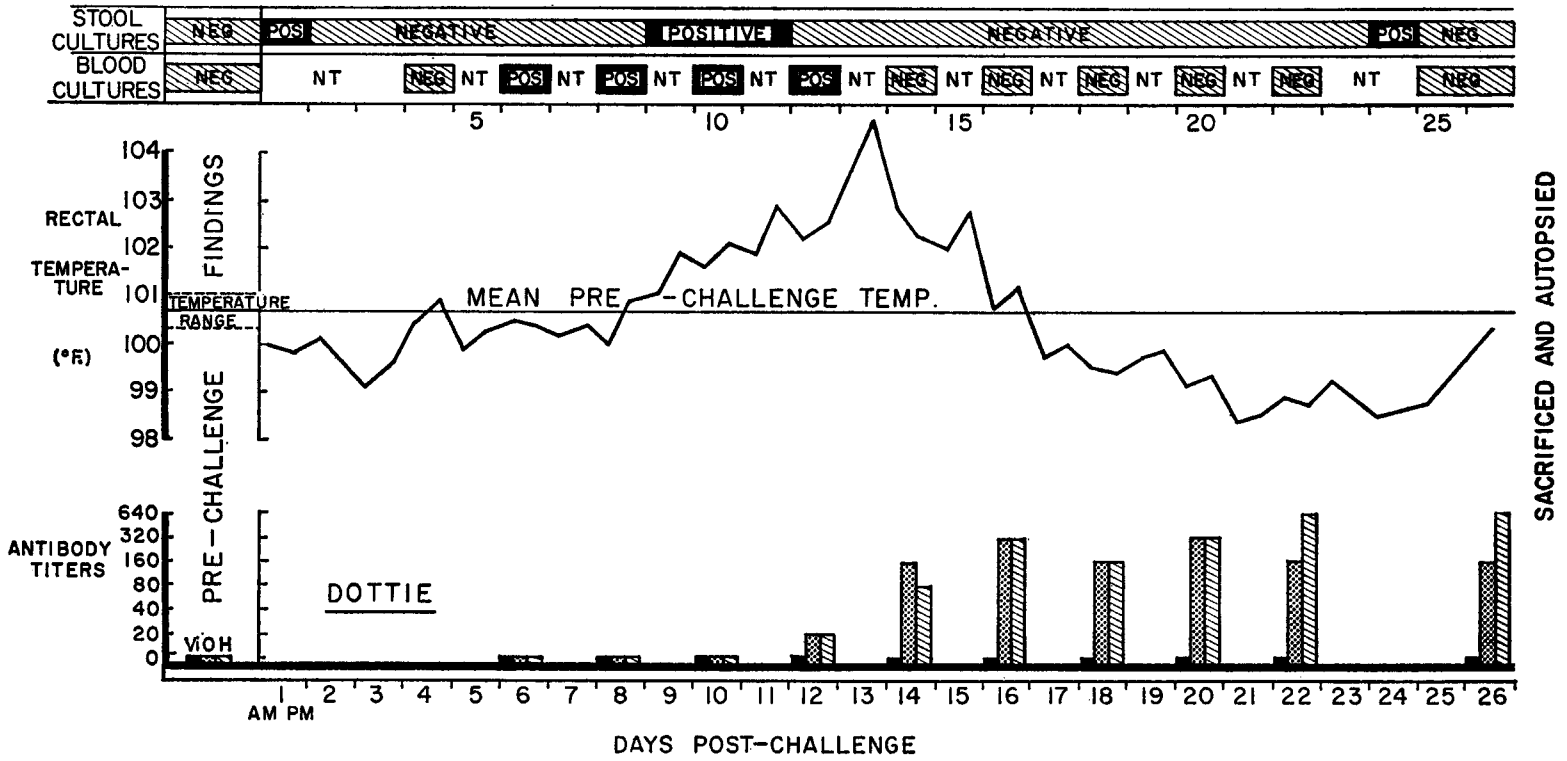
Chimpanzee Dottie, (Text-fig. 3), a 30 pound female, received the same challenge as Mickey. Her first manifestation of infection was a positive blood culture on the 6th day. Three days later the temperature began a gradual rise which reached a peak in 4 more days, falling then by lysis over the next few days. A series of three successive positive stool cultures was obtained simultaneously with the onset of fever; only one more positive stool was obtained, later in convalescence. A series of positive blood cultures occurred ending with the peak of the fever; none were obtained thereafter. H and O antibodies began to rise on the 12th day after challenge and reached high titers during convalescence; no Vi antibodies were detected up to the day of sacrifice. Clinically Dottie showed little change either in appetite, general appearance, or her usual ill temper. Autopsy on the 26th day after infection showed no remarkable gross changes, and all cultures were negative for typhoid bacilli .

A characteristic case of afebrile infection was seen in *Chimpanzee June*, (Text-fig. 4), an 11 pound female. Following the ingestion of approximately 0.425 billion *S. typhosa*, her stools were consistently negative except for the usual positive 24 hour specimen. A single positive blood culture, 4 days after infection, was obtained, and her temperature never rose significantly above her normal. Yet by the 10th day after ingestion of the challenge she had developed H and O antibody titers of 80 and 160 respectively; the titers doubled in the next 4 days, and then fell somewhat by the 19th day after infection, at which time the experiment was terminated. Clinically this animal remained completely normal throughout the period of observation.

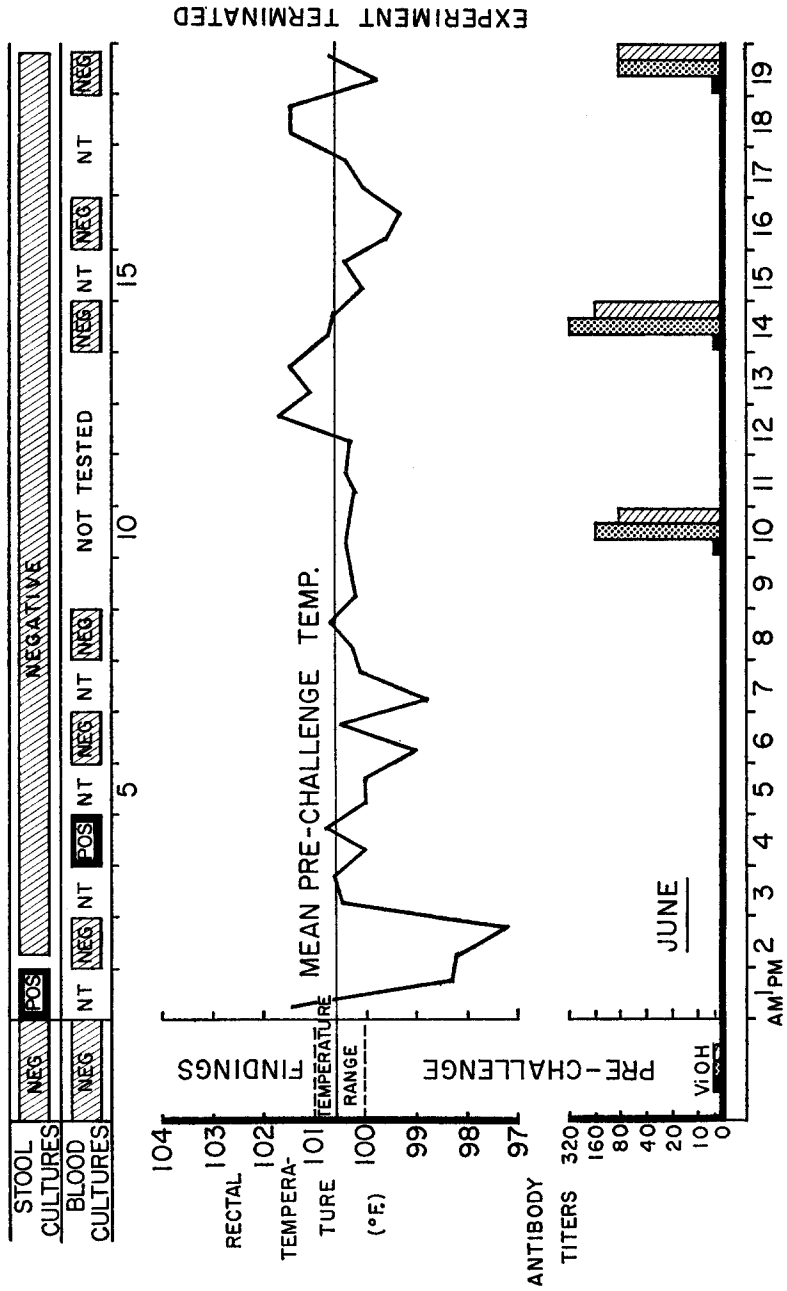
The Carrier State.—Two chimpanzees in the studies reported here, and one in another study, developed the carrier state for relatively long periods following challenge. One of the three, Flint, a 21 pound male, was infected in the same experimental group that included June, described above, and received the same dose of *S. typhosa*. For the first 18 days following challenge, Flint showed no



TEXT-FIG. 2. Chimpanzee Mickey: response to oral challenge with 4.5×10^9 *S. typhosa* Ty2.



TEXT-FIG. 3. Chimpanzee Dottie: response to oral challenge with 4.5×10^9 *S. typhosa* Ty2.



TEXT-FIG. 4. Chimpanzee June: response to oral challenge with 0.425×10^9 *S. typhosa* Ty2.

bacteriological, clinical, or serological evidence of infection. On the 19th day, however, a positive stool culture was found. Daily stool specimens were examined for more than 8 months, during which time 85 positive specimens were obtained, the last one on the 169th postchallenge day. Bile, cultured 41 and 201 days after infection, contained large numbers of typhoid bacilli, but was negative on the 246th day.[5] Blood cultures taken periodically during the observation period were consistently negative. With the onset of positive stool cultures, H and O antibody titers of moderate level appeared, but Vi hemagglutinins never rose above occasionally observed levels of 1:7.5 or 1:15.

Another carrier, Jet, a 25 pound male, was one of ten chimpanzees (described in a subsequent report (31)) which had been injected with 3 doses of acetone-killed and dried typhoid vaccine 3 months before, and a booster dose 2 weeks prior to being challenged with 5 billion *S. typhosa* Ty2. His first positive stool after the usual 24 hour "passage" positive, was obtained 14 days after challenge, and during the next 4½ months 23 additional positive stool cultures were obtained out of 144 tested; the last positive specimen occurred 90 days after challenge. A third animal, Rona, an unimmunized 18 pound female in Experiment 13, continued to pass typhoid bacilli for approximately 2 months after challenge in contrast to the usual 1 month or less of intermittent shedding seen in most chimpanzees.

No clear cut obvious connection between challenge dose, immune response, and the duration of the carrier state could be observed. The two most persistent carriers, Flint and Jet, had experienced either subclinical or no demonstrable infection, whereas the animal exhibiting the briefest carrier state (Rona) had a febrile response lasting 9 days, with a peak of 4.9°F. above normal, and 3 positive blood cultures.

Serologic Findings.—Of 37 unimmunized animals exposed to challenges of varying quantities with either strain Ty2 or 2593, and observed for 10 days or longer, only 2 animals failed to show at least a fourfold O and H antibody rise, and these developed a twofold increase. Thus a serologic response to the somatic and flagellar antigens of live *S. typhosa* was a virtually universal finding, regardless of which strain of the organism was administered or whether the dose was as small as 0.425 billion organisms or as large as 100 billion organisms. Vi antibodies, however, were found to develop in only 13 of the 37 animals following infection, and in only two of these animals did the titer exceed 1:60.

The peak responses to O, H, and Vi antigens, following infection, are shown in Table IV.

DISCUSSION

The experiments described above clearly confirm the observations of Grünbaum, and of Metchnikoff and Besredka, that a disease bearing a marked

[5] We are indebted to Dr. H. H. Balch (then Major, MC) for performing the cholecystopexy which facilitated the procurement of bile samples.

resemblance to human typhoid fever can be produced in chimpanzees following the ingestion of typhoid bacilli. Although the findings from animal to animal varied, several essential generalizations can be made regarding the response of chimpanzees to oral infection with *S. typhosa*. A great majority of the animals exhibited typhoid bacteriemia usually from 4 to 10 days after oral challenge; most exhibited from 1 to 9 days of fever, generally coincident with bacteriemia, although there was a tendency for bacteriemia to appear first; about half excreted typhoid bacilli in the feces, and this excretion was in practically every instance preceded by an "eclipse" period following the usual transient appearance of the surviving organisms the day after challenge; anti-typhoid O and H antibodies were present a few days after the onset of demonstrable infection in every animal; gross and microscopic changes characteristic of human typhoid fever were observed in intestinal and mesenteric lymphatic tissues, spleen, liver, and gallbladder; the carrier state was observed to develop in a small proportion of the infected animals, and the biliary tract was shown to be a source of persisting infection in one such animal.

The principal apparent differences between typhoid fever as seen in our chimpanzees and in man were the incubation period, which was relatively short in the chimpanzees, and the clinical course of the disease, which in the chimpanzees was relatively mild and brief. Only a few animals in the whole series appeared seriously ill during the course of the infectious process. The hypertoxicity, typhoid facies, stupor, extreme lethargy, etc., which are so generally associated with the disease in man were not discernible in our infected chimpanzees. Finally, the pathological changes, although wholly typical of mild typhoid fever in man, did not include, in any of the animals examined, any evidence of ulceration of the Peyer's patches, or any of the other complications of typhoid fever.

However, it must be borne in mind that not all human typhoid fever is severe or "typical," and that particularly in young children the disease is frequently mild or even abortive. Since the great majority of our chimpanzees were in a stage of maturation comparable to the child under 10 years of age, it seems reasonable to assume that their response to typhoid infection could best be compared, both clinically and pathologically, to pediatric rather than to adolescent or adult typhoid fever in man. Consequently, the relatively mild course of the disease in chimpanzees, and the lack of ulceration of the Peyer's patches, do not necessarily set apart the chimpanzee syndrome from that seen in man. Moreover, it has long been recognized that a significant number of swollen Peyer's patches, even in adults suffering from typhoid fever, never come to ulceration, but resolve as convalescence proceeds (32).

One of the features of the disease in chimpanzees, as is also true in man, was its striking lack of uniformity from animal to animal, ranging from moderately severe systemic and localized illness, to wholly inapparent infection detectable only by serologic means. Murphy *et. al* (33) have described an outbreak of

typhoid fever in a small community in which the entire exposed population could be studied. Of the 80 individuals who yielded bacilli on examination, 38 were moderately or severely ill, 20 were confined to bed but mildly ill, 11 had some symptoms but remained ambulatory, and 11 were wholly asymptomatic. Bradley *et al.* (34) have reported essentially similar observations in a hospital outbreak affecting a "closed" population. Other examples could be cited, but the principle remains, that typhoid fever as seen in a population group is a protean disease. The chimpanzee observations, however, add one component to our perspective of this variation, for in human groups it has never been possible to exclude great differences either in infectious dose, or in pre-existing immunity, as the cause of the wide spectrum of response to infection so commonly seen. In the chimpanzees, however, the infectious dose was carefully controlled, and the pre-existing immunity status was fairly uniform as judged by serologic evidence, the youth of the infected animals, and their relatively limited opportunities to acquire previous experience with enteric fevers. Thus the chimpanzee data support the widespread impression that in typhoid fever, as in many other infectious disease entities, exact replication of the clinical, laboratory, and pathological picture from animal to animal (or man to man) is the exception rather than the rule. The possible causes of this difficulty in replication may be numerous; however, two which have impressed us as worthy of mention are the variety in degree and kind of parasitism seen in chimpanzees, and the variation in the inhibitory properties of chimpanzee stools toward *S. typhosa*, noted earlier in this paper. Indeed the variations in the ecological situation in the intestinal tract, in the physiological state of the gut, and in many less well understood factors in the host, are so numerous that it would be impractical to discuss them all in detail, in the absence of more concrete information.

Published reports of the frequency of occurrence of Vi antibodies in typhoid fever are not in agreement. Felix *et al.* (35), Almon and Stovall (36), and Almon, Read, and Stovall (37), using the classical bacterial suspensions, found Vi antibodies in less than half of the patients with typhoid fever. Bhatnagar (38) on the other hand, detected such antibodies in all of 78 unimmunized cases studied. Potuzník and Gavlík (39), using a hemagglutination technique, found Vi antibodies of low titer in most of their patients but only late in the disease. It was therefore of interest to note that Vi hemagglutinating antibodies were detected in only one-third of our chimpanzees. The possibility occurred to us that the immunological inexperience of the young animals under study might have influenced the degree of their response to the Vi antigen. However, the recent study by Huang (40) on the relation of age to Vi antibody response in man, does not support this concept. We feel that the Vi response of chimpanzees after infection with *S. typhosa* is essentially comparable to that of man.

The consistently vigorous responses to O and H antigens were similar to those seen in man, and provided further evidence of the high antigenicity of

these components of the typhoid bacillus. Even in afebrile infection—where there was presumably insufficient production of O antigen (endotoxin) to induce a demonstrable fever—these antibodies were developed. Indeed, the available data do not provide any clear cut evidence of a relationship between severity of infection and height or persistence of antibody response.

The development of the carrier state in 3 chimpanzees, lasting from 2 to 6 months, was an unexpected and gratifying simulant of the spectrum of typhoid infection in man, particularly in the case of one animal, from whose bile viable *S. typhosa* was recovered a month after these organisms ceased to be detectable in the feces. Although one can hardly generalize on the basis of 3 animals, it is of interest that the two carriers of the longest duration did not exhibit any other evidence of infection. Thus it is clear that a carrier state can follow minimal infection of the host. This observation is, of course, in accordance with the long recognized occurrence of the carrier state in a significant proportion of human beings who have no identifiable past history of clinical typhoid fever.

A theoretical major difference between chimpanzee and human typhoid is the presumably large dose of viable *S. typhosa* required for the induction of clinical infection in the chimpanzee. With strain Ty2 it appeared that less than 1 billion organisms would not regularly induce febrile disease. With strain 2593—despite the fact that it was essentially indistinguishable from strain Ty2 by the usual laboratory tests—the minimal dose for production of febrile disease appeared to be even greater than with strain Ty2. These figures are very much higher than the generally assumed threshold dose required for producing typhoid fever in man. Although this assumption is hard to validate with actual data, nevertheless there are scattered experiences which support the belief that the naturally infecting dose for man may at times be closer to a few thousands, rather than billions of organisms (41, 42). We can only speculate that great differences in the infectious dose may be required under different circumstances. Thus human clinical disease resulting from small doses of typhoid bacilli may be dependent upon the chance presence of a particularly favorable bacterial intestinal flora in the exposed subject, or upon non-specific resistance factors which are at times deficient. The inability to reproduce these undefined conditions in the chimpanzee would then mean that much larger doses of viable organisms must be introduced, in order to insure survival, in the receptive tissues, of the relatively few cells that are actually required to initiate the disease.

The other most prevalent explanation for the discrepancy in the size of the infecting dose—that laboratory-maintained cultures are likely to be less virulent than "wild" strains—is less acceptable in the case of typhoid fever, not only because the natural habitat of infective strains of *S. typhosa* includes water, ice, milk, cheese, and various other environments not unlike that of the laboratory, but also because laboratory infections, even with "laboratory strains," are by no means unheard of. At all events it is at present impossible

to interpret clearly the significance of the high infecting dose required for chimpanzees. However, the general similarity of the animal disease to that seen in man supports the belief that the major difference in the two infectious processes may be quantitative rather than qualitative.

The apparent difference in infectivity between strains Ty2 and 2593 was of particular interest because of their similarity on the basis of laboratory tests. The difference in their capacity to induce febrile disease in chimpanzees can be regarded as supporting the hypothesis that, even in an organism as thoroughly studied as *S. typhosa*, there may be critical unidentified factors, varying from strain to strain, which contribute to the invasiveness, virulence, and survival capacity of the organism.

The results of these studies have shown that it is possible to establish in chimpanzees a disease closely resembling typhoid fever, produced by ingestion of well defined strains of *S. typhosa* administered in reproducible fashion and in fairly accurately quantitated doses. This in turn has provided a basis for a more precise description of certain aspects of the pathogenesis of typhoid fever and the factors involved in immunity to the disease. Information concerning pathogenesis and immunity will be presented in the succeeding papers (31, 43, 44). It is clearly recognized that the analogy between typhoid fever in the chimpanzee and in man is necessarily limited, and that the study of this disease by infecting primates with laboratory strains of *S. typhosa* might well give information not applicable to naturally acquired human disease. Nevertheless it is felt that the controlled, reproducible induction of typhoid fever in the chimpanzee by oral ingestion of the organisms provides a tool superior to any heretofore used, for the study of the characteristics of this disease as it is seen in man.

SUMMARY

A disease resembling human typhoid fever has been induced by feeding live cultures of *Salmonella typhosa* to young chimpanzees, thus confirming the classical reports of Grünbaum and of Metchnikoff and Besredka.

Detailed clinical observations, results of stool and blood cultures, and serological studies have confirmed the impression that the disease produced in chimpanzees closely resembles the mild form of human typhoid fever frequently seen in childhood.

Gross and histologic examination of intestines, mesenteric lymph nodes, liver, spleen, and other organs of orally infected chimpanzees has demonstrated that the pathological findings are essentially indistinguishable from those seen in mild typhoid fever in man.

The clinical spectrum of disease seen in chimpanzees ranged from moderately severe illness, through transitory illness, to afebrile infection with or without bacteriemia (but invariably with an antibody response), occasionally leading to the development of persisting biliary infection and the carrier state. Thus the

range of illness observed in chimpanzees resembled that seen in man, except that the severe and complicated forms of typhoid fever were not observed in the chimpanzee. A reason for this difference is proposed and discussed.

In contrast to the limitations imposed upon the interpretation of human epidemiologic observations, it has been possible to demonstrate in the chimpanzee that clinical variation in disease pattern from animal to animal may occur despite the administration of the same dose of the same bacterial strain simultaneously to an entire group of animals under study; in other words, variation in clinical pattern is dependent on inherent, non-specific host factors as well as on dose, strain or preceding state of immunity.

Variation in dose and in challenge strain of *S. typhosa* employed also appeared to have an effect upon the likelihood of producing febrile as against afebrile infection in chimpanzees. The dose required to produce clinical disease, even with the more virulent strain, was excessively large compared to what is believed to be the dose required to produce illness in man; the limitations of this assumption, and suggested explanations for the findings, are discussed.

The production of the spectrum of typhoid fever in the chimpanzee has made possible the study of basic problems in this disease which are not amenable to definitive study through the use of prevailing laboratory techniques.

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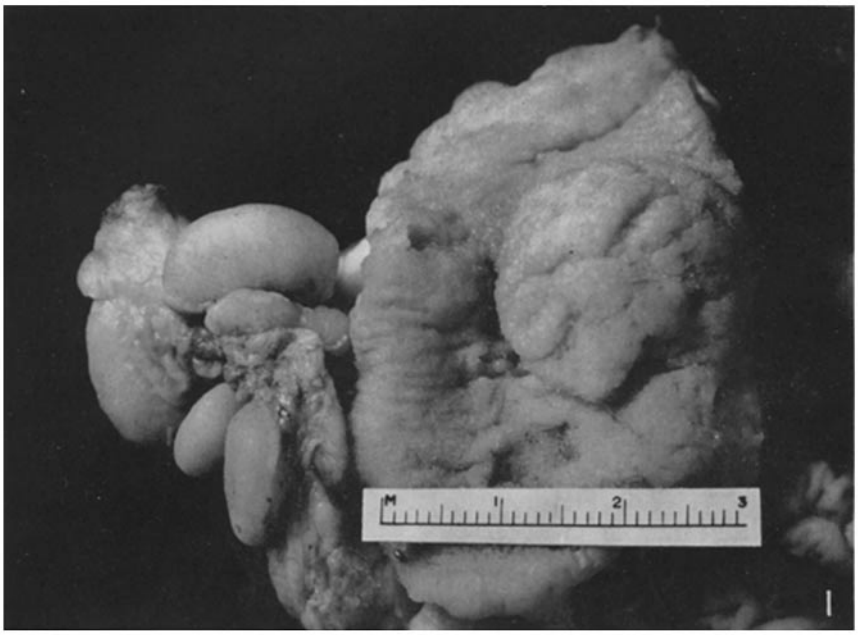
EXPLANATION OF PLATES

PLATE 6

FIG. 1. Photograph of terminal ileum and ileocecal valve. A group of swollen ileocecal nodes is seen at left. The cecum is barely visible in the shaded right lower corner. Note the marked swelling of the terminal Peyer's patch and the granulosity and edema of the mucosa of the terminal ileum.

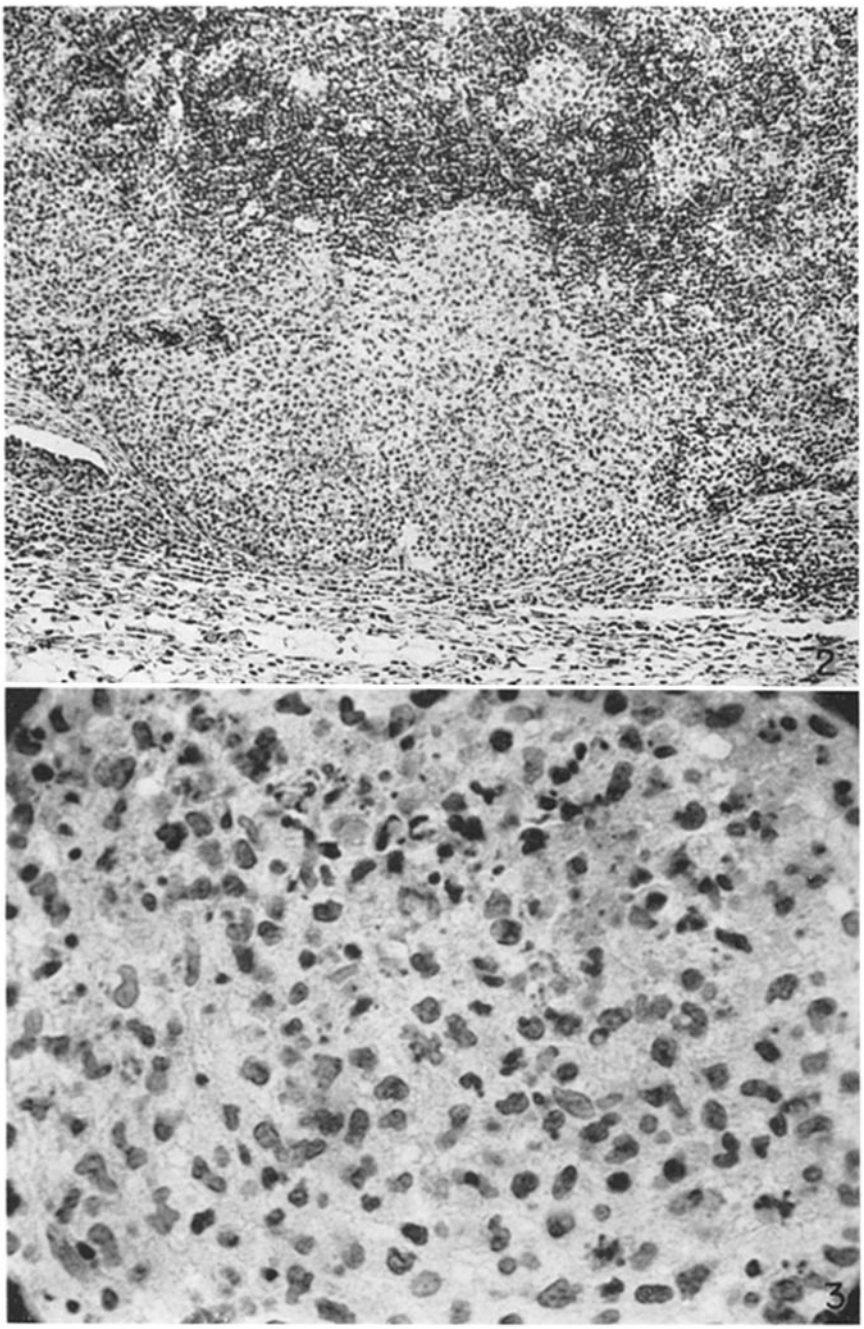


(Edsall *et al.*: Infection and immunity in typhoid fever. I)

PLATE 7

FIG. 2. Photomicrograph of mesenteric lymph node illustrating the marked proliferation of pale mononuclear cells starting in the peripheral sinus.

FIG. 3. High power photomicrograph of a field from the area of mononuclear cell proliferation shown in Fig 2. Note the sheet of hyperplastic reticulum cells. Necrobiosis of individual cells and phagocytosis of cellular debris is evident.



(Edsall *et al.*: Infection and immunity in typhoid fever. I)