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Chemical Composition and Biological Properties of the Endotoxin of *Brucella abortus*

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ABSTRACT

BAKER, PHILLIP J. (University of Wisconsin, Madison), AND J. B. WILSON. Chemical composition and biological properties of the endotoxin of *Brucella abortus*. J. Bacteriol. 90:895-902. 1965.—The ability of endotoxin to induce hypoferremia in mice was used to measure the specific activity of various endotoxin preparations of *Escherichia coli* and *Brucella abortus* and to determine the endotoxin content of several strains of *B. abortus* differing in virulence for guinea pigs. The endotoxin preparations from *E. coli* possessed greater biological activity than those from *B. abortus*. The same types of *B. abortus* endotoxin preparations, whether obtained from strains of high or low virulence, had about the same activity. Although differences in endotoxin content were noted among several strains of *B. abortus* of different virulence, there appeared to be no correlation between endotoxin content and virulence. Chemical analyses of *B. abortus* and *E. coli* endotoxins, based upon nitrogen, phosphorus, fatty acid ester, fatty acid amide, total fatty acids, hexose, and hexosamine content, revealed differences in chemical composition; however, these differences could not be correlated with differences in biological activity. The same types of endotoxin preparations, whether obtained from strains of *B. abortus* of high or low virulence, were similar in chemical composition and in biological activity. A correlation between the ability of endotoxin to induce hypoferremia and its lethal effects in mice is suggested. In general, endotoxin preparations of high hypoferremic activity had low mouse LD_{50} values.

The many pharmacological properties of bacterial endotoxins are well documented in the reviews by Thomas (1954), Bennett and Cluff (1957), Atkins (1960), Rosen (1961), and Landy (1962). The monograph on bacterial endotoxins, edited by Landy and Braun (1964), presents in detail much of the most recent work in this active area of research. The work to be described in this paper is a continuation of the investigations concerning factors related to virulence in *Brucella abortus* (Wilson and Dasinger, 1960; Baker, Wyly, and Wilson, 1964).

In view of the many pharmacological effects produced by endotoxin, its role as a component of virulence has been considered (Spink and Anderson, 1954; Cameron, Holtman, and Jefferies, 1960). Endotoxin can be obtained from nonpathogenic gram-negative bacteria as well as from pathogenic species (Westphal and Lüderitz, 1954). Moreover, the work of Boivin and Mesrobeanu (1935, 1936), Westphal and Lüderitz (1954), and Spink and Anderson (1954) has shown that, among the gram-negative pathogens, endotoxin is present in larger amounts in the smooth virulent forms than in the rough avirulent forms. Spink and Anderson (1954) found no difference in lethal effects between Boivin endotoxins obtained from smooth strains of *Brucella* species of high and low virulence when tested in mice. Wilson, Kolbye, and Baker (1964) found no differences in the numbers of heat-killed or acetone-killed cells required to obtain 1 LD_{50} in mice when *B. abortus* strains of different virulence were compared. Although these studies suggest that strains of high and low virulence contain about the same amount of endotoxin, a more sensitive means of bioassay would be desirable to refine these data and to establish this point firmly.

Since intracellular parasitism is a prominent feature of brucellosis (Spink, 1956), the possibility of a significant difference in the rate of intracellular growth between strains of high and low virulence has been explored. Spink and Anderson (1954) found that mice given intravenous injections of highly virulent *B. abortus* died in 1 to 2 days, whereas mice injected with a strain of *B. abortus* of low virulence died in 6 to 7 days. Differences in the rate and extent of

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intracellular growth between strains of *B. abortus* of high and low virulence have been noted in tissue-culture systems by Holland and Pickett (1956, 1958), Braun, Pomales-Lebron, and Stinebring (1958), and Bessudo and Wilson (1962). Clearly, if there is essentially no difference in the amount of endotoxin present per cell between strains of high and low virulence, then the rate and extent of intracellular growth would be the limiting factor in the amount of endotoxin produced within the host. Alternatively, with strains having a relatively large amount of endotoxin per bacterial cell, limited intracellular multiplication might produce the same effects as those obtained with the extensive intracellular multiplication of strains with a lesser content of endotoxin. It is the purpose of these investigations to determine whether strains of *B. abortus* of high virulence have a greater endotoxin content than do strains of low virulence.

MATERIALS AND METHODS

Cultures. Some of the strains of *B. abortus* used in these studies and their virulence as determined by guinea pig titration are listed below. The acriflavine test of Braun (1947) and the crystal violet test of White and Wilson (1951) were used as tests for colonial morphology.

The principal strains used were: strain 11 (low virulence for guinea pigs), 2308 (high virulence for guinea pigs and cattle), 544 (high virulence for guinea pigs), 544A (low virulence for guinea pigs), 45/0 (low virulence for guinea pigs), and 19 (medium to low virulence for guinea pigs).

In addition to the above strains, independently occurring streptomycin-resistant (SR) and "reverse" streptomycin-sensitive (SS) mutants of a streptomycin-dependent mutant of *B. abortus* 2308 were obtained by selection and enrichment in streptomycin-free Trypticase Soy Broth. It was reported by Simon (1955) that such mutants differ from their respective parent strains in virulence. Although a series of independently occurring mutants were obtained by this method, only mutants 2308-SR-4, and 2308-SS-6, having high virulence for guinea pigs, and 2308-SS-1, having low virulence, were employed in these studies.

Another series of streptomycin mutants, obtained from *B. abortus* 2308 by selection on solid media, were supplied through the courtesy of Lois M. Jones. These mutants are numbered 2308-2A-1, 2308-2A-2, 2308-2F-1, 2308-2F-2, and 2308-2F-Er.

Two rough mutants, *B. abortus* 2308 R and *B. abortus* 11 R, were isolated as phage-resistant mutants on lawns of their respective smooth parent strains. No experimental information is available on the virulence of these two mutants, but, in general, rough strains are of low virulence (Spink, 1956).

Cultures of *Escherichia coli* Rolf and *E. coli* ICR were isolated from the Rolfsmeyer Swiss Webster and the A. R. Schmidt ICR strains of mice, respectively, by conventional methods.

Cultivation of cells for the extraction of endotoxin. Trypticase Soy Agar and Trypticase Soy Broth (BBL) were used exclusively for the cultivation and maintenance of all cultures; 48-hr broth cultures of *B. abortus* and 12-hr cultures of *E. coli* were used as the source of cells. The cultures were incubated on a rotary shaker at 37 C. The cells were harvested by centrifugation at 10,000 $\times$ *g* for 20 min at 4 C. The harvested cells were washed three times with cold saline, and then were suspended in distilled water for the extraction of endotoxin. Because *E. coli* and *B. abortus* have different growth rates, harvest at 24 hr and 48 hr, respectively, represents comparable physiological age, as both were in the early stationary phase of growth.

Heat-killed cell preparations. Freshly harvested and washed viable-cell suspensions were adjusted turbidimetrically and by direct microscopic count to contain 10^9 cells per milliliter. This cell suspension was kept at 65 C for 1 hr, after which time it was cooled, diluted, and stored at 4 C until used. Viability tests were made with Trypticase Soy Agar to determine the effectiveness of the heat-killing process.

Experimental animals. Swiss Webster female mice, weighing 22 to 25 g, obtained from the Rolfsmeyer Rat and Mouse Farm, Madison, Wis., were used exclusively. The mice were fed Purina Laboratory Chow and were given water *ad libitum*. The temperature of the animal rooms was maintained at 25 C.

Determination of mouse toxicity. Serial dilutions of various concentrations of endotoxin, contained in 0.2 ml of pyrogen-free saline, were administered to mice intravenously. The number of deaths within each dosage group, which contained six to eight mice, were recorded over a 24-hr period. The data were then plotted as log dose of endotoxin versus probit of the per cent mortality at 24 hr. From the data, the LD_{50} was calculated (Boyd, 1956).

Preparation of endotoxins. The methods used for the preparation of Boivin (TCA) and Westphal (PW) types of endotoxins, as well as the commercially available endotoxin preparations used in these studies, are those described by Baker and Wilson (1965). In addition, endotoxins were prepared by the ether-water (EW) extraction procedure of Ribi, Milner, and Perrine (1959).

An erythrocyte-sensitizing substance (ESS), used for passive hemagglutination studies, was also tested for endotoxic activity. The material was prepared from *B. abortus* strains 2308 and 11 by first autoclaving a washed viable-cell suspension for 30 min at 121 C and then precipitating the solubilized antigen from the cooled, cell-free supernatant liquid by the addition of three volumes of ethyl alcohol. The precipitated material was dissolved in saline and was reprecipitated two additional times with ethyl alcohol. The final product was lyophilized and stored in a desiccator until used.

Attempt to extract endotoxin from rough strains of B. abortus. An attempt was made to extract endotoxin from two rough strains of *B. abortus*, 2308 and 11, according to the method described by Raynaud, Digeon, and Nauciel (1964).

Determination of the serum-iron levels of mice. The procedure used for the collection of serum is described in the accompanying publication (Baker and Wilson, 1965). Serum-iron levels were determined by the method of Schade et al. (1954).

Statistical methods. A randomized complete-block experimental design, with the *F*-test criterion to detect differences among treatments at the 5% level of significance, was used to compare the specific activities of various endotoxin preparations or heat-killed cell preparations. Tukey's W procedure was used to evaluate and judge the significance of all differences among mean serum-iron responses at the 5% level of significance. The procedure of Cochran and Cox was used to determine the number of replicates required to detect a reduction in the serum-iron level of 50 μg/100 ml at the 5% level of significance with 90% assurance. All of these statistical methods are described by Steel and Torrie (1960).

Chemical methods of analysis. Hexose was determined by the method of Dische (1955), esterified fatty acids by the method of Snyder and Stephens (1959) as modified by Tauber (1960), fatty acid amides by the method of Haskins (1961), nitrogen by the method of Johnson (1941), hexosamine by the method of Rimington (1940), and phosphorus by the method of Dryer, Tammes, and Routh (1957). The selection of appropriate standards and slight adaptations of the methods for use with bacterial endotoxins were made as suggested by Ribi et al. (1961).

For hexose determinations, a mixed standard containing equal amounts of glucose, mannose, galactose, and rhamnose was used. Fatty acid amide, fatty acid ester, and total fatty acid contents were calculated as palmitic acid.

RESULTS

Ability of various endotoxin preparations to induce hypoferremia in mice. The values listed in Table 1 represent reductions in the serum-iron level (micrograms or milligrams of iron per 100 ml of serum) obtained by subtracting the serum-iron levels of mice receiving 100 μg of the various endotoxin preparations from the mean serum-iron level of the saline control group (303 μg/100 ml). An analysis of variance, by use of the *F*-test criterion, revealed that there were significant differences in the response of mice to the various endotoxin preparations.

To compare statistically the difference between the mean response elicited by a given endotoxin with the mean response produced by any other endotoxin, Tukey's W procedure, at the 5% level of significance, was employed (Fig. 1). All mean values which are underlined by the same line do not differ from each other significantly at the 5% level of significance. Endotoxin preparations obtained from *E. coli* did not differ significantly from each other in activity, and these endotoxin preparations possessed the highest degree of hypoferremic activity. Among the different *B. abortus* endotoxins tested, fraction 5 had the highest activity and did not differ significantly in activity from most of the *E. coli* endotoxin preparations. Fraction 3, which appeared in the aqueous phase after cells of *B. abortus* were extracted with hot phenol-water, appeared to

TABLE 1. *Reduction in the serum-iron levels (μg/100 ml) of mice given 100 μg of various endotoxin preparations**

Endotoxin prepn	Replicates					Mean
	1	2	3	4	5	
Brucella abortus 2308-TCA	106	136	152	110	134	127
B. abortus 11-TCA	172	139	150	176	81	143
Escherichia coli O55:B5-TCA	237	231	220	241	226	231
E. coli O55:B5-PW	211	221	188	203	211	206
B. abortus 2308-fraction 5	206	244	172	196	190	201
B. abortus 11-fraction 5	173	153	141	198	169	166
B. abortus 2308-fraction 3	62	49	54	57	29	50
B. abortus 11-fraction 3	94	123	117	65	78	95
B. abortus 2308-EW	117	110	70	107	120	104
B. abortus 11-EW	79	170	125	165	97	127
B. abortus 2308-ESS	130	113	62	83	100	97
B. abortus 11-ESS	105	120	123	167	92	121
E. coli Rolf-TCA	253	146	240	228	220	217
E. coli Rolf-EW	190	220	187	217	200	202

* The reduction in the serum-iron level was determined by subtracting the level for each replicate from the mean level (303 μg/100 ml) of the saline controls. Tabular $F_{05} = 2.54$; experimental $F_{05} = 23.17$.

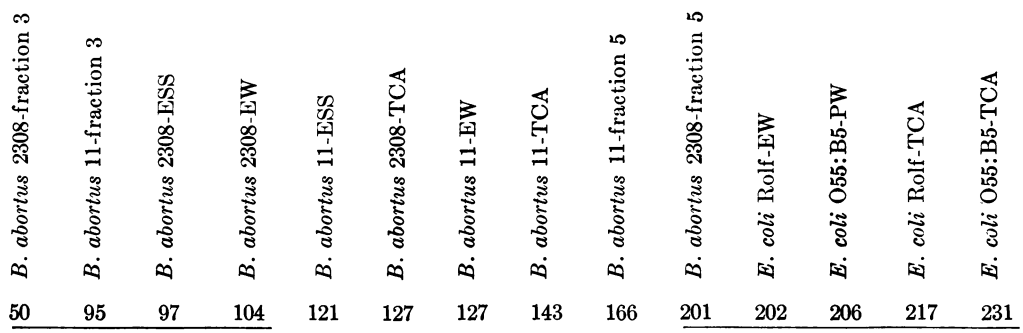

FIG. 1. *Summary of significant differences in mean reduction of serum-iron levels (μg/100 ml) of mice given 100 μg of various Brucella abortus and Escherichia coli endotoxin preparations. Mean values underlined by the same line do not differ from each other significantly at the 5% level of significance. To be significant at the 5% level, the difference between any two means must be greater than 57.2 μg/100 ml.*

have the lowest activity. Endotoxin preparations of the same type, whether obtained from *B. abortus* 2308 (high virulence) or *B. abortus* 11 (low virulence), did not differ from each other significantly in specific activity.

An estimate of the number of replicate serum samples required to detect differences in the serum-iron level of 50 μg/100 ml, which are significant at the 5% level with 90% assurance, was made beforehand according to the method of Cochran and Cox (Steel and Torrie, 1960). In a randomized complete-block experimental design with 14 endotoxin preparations, five replicates were sufficient to satisfy this criterion.

Mouse toxicity. Because of an insufficient amount of some of the endotoxin preparations, not all of the preparations could be assayed for the LD_{50}. The endotoxins obtained from *E. coli* were the most toxic, and gave the lowest LD_{50} values (Table 2). Fraction 5 endotoxin preparations, obtained from *B. abortus* 2308 and 11, were next in potency. These same preparations were also the most active in the hypoferremia experiments (Fig. 1 and Table 2). Preparations with low hypoferremic activity, in general, gave the highest mouse LD_{50} values. This indicates that a correlation may exist between the lethal effects of endotoxin and its ability to induce hypoferremia; however, the wide differences in the relative sensitivity of the two systems of bioassay (Baker and Wilson, 1965) would present some difficulties in establishing this point.

Serum-iron levels in mice given heat-killed cells of B. abortus. The serum-iron levels of mice after the administration of 10^8 heat-killed cells of various strains of *B. abortus* of different virulence were determined (Table 3). In group I are placed strains of *B. abortus* of high virulence, and in group II are placed strains of low virulence; group III contains strains of *B. abortus* of unknown virulence. On the basis of the *F*-test criterion (Table 3), there were significant differences in the serum-iron levels of mice after the administration of the various heat-killed cell preparations. From the summary of a multiple-comparisons test with mean serum-iron levels obtained in the experiment (Fig. 2), it can be noted that there were significant differences in the serum-iron responses to the various heat-killed cell preparations tested. Presumably, these differences are due to differences in endotoxin content.

B. abortus strains 2308 R and 11 R produced little if any changes in the serum-iron levels of mice in comparison to the serum-iron level of the controls given pyrogen-free saline (303 μg/100

TABLE 2. *Mouse toxicity and mean reductions in serum-iron levels obtained with various endotoxin preparations**

Endotoxin prepn	LD_{50}	Mean reduction in serum iron
	μg	μg/100 ml
Escherichia coli Rolf-TCA	<100	217
E. coli Rolf-EW	209	202
Brucella abortus 11-fraction 5	393	166
B. abortus 2308-fraction 5	429	210
B. abortus 2308-EW	621	104
B. abortus 2308-TCA	621	127
B. abortus 11-EW	740	127
B. abortus 11-TCA	872	143
B. abortus 11-ESS	3,000	121
B. abortus 2308-ESS	6,000	97

* The mean reduction in serum iron represents the decrease in the serum-iron level obtained after the administration of 100 μg of each of the endotoxin preparations.

TABLE 3. *Serum-iron levels (μg/100 ml) of mice after administration of 10^8 heat-killed cells of various strains of Brucella abortus**

Group†	Strain	Replicates					Mean
		1	2	3	4	5	
I	2308-SS-6	232	238	222	222	190	220
	2308-SR-4	208	293	203	196	251	230
	2308	215	169	151	177	156	173
	544	250	258	319	307	255	277
II	11	281	286	279	206	246	259
	544A	193	311	260	325	276	273
	45/0	237	272	233	220	347	261
	2308-SS-1	269	275	317	237	293	278
	2308-2A-2	129	242	177	219	175	188
	2308-2F-1	310	309	239	316	322	299
	2308-2F-2	287	279	265	239	258	265
	2308-2F-1-Er	221	230	194	263	192	220
	US 19	141	281	216	223	216	215
III	2308 R	310	333	310	325	214	298
	11 R	302	357	257	303	308	305
	2308-2A-1	177	271	137	225	244	210

* Tabular $F_{05} = 2.53$; experimental $F_{05} = 6.19$.

† Group I, strains of high virulence; group II, strains of low virulence; group III, strains of unknown virulence.

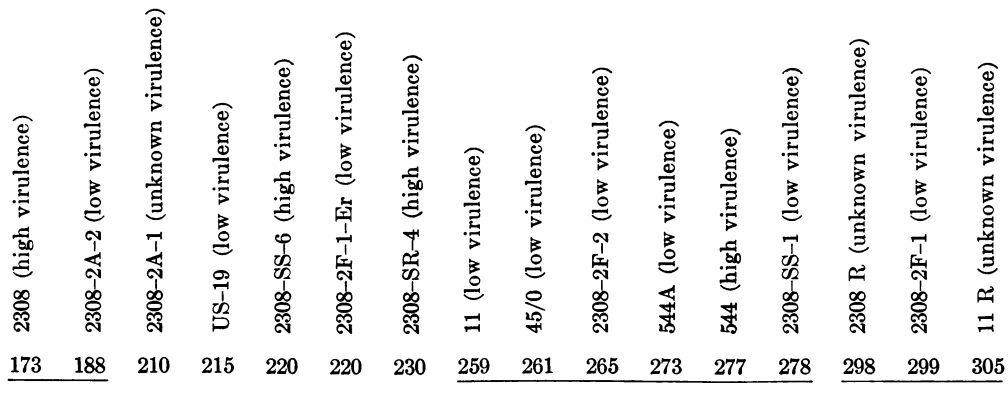

FIG. 2. *Summary of the significant differences in mean serum-iron levels (μg/100 ml) of mice given 10^8 heat-killed cells of various strains of Brucella abortus. Mean values underlined by the same line do not differ from each other significantly at the 5% level of significance. To be significant at the 5% level, the difference between any two means must be greater than 28.3 μg/100 ml.*

ml). When these rough strains were extracted for endotoxin by the method of Raynaud et al. (1964), no endotoxin could be obtained.

Reproducibility of the hypoferremia obtained with heat-killed cells. To determine the reproducibility of the hypoferremic response obtained with different lots of heat-killed cell preparations of *B. abortus* 2308, four different lots of heat-killed cells were prepared independently and in the same manner. An analysis of variance of the data (Table 4) revealed that the different lots of heat-killed cell preparations did not differ from each other significantly in respect to their ability to induce hypoferremia.

Chemical composition of the endotoxins. A summary of the data on the chemical composition of the various endotoxin preparations is given in Table 5. The highest values for hexose content were obtained with the *E. coli* endotoxin preparations. These were the endotoxins which were the most active in respect to mouse lethality and in their ability to induce hypoferremia; however, among the *B. abortus* endotoxin preparations, those having the highest biological activity

(fraction 5) were low in hexose content or did not differ appreciably in hexose content from some of the *B. abortus* endotoxins of lower biological activity (TCA and EW preparations). The ESS preparations of *B. abortus*, although having the highest hexose content, were among the lowest in biological activity. From these findings, it is difficult to relate biological activity to hexose content.

The differences observed in the fatty acid ester, fatty acid amine, and total fatty acid content could not be correlated with differences in biological activity. Although low values for fatty acid ester content were obtained with the ESS and fraction 3 preparations of endotoxin, which were relatively low in biological activity, low values in fatty acid ester content were also evident in the *E. coli* Rolf-EW and *E. coli* O55:B5-PW endotoxins which had a high degree of biological activity. Similarly, differences in fatty acid amide or total fatty acid content could not be correlated with differences in biological activity. Neither nitrogen content nor phosphorus content could be related to biological activity.

Those endotoxin preparations which appeared to have the lowest hexosamine content (*B. abortus* ESS), in general, also appeared to have the lowest degree of biological activity. A high hexosamine content appeared to be associated with a high degree of biological activity, although preparations of similar hexosamine content did not possess similar biological activity. These findings suggest that, although hexosamine content might in some way be related to biological activity, other factors are involved.

Similar types of endotoxin preparations, whether obtained from *B. abortus* 2308 (high virulence) or *B. abortus* 11 (low virulence), resembled each other very closely in chemical composition and in biological activity.

TABLE 4. *Serum-iron levels (μg/100 ml) of mice given different lots of heat-killed cells of Brucella abortus 2308**

Lot	Replicates					Mean
	1	2	3	4	5	
1	153	136	222	128	186	165
2	157	195	224	179	163	183
3	172	193	153	166	178	172
4	215	169	151	177	156	173

* Tabular $F = 3.26$; experimental $F = 0.31$.

DISCUSSION

When various endotoxin preparations were compared for their ability to induce hypoferremia in mice, significant differences were noted. The endotoxin preparations obtained from *E. coli* had the highest activity. Among the *B. abortus* endotoxin preparations, fraction 5 was the most active and did not differ significantly in activity from most of the *E. coli* endotoxins. The least active endotoxins were *B. abortus* fraction 3 and ESS preparations. Endotoxin preparations of the same type, whether obtained from *B. abortus* 2308 (high virulence) or *B. abortus* 11 (low virulence), did not differ significantly in hypoferremic activity or in their lethal effects in mice. These data support the work of Spink and Anderson (1954), who could find no differences in lethal effects between endotoxins obtained from strains of brucellae of high and of low virulence.

Since heat-killed cells were capable of inducing a hypoferremia in mice, and this biological response appeared to be specific and dose-related (Baker and Wilson, 1965), the endotoxin content

TABLE 5. *Chemical composition (per cent) of various endotoxin preparations*

Endotoxin prepn	Hexose	Fatty acid esters*	Fatty acid amines*	Total fatty acids*	Hexosamine	Nitrogen	Phosphorus
Brucella abortus 2308-TCA	15.3	13.3	1.4	14.7	2.9	3.6	0.70
B. abortus 11-TCA	16.1	12.2	2.9	15.1	4.0	3.4	0.61
Escherichia coli Rolf-TCA	35.9	13.1	11.8	24.9	17.8	2.2	1.53
E. coli O55:B5-TCA	21.9	9.3	5.4	14.7	—	—	—
B. abortus 2308-ESS	29.2	4.2	7.1	11.3	1.5	6.6	1.47
B. abortus 11-ESS	23.4	4.3	8.7	13.0	1.1	7.8	1.42
B. abortus 2308-fraction 3	11.4	1.6	2.4	4.0	—	—	—
B. abortus 2308-fraction 5	13.4	14.2	4.2	18.4	4.2	5.2	0.42
B. abortus 11-fraction 5	14.1	14.6	3.4	18.0	4.6	4.2	0.39
B. abortus 2308-EW	16.6	18.4	0.1	17.3	4.1	1.8	1.27
B. abortus 11-EW	13.5	19.4	0.3	19.7	4.9	3.9	1.02
E. coli Rolf-EW	12.0	4.5	18.7	23.2	4.2	8.8	1.61
E. coli O55:B5-PW	25.0	4.4	3.8	8.2	—	—	—

* Calculated as palmitic acid.

of various strains of *B. abortus* of different virulence could be determined by using equal numbers (10^8) of heat-killed cells. Significant differences in the activities of the various heat-killed cell preparations were found. Although there were differences in activity, presumably due to differences in endotoxin content, the differences could not be correlated with virulence. The strains of high virulence did not always possess the highest activities, and consequently did not have a greater endotoxin content than the strains of low virulence. For example, *B. abortus* 544 did not differ significantly in activity, hence, endotoxin content, from *B. abortus* 544A, *B. abortus* 11, or other strains of low virulence. Also *B. abortus* 2308 (high virulence) did not differ significantly in endotoxin content from *B. abortus* 2308-2A-2 (low virulence). *B. abortus* 544 (high virulence) had the same activity as *B. abortus* 2308 R, a rough strain from which no endotoxin could be extracted by the method of Raynaud et al. (1964). It is concluded from these data that, although the strains of *B. abortus* used differed in endotoxin content, there appears to be no relationship between endotoxin content and virulence. These findings are in agreement with the results of Gilardi (1956), who compared the lethal effects in mice of heat-killed and acetone-killed cells of strains of *B. suis* of different virulence and could find no relationship between endotoxin content and virulence.

Cameron et al. (1960) reported that, when strains of *Salmonella pullorum* were extracted for endotoxin by the method of Boivin and Mesrobeanu (1935, 1936), more endotoxin was obtained from strains of high virulence than from strains of low virulence. Although this finding shows differences in endotoxin content between strains of high and low virulence, it has been our experience (Baker, 1962) and the experience of others (Ribi et al., 1964) that endotoxin can not be extracted quantitatively from intact cell preparations, even if the extraction process is repeated several times. Endotoxin can be extracted quantitatively, however, if cell walls rather than intact cells are employed (Ribi et al., 1964).

To determine whether the data obtained for the heat-killed cells were reproducible, four different lots of heat-killed cells of *B. abortus* 2308 were prepared independently, and the activities of the preparations were compared. Under the conditions used in our studies, no significant differences in hypoferremic activity, hence endotoxin content, were noted. Ribi et al. (1964), however, reported that the endotoxin content of strains of *Salmonella enteritidis* varied according to the method of cultivation and the extraction procedure employed.

A comparison of the LD_{50} values with the specific activities of the various endotoxin preparations used in these studies shows that there is a relationship between lethal effects and the ability of endotoxin to induce hypoferremia. In general, as toxicity decreases, the ability of endotoxin to induce hypoferremia also decreases; however, it would be necessary to obtain more LD_{50} values to establish a statistical correlation. Comparisons of the lethal effects with ability to induce hypoferremia by use of detoxified endotoxins (Baker and Wilson, 1965) also suggest a relationship between lethal effects and the ability to induce hypoferremia.

Although differences in the chemical composition of the endotoxin preparations used in these studies were noted, these differences could not be correlated with differences in biological activity. This finding is in agreement with the data reported by Nowotny et al. (1963), who could find no relationship between chemical composition and biological activity among the endotoxins of *Serratia marcescens*, *Salmonella typhosa*, and *E. coli* prepared by six different extraction procedures. They concluded that either the biologically active centers of the endotoxin complex could not be detected by gross chemical analyses or that the biologically active centers required a highly specific steric arrangement of the structure in which the individual "building blocks" played only a secondary role.

The same types of endotoxin preparations, whether obtained from *B. abortus* 2308 (high virulence) or *B. abortus* 11 (low virulence), were virtually identical in chemical composition. These data suggest that there are no chemical differences between the endotoxins of strains of *B. abortus* of different virulence.

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