Tolerance and Other Biological Properties of Vibrio parahaemolyticus Endotoxins

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ABSTRACT

Biological properties of endotoxins prepared from three strains of Vibrio parahaemolyticus were compared with reference to commercially prepared Salmonella typhi endotoxin. Endotoxin assays performed in rabbits included dermal Shwartzman reactivity, pyrogenicity, heat stability, and ability to induce tolerance as well as cross-tolerance. Mice were used for endotoxin LD50 determinations. Results showed V. parahaemolyticus endotoxins were similar to that of S. typhi strain O901. Induction of tolerance to V. parahaemolyticus strain 11590 endotoxin resulted in complete cross-tolerance to S. typhi endotoxin, and vice versa. Partial cross-tolerance to S. typhi endotoxin was demonstrated with rabbits rendered tolerant to endotoxin from V. parahaemolyticus strains Sak-3 and FC1011. Absorption spectra, nitrogen, phosphorus and carbohydrate analyses revealed additional similarities between endotoxins from V. parahaemolyticus and endotoxin from a member of the Enterobacteriaceae.

Known in Japan for two decades as a causative agent of large outbreaks of seafood-associated poisoning, Vibrio parahaemolyticus has recently been found associated with similar outbreaks in the U.S.A. [1, 4, 7, 8, 14, 26, 40, 42, 43], and other countries [2, 3]. Nevertheless, the mode of pathogenesis of V. parahaemolyticus is poorly understood. Strains isolated from clinical specimens and displaying hemolysis on selected media (Kanagawa-phenomenon positive) are considered to be enteropathogenic [30, 39, 49], whereas nonhemolytic (Kanagawa-phenomenon negative) seafood or seawater isolates are generally considered non-enteropathogenic [19, 37, 38]. The problem is further complicated by the frequent isolation of several different serotypes of V. parahaemolyticus during an outbreak of food poisoning [7, 8]. Biologically active components (hemolysins) from selected strains of V. parahaemolyticus have been isolated which do not, however, account for the spectrum of gastroenteritis symptoms observed in clinical cases [12, 17, 19, 25, 28, 29, 31, 36, 50, 51]. A single case of endotoxin shock and leg gangrene due to infections by V. parahaemolyticus following an attack of gastroenteritis has been demonstrated [36].

Endotoxins from V. parahaemolyticus have not previously been studied, although some chemical analyses of O antigens [44, 45] and K antigens have been reported [32, 33]. The purpose of this research was to study the biological properties of V. parahaemolyticus endotoxins and to determine if they are similar to endotoxins classically prepared from selected members of the Enterobacteriaceae, such as Salmonella typhi.

MATERIALS AND METHODS

V. parahaemolyticus strains employed. Strain Sak-3 (serotype O2:K3) was obtained through the courtesy of Dr. R. Sakazaki,
National Institute of Health, Tokyo, and had been isolated from a Japanese patient suffering gastroenteritis. Strain 11590 (serotype O4:K11) was isolated from a gastroenteritis patient in Bainbridge, Maryland [26] and was obtained through the courtesy of the late Dr. M. Fishbein, Food and Drug Administration, H.E.W., Washington, D.C. Strain FC 1011 (serotype O7:K38) was isolated from a normal Chesapeake Bay Blue Crab by Dr. R.R. Colwell.

**Media.** Brain heart infusion (BHI) was obtained from Difco Labs., Detroit, Michigan and thiosulfate citrate bile salts sucrose agar (TCBS) from BBL, division of Bioquest, Cockeysville, Maryland. Modified seawater yeast extract medium (MSWYE) and seawater yeast extract medium (SWYE) were prepared as described [6].

**Experimental animals.** Female or male New Zealand white rabbits weighing 2–3 kg were obtained from Camm Research Institute, Wayne, New Jersey. Female BALB/c mice, weighing 15–18 g, were obtained from Cumberland View Farms, Clinton, Tennessee.

**Reference endotoxin.** Salmonella typhi strain O901 endotoxin, lot no. 3124, a phenol-water preparation, was purchased from Difco Labs.

**Growth and harvest of cells for endotoxins.** Cells were grown in 5 and 10-liter volumes of SWYE in a Microferm fermentor (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey). After 6 hr of incubation with aeration and agitation at 37 C, bacterial cells were harvested at 15 000 rpm in a Sorvall model KSB continuous flow apparatus. Harvested cells were lyophilized in a Virtis model 10–800 lyophilizer (Virtis Co., Gardiner, New York), and refrigerated at 4 C until used.

**Extraction of endotoxins.** Five grams (dry weight) of each strain of *V. parahaemolyticus* described above were extracted using phenol-water [48], trichloroacetic acid [5], in a modified procedure [29] and saline-ether [35]. Extracts were concentrated in the cold to one-third volume with hydroscopic gel (Aquacide, I, Calbiochem, La Jolla, California) and lyophilized as above.

**Dermal Shwartzman reactions.** Two female rabbits were used per experiment. Preparative injections consisted of 0.2–1.0 mg graded doses of *V. parahaemolyticus* endotoxins in 0.2 ml pyrogen-free, sterile saline administered intradermally in depilated rabbits. Positive controls consisted of rabbits into which 20 and 50 µg *S. typhi* endotoxin had been similarly administered and for negative controls, rabbits received injections of 0.2 ml pyrogen-free saline. Animals were given a provocative dose the following day, consisting of 20 µg *S. typhi* endotoxin.

In a similar experiment, phenol-water extracts (PHE) from *V. parahaemolyticus* strains Sak-3 and FC 1011 were tested for ability to provoke the dermal Shwartzman reaction. In this case, two rabbits were injected intradermally. The following day, animals were challenged, respectively, with 50 µg of strain FC 1011 and strain Sak-3 endotoxins administered intravenously in pyrogen-free saline, (U.S.P., Cutter Laboratories, Berkeley, California).

**Pyrogenicity.** Twelve acclimatized rabbits, demonstrating less than 0.5 C variability in rectal temperature as determined by daily measurements with a B-D rectal thermometer (Becton, Dickinson Co., Rutherford, New Jersey), were grouped and treated as follows: Group 1 was injected intravenously with 0.2 µg *S. typhi* endotoxin in 0.2 ml pyrogen-free saline. Group 2 received *V. parahaemolyticus* strains 11590 PHE endotoxin similarly administered. Group 3 received 0.2 ml pyrogen-free saline and Group 4 received no treatment. Temperature of all animals were taken at hourly intervals 3 hr prior to endotoxin injections. Temperature readings were initiated immediately after injection of endotoxin preparations, with animals randomized to avoid bias. Temperatures were measured at 1/2-hr intervals for 6 hr following injections. In a similar experiment, PHE preparations of strains Sak-3 and FC 1011 were tested for pyrogenicity, omitting the “no treatment” group and limiting the saline-injected control group to two animals.

**Heat stability of endotoxins.** Samples of endotoxin preparations were heated to 100 C for 1 hr, and 0.2 µg injected intravenously into acclimatized rabbits. Temperatures were measured prior to and for 6 hr following injections, as for pyrogenicity studies.

**LD50 assays in BALB/c mice.** Endotoxin
from V. parahaemolyticus strain 11590 (PHE) and S. typhi strain O901 (Difco) were compared for LD<sub>50</sub> in mice. Endotoxins were diluted in sterile pyrogen-free saline and injected intraperitoneally into groups of five mice. Injection dosages ranged from 0.062 to 1.0 mg for S. typhi and 1.25 to 10 mg for V. parahaemolyticus strain 11590. Mice were observed over a 24-hr period and the number of deaths in each group was tallied. The 50% endpoint was then calculated [34] and plotted [10].

**Tolerance induction.** Two groups of five acclimatized female rabbits were given daily intravenous injections of either V. parahaemolyticus strain 11590 PHE or S. typhi strain O901 endotoxin. Temperatures of animals were measured rectally, as for pyrogenicity assays, with daily temperature profiles for each group prepared. Control animals were injected with pyrogen-free saline and temperature profiles similarly determined. Animals were considered tolerant when a minimal pyrogenic response was observed, together with a rapid return to the base line reading. The experimental protocol was as described above, except that two groups of three rabbits were injected with endotoxin preparations. Control animals were injected with saline. Temperature profiles for all groups were prepared and the mean and standard deviations calculated and data were graphed. Fever indices were obtained by planimeter.

**Assays for cross-tolerance.** Upon achieving the tolerant state, all animals previously injected with V. parahaemolyticus endotoxins were challenged with 0.2 µg S. typhi strain O901 endotoxin in pyrogen-free saline, administered intravenously. Animals rendered tolerant to S. typhi strain O901 endotoxin were challenged with 0.2 µg V. parahaemolyticus strain 11590 PHE, prepared and administered as above. Temperature profiles of injected animals were prepared, mean and standard deviations plotted and the data graphed. Experiments were also performed with endotoxins prepared from V. parahaemolyticus strains FC 1011 and Sak-3.

**Absorption characteristics.** Endotoxin samples were dissolved in 0.05 mM phosphate buffer adjusted to pH 7.2. Scans ranging from 600 to 240 nm were made on a Beckman D-B spectrophotometer (Beckman Instruments, Palo Alto, California). Scans were initiated at 600 nm, using the tungsten lamp. Readings from 320 to 240 nm were made using the hydrogen lamp. Endotoxin samples were run at 1.0 mg/ml sample. For reference spectra, readings were taken of S. typhi endotoxin, DNA from V. parahaemolyticus strain FC 1011 (courtesy of Dr. Thomas E. Staley) at a concentration of 30 µg/ml, RNA (Nutritional Biochemicals, Cleveland, Ohio) 25 µg/ml, and ovalbumin (Nutritional Biochemicals) 1.0 mg/ml. Estimates of nucleic acid contamination in endotoxin samples were made from standard curves prepared from the DNA samples.

**Carbohydrate determination.** The phenol-sulfuric acid method was used for carbohydrate determination [9]. Standards consisted of glucose at concentrations of 2.5, 5, 10, 20, 40 and 60 µg, DNA concentrations of 2.5, 5, 10, 30, 40, 80, and 100 µg and xylose at 2.5, 5, 10, 50, and 80 µg. Endotoxin preparations were employed at a concentration of 0.4 mg. Absorbance was read at 490 nm using a Spectronic 20 spectrophotometer (Bausch and Lomb Optical Co., Rochester, New York). Carbohydrate determinations were based on glucose. DNA was found to give 20% of the color value of glucose at similar concentrations; xylose yielded 72% of the glucose value. Carbohydrate concentrations of endotoxin samples, were determined by reading directly from the standard curve prepared for glucose.

**Phosphorus determination.** The method used was as described elsewhere [11]. Phosphorus standards were prepared using potassium phosphate, 2.5 µg P to 20 µg P, and provided a standard curve. Absorbances of standards and unknowns were determined at 660 nm in a Spectronic 20 spectrophotometer. Concentrations of unknowns were determined by reading absorbances directly from the standard curve.

**Nitrogen determination.** The method employed was as described elsewhere [21]. Ammonium sulfate, used as a standard, was diluted in distilled water, providing 2.5, 5, 10, 15 and 20 µg of nitrogen. Absorbances of samples were read at 420 nm in a Spectro-
Table 1. Yields and composition of endotoxins, obtained by three different extraction methods applied to 5 g of dry cells of \textit{V. parahaemolyticus} strains 11590, FC 1011 and Sak-3

<table>
<thead>
<tr>
<th>\textit{V. parahaemolyticus} strain</th>
<th>Yield of crude endotoxin in g/5g dry cells</th>
<th>Carbohydrate (\mu g/mg)</th>
<th>N (\mu g/mg)</th>
<th>P (\mu g/mg)</th>
<th>Protein (\mu g/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11590</td>
<td>PHE</td>
<td>1.10</td>
<td>70.0</td>
<td>58.8</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>TCA</td>
<td>.08</td>
<td>60.0</td>
<td>15.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>S/E</td>
<td>.12</td>
<td>30.0</td>
<td>92.5</td>
<td>1.9</td>
</tr>
<tr>
<td>FC 1011</td>
<td>PHE</td>
<td>.71</td>
<td>170.0</td>
<td>25.0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>TCA</td>
<td>.09</td>
<td>150.0</td>
<td>27.5</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>S/E</td>
<td>.06</td>
<td>165.0</td>
<td>33.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Sak-3</td>
<td>PHE</td>
<td>.83</td>
<td>150.0</td>
<td>18.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>TCA</td>
<td>.03</td>
<td>58.0</td>
<td>0.25</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>S/E</td>
<td>.10</td>
<td>35.0</td>
<td>60.0</td>
<td>1.3</td>
</tr>
<tr>
<td>\textit{S. typhi} O901 (Difco)</td>
<td></td>
<td></td>
<td>183.0</td>
<td>28.8</td>
<td>9.4</td>
</tr>
</tbody>
</table>

PHE = phenol-water, TCA = trichloroacetic acid, S/E = saline-ether.

**RESULTS**

Table 1 lists the yields of endotoxin preparations per 5.0 g dry cells and the nitrogen, phosphorus, protein and carbohydrate content per mg of endotoxin. Absorption spectra of endotoxins, including spectra of reference endotoxin and DNA, are shown in Figure 1. Figure 1a shows the spectra of \textit{V. parahaemolyticus} strain 11590 phenol-water extract (PHE), trichloroacetic acid extract (TCA) and saline ether extract (S/E), as compared with \textit{S. typhi} strain O901 (Difco; a phenol-water preparation). Figure 1b shows the spectra of similar preparations from \textit{V. parahaemolyticus} strains Sak-3 with DNA from \textit{V. parahaemolyticus} strain FC 1011 for reference. Figure 1c shows the spectra obtained with preparations from strain FC 1011 in comparison with homologous DNA. The spectra, in general, are directly compa-

Fig. 1. Absorption spectra for \textit{V. parahaemolyticus} endotoxin preparations. a, Comparison of PHE, S/E and TCA extracts of strain 11590 with \textit{S. typhi} O901 (Difco) endotoxin. b, Comparison of preparations from strain Sak-3. Reference DNA from strain FC 1011. c, Comparison of preparations from strain FC 1011. Reference DNA from strain FC 1011. Concentrations of all preparations = 1.0 mg/ml; that of FC 1011 DNA = 30 \(\mu g/ml\).
V. PARAHAEMOLYTICUS ENDOTOXINS

Fig. 2. Dermal Shwartzman reactions.Injected sites are marked and were injected with endotoxin ranging from 0.05–0.4 mg endotoxin. Sites 1–5 were injected with V. para- haemolyticus strain 11590 TCA extract, 6–10, phenol-water extract, 11 and 12, S. typhi strain O901 endotoxin (positive control) and 13, saline (negative control), preparative injections. The provocative injection, given 24 hr later, consisted of 20 μg S. typhi strain O901 endotoxin. The reactions shown here were observed 3 hr after the provocative injection. Injection sites were marked with felt-tipped pen.

Results of Biological Assays

Figure 2 presents results of the dermal Shwartzman assay employing V. para- haemolyticus strain 11590 PHE, TCA and S/E preparations administered intradermally as preparative injections. S. typhi endotoxin was used as a positive control and saline as a negative control. The reactions were provoked by a single intravenous injection of 20 μg of S. typhi endotoxins administered 24 hr later.

Shwartzman Reaction of Endotoxin Preparations from V. paraahaemolyticus

Dermal reactivity of preparations from V. paraahaemolyticus strains Sak-3 and FC 1011 differed significantly. Results are shown in Table 2. Extracts of strains FC 1011 and Sak-3 failed to demonstrate the dermal Shwartzman reactivity of S. typhi by intravenous challenge with S. typhi endotoxin. Successful reactivity was observed only on challenge with homologous endotoxin in the case of strain FC 1011, although FC 1011 PHE was able to provoke Shwartzman reactivity in skin areas preparatively injected with either 11590 PHE or S. typhi. In contrast, the phenol-water extract of strain Sak-3 was able to provoke skin reactivity in skin areas preparatively injected with FC 1011, 11590, Sak-3 and S. typhi O901.

Pyrogenicity in Rabbits

Fever curves were obtained and plotted for all groups of rabbits injected with V. paraahaemolyticus and S. typhi. Figures 3–6, parts a, show the average fever index in groups of rabbits intravenously administered endotoxin preparations. Fever indices of V. paraahaemolyticus strain 11590 and S. typhi strain O901 were very similar. Figures 7 and 8, (dashed lines) in both parts a and b, indicate day one mean responses and standard deviations in groups of rabbits adminis-
Fig. 3. Demonstration of cross-tolerance to *V. parahaemolyticus* strain 11590 endotoxin by rabbits tolerant to *S. typhi* endotoxin. a, Fever index obtained as an average of a group of five rabbits injected with 0.2 µg endotoxin. b, Average fever index in the same group of rabbits after nine daily injections of 0.2 µg endotoxin. c, Average fever index in the same group of rabbits following challenge with 0.2 µg *V. parahaemolyticus* 11590 endotoxin.

Fig. 4. Demonstration of cross-tolerance to *S. typhi* O901 endotoxin by rabbits tolerant to *V. parahaemolyticus* strain 11590 endotoxin (phenol-water preparation). a, Average fever index obtained in a group of five rabbits injected with 0.2 µg 11590 endotoxin. b, Average fever index in the same animals after nine daily injections of 0.2 µg endotoxin. c, Average fever index in the same group of animals following challenge with 0.2 µg *S. typhi* O901 endotoxin. A synergistic response is observed.

Table 2. Dermal Shwartzman reactions in rabbits

<table>
<thead>
<tr>
<th>Provocative injection</th>
<th>Preparative injection 0.2 µg</th>
<th>Three-hour reaction in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sak-3</td>
<td>PHE</td>
<td>1.0</td>
</tr>
<tr>
<td>FC 1011 PHE</td>
<td>TCA</td>
<td>1.0</td>
</tr>
<tr>
<td>FC 1011 TCA</td>
<td>S/E</td>
<td>1.0</td>
</tr>
<tr>
<td>FC 1011 S/E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11590 PHE</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td><em>S. typhi</em> O901</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Saline control</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC 1011, 50 µg</td>
<td>PHE</td>
<td>0.0</td>
</tr>
<tr>
<td>FC 1011 TCA</td>
<td>TCA</td>
<td>0.0</td>
</tr>
<tr>
<td>FC 1011 S/E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11590 PHE</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td><em>S. typhi</em> O901</td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>Saline control</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

Endotoxins from *V. parahaemolyticus* strains FC 1011, 11590 and Sak-3 (experimentals) and endotoxin from *S. typhi* strain O901 (positive control) administered as preparative injections. Provocative injections administered intravenously 24 hr later were 50 µg of phenol-water extracts of either strains FC 1011 or Sak-3.
Fig. 5. Demonstration of cross-tolerance to endotoxin from *S. typhi* O901 in rabbits tolerant to endotoxin from *V. parahaemolyticus* strain FC 1011 (phenol-water extract). 

- a. Average fever index in a group of three rabbits injected with 0.2 μg FC 1011 endotoxin.
- b. Average fever index in the same group of animals after 5 daily injections of 0.2 μg endotoxin.
- c. Average fever index in the same group of animals following challenge with 0.2 μg *S. typhi* endotoxin. Although the response is elevated over the day 1 response of the same group of animals, the overall fever index is well below that observed with a similar dose of *S. typhi* on day 1. (See Fig. 3a and Fig. 8b, and discussion).

Fig. 6. Demonstration of cross-tolerance to endotoxin from *S. typhi* O901 in rabbits tolerant to endotoxin from *V. parahaemolyticus* strain Sak-3 (phenol-water extract). 

- a. Average fever index in a group of three rabbits injected with 0.2 μg Sak-3 endotoxin.
- b. Average fever index in the same group of rabbits after 7 days of daily injections of 0.2 μg endotoxin.
- c. Average fever index in the same group of animals following challenge with 0.2 μg *S. typhi* endotoxin. Although the response exceeds the day 1 response to Sak-3 endotoxin, the overall fever index is well below that observed with a similar dose of *S. typhi* endotoxin on day 1. (See Fig. 3a and Fig. 8a, and discussion).

11590 are almost identical; this is also seen in Figure 7 (solid lines; mean and standard deviations are shown). The data for endotoxins from *V. parahaemolyticus* strains Sak-3 and FC 1011 are quite different. Figures 5 and 6, parts c, show clearly that animals tolerant to Sak-3 and FC 1011 demonstrated a more elevated fever index in response to *S. typhi* challenge than the same group had on day one injections with homologous endotoxins (Figs. 5 and 6, parts a). This puzzling result is resolved, however, when mean fever responses of the same animals are compared with mean fever responses of animals injected with *S. typhi* for the first time; clear indication of cross-tolerance, albeit at a low level, is demonstrated. This is shown in Figure 8 (solid lines, parts a and b) showing mean responses and standard deviations in groups of rabbits tolerant to Sak-3 and FC 1011, respectively, challenged with *S. typhi* endotoxin. The demonstration that none of the means and standard deviations show any overlap, particularly in the critical 3-hr post-injection period, is interpreted as a degree of cross-tolerance.

**Comparative LD50 of *V. parahaemolyticus* Strain 11590 and *S. typhi* O901**

Because of similarities of fever indices and tolerance effects as well as Shwartzman reactivity shown by animals injected with endotoxin from *V. parahaemolyticus* strain 11590 and *S. typhi* strain O901, lethality assays in mice were limited to those two endotoxin preparations. Results of LD50 assays in BALB/c mice are shown in Figure 9. The LD50 for 15–18 g female mice, employing strain 11590 was 2.1 mg; the comparable figure for *S. typhi* was 0.53 mg. Saline-injected controls showed no effect. The
parallelism exhibited by the curves for S. typhi endotoxin-injected mice and V. parahaemolyticus endotoxin-injected mice indicates the overall biological similarity of the two preparations despite the difference in potency [10], i.e., S. typhi endotoxin demonstrated an approximately 2.5 × greater effect in mice than did V. parahaemolyticus.

**DISCUSSION**

Biological properties of V. parahaemolyticus endotoxins have not heretofore been described. Results of the present studies indicate that V. parahaemolyticus endotoxins, prepared by classic methods, are capable of inducing response in susceptible animals. Endotoxin from V. parahaemolyticus strain 11590, in particular, elicits biological responses very similar to those of S. typhi endotoxin. Endo-
V. parahaemolyticus strains Sak-3 and FC 1011 appear to be less potent, by comparison, at least under the conditions employed in the present studies. V. parahaemolyticus strains, Sak-3 and FC 1011 endotoxin preparations were less effective in eliciting the dermal Shwartzman reaction, as evidenced by failure of preparative injections of those endotoxins to be provoked by S. typhi endotoxin, in contrast with endotoxin from strain 11590. Successful Shwartzman reactivity could be demonstrated with Sak-3 and FC 1011 only if homologous endotoxins were employed for provocation of the dermal Shwartzman. In addition, while significant cross-tolerance to S. typhi endotoxin was demonstrated, employing Sak-3 and FC 1011 endotoxin preparations, the degree of tolerance, as measured by depression of the pyrogenic response, was not as dramatic as that achieved employing S. typhi endotoxin challenge against V. parahaemolyticus 11590-tolerant animals and vice versa.

Demonstration of cross-tolerance does not indicate immunological cross-reactivity. With small doses of endotoxins, the early state of refractoriness is specific, whereas continuous infusion of endotoxins leads to pyrogenic unresponsiveness, based on cellular refractoriness [15, 16, 24]. As the intervals of time between endotoxin challenge are lengthened, the cellular refractory state wanes and resistance then is dependent upon circulating antibodies which assist in endotoxin clearance [15, 16, 24]. Although antibody studies were not included in the present studies, the patterns of tolerance exhibited by animals injected with endotoxins of V. parahaemolyticus are in agreement with observations of others, and it would be anticipated that the continuous infusion with endotoxins would prevent emergence of antibody. Further studies concerning the role of antibodies in the early and late tolerance effects, employing V. parahaemolyticus endotoxins, are in progress.

Absorption spectra of all endotoxin preparations, i.e., TCA, PHE and S/E, were similar. Yields of carbohydrates, however, tended to vary, with values for strains of FC 1011 most closely approximating that of S. typhi. Nitrogen, phosphorus, carbohydrate and protein values obtained for the different preparations of V. parahaemolyticus endotoxins were well within the range documented in the literature for members of Enterobacteriaceae [13] and for some Vibrio sp. [27, 42].

LD_{50} values obtained in this study, employing 15 to 18 g BALB/c mice, are in reasonable agreement with those reported [22] for mice of the Bagg strain, antecedents of the BALB/c strain [41], but generally lower than for V. cholerae [20]. Thus the biological activities of V. parahaemolyticus strain 11590 endotoxin, prepared by the phenol-water method, strongly resemble those of S. typhi O901 endotoxin (Difco). It may be conjectured that the lesser degree of potency displayed by the V. parahaemolyticus 11590 endotoxin might be due to less carbohydrates, in comparison with S. typhi, since there is some evidence for correlation of carbohydrate content and/or type with potency in endotoxin [46, 47].

In conclusion, biological assays of endotoxins from V. parahaemolyticus strains, when compared in animals and employing different tests for reactions characteristic for endotoxins, demonstrated that the V. parahaemolyticus endotoxins resembled those of the Enterobacteriaceae. V. parahaemolyticus strain 11590 endotoxin prepared as a phenol-water extract, in particular, resembled S. typhi endotoxin in
the dermal Shwartzman reaction, fever index, and induction of tolerance and cross-tolerance effects in rabbits. *V. parahaemolyticus* 11590 and *S. typhi* O901 endotoxin produced overall parallel curves in the LD$_{50}$ test using BALB/c mice, with *V. parahaemolyticus* demonstrating a lesser potency, in agreement with other work [18]. Although these studies do not define the pathogenicity of *V. parahaemolyticus*, they do, nevertheless, provide an understanding of some fundamental properties of the endotoxins of this organism which may, in conjunction with future studies, elucidate the mechanisms of pathogenesis.

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REFERENCES


[2] Barrow, G.I., and Miller, D.C. 1972. *Vibrio parahaemolyticus*: A potential pathogen from *Vibrio* strains employed in this study. We wish to thank Dr. Y. Takeda of Osaka University, Osaka, Japan, for his generous help in reading and commenting on this manuscript.


