JOURNAL OF BACTERIOLOGY, Mar., 1965
Vol. 89, No. 3

Use of Ligated Segments of Rabbit Small Intestine in Experimental Shigellosis[1]

H. G. ARM,[2] T. M. FLOYD,[3] J. E. FABER, AND J. R. HAYES

Naval Medical Research Institute, Bethesda, and Department of Microbiology, University of Maryland, College Park, Maryland

Received for publication 14 September 1964

ABSTRACT

ARM, H. G. (Naval Medical Research Institute, Bethesda, Md.), T. M. FLOYD, J. E. FABER, AND J. R. HAYES. Use of ligated segments of rabbit small intestine in experimental shigellosis. J. Bacteriol. **89**:803-809. 1965.—Inoculation of ligated segments of small intestine in living rabbits with broth cultures or resting-cell suspensions of recently isolated strains of *Shigella* caused distension of the segments by accumulation of exudate within 12 hr. Histological changes characteristic of an inflammatory response were similar to those of human bacillary dysentery. Tissue response and accumulation of exudate preceded demonstrable increase in numbers of shigellae inoculated as 2×10^{10} resting cells. Capability of shigellae to provoke intestinal response was not related to any particular serological group. The active principles concerned with eliciting intestinal response were associated only with preparations containing living organisms. Ability of recently isolated strains to elicit response diminished rapidly during culture on artificial media. The capability was preserved indefinitely by lyophilization soon after isolation from acute bacillary dysentery infections of man. Advantages of using shigellae recently isolated for investigating possible mechanisms of pathogenesis were indicated. During the summer months, the rabbit small intestine was refractory to the activity of shigellae, and positive responses were not observed. Use of ligated segments of rabbit small intestine qualified as an indicator of virulence for the rabbit; and, virulence for the rabbit showed a high degree of correlation with a short period of culture of shigellae on artificial media after isolation from human bacillary dysentery infections.

Until recently the search for mechanisms involved in pathogenesis of *Shigella* has been hindered by the lack of a susceptible laboratory animal. However, Freter (1956) succeeded in producing a long-term nonfatal infection with a streptomycin-resistant strain of *S. flexneri* 2a in guinea pigs treated with streptomycin to alter normal intestinal flora. Formal et al. (1958, 1959) rendered guinea pigs susceptible to fatal infections by acute starvation or by subcutaneous injection of carbon tetrachloride prior to oral challenge. This report concerns use of ligated segments of rabbit small intestine for studying characteristics of virulence in *Shigella*.

The technique, described by De and Chatterje (1953), consisted of injecting bacterial preparations into ligated segments of living rabbit small intestine. Within 18 to 24 hr, some bacterial strains elicited response characterized by accumulation of a protein-rich exudate causing gross dilatation of the inoculated segment. Although the technique has been frequently used in the study of enteropathogenicity of *Vibrio cholerae* (De and Chatterje, 1953; Jenkin and Rowley, 1959) and *Escherichia coli* (De, Bhattacharya, and Sarkar, 1956; Taylor, Maltby, and Payne, 1958; McNaught and Roberts, 1958), only preliminary investigations with *Shigella* have been reported.

Taylor and Wilkins (1961) noted in a small series that recently isolated strains of *S. sonnei* consistently gave a positive intestinal response, whereas five serotypes of *S. flexneri* obtained

[1] From the Bureau of Medicine and Surgery Navy Department, Research Task MR005.09-1100.01. Some of the data in this paper are taken from a dissertation submitted by H. G. Arm to the Faculty of the Graduate School of the University of Maryland in partial fulfillment of the requirements for the Ph.D. degree. A preliminary account was presented at the 62nd Annual Meeting of the American Society for Microbiology, Kansas City, Mo., 6-10 May 1962.

[2] Present address: Naval Medical Research Unit No. 2, Taipei, Taiwan.

[3] Present address: Naval Preventive Medicine Unit No. 6, Oahu, Hawaii.

from different sources produced variable results. Floyd and Arm (1962) also observed that freshly isolated strains of *Shigella*, or strains lyophilized soon after isolation, were more active within intestinal segments than were laboratory cultures maintained on artificial media.

The present study was undertaken to ascertain whether the method of De and Chatterje is applicable to investigations concerning pathogenicity of *Shigella* and to determine factors that may influence use of the ligated intestinal-segment technique.

MATERIALS AND METHODS

Shigellae. Forty-six lyophilized strains of *Shigella* were selected at random from the Naval Medical Department Reference Collection of Enterobacteriaceae. These strains, received as reference cultures from epidemics and from individual patients, represented seven serotypes of *S. dysenteriae*, eight serotypes of *S. flexneri*, seven serotypes of *S. boydii*, and *S. sonnei*. The length of time the strains were maintained on artificial media prior to lyophilization varied from 48 hr to 2 years. All cultures were smooth by the following criteria: colonial appearance, turbidity in broth culture, stability in 0.85% saline, stability in 1:500 dilution of acriflavine, and serological specificity with group- and type-specific antisera.

Preparations for injection. Broth cultures containing from 10^8 to 10^9 organisms per milliliter, obtained by growing shigellae in Brain Heart Infusion (Difco), were inoculated in 1-ml amounts. Culture filtrates were prepared by sedimenting the organisms by centrifugation at 3,000 $\times$ *g* for 15 min at 4 C and by filtration of the supernatant fluids through Seitz filters (no. 6) under suction. Culture filtrates were inoculated in 2-ml amounts.

Resting-cell suspensions in physiological saline were prepared from 18-hr cultures on Brain Heart Infusion Agar (Difco), and were stored at 4 C for 48 hr prior to use. Inocula consisted of 1 ml containing 2×10^{10} viable organisms. Resting cells treated with cold acetone or heated in a boiling-water bath for 2 hr were inoculated as the equivalent of 1 ml of resting-cell suspension.

Cell-free extracts were prepared from resting cells by disintegration in a sonic oscillator (Raytheon, 10 kc/sec, 250 w at 0 C for 30 min). The sonically treated suspensions were inoculated as the equivalent of 1 ml of resting-cell suspension.

Endotoxin of the Boivin type was prepared by extraction of resting cells with trichloroacetic acid and ethanol as outlined by Webster et al. (1955). The extracted somatic antigen was dissolved in sterile distilled water, and was inoculated as the equivalent of 5 mg (dry weight).

Animals. Inbred Naval Medical Research Institute (NMRI) New Zealand albino rabbits and white rabbits randomly selected from different breeding stocks were used. Animals of both sexes, weighing between 1.5 and 2.5 kg, were individually caged in a room maintained at 72 C. A pelleted diet produced by the Gleco Milling Corp., Charlottesville, Va., was provided. Food was withheld for 24 hr prior to use and during use.

Surgical procedure. The technique of preparing intestinal segments was similar to that described by Taylor et al. (1958). After exposure of the small intestine, ligatures of braided black silk thread (no. 1) were prepared with care so as to avoid disruption of mesenteric capillaries. A ligature was placed in the jejunum, approximately 30 cm below the stomach, to retain gastric juices. The intestine was ligated to form four 15-cm segments separated by three 15-cm gaps. Inocula were introduced slowly into the segments by means of a syringe fitted with a 25-gauge needle. The inoculated intestine was replaced in the abdominal cavity, and the incision was closed in two layers by continuous suturing. After 18 to 24 hr, the animals were killed by air embolism, and the abdominal cavity was reopened. The appearance of the segments was recorded, and the amount of fluid in each segment was determined.

Each rabbit contained an intestinal segment inoculated with organisms known to elicit a positive response and a segment that had received a preparation known to be incapable of eliciting response. Each strain was tested in a minimum of three rabbits, with the segment assigned to a certain strain being in a different anatomical position in each rabbit. Rabbits from NMRI and from commercial sources were paired in each test.

Histopathological evaluation. Appropriate segments of small intestine were carefully excised from rabbits killed at 3-hr intervals after the operation. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μ. Sections were stained with Mayer's hematoxylin stain with 1% alcoholic eosin as a counterstain and by the Brown and Brenn modification of the Gram stain for bacteria in tissue. The gross appearance of the tissue response was recorded.

Enumeration of bacteria in ligated segments. The volume of fluid in positive intestinal segments was determined by measurement of the fluid aspirated with a syringe fitted with an 18-gauge needle. Total numbers of bacteria per segment were determined by the pour-plate method in nutrient agar (Difco) and the spread plate method of Miles and Misra (1938) on MacConkey Agar (Difco). Numbers of viable shigellae in negative segments, and the normal flora counts in uninoculated gaps adjacent to inoculated segments, were determined by injecting 2 ml of sterile physiological saline into the lumen and gently massaging the fluid to rinse the mucosal surfaces. Rinsing fluids were aspirated, and 1 ml was used to determine the bacterial count.

Growth of shigellae in vivo was determined at 3-hr intervals for 12 hr. Four different resting-cell suspensions were evaluated simultaneously by inoculating four ligated segments in each rabbit. Rabbits were killed every 3 hr, and the sizes of bacterial populations in each respective segment and uninoculated gaps adjacent to the segments

were measured. Normal flora counts were deducted from the total bacterial count.

RESULTS

Introduction of shigellae into the lumen of ligated segments of small intestine in the living rabbit frequently resulted in an accumulation of exudate, causing dramatic dilatation of the segment. Histological examinations made at 3-hr intervals revealed that the sequence of events following injection of resting-cell suspensions was characteristic of an inflammatory response of progressively increasing severity.

Early stages of an inflammatory response were detected at 3 hr after inoculation (Fig. 1). The main event was tissue edema with prominent distension of lacteals. Mild submucosal edema was noted. Gross appearance of the segment at this time was comparable to collapsed noninoculated segments, and no exudate was observed within the lumen. At 6 hr, lacteals were distended so as to create intravillous vesicles, and submucosal edema was prominent (Fig. 2). Margination of polymorphonuclear leukocytes in the vessels, and infiltration of the mucosa, were observed. Some focal ulceration was present. Gross examination revealed an accumulation of exudate within the lumen, causing severe distension of the intestinal walls.

Prominent histological changes at 9 hr were erosion of several villi, increased areas of superficial ulceration, and infiltration of the submucosa by polymorphonuclear leukocytes. Fibrin-enmeshed polymorphonuclear cells were present in the submucosa. The lumen was filled with exudate, and the segment was grossly distended.

The most salient feature at 12 hr was an intense mucosal hyperemia (Fig. 3). Mucosal and submucosal hemorrhages were observed, and the larger submucosal vessels were distended with blood. Superficial ulceration was extensive, leaving large areas denuded except for intact crypts in a stroma infiltrated with inflammatory cells and debris. Numerous accumulations of fibrinous material containing many leukocytes were observed in the submucosa. The muscularis and peritoneal layers contained focal collections of acute inflammatory cells. The segment was extremely dilated by a pink exudate containing mucus and fibrin shreds.

Gram-negative bacilli were observed within the lumen of segments inoculated with resting-cell suspensions of both organisms capable of eliciting response and those incapable of provoking the response. There was no evidence of tissue invasion.

Shigellae not capable of eliciting the positive response did not induce inflammatory changes in the tissue. Histological findings showed essentially normal bowel structure without edema or cellular infiltration. Macroscopically, the segments resembled noninoculated segments. The numbers of viable shigellae within the intestinal segments were determined at 3-hr intervals after inoculation with resting-cell suspensions (Table 1). The shigellae, regardless of subsequent events, exhibited a slight decrease in numbers during the first 3-hr period.

Changes in population sizes of strains capable of eliciting intestinal response were similar. During the first 9 hr after inoculation, the populations remained remarkably stable. A marked increase, approximately 300-fold, was observed at 12 hr. Therefore, the positive intestinal response readily detected at 6 hr by gross examination preceded demonstrable proliferation. Increased amounts of mucus and fibrin shreds in the fluid at periods greater than 12 hr caused difficulty in determining the numbers of organisms at later intervals. At 12 hr the population size of strain B902, incapable of provoking response, was only slightly more than double the number of organisms inoculated.

A survey of *Shigella* strains clearly indicated that the capability of organisms in broth cultures to elicit a positive response in segments of rabbit small intestine could be correlated with the length of time that strains were maintained on artificial media after isolation and prior to lyophilization (Table 2). The intestinal response could not be related to any particular serological group.

Organisms lyophilized soon after isolation from acute bacillary dysentery infections had the capability of eliciting the intestinal response regardless of the length of time they were stored in the lyophilized state. Strains such as these were capable of provoking dramatic response after 17 years in the lyophilized state.

Experiments were performed with two cultures of *S. flexneri* 2a lyophilized immediately after isolation from human dysentery infections. The strains were transferred daily on Brain Heart Infusion Agar, and resting-cell suspensions were prepared at 7-day intervals. Suspensions from the seventh transfer elicited response in each of 10 rabbits inoculated. Organisms of the 14th and the 20th transfers each produced response in 1 of 10 rabbits inoculated. One of the strains was maintained at room temperature in agar deeps for 30 days. At the end of this period, resting cells from the culture produced one positive response in 10 rabbits.

Results of experiments to determine activity of different preparations in intestinal segments are summarized in Table 3. Five strains of *S.*

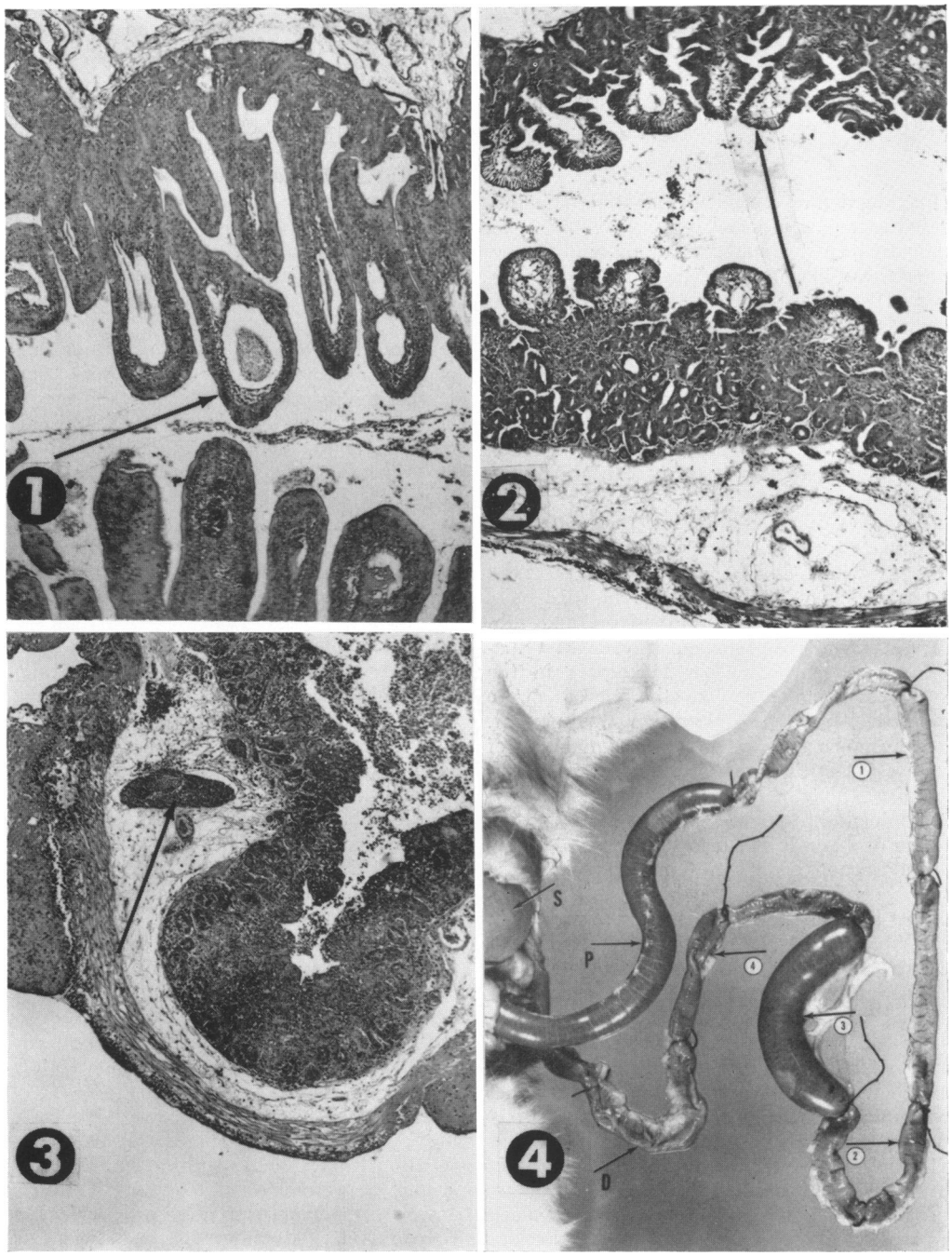

FIG. 1. *Section of rabbit small intestine 3 hr after inoculation with strain 8822. Lacteals are distended (arrow), causing prominent distortion of villi. Mild submucosal edema is present. Hematoxylin (H) and Eosin (E).* × *50.*

FIG. 2. *Section after 6 hr. Intravillous vesicles are present (arrow). Submucosal edema is prominent. H and E.* × *50.*

FIG. 3. *Section after 12 hr. Acute inflammatory reaction with marked edema and hyperemia is prominent. Extensive degeneration and hemorrhagic necrosis of villi are present. Numerous inflammatory cells are collected in submucosa (arrow). H and E.* × *50.*

FIG. 4. *Assay of activity by rabbit intestinal response. Stomach (S) and intestine proximal to stomach (P) are distended with gastric juices. Negative segments (1, 2, and 4) were injected with known negative bacterial suspension, unknown bacterial suspension, and endotoxin of the unknown, respectively. Segment 3 was injected with a known positive bacterial suspension. Distal portion of intestine (D) is collapsed.*

TABLE 1. *Geometric means and ranges of counts of Shigella flexneri 2a within ligated segments of rabbit small intestine*

Strain*	No. of viable organisms per segment at			
	3 hr	6 hr	9 hr	12 hr
1047†	7.9×10^9 (4.0×10^9–1.2×10^{10})	3.9×10^{10} (2.8×10^{10}–6.2×10^{10})	2.3×10^{10} (1.8×10^{10}–2.9×10^{10})	2.5×10^{12} (9.8×10^{11}–4.9×10^{12})
8822†	8.7×10^9 (6.2×10^9–1.4×10^{10})	2.7×10^{10} (1.8×10^{10}–5.1×10^{10})	1.6×10^{10} (8.7×10^9–3.1×10^{10})	6.0×10^{12} (4.0×10^{12}–8.4×10^{12})
1015†	5.8×10^9 (4.2×10^9–7.1×10^9)	1.8×10^{10} (9.6×10^9–3.7×10^{10})	2.2×10^{10} (1.2×10^{10}–3.0×10^{10})	1.0×10^{13} (9.4×10^{12}–3.7×10^{13})
B902	9.1×10^9 (8.6×10^9–1.0×10^{10})	1.5×10^{10} (1.1×10^{10}–2.2×10^{10})	3.6×10^9 (1.4×10^8–3.6×10^{10})	4.9×10^{10} (3.0×10^{10}–9.2×10^{10})

* Inoculated as resting-cell suspensions containing 2.0×10^{10} viable organisms.
† Positive intestinal response in each of three rabbits examined at 6, 9, and 12 hr, respectively.

TABLE 2. *Relationship of in vitro cultivation after isolation to capability of Shigella to elicit response in ligated segments of rabbit small intestine*

Organisms*	Time after isolation (months)	Strains tested	Positive responses/total no. of tests
S. dysenteriae	<1	2	5/6
	>1	9	2/29
S. flexneri	<1	7	84/101
	>1	9	21/103
S. boydii	<1	3	11/11
	>1	8	4/25
S. sonnei	<1	3	9/10
	>1	5	0/15

* Inoculated as 1 ml of broth culture (10^8 to 10^9 organisms/ml).

TABLE 3. *Response of ligated segments of rabbi small intestine to preparations obtained from strains of Shigella flexneri 2a*

Strain	Inoculum						
	Broth culture	Resting-cell suspension	Culture filtrate	Cell-free extract	Endotoxin	Acetone-dried cells	Heat-killed cells
1047	17/18*	28/28	0/9	0/6	0/6	0/3	0/3
4309	9/11	23/24	0/9	0/9	0/9	0/6	0/6
8822	9/9	29/29	0/3	0/4	0/14	0/4	0/4
2143	6/6	15/15	0/6	0/3	0/6		
1015	6/6	15/15	0/6	0/5	0/6		
B902	0/18	5/39	0/9	0/6	0/10	0/3	0/3

* Positive responses/total segments inoculated.

flexneri serotype 2a recently isolated from acute bacillary dysentery infections, and one strain used in laboratory investigations, were selected for this study. All broth cultures and resting cells prepared with the recently isolated strains elicited response in the small intestine (Fig. 4). Laboratory strain B902 did not provoke response in a significant number of animals. Forty-two segments inoculated with culture filtrates did not respond, nor did 33 segments inoculated with cell-free extracts, 51 segments inoculated with endotoxin, 16 segments inoculated with acetone-dried cells, or 16 segments inoculated with heat-killed cells. These data indicate that activity responsible for eliciting intestinal response was associated with living organisms.

Of 183 rabbits, 55 failed to respond to the injection of organisms known to be capable of eliciting intestinal response. Such animals were eliminated from the studies. Tests conducted during June and July for two consecutive summers accounted for elimination of 41 of the 55 rabbits. During these periods, six different resting-cell suspensions prepared from recently isolated strains of *S. flexneri* 2a were used. Only 7 of 48 rabbits tested responded. With the resumption of tests each fall, organisms similarly prepared from the same lyophilized stock elicited intestinal response.

DISCUSSION

Although there is no distinct criterion for measuring virulence, except by inoculation of the susceptible host, results obtained with shigellae in the experimental system of De and Chatterje (1953) resemble, in some aspects, natural shigellosis in man. The tissue response and the histological appearance of lesions produced within ligated segments of rabbit small intestine are consistent with classical descriptions of human bacillary dysentery.

Macroscopically and microscopically, the positive response of rabbit small intestine to shigellae resembles that observed when ligated segments are inoculated with pathogenic strains of *E. coli*

(Taylor et al., 1958) or cholera vibrios (Formal et al., 1961*b*). The histopathology of guinea pig small intestine after inoculation with *Shigella* is similar (Formal et al., 1963).

Other investigators using the ligated-segment technique did not compare bacterial counts with histopathological changes. The results of this study, in which an inoculum of 2×10^{10} resting cells was used, indicate that increases in bacterial population lag behind inflammatory changes and occur only after the accumulation of fluid. This observation differs from that reported by Formal et al. (1963). Using the guinea pig as the experimental animal, they placed a single ligature at the terminal ileum and inoculated the duodenum with approximately 2×10^4 shigellae in broth. Examinations at 4-hr intervals revealed inflammatory changes paralleling bacterial counts. However, in guinea pigs, accumulation of gastric and intestinal juices would obscure fluid formation; the juices themselves may provide the milieu necessary for rapid proliferation.

In ligated segments of rabbit small intestine, the early tissue response may be due to the larger numbers of organisms inoculated and to the presence of a diffusible factor capable of initiating the response. Failure to demonstrate a parallel increase in numbers may be the result of technical failure to recover the organisms, death rates equaling the concentrations of surviving bacteria, phagocytosis, or failure to recover the organisms because of tissue invasion. Although organisms were not seen within the tissues when stained by Gram's method, tissue invasion may occur; LaBrec and Formal (1961) detected shigellae by the fluorescent-antibody technique in lamina propria of villi in ileum of pretreated guinea pigs 8 hr after oral inoculation. A similar study would be of value in clarifying events occurring within the rabbit intestinal segment.

Virulence may be considered as the ability of microorganisms to multiply in the host and to cause progressive pathological changes. Therefore, the use of ligated segments of rabbit small intestine qualifies as an indicator of virulence for the rabbit; and, virulence for the rabbit shows a high correlation with a short period of culture of shigellae on artificial media after isolation from human bacillary dysentery infection.

The capability of shigellae to elicit response in rabbit small intestine is rapidly lost during cultivation on artificial media. However, lyophilization immediately after isolation appears to preserve for indefinite periods of time the factors concerned with provoking the intestinal response. The ramifications and advantages of using shigellae recently isolated from acute dysentery infections for investigating possible mechanisms of pathogenesis or for the production of possible vaccines are obvious.

The finding that the capability of *Shigella* to elicit intestinal response is associated with living organisms is in agreement with the observations of Taylor et al. (1958) concerning enteropathogenic *E. coli*. These investigators postulated that intestinal response may be elicited by a diffusible factor produced by living organisms confined to the lumen. Jenkin and Rowley (1959), working with cholerae vibrios, speculated that metabolic products formed during growth of the organisms may be involved. It is not known whether shigellae initiate an inflammatory tissue response by invasion of the superficial mucosal layers or by production of a diffusible factor that may act as a tissue irritant. These possibilities are too speculative for discussion at this time and deserve further investigation.

The use of resting-cell suspensions provides the opportunity to conduct several experiments in vitro with a stable population known to be capable of provoking response in rabbit small intestine.

The effect of seasonal variation on the host response is striking. Although the rabbits were maintained in a constant-temperature environment, provided the same diet throughout the year, and subjected to identical experimental procedures, positive intestinal responses were not observed during the summer months. This finding is similar to that reported by Formal et al. (1961*a*) concerning experimental shigellosis in modified guinea pigs. No differences were observed between the inbred rabbit strain and strains of unknown lineage.

The technique of injecting bacterial preparations into ligated segments of living rabbit small intestine appears to be suited for the study of pathogenesis of *Shigella*. This in vivo system confines the organisms, and possibly some of their products, to a localized area within the alimentary tract, and lends itself to the inclusion of experimental controls within the same animal.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Mrs. M. C. Babcock, A. F. Grebey, F. H. Spofford, V. V. Hernandez, and R. R. Riggle. We thank F. B. Gordon for his advice in the preparation of the manuscript, and the Photographic Department of the Armed Forces Institute of Pathology for the photomicrographs used in this study.

LITERATURE CITED

DE, S. N., K. BHATTACHARYA, AND J. K. SARKER. 1956. A study of the pathogenicity of strains of

Bacterium coli from acute and chronic enteritis. J. Pathol. Bacteriol. **71**:201–209.

DE, S. N., AND D. N. CHATTERJE. 1953. An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. J. Pathol. Bacteriol. **66**:559–562.

FLOYD, T. M., AND H. G. ARM. 1962. Use of the isolated segment of rabbit or guinea pig small intestine as an *in vivo* model for experimental shigellosis. Bacteriol. Proc., p. 99.

FORMAL, S. B., G. D. ABRAMS, H. SCHNEIDER, AND H. SPRINZ. 1963. Experimental *Shigella* infections. VI. Role of the small intestine in an experimental infection in guinea pigs. J. Bacteriol. **85**:119–125.

FORMAL, S. B., G. J. DAMMIN, E. H. LABREC, AND H. SCHNEIDER. 1958. Experimental Shigella infections: characteristics of a fatal infection produced in guinea pigs. J. Bacteriol. **75**:604–€10.

FORMAL, S. B., G. J. DAMMIN, H. SCHNEIDER, AND E. H. LABREC. 1959. Experimental Shigella infections. II. Characteristics of a fatal enteric infection in guinea pigs following the subcutaneous inoculation of carbon tetrachloride. J. Bacteriol. **78**:800–804.

FORMAL, S. B., G. DAMMIN, H. SPRINZ, D. KUNDEL, H. SCHNEIDER, R. E. HOROWITZ, AND M. FORBES. 1961*a*. Experimental Shigella infections. V. Studies in germ-free guinea pigs. J. Bacteriol. **82**:284–287.

FORMAL, S. B., D. KUNDEL, H. SCHNEIDER, N. KUNEV, AND H. SPRINZ. 1961*b*. Studies with *Vibrio cholerae* in the ligated loop of the rabbit intestine. Brit. J. Exp. Pathol. **42**:504–510.

FRETER, R. 1956. Experimental enteric *Shigella* and *Vibrio* infections in mice and guinea pigs. J. Exp. Med. **104**:411–418.

JENKIN, C. R., AND D. ROWLEY. 1959. Possible factors in the pathogenesis of cholera. Brit. J. Exp. Pathol. **40**:474–481.

LABREC, E. H., AND S. B. FORMAL. 1961. Experimental *Shigella* infections. IV. Fluorescent antibody studies of an infection in guinea pigs. J. Immunol. **87**:562–572.

MCNAUGHT, W., AND G. B. S. ROBERTS. 1958. Enteropathogenic effects of strains of *Bacterium coli* isolated from cases of gastro-enteritis. J. Pathol. Bacteriol. **76**:155–158.

MILES, A. A., AND S. S. MISRA. 1938. The estimation of the bactericidal power of the blood. J. Hyg. **38**:732-748.

TAYLOR, J., M. P. MALTBY, AND J. M. PAYNE. 1958. Factors influencing the response of ligated rabbit-gut segments to injected *Escherichia coli*. J. Pathol. Bacteriol. **76**:491–499.

TAYLOR, J., AND M. P. WILKINS. 1961. The effect of *Salmonella* and *Shigella* on ligated loops of rabbit gut. Indian J. Med. Res. **49**:544–549.

WEBSTER, M. E., J. F. SAGIN, M. LANDY, AND A. G. JOHNSON. 1955. Studies on the O antigen of *Salmonella typhosa*. I. Purification of the antigen. J. Immunol. **74**:455–465.