Comparisons of serologic and physiologic groups of Vibrio fetus
by Russell Lowell Berg

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in MICROBIOLOGY
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Abstract:
Various serologic and physiologic characteristics of 62 Vibrio fetus isolates were compared.

The Marsh and Firehammer serotypes were determined by agglutination reactions between whole-cell antigens and antisera prepared against specific whole-cell antigens® Somatic serotypes were determined by agglutination reactions between boiled antigens and absorbed whole-cell antisera® Heat-labile antigens were determined by agglutination reactions between whole-cell antigens and antisera which had been absorbed with homologous boiled antigen and also with specific whole-cell antigens® Glycine tolerance was indicated, if growth occurred in medium containing 1% glycine® Hydrogen sulfide production was measured by suspending filter paper strips impregnated with lead acetate over a medium containing 0.02% cysteine.

The Marsh and Firehammer (MF) serotyping system divides V. fetus into four serotypes. Serotype III (MF) consists of V. fetus var. venerealis (glycine intolerant. Serotypes I, II, and V consist of V. fetus var. intestinalis (glycine tolerant).

Three somatic serotypes were demonstrated. Somatic serotype A of Morgan is identical to somatic serotype 1 of Mitscherlich and Liess and contains both serotype III and V (MF) isolates. Somatic serotype B of Morgan is identical to somatic serotype 2 of Mitscherlich and -Liess and consists of serotype II (MF) isolates. Somatic serotype C consists of serotype I (MF) isolates® The glycine tolerance test is reliable for separating the venerealis variety of somatic serotype A isolates from the intestinalis variety of somatic serotype A V. fetus isolates.

Physiologic type 1 (glycine intolerant, H2S negative) consists of serotype III (MF) isolates. Physiologic subtype 1 (glycine intolerant, H2S positive) predominantly consists of serotype III (MF) isolates. Physiologic type 2 (glycine tolerant, H2S positive) consists of serotype I, serotype II and serotype V (MF) isolates.

All serotype I (MF) isolates grew at 45°C, but were the only isolates which grew at this high temperature. All serotype II (MF) isolates grew at 42°C. Some of the serotype V (MF) isolates grew at 42°C, while others failed to grow at 42°C, but grew at 37°C, Isolates which were classified both as serotype III (MF) and as physiologic type 1 grew at 37°C, but hot at 42 or 45°C, All physiologic type 1 isolates grew at 37°C, but failed to grow at 42 or 45°C Physiologic subtype 1 and type 2 isolates grew at all three temperatures.

At least seven heat-labile antigens are present in V. fetus isolates. These antigens may be immunogenically important and may lead to a practical means of diagnosing vibriosis®

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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ABSTRACT

Various serologic and physiologic characteristics of 62 Vibrio fetus isolates were compared.

The Marsh and Firehammer serotypes were determined by agglutination reactions between whole-cell antigens and antisera prepared against specific whole-cell antigens. Somatic serotypes were determined by agglutination reactions between boiled antigens and absorbed whole-cell antisera. Heat-labile antigens were determined by agglutination reactions between whole-cell antigens and antisera which had been absorbed with homologous boiled antigen and also with specific whole-cell antigens. Glycine tolerance was indicated if growth occurred in medium containing 1% glycine. Hydrogen sulfide production was measured by suspending filter paper strips impregnated with lead acetate over a medium containing 0.02% cysteine.

The Marsh and Firehammer (MF) serotyping system divides V. fetus into four serotypes. Serotype III (MF) consists of V. fetus var. venerealis (glycine intolerant). Serotypes I, II, and V consist of V. fetus var. intestinalis (glycine tolerant).

Three somatic serotypes were demonstrated. Somatic serotype A of Morgan is identical to somatic serotype 1 of Mitscherlich and Liess and contains both serotype III and V (MF) isolates. Somatic serotype B of Morgan is identical to somatic serotype 2 of Mitscherlich and Liess and consists of serotype II (MF) isolates. Somatic serotype C consists of serotype I (MF) isolates. The glycine tolerance test is reliable for separating the venerealis variety of somatic serotype A isolates from the intestinalis variety of somatic serotype A V. fetus isolates.

Physiologic type 1 (glycine intolerant, H_2S negative) consists of serotype III (MF) isolates. Physiologic subtype 1 (glycine intolerant, H_2S positive) predominantly consists of serotype III (MF) isolates. Physiologic type 2 (glycine tolerant, H_2S positive) consists of serotype I, serotype II and serotype V (MF) isolates.

All serotype I (MF) isolates grew at 45 C, but were the only isolates which grew at this high temperature. All serotype II (MF) isolates grew at 42 C. Some of the serotype V (MF) isolates grew at 42 C, while others failed to grow at 42 C, but grew at 37 C. Isolates which were classified both as serotype III (MF) and as physiologic type 1 grew at 37 C, but not at 42 or 45 C. All physiologic type 1 isolates grew at 37 C, but failed to grow at 42 or 45 C. Physiologic subtype 1 and type 2 isolates grew at all three temperatures.

At least seven heat-labile antigens are present in V. fetus isolates. These antigens may be immunogenically important and may lead to a practical means of diagnosing vibriosis.

CHAPTER I

INTRODUCTION

Classification of *Vibrio fetus* has been confusing because many serologic and physiologic typing systems were developed by different workers who did not correlate results.

Vibrio fetus causes important reproductive diseases in cattle and sheep and occasionally infects man. Recent surveys (Hoerlein et al., 1964) indicate that vibriosis is rapidly spreading through cattle herds in the United States. Control and diagnosis of this disease requires more knowledge of the characteristics of *V. fetus*. This knowledge may lead to practical diagnostic procedures and more effective control through therapeutic agents.

The purpose of the present study was to investigate and compare serologic and physiologic types of numerous *V. fetus* isolates.

CHAPTER II

REVIEW OF LITERATURE

Identification

For many years physiologic criteria for identification of *V. fetus* remained loosely defined and early attempts to identify this species by serologic reactions were unsatisfactory. In recent years more rigid criteria have been adopted which better characterize pathogenic and saprophytic species of *Vibrio*.

Florent (1953) described a *Vibrio* isolated from bull semen and cow vaginas which was more anaerobic than *V. fetus* and produced an abundance of H_2S*. Thouvenot and Florent (1954) proposed that this organism be called *Vibrio bubulus*.

Bryner and Frank (1955) studied the physiologic characteristics of vibrios isolated from bovine fetuses and reproductive organs of bulls and cows. They concluded that catalase-positive vibrios were *V. fetus*, while catalase-negative vibrios were not.

Firehammer (1965) described another catalase-positive *Vibrio* and proposed that this organism, which was isolated from sheep feces, be called *Vibrio fecalis*. In contrast to *V. fetus*, this species is H_2S* positive. It resembles *V. bubulus* in many respects, but differs from it by producing large amounts of catalase.

On the basis of catalase and H_2S tests, these three species can be

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* H_2S production measured by an "insensitive" method such as SIM (Difco Laboratories, Detroit, Michigan) or Triple-Sugar Iron Agar (Difco) or by suspending a strip of filter paper saturated with lead acetate over a medium which does not contain an added source of sulfur.

differentiated as follows: V. bubulus (catalase-negative, H_2S* positive), V. fetus (catalase-positive, H_2S* negative), V. fecalis (catalase positive, H_2S* positive). All three species are gram-negative, curved rods. They are all microaerophilic, and all reduce nitrates to nitrites. Because of the biochemical inertness of these species, other usual methods of classification cannot be used except that V. fetus is indicated by lack of activity. Of these three species V. fetus is the only one considered pathogenic.

Physiologic groups of V. fetus

Akkermans et al. (1956) isolated two varieties of V. fetus from cattle. One variety was weakly positive for H_2S** and was found only in sporadic cases of abortion; the second variety was H_2S** negative and was isolated from the genitals of cows and bulls involved in outbreaks of vibriosis resulting in infertility.

Florent (1959) studied both varieties and suggested that the variety causing epidemics of infertility be named V. fetus var. venerealis and that the other variety be named V. fetus var. intestinalis. He was able to isolate V. fetus var. intestinalis from the intestinal tracts of cattle, sheep, and pigs.

Leece (1958) reported that V. fetus isolates from sheep tolerated 0.8%

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* H_2S measured by an "insensitive" method.
** H_2S measured by a "sensitive" method. A strip of filter paper saturated with lead acetate is suspended over a growth medium containing an added source of sulfur such as 0.02% cystine.

glycine in the growth medium, while the majority of isolates from cattle did not. Ringen and Frank (1963) found the glycine tolerance test to be the only reliable laboratory method for differentiating V. fetus var. venerealis (glycine intolerant) from V. fetus var. intestinalis (glycine tolerant).

Both Bryner et al. (1962) and Mohanty et al. (1962) reported that V. fetus isolates from cattle could be divided into three groups on the basis of H_2S* production and ability to tolerate 1.0% glycine. Bryner et al. called the three groups type 1 (B)[1], subtype 1 (B) and type 2 (B). Type 1 (B) was H_2S* negative and could not tolerate 1.0% glycine. Subtype 1 (B) differed from type 1 (B) in that it produced H_2S*. Type 2 (B) produced H_2S* and differed from both type 1 (B) and subtype 1 (B) by tolerating 1.0% glycine. Type 2 (B) was identical with V. fetus var intestinalis, while the other two types (B) were considered to be V. fetus var. venerealis. Experimental results (Bryner et al., 1964) indicated that type 1 (B) and subtype 1 (B) were not able to survive a gastrointestinal environment. After oral inoculation of all three types (B) into different lots of cattle, only type 2 (B) isolates could be recovered from the feces and digestive tracts.

It is generally agreed that V. fetus var. venerealis is spread venereally, is the most common cause of vibrionic infertility in cattle and only

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[1] The physiologic types of Bryner et al., (1962).
* Detected only if measured by the "sensitive" method.

occasionally causes bovine abortion. V. fetus var. intestinalis is undisputedly the only natural cause of vibriosis in sheep. However, there is controversy as to its significance in cattle vibriosis. Most authors agree that this variety can cause sporadic abortions in cattle. Akkermans et al. (1956), Terpstra (1956), Bryner et al. (1964) and Hoppe and Ryniewicz (1961) found that V. fetus var. intestinalis does not cause infertility. Florent (1959), Hoerlein and Kramer (1963), Bryner et al. (1964) and Wagner et al. (1961) found that V. fetus var. intestinalis could not survive for long periods in genital tracts of cattle. Therefore, all these authors concluded that this variety did not cause the common, rapidly spreading, venereal type of vibriosis.

Park et al. (1962) found that V. fetus var. intestinalis (obtained from bulls) could be readily transferred venereally; it could also be transferred by intrauterine inoculation with vaginal mucus or with Vibrio cultures obtained from a previously inoculated animal. V. fetus var. intestinalis could be isolated from many of these animals 12 months after inoculation. Florent (1963) worked with isolates obtained from Park and conceded that Park's work was correct. He felt that these V. fetus var. intestinalis isolates were somewhat different from those commonly encountered.

Vinzent et al. (1947) published the first report of an abortion in man due to V. fetus. Since then V. fetus has been recognized as a cause of many human infections of different types (King, 1962). King (1957) reported 11 cases of human vibrionic infections. According to her, V. fetus was isolated from seven infections, and four infections were caused by a

"closely related Vibrio species", which she designated as "related vibrios". In temperature tolerance studies, she found that V. fetus isolates grew well at 25 C, but failed to grow at 42 C. The "related vibrios" grew well at 42 C, but failed to grow at 25 C. The "related vibrios" were also antigenically different.

Firehammer and Berg (1965) studied the temperature tolerance of 16 serotypes I (MF)[2] isolates, 24 serotype III (MF) isolates, 10 serotype V (MF) isolates, and four isolates of "related vibrios"[3]. Both serotype III (MF) and serotype V (MF) isolates were divided into two temperature tolerance groups. One group grew at 25, 37, and 42 C; the other group failed to grow at 42 C. The serotype I (MF) isolates and the "related vibrios" had the same temperature tolerances. They grew well at 37 and 42 C, but did not grow at 25 C.

Three of the four "related vibrios" were serotyped according to the system of Marsh and Firehammer (1953). Of these three, two[4] were serotype I (MF) and one did not fit any of the types of this system. No significant morphologic, biochemical, or physiologic differences were observed between the "related vibrios" and the serotype I (MF) isolates.

Serologic groups

The discovery by McFadyean and Stockman (1913) that V. fetus was the

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[2] The serologic typing system of Marsh and Firehammer (MF) (1953).
[3] The "related vibrios" were supplied through the courtesy of the late Miss E. O. King, Communicable Disease Center, Atlanta, Georgia.
[4] One did not agglutinate at serum dilutions greater than 1/50 and was considered negative in the original paper.

causative agent of epidemic abortions in sheep prompted many workers to investigate the serologic properties of this species. Much of the work was done in attempts to identify the species or in attempts to develop a serologic means of diagnosing vibriosis.

Smith and Taylor (1919) examined 22 Vibrio isolates from cattle fetuses and two from calves. Results of agglutination tests indicated that 21 of the isolates from fetuses were identical, while one deviated slightly. The two isolates from calves were distantly related to the isolates from fetuses. Smith and Orcutt (1927) investigated by the agglutination process five Vibrio cultures isolated from cattle fetuses and two isolated from calves. They concluded that these seven Vibrio cultures had at least four different antigenic factors of which each isolate could possess three.

Blakemore and Gledhill (1946) worked with four V. fetus isolates from sheep and found three somatic serotypes. Levi (1950) found that serum from infected sheep produced high agglutination titers when mixed with antigens prepared with isolates from the same sheep. Plastridge et al. (1951) concluded that V. fetus isolated from cattle belonged in a different serotype than those isolated from sheep. Gallut (1952a) investigated four V. fetus isolates from man and six V. fetus isolates from animals. He found six different antigens and six haptenes.

Gallut (1952b) used a precipitation test to differentiate V. fetus. For each of ten isolates investigated, he obtained both a phenol soluble and a phenol insoluble fraction. The purified phenol soluble fraction appeared to be protein in character and was precipitated by antiserum specific for V. fetus. The phenol insoluble fraction appeared to be

polysaccharide in character. It was precipitated in high titer by homologous V. fetus serum, but only irregularly by heterologous V. fetus serum.

Marsh and Firehammer (1953) performed cross-agglutination studies on three V. fetus isolates from cattle and 23 V. fetus isolates from sheep. They found that the sheep isolates belonged in four serotypes (I (MF), II (MF), IV (MF), and V (MF), while the bovine isolates belonged in a fifth (serotype III (MF)). Serotype I (MF) was distinctly differentiated from the other four types. The other four types, three containing sheep isolates and one containing cattle isolates, cross-reacted with each other.

Price et al. (1955), using the agglutination test, found four somatic serotypes when 14 antisera prepared from 14 boiled antigens were absorbed with different combinations of the boiled antigens. A boiled antigen of ovine origin was serologically unrelated to the isolates of bovine origin.

Bryner and Frank (1955) immunized 25 rabbits with catalase-positive isolates and 12 rabbits with catalase-negative isolates. In an agglutination test, serum which had been obtained through immunization with catalase-negative isolates reacted only with catalase-negative isolates and not with catalase-positive isolates. Serum from rabbits immunized with catalase-positive isolates reacted strongly with catalase-positive isolates and only slightly or not at all with catalase-negative Vibrio.

Wiidik and Hildar (1955) observed that different antigens prepared in the same manner from the same V. fetus isolate produced different agglutination titers with the same homologous serum. They believed that these bacteria had a capsular K-antigen in addition to the thermolabile H-antigen and the thermostabile somatic O-antigen. In their opinion the

differences in agglutination titers could have resulted from quantitative differences in occurrence of the three different antigens.

Biberstein (1956) studied 47 V. fetus isolates and concluded that 44 belonged in one serotype, while each of the other three belonged in different serotypes.

Amell and Stockton (1956), using the complement fixation test, proved that cows vaccinated with V. fetus possessed antibodies specific for V. fetus.

Ristic et al. (1956) worked with both smooth and rough colonial types of V. fetus. Strain specificity was observed among smooth colonial types, while cross reactions were noted among rough colonial variants of homologous and heterologous strains. The rough types had negligible catalase activity. Ristic et al. (1957) demonstrated the presence of a thermolabile superficial antigen on smooth V. fetus cells. Boiling for two hours destroyed or removed the superficial antigen of V. fetus and only partly degraded or removed a superficial antigen of saprophytic Vibrio. Serologic heterogeneity of the rough variants was considerably minimized when heat treated antigens were used for production of antibodies as well as in agglutination tests. Ristic et al. (1958) proposed that colonial types other than rough variants also possessed antigens different from those of smooth colonial types. In addition they found that homologous formalin-killed antigen did not react with antibodies in the serum of a cow that aborted due to V. fetus, while heat-treated, homologous antigen did.

Te Punga (1958) developed an indirect hemagglutination test. He modified sheep erythrocytes by heating them in the presence of material derived

from V. fetus cells. He found this test to be a more sensitive indicator of V. fetus antibodies in rabbit serum than bacterial agglutination tests. He also found that this hemagglutination test could be applied to the detection of antibodies in bovine vaginal mucus, but was concerned as to whether all serotypes causing vibriosis could be detected by this method.

Using 15 V. fetus isolates Mitscherlich and Liess (1958) found two somatic serotypes (1 (Mit)[5] and 2 (Mit)) when the complement fixation test was used to detect reactions between phenol soluble antigens and antisera produced against whole-cell antigens.

Morgan (1959) worked with 25 V. fetus isolates and found two somatic serotypes (A (M)[6] and B (M)) by studying agglutination reactions between boiled antigens and antisera produced against either boiled or whole-cell antigens. Isolates from cattle and sheep were found in both serotypes (M). Absorption with antigens of the homologous group completely removed all agglutinins whereas absorption with antigens of the heterologous group left agglutinins for homologous antigens. Also, by conducting extensive absorptions, Morgan found one common flagellar component and eight specific flagellar components. He reported that there was no correlation between somatic serotypes and the flagellar components.

A heat-stabile, water-soluble substance, termed "HS", apparently partly polysaccharide in nature, was isolated from smooth V. fetus cells by Ristic and Brandly (1959a). This fraction was serologically active in

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[5] The serologic typing system of Mitscherlich and Liess (Mit) (1958).
[6] The serologic typing system of Morgan (1959).

gel precipitation tests and was capable of inhibiting a specific agglutination reaction. Ristic and Brandly (1959b) agglutinated sheep erythrocytes sensitized with the "HS" polysaccharide fraction of V. fetus with V. fetus-specific rabbit sera and with sera from naturally infected bulls. They believed this polysaccharide fraction represented a type-specific O antigen common to a number of individual strains, rather than a species-wide antigen. Ristic and Walker (1960), using a hemolytic test, showed that sheep erythrocytes sensitized with the "HS" polysaccharide fractions of two V. fetus isolates were lysed by 21 specific sera. These sera were produced by inoculating rabbits with V. fetus isolates of bovine, ovine, and human origin. This hemolytic test was found to be superior to the tube-agglutination test as a tool for detecting small quantities of antibody. Treatment of sheep erythrocytes with one of the antigens did not block the simultaneous or subsequent absorption of the second.

Using the rapid slide gel diffusion technique, Ristic and Murty (1961) performed cross-precipitation reactions between V. fetus polysaccharide "HS" antigens and V. fetus specific antisera. A cross-precipitation test between 16 V. fetus antisera and two antigens revealed that the two antigens were different. One reacted with 12 of the antisera, and the other reacted with the remaining four, as well as with three which had reacted with the first antigen. In another cross- precipitation test, seven V. fetus antisera were tested with four "HS" antigens. One of the antigens reacted with five of the antisera, a second and a third antigen reacted with four, including the two which did not react with the first. The

fourth antigen reacted only with the antisera which reacted with all three of the other antigens. Different results were obtained if rough variants were used. In addition, these authors found that the first antigen yielded precipitin lines with the sera of 12 cattle artificially infected with V. fetus. Specificity of the gel-precipitation technic using this antigen fraction was indicated by the absence of precipitin reactions with control samples of serum from V. fetus-free cattle and rabbit antisera specific for Leptospira and Brucella. Comparison of the serologic results obtained with the gel-precipitation, hemolysis, and whole-cell agglutination tests indicated that the latter two tests were inferior in accuracy to the gel-precipitation test.

Kamel (1960), using the techniques described by Mitscherlich and Liess (1958), found a third somatic serotype of V. fetus (serotype 7 (Mit). Mitscherlich (1961) verified Kamel's results. In addition he correlated types 1 (Mit) and 2 (Mit) with pathogenicity and compared them with the physiologic varieties (V. fetus var. intestinalis and V. fetus var. venerealis) of Florent. He came to the conclusion that serotype 2 (Mit) was responsible for epidemic abortions in sheep and sporadic vibrionic abortions in cattle, and that serotype 1 (Mit) was the causative agent of vibriosis of cattle characterized by both infertility and abortion. He further concluded that serotype 1 (Mit) was identical to V. fetus var. venerealis and serotype 2 (Mit) to V. fetus var. intestinalis. Thirty-seven per cent of the serotype 1 (Mit) organisms tolerated glycine. (According to the work of Ringen and Frank (1963), these glycine tolerance results indicate that Mitscherlich's latter conclusion was incorrect.)

Söderlind (1961) compared the agglutination method used by Morgan[7] (1959) with the complement fixation test used by Mitscherlich and Liess (1958). Of 47 isolates belonging to either serotype 1 (Mit) or 2 (Mit), 30 were satisfactorily divided into two serotypes by both methods. The other 17 were clearly split into the two serotypes only by the complement fixation test. Four isolates belonging to the third serotype (Kamel, 1960) could not be differentiated by the agglutination test. Söderlind made no attempt to specifically correlate the type designations of Morgan's somatic serotypes with those of Mitscherlich and Liess.

Winter and Dunne (1962) worked with 26 V. fetus and seven V. bubulus isolates. Phenol and ultrasonic extracts were obtained from several isolates. Ultrasonic extracts from one V. fetus isolate contained antigenic materials which reacted in the indirect hemagglutination test with whole-cell antisera of all the V. fetus isolates and all but one of the V. bubulus isolates. When ultrasonic extracts from one V. fetus isolate was tested against all 33 whole-cell antisera in the agar gel-precipitation test, one line of identity of a heat-labile component was formed between all the V. fetus and two of the V. bubulus antisera; other lines of identity were formed between smaller numbers of antisera. In addition two different lines of identity of heat-stabile components were formed. One was formed between 20 V. fetus antisera (the major V. fetus 0 group) and the second between three V. fetus antisera (the minor V. fetus 0 group). Three of the

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[7] Söderlind did not absorb the antisera used in the agglutination test, whereas Morgan did.

V. fetus antisera did not react with this heat-stabile antigen.

Winter (1963), using chromatographic separation, found nine precipitating antigens in one V. fetus isolate. Eight of the antigens were heat-labile, and one of these was considered by Winter to be a species specific antigen. (However, in addition to reacting with all the rabbit anti-V. fetus sera, it reacted with some of the rabbit anti-V. bubulus sera.) The ninth antigen, the heat-stabile antigen responsible for the lines of identity of the major and minor O groups of V. fetus, was found in cell-free suspensions of flagella.

Nageswararao and Blobel (1963) found acid precipitable antigenic materials in the filtrates of 19 broth cultures of V. fetus. When this antigen was injected into rabbits, antibodies were formed. These antibodies and the acid precipitable antigenic materials produced precipitin lines in a double-diffusion precipitin test. They also agglutinated whole-cell and lysed V. fetus antigens. Some heterogeneity between the acid precipitable materials of different isolates was demonstrated.

O'Berry (1964), using ethanol, ammonium sulfate, and ethodin to fractionate antisera demonstrated that fluorescent antibody conjugates capable of producing fluorescence on V. fetus cells can be prepared. The two V. fetus var. venerealis isolates used to immunize cows and rabbits gave homologous agglutination titers of 1:3200 and 1:1600; heterologous titers of 1:100 were demonstrated. Only slight differences were noted between homologous and heterologous fluorescent staining when conjugated ammonium sulfate serum fractions were used. The fluorescent staining of the other two conjugated serum fractions varied with the isolate used for immunization

and the species of animal immunized.

Belden and Robertstad (1965), using cultures representing serotypes I (MF), II (MF), and V (MF) and fluorescent antibody technics, typed 33 V. fetus isolates. They found no cross reaction between serotype I (MF) and the other serotypes. Because reactions did not occur with serotype III (MF) that did not also occur with serotypes II (MF) or V (MF), only reactions occurring with types I (MF), II (MF) and V (MF) were used to establish their serogroups. However, no absorptions were conducted and it is therefore impossible to determine from this study whether serotype III (MF) is significant.

Winter (1966) purified the antigen responsible for the lines of identity of the major and minor O groups of V. fetus (Winter and Dunne, 1962). This heat-stabile polysaccharide endotoxin was comparable to the endotoxins from other species. It had a lethal effect in mice, and produced a biphasic febrile response and the generalized Shwartzman reaction in rabbits. After treatment in a basic solution, the polysaccharide absorbed readily onto red blood cells to serve as an antigen in a passive hemagglutination test. The substance was capable of stimulating formation of precipitating antibody in rabbits. The endotoxin of an isolate of V. fetus var. venerealis and an isolate of V. fetus var. intestinalis formed a line of identity in agar gel-precipitation tests when the antiserum from either isolate was used.

Winter (1965) separated 33 serum samples into two fractions (F1 and F2) by centrifugation in a sucrose gradient. These samples were obtained from cattle ranging in age from two weeks to 12 years. Ten were paired samples

from five heifers drawn before and after experimental infection with V. fetus var. venerealis. Serum from a heifer which aborted in the sixth month of pregnancy was also studied.

Antibodies for the V. fetus polysaccharide endotoxin were found in the majority of sera from normal cattle over seven months of age. The antibody necessary for activity in a passive hemagglutination test was found in the F1 fraction and was associated almost exclusively with the gamma-1 macroglobulin serum component. The antibody necessary for activity in a double diffusion precipitin test was found in the F2 fraction, which contained the characteristic gamma-2 globulin line as well as several other immunoelectrophoretic lines.

Serum from the heifer which aborted due to V. fetus var. intestinalis "unequivocally" contained gamma-2 globulin antibody for V. fetus endotoxin.

Serum drawn from five heifers after experimental infection had developed, contained heat-stabile precipitins (65 C for 30 min), whereas sera drawn prior to the infection did not. Heat lability of the precipitins in the F2 serum fraction of normal cattle was considered characteristic of "nonspecific" agglutinins, (gamma-1 globulin) but not characteristic of "specific" agglutinins (gamma-2 globulin).

The absorption of sera from three normal cattle with V. fetus endotoxin removed all the agglutinins and precipitins for V. fetus endotoxin from these sera. However, absorption with Escherichia coli endotoxin or Salmonella enteriditis endotoxin had little or no effect on hemagglutinating or precipitating activity.

CHAPTER III

MATERIALS AND METHODS

Isolates

The majority of isolates used in this study were furnished by the Montana Livestock Sanitary Board Diagnostic Laboratory and the Montana Veterinary Research Laboratory. Dr. E. Mitscherlich[8] supplied seven isolates each of serotypes 1 (Mit) and 2 (Mit). Dr. B. Morgan[9] supplied five serotype A (M) and three serotype B (M) isolates. Other isolates came from Leeds, England[10], Idaho[11], Utah[12], New Zealand[13], the National Animal Disease Laboratory, Ames, Iowa[14], and the Communicable Disease Center, Atlanta, Georgia[15]. All isolates were frozen in defibrinated bovine blood and stored at -30 C until used.

Media

A basic aqueous medium containing 2.8% Brucella broth[16], 0.5% yeast extract[17], and 0.1% agar[17] was used for growing inoculum and for all

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[8] Tierärztliches Institut, Universitat Gottingen, Gottingen, Germany.
[9] The Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.
[10] Courtesy Dr. W. A. Watson, Veterinary Investigation Centre, Ministry of Agriculture, Fisheries and Foods.
[11] Courtesy Dr. F. W. Frank, Dr. D. G. Waldhalm, and Mr. W. A. Minershagen, University of Idaho Branch Experiment Station.
[12] Courtesy Dr. J. Storz, Utah State University, Logan, Utah.
[13] Courtesy Dr. W. A. Te Punga, Wallaceville Animal Research Station, Dept. of Agriculture, Private Bag, Wellington.
[14] Courtesy Mr. J. Bryner and Dr. P. O'Berry.
[15] Courtesy the late Miss E. O. King.
[16] Albimi Laboratories, Flushing, New York.
[17] Difco Laboratories, Detroit, Michigan.

physiologic tests except for the "insensitive" H_2S test. For the glycine tolerance test, 1.0% glycine[18] was added to the above medium; for the "sensitive" H_2S test, 0.02% cysteine[18] was added. The media were dispensed seven ml per tube, in 16 mm screw-cap tubes. All tubes of semisolid medium used in physiologic tests were inoculated with two drops of 48-hour growth. All physiologic tests were conducted in duplicate. The incubation period was five days for all tests except for the temperature tolerance tests at temperatures below 20 C, for which a 10-day incubation period was used. All tests were conducted in an atmosphere containing 3.0% oxygen, 5.0% carbon dioxide, and 92% nitrogen. With the exception of temperature tolerance tests, an incubation temperature of 37 C was used.

Hydrogen sulfide production

Two methods were used to determine H_2S production. The "insensitive" H_2S test consisted of inoculating SIM stabs[19] with a large loop of 48-hour growth. If the stabs became black, the isolate was considered H_2S positive by the "insensitive" method.

The "sensitive" H_2S test was conducted by suspending filter paper strips saturated with lead acetate from tops of tubes containing the medium with cysteine added. If approximately 20 mm of the lower end of the portion of filter paper became black, a 4 + reading was given; isolates which produced approximately 10 mm of blackness received a 3 + reading. Two

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[18] Nutritional Biochemicals Corporation, Cleveland, Ohio.
[19] Difco Laboratories, Detroit, Michigan.

plus (2 +) readings were given for isolates producing approximately 5 mm of blackness. If approximately 2 mm of the paper strip became black, a 1 + reading was given; if the color produced was dark brown instead of black and only at the lower end of the strip, a ± reading was given. Only isolates receiving negative readings were considered H_2S negative by the "sensitive" H_2S method.

Glycine tolerance test

If growth occurred throughout the entire upper portion of tubes containing the basic medium with glycine added, a 4 + reading was given. Isolates producing less growth were given a 3 + or a 2 + reading, according to amount of growth present. If only a small amount of growth occurred in the center of the tube a 1 + reading was given. (Because the inoculum, two drops of 48-hour growth, was visible, it was occasionally difficult to determine whether growth had occurred or whether the inoculum had spread.) If it could not be determined with certainty that growth had occurred, a ± reading was given. A negative reading was given if no growth was present. Isolates which received a ± or a negative reading were considered glycine negative.

Catalase test

The catalase test consisted of dropping a 3.0% solution of hydrogen peroxide on growth from each isolate. If bubbling occurred, the isolate was considered catalase positive (+). Isolates which did not bubble immediately after hydrogen peroxide was added, but within a two minute period were designated as delayed (D). The amount of growth in each tube

was recorded according to the procedure described previously for the glycine tolerance test.

Temperature tolerance test

Medium was inoculated at room temperature. Immediately after inoculation duplicate tubes were incubated at each of the following temperatures: 45,42,37, and 20 C. Readings were made according to the procedure described previously for the glycine tolerance test. However, because growth readings below 2 + were not observed in the control tubes, growth readings of 1+ were considered questionable and recorded as trace (T). This trace reading of growth may have occurred before the temperature of the medium adjusted to the temperature of the various incubators. Isolates which grew well at 20 C were later tested at 17 C and at temperatures fluctuating from 10 C to 13 C.

Antigen production

Growth for production of antigens was obtained according to the method used by Firehammer and Berg (1966).

For production of boiled antigens, the growth was centrifuged at 4,080 X g for 20 min, the cells were resuspended in distilled water and boiled for two hours under a reflux condenser. After removing the distilled water by centrifugation, the cells were suspended in a 0.4% saline solution in proportions that will be described later.

For production of whole-cell antigens, 3.75 ml of 37% formalin was added to the 1,250 ml of growth and allowed to stand for 12 hours. These formalinized cells were removed from the medium by centrifugation. Both

the boiled cells and the formalinized cells were used in various concentrations. A portion of the packed cells of each antigen to be used in absorption studies was diluted in an equal volume of 0.4% saline solution. For the slide agglutination test, a second portion was diluted with saline until an optical density (O. D.) of 0.8 was obtained on a Lumetron[20]. A third portion was diluted to an O. D. of 0.15 for use with the tube agglutination test. If the antigen was to be used to inoculate rabbits for production of antisera, an additional portion was adjusted to 0.4 O. D. on the Lumetron. All adjustments were made using the red (# 650) Lumetron filter. All antigens were stored at 4 C.

Production of antiserum

Antisera were produced by inoculating rabbits intravenously with 15 ml of antigen. Beginning with 0.5 ml, the amount of inoculum was increased by 0.5 ml until 2.5 ml was given at each inoculation. Inoculations were given at three to four day intervals. The rabbits were exsanguinated five to eight days after the last injection.

Absorptions

Absorptions with both boiled and whole-cell antigens were achieved by mixing antigen with serum at a ratio of one part antigen to four parts serum. The mixture was incubated at 37 C for 3 hours and then placed in a refrigerator at 4 C for several hours. The chilled mixture was centrifuged at 17,300 X g for 15 min. The supernatant was tested for antibodies against the antigen used in absorption by an agglutination process. If a

[20] Photovolt Corporation, New York, New York.

reaction occurred, the absorption was repeated.

Agglutination reactions

Agglutination reactions were detected by the tube agglutination test and slide agglutination tests.

The tube agglutination test was conducted according to the method described by Marsh and Firehammer (1953).

The slide agglutination test was conducted by pipetting 0.08, 0.04, 0.02, 0.01, 0.005, and 0.0025 ml of antiserum into six squares (1.25 inches) arranged in a row on a 7.5 X 15 inch sheet of plate glass. Approximately 0.03 ml of antigen was dropped on each portion of serum and the liquids were mixed and spread over the area with a clean applicator stick[21]. Further mixing was achieved by slowly rotating the glass. The titers of the mixtures of antiserum and antigen on the squares of the glass plate were determined through comparisons with the tube agglutination test. Mixing 0.03 ml of antigen with the first amount of antiserum (0.08 ml) resulted in a titer of 1/5. The remaining portions of antiserum resulted in titers of 1/10, 1/12, 1/40, 1/80, and 1/160 respectively. Diluting the antisera 1/10 resulted in titers of 1/50, 1/100, 1/200, 1/400, 1/800, and 1/1600 respectively. Antisera were diluted to desired concentrations with 0.04% saline solution and the titer was considered to be proportional to the amount of dilution. In instances where titers were not determined, only the 0.08 ml portion of the desired concentration of antiserum was pipetted onto the glass plate and 0.03 ml of antigen was added to this portion.

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[21] Diamond National Corporation, New York, New York.

RESULTS

All isolates used in this study met the criteria for V. fetus as designated by workers in this field and outlined in the "Review of Literature". They were curved Gram-negative rods which were microaerophilic and produced catalase, but not H_2S when measured by the "insensitive" method (table I).

According to the physiologic classification system of Bryner et al. (1962), 38 of the isolates were type 2 (B) (V. fetus var. intestinalis), 15 were type 1 (B) (V. fetus var. venerealis), and nine were subtype 1 (B) (V. fetus var. venerealis) (table I).

The isolates were serotyped according to the system of Marsh and Firehammer (1953). Thirteen of the isolates were serotype I (MF), 10 agglutinated predominantly[22] with type II (MF) antiserum, 14 agglutinated predominantly with type V (MF) antiserum, and 21 isolates agglutinated predominantly with type III (MF) antiserum (table II). Eight isolates were not agglutinated by any of the antisera in the Marsh and Firehammer typing system, and one isolate was not serotyped.

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[22]This applies when the antigen was agglutinated by more than one of the antisera in the Marsh and Firehammer typing system and means that the best agglutination resulted with the antiserum indicated, regardless of whether a strong agglutination occurred with any of the antisera in the typing system.

TABLE I

The metabolic properties and physiologic types of 62 *Vibrio fetus* isolates

Isolate	Catalase test		"Insensitive" H_2S test		"Sensitive" H_2S test		Glycine tolerance test	Physiologic type	Variety of *V. fetus*
Mont. V. R. L. number	Growth in medium	Bubbling	Growth in SIM[a]	Blackness in SIM	Growth in .02% cysteine	Blackness of paper strip[b]	Growth in 1% glycine	Nomenclature of Bryner et al., 1962	Based on glycine results
14901	++++	+	++++	–	++++	+++	++	Type 2	int[c]
14902	++++	+	++++	–	+++	+++	+	Type 2	int
14903	++	+	++++	–	++	–	–	Type 1	ven[d]
14904	++++	+	++++	–	++++	–	–	Type 1	ven
14905	++++	+	++++	–	+++++	++	+++	Type 2	int
14906	++++	+	++++	–	++++	++++	++	Type 2	int
14907	+++	+	++++	–	++++	+	++	Type 2	int
14908	++++	+	++++	–	++++	++++	+++	Type 2	int
15107	++++	+	++++	–	++++	++++	+	Type 2	int
14864	+++	+	++++	–	++	–	–	Type 1	ven
14863	++++	+	++++	–	++	–	–	Type 1	ven
14865	++++	+	++++	–	+++	+++	+++	Type 2	int
14866	++++	+	++++	–	+++	++	++	Type 2	int
15108	++++	+	++++	–	++++	–	–	Type 1	ven
15109	++++	+	++++	–	++++	–	–	Type 1	ven
15110	+++	+	++++	–	++	+++	++	Type 2	int
15111	+++	+	++++	–	++	++	+++	Type 2	int
15112	+++	+	++++	–	++	++	+++	Type 2	int
15113	+++	+	++++	–	++	–	+++	Type 2	int
2315	++++	+	++++	–	++++	–	–	Type 1	ven
13925	+++	+	++++	–	+	–	–	Type 1	ven
14071	++++	+	++++	–	+++	+++	–	Subtype 1	ven

continued

TABLE I
(continued)

Isolate	Catalase test		"Insensitive H_2S test		"Sensitive" H_2S test		Glycine tolerance	Physiologic type	Variety Of *V. fetus*
Mont. V. R. L. number	Growth in medium	Bubbling	Growth in SIM[a]	Blackness in SIM	Growth in .02% cysteine	Blackness of paper strip[b]	Growth in 1% glycine	Nomenclature of Bryner et al., 1962	Based on glycine results
15124	++	+	+++	-	+++	+	++	Type 2	int[c]
15126	+++	+	++++	-	++	++	++	Type 2	int
15122	++++	+	++	-	++++	-	-	Type 1	ven[d]
15120	++++	+	++++	-	++++	+	-	Subtype 1	ven
13140	+++	+	++++	-	+++	++	++	Type 2	int
8916	+++	D[e]	++	-	+++	++++	+	Type 2	int
13316	+++	+	++++	-	+	++++	+++	Type 2	int
13136	+++	D	++++	-	++++	+++	+	Type 2	int
13161	+++	+	++++	-	+++	+	+++	Type 2	int
13014	++++	+	+++	-	++++	+++	+++	Type 2	int
13641	++++	+	++++	-	+++	++	+++	Type 2	int
13049	++++	+	++	-	+++	++	++	Type 2	int
13930	+++	+	++++	-	+++	+	++	Type 2	int
14539	+++	+	++++	-	++++	++	+++	Type 2	int
14698	+++	+	++++	-	++	++++	+++	Type 2	int
13262	±	-	++++	-	++	+++	++	Type 2	int
14185	+++	+	++++	-	++	++	++	Type 2	int
13350	++++	+	++++	-	++++	++++	++++	Type 2	int
15103	+++	+	++++	-	++	-	-	Type 1	ven
13924	+++	+	++++	-	+++	-	-	Type 1	ven
13797	++++	+	++++	-	+++	-	-	Type 1	ven
15550	+++	+	++++	-	+++	-	-	Type 1	ven

continued

TABLE I
(continued)

Isolate	Catalase test		"Insensitive H_2S test		"Sensitive" H_2S test		Glycine tolerance	Physiologic type	Variety of *V. fetus*
Mont. V. R. L. number	Growth in medium	Bubbling	Growth in SIM[a]	Blackness in SIM	Growth in .02% cysteine	Blackness of paper strip	Growth in 1% glycine	Nomenclature of Bryner et al., 1962	Based on glycine results
13831	++++	+	++++	–	++++	++	–	Subtype 1	ven[d]
14694	++	+	++++	–	++	–	–	Type 1	ven
13841	+++	+	++++	–	+++	+	–	Subtype 1	ven
13011	++++	+	++++	–	++++	+	++	Type 2	int[c]
13823	++++	+	++++	–	+++	–	–	Type 1	ven
7418	+++	+	++	–	+++	+	+++	Type 2	int
14544	++++	D[e]	++++	–	++++	++++	++	Type 2	int
14586[g]	++++	+		–	++++	++++	–	Subtype 1[f]	
13036[g]		+		–					
11148[g]		+		–					
12351[g]		+		–					
5854[g]		+		–					
14634	++++	+	++++	–	+++	++++	+	Type 2	int
9437	++++	+	++++	–	+++	++++	++	Type 2	int
14664	+++	+	++++	–	++++	+++	–	Subtype 1	ven
4440	+++	+	++++	–	+++	+++	++	Type 2	int
14688	++++	+	++++	–	+++	++++	++	Type 2	int
14840	+++	+	++++	–	++	++	–	Subtype 1	ven
7904	++++	+	++++	–	+++	++	++	Type 2	int
61[h]	++++	–	++++	+++	++++	++++			
15121	+++	+	++++	–	++	+	–	Subtype 1	ven
15125	+++	+	++++	–	++++	++	–	Subtype 1[f]	
15123	+++	+	++++	–	++	+++	++	Type 2	int

continued

TABLE I
(continued)

[a] Difco Laboratories, Detroit, Michigan.
[b] Filter paper strip impregnated with lead acetate.
[c] Intestinalis.
[d] Venerealis.
[e] D = delayed bubbling.
[f] Not classified as V. fetus var. venerealis because there is no evidence that these isolates are capable of causing infertility.
[g] Results supplied by Mr. D. Firehammer.
[h] Vibrio bubulus isolate used as a control.

TABLE II

The Marsh and Firehammer serotypes[a] of 65 *Vibrio fetus* isolates

Antigen	Antisera directed against serotype I	Antisera directed against serotype II	Antisera directed against serotype III	Antisera directed against serotype V	Marsh and Fire-hammer serotype
13036[b]	50[c]	-	-	-	I
13011	-	-	-	200[d]	V
13140	-	-	-	200	V
13136	25	-	-	-	I
13161	-	-	-	200	V
13316	-	200	-	100	II-V
13350	50	-	-	-	I
9437	200	-	-	-	I
13039	-	-	-	100	V
13014	-	-	-	200	V
13641	-	-	-	50	V
13930	-	25	-	200	V
8916	200	-	-	-	I
14544	200	-	-	-	I
14549	-	200	-	-	II
14185	-	-	100	100	V-III
13841	-	200	50	-	II-III
14586	100	-	-	-	I
13831	-	-	100	-	III
14688	-	-	-	-	?[e]
15103	-	50	200	-	III-II
15120	-	-	100	-	III
15121	-	100	200	-	III-II
15122	-	50	50	50	V-III-II
15124	-	-	-	-	?
15123	-	-	-	-	?
15126	-	200	-	-	II
15125	50	-	-	-	I
15109	-	-	200	-	III
14863	-	-	100	-	III
14864	-	100	200	-	III-II
14865	-	50	-	-	II
15111	-	100	-	-	II

continued

TABLE II
(continued)

Antigen	Antisera directed against serotype I	Antisera directed against serotype II	Antisera directed against serotype III	Antisera directed against serotype V	Marsh and Fire-hammer serotype
15110	-	-	-	-	?[e]
15112	-	-	-	-	?
15113	-	-	-	-	?
15107	-	-	-	-	?
15108	-	-	100	-	III
14901	-	-	50	-	III
14866	-	100	-	-	II
14904	-	-	200	100	III-V
14903	-	-	200	-	III
14906	-	200	-	-	II
14905	-	100	25	200	V-II
14908	-	200	-	100	II-V
14907	-	200	200	-	III-II
14902	-	200	-	25	II-V
13262	200	-	-	-	I
14698	100	-	-	-	I
14071	-	200	200	-	III-II
13925	-	-	200	200	V-III
14634	100	-	-	-	I
14664	-	200	-	200	V-II
14840	-	-	200	50	III-V
14694	-	25	25	-	III-II
13823	-	-	100	-	III
13797	-	200	200	100	III-II-V
13924	-	50	200	25	III-II
5854	200	-	-	-	I
11148	200	-	-	-	I
12351	-	-	-	200	V
7418	-	-	-	25	V
7904	-	-	-	-	?
4440			Original Typing Isolate		II
2315			Original Typing Isolate		III

[a] Much of the data for this table was furnished by B. D. Firehammer.
[b] Isolate numbers were also used to identify antigens.
[c] Number = reciprocal of serum dilution.
[d] Titers higher than 1/200 were not determined for most isolates. Therefore, titers higher than 1/200 were not recorded in this table.
[e] Little or no agglutination with any of the antisera.

Somatic Antigens

Agglutination reactions between 16 boiled antigens and 15 antisera produced against whole-cell antigens revealed three somatic antigens (table III). Nine isolates contained only somatic antigen A. Four isolates contained only somatic antigen C. Although some cross agglutination with antisera produced against antigens A and C was evident, it appeared that two isolates, 14688 and 4440, predominantly contained somatic antigen B. Isolate 14664 appeared to contain considerable amounts of both somatic antigens A and B.

Investigation revealed that most normal rabbit sera contain antibodies which agglutinate boiled V. fetus antigens at titers as high as 1/80. In order to remove these non-specific agglutinins, it was necessary, in many cases, to absorb antisera produced against one somatic serotype with antigen of another somatic serotype. Using the information from the results presented in table III, it was possible to identify antigens and in some cases antisera which were specific for the three somatic serotypes. Other specific antisera were obtained by absorption.

The agglutination results between 34 boiled V. fetus antigens and 10 specific antisera are presented in table IV. Four of the antisera (13823, 7418, 13011, and 13831) were specific for somatic serotype A, another four (14908, 14907, 15110, and 16001) were specific for somatic serotype B, and two (8916 and 14544) were specific for somatic serotype C. Many cross agglutinations occurred with antisera 9437 which was produced against somatic serotype C. This antiserum was not absorbed and, therefore, could have contained a considerable amount of non-specific agglutinins. This

TABLE III

Somatic serotypes established by agglutination* of boiled *Vibrio fetus* antigens produced against whole-cell antigens

	Antisera														
	Somatic serotype A**								Somatic Serotype B**		Somatic serotype C				
	13011	7418	13831	13925	14071	14691	14694	13841	4440	14688	11148	14586	14544	5854	9437
Antigen															
13011	800***	800	800	400	400	200	800	–	–	–	–	–	–	–	–
7418	–	400	400	400	400	400	200	25	25	–	–	–	–	–	–
13831	–	200	800	200	800	400	400	100	–	–	–	–	–	–	–
13925	50	400	800	200	400	400	400	50	–	–	–	–	–	–	–
14071	–	400	400	200	800	200	200	50	–	–	–	–	–	–	–
14691	100	200	200	200	200	400	400	–	–	–	–	–	–	–	–
13823	100	400	400	400	400	400	400	400	–	–	–	–	–	–	–
14694	50	400	400	200	400	400	200	100	–	–	–	–	–	–	–
13841	100	400	800	400	800	800	800	400	–	–	–	–	–	–	–
4440	–	–	–	–	–	–	200	50	200	100	50	–	–	–	–
14688	–	–	–	–	–	–	–	50	200	800	50	–	–	–	–
14664	–	–	–	–	–	50	100	400	400	–	–	50	25	25	–
14586	–	–	–	–	–	–	–	–	–	–	–	100	200	25	50
14544	–	–	–	–	–	–	–	–	–	–	100	–	400	200	200
14634	–	–	–	–	–	–	–	–	–	–	–	200	–	–	200
9437	–	200	–	–	–	–	–	–	–	–	50	50	25	200	200

* The tube agglutination test was used.

** Somatic serotype A and somatic serotype B are considered to be identical to somatic serotype A and B of Morgan, 1959.

*** The number given equals the reciprocal of highest serum dilution giving a + reading. Antisera were not diluted more than 1/800.

TABLE IV

Somatic serotypes established by agglutination* of boiled *Vibrio fetus* antigens by antisera produced against whole-cell antigens

	Antisera											
	Somatic serotype A				Somatic serotype B					Somatic serotype C		
	13823	7418	13011	13831	14908	14907	15110	16001**	16001**	9437	8916	14544
Absorbed With				14908 B***		15109 A***	15109 A	15109 A	14908 B		15109 A	15109 A
Antigen												
12351	4000	800	200	800	–	–	–	–	–	–	–	–
13011	1000	200	100	400	–	–	–	–	–	–	–	–
13823	4000	1000	200	800	25	–	–	–	–	25	–	–
14864	8000	1000	400	1000	–	–	–	–	–	–	–	–
14694	8000	800	200	1000	–	–	–	–	–	–	–	–
14071	1000	400	50	400	–	–	–	–	–	–	–	–
15109	2000	400	100	400	–	–	–	–	–	–	–	–
15108	2000	800	100	400	–	–	–	–	nd****	–	–	–
14904	2000	800	50	200	–	–	–	–	nd	–	–	–
2315	1000	400	100	400	–	–	–	–	–	–	–	–
7418	4000	400	100	400	–	–	–	–	–	–	–	–
14901	4000	800	200	800	–	–	–	–	–	100	–	–
13140	4000	500	200	800	–	–	–	–	–	–	–	–
13316	2000	800	200	800	–	–	–	–	–	–	–	–
13093	4000	800	100	400	–	–	–	–	–	–	–	–
13930	2000	400	100	400	–	–	–	–	–	–	–	–
15107	4000	1000	200	400	–	–	–	–	–	–	–	–
14902	4000	1000	400	800	800	100	–	–	–	50	–	–
13161	4000	800	200	400	800	800	–	–	–	50	–	–
14863	4000	400	400	800	1000	200	200	–	–	100	100	–
15111	25	25	–	–	1000	400	400	2000	–	100	25	–

continued

TABLE IV
(continued)

	Antisera											
	Somatic serotype A				Somatic serotype B					Somatic Serotype C		
	13823	7418	13011	13831	14908	14907	15110	16001**	16001**	9437	8916	14544
Absorbed With				14908 B***		15109 A***	15109 A	15109 A	14908 B		15109 A	15109 A
Antigen												
14907	-	-	-	-	2000	100	500	4000	-	50	-	25
14866	-	-	-	-	800	400	400	2000	-	-	-	-
14539	-	-	-	-	1000	200	400	2000	-	25	-	-
15126	-	-	-	-	800	400	200	400	-	-	-	-
14688	-	-	-	-	400	25	400	1000	-	-	-	-
4440	-	-	-	-	400	200	100	-	-	-	-	-
14664	-	-	50	-	400	50	-	-	-	-	-	-
14908	-	-	-	-	800	100	800	4000	-	50	-	-
14906	-	50	50	-	800	800	400	2000	-	-	-	-
15123	-	-	-	-	500	400	-	-	-	100	-	-
14865	-	-	-	-	800	400	400	800	-	-	-	-
14544	-	-	-	-	-	-	-	-	-	250	200	200
13036	-	-	-	-	-	-	-	-	-	200	400	50

* The slide agglutination test was used.
** Antisera produced against somatic serotype 7 of Mitscherlich (Kamel, 1960) and supplied by Dr. W. Winkenwerder, Hanover, Germany.
*** The letter A, or B indicates the somatic serotype of the antigen used to absorb the antiserum under which the number of the antigen and the letter are located.
**** nd = not done.

interpretation is in agreement with the fact that no significant amount of cross agglutination occurred between antigens of either serotype A or B and the other two antisera (8916 and 14544) directed against antigen C, which had been absorbed. Adequate agglutination occurred between these two antisera and serotype C antigens (13036 and 14544).

Seventeen isolates contained only antigen A, 11 contained only antigen B, and two contained only antigen C. Two isolates (14902 and 13161) contained both antigens A and B, and isolate 14863 appeared to contain all three somatic antigens (A, B, and C). The results obtained for isolate 14664 were inconclusive.

Table IV also shows the results of absorbing two separate portions of an antiserum[23] produced against somatic serotype 7 (Mit) (Kamel, 1960) with serotype A and serotype B antigens, respectively. Prior to absorption, this antiserum agglutinated both serotype A and serotype B antigens. After absorbing with serotype A antigen, serotype 7 (Mit) antiserum agglutinated all but two of 11 serotype B antigens. No agglutinations occurred with any of the antigens of the three somatic serotypes after this antiserum had been absorbed with serotype B antigen. Thus it appears that somatic serotype 7 (Mit) is a subserotype of somatic serotype B. This conclusion is in agreement with the fact that Söderlind (1961) and Mitscherlich (1961) demonstrated that this serotype could not be detected by the agglutination test.

The somatic serotype was determined for six isolates for which an antiserum, but no homologous boiled antigen was available. Different

[23] Supplied by Dr. Winkenwerder, Hanover, Germany.

TABLE V

Determination of the somatic serotype when antiserum, but no homologous antigen, is available by absorbing and agglutinating with antigens of known serotypes.

	Antisera															
	14903	14903	13797	13797	13925	13925	13841	13841	7904	7904	15112	15112	15113	15113	5854	5854
Absorbed with	13140 A**	14908 9437 B & C	13140 A	14908 9437 B & C	13140 A	14908 9437 B & C	13140 A	14908 9437 B & C	13140 A	14908 9437 B & C	14908 B	13140 9437 A & C	14908 B	13140 9437 A & C	9437 C	13140 14908 A & C
Antigen																
15108 A**	-	160***	-	160	-	160	-	160	-	80	80	-	80	-	-	-
14866 B	-	-	-	-	-	-	-	-	-	-	-	80	-	80	-	-
14544 C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	160

* The slide agglutination test was used.

** The letters A, B, and C indicate the somatic serotype of the antigens used to absorb the antisera and also the somatic serotype of the antigens used in the agglutination test.

*** Antisera were not diluted higher than 1/160.

portions of the antiserum for each isolate were absorbed with different combinations of antigens of known serotypes. Somatic serotypes were determined by agglutination reactions between the absorbed antisera and antigens of the three somatic serotypes (table V).

Heat-labile antigens

Ten antisera produced against whole-cell antigens were examined for antibodies against specific heat-labile antigens. Antisera used to determine the Marsh and Firehammer serotypes and six other antisera which agglutinated antigens not agglutinated by any of the Marsh and Firehammer serotyping antisera (table VI) were examined for antibodies against specific heat-labile antigens.

TABLE VI

The demonstration of *Vibrio fetus* antigens not found in the Marsh and Firehammer (MF) serotyping system

	Antisera									
	From (MF) serotyping system				Not from (MF) serotyping system					
	14544	4440	13823	12351	14071	14694	14688	7904	14691	14664
(MF) Sero-type	I	II	III	V	III-II	II-III	?*	V-III	III-V	V-II
Antigen										
15110	-	-	-	-	25**	200***	200	200	50	-
15112	-	-	-	-	25	200	200	50	200	200
15113	-	-	-	-	100	50	-	-	-	-

* ? = not agglutinated by any of the antisera in the Marsh and Firehammer serotyping system.
** The tube agglutination test was used for all tests.
*** Antisera were not diluted more than 1/200.

Absorption studies revealed that all the antisera used to determine the Marsh and Firehammer serotypes and three of the other six antisera contained antibodies for a specific antigen (table VII).

TABLE VII

The homologous agglutination* titer of ten antigens following absorption with various combinations of the whole-cell antigens

	Antisera									
	From (MF)** serotyping system				Not from (MF) serotyping system					
	14544	4440	13823	12351	14071	14694	14688	7904	14691	14664
Absorbed with antigens	4440 13823 12351 14071 14694 14688	14544 13823 12351 14688	14544 4440 12351 14688	14544 4440 13823 14688	14544 4440 13823 12351 14694 14688	14544 4440 13823 12351 14071	14544 4440 13823 12351 14071 14694	14544 4440 13823 12351 14071	14544 4440 13823 12351	14544 4440 13823 12351
Titer	25	400	400	400	800***	200	100	-	-	-

* The tube agglutination test was used.
** The serotyping system of Marsh and Firehammer, 1953.
*** Antisera were not diluted higher than 1/800.

In order to demonstrate heat-labile antigens, the seven antisera which appeared to contain antibodies for a specific antigen were absorbed with homologous boiled antigen and tested for agglutination with the seven whole-cell antigens used to produce the antisera. All of the antisera demonstrated homologous agglutinations (table VIII).

TABLE VIII

Heat-labile antigens demonstrated by cross agglutinations* between seven whole-cell antigens and antisera

	Antisera**						
	From (MF)*** serotyping system				Not from (MF) serotyping system		
	14544	4440	13823	12351	14071	14694	14688
Antigen							
14544	+	-	-	-	-	-	-
4440	-	+	-	±	±	±	±
13823	-	±	+	-	+	+	-
12351	-	-	-	++	-	-	-
14071	-	++	+	-	++	±	-
14694	-	±	±	-	+	++	+
14688	-	±	±	±	±	+	+

* The slide agglutination test was used.
** All antisera were absorbed with homologous boiled antigen.
*** The serotyping system of Marsh and Firehammer, 1953.
\+ Good agglutination at a titer of 1/10.
++ Exceptional agglutination at a titer of 1/10.
± Doubtful agglutination at a titer of 1/10.

Table VIII also shows that a considerable amount of cross agglutination occurred. By absorbing the seven antisera with many different combinations of whole-cell antigens, the minimum amount of absorption necessary to obtain antisera specific for seven heat-labile antigens was demonstrated (Table IX). The specific heat-labile antigens determined by absorbing the antisera used in the Marsh and Firehammer serotyping system were given Arabic numbers (1, 2, 3, and 5) corresponding to their Roman Numeral (MF) serotype numbers. The other heat-labile antigens were given three consecutive Arabic numbers beginning with number 6 (table IX).

TABLE IX

Minimum amount of absorptions necessary to demonstrate specific heat-labile antigens of seven isolates

	Antisera*								
	From (MF) serotyping system				Not from (MF) serotyping system			Heat labile antigen number	(MF)*** serotype
	14544	4440	13823	12351	14071	14694	14688		
Absorbed with whole-cell antigens	none	14071 14688	14071 14688	4440	13823	13823 14688	13823 14694		
Antigen									
14544	+**	-	-	-	-	-	-	1	I
4440	-	+	-	-	-	-	-	2	II
13823	-	-	++	-	-	-	-	3	III
12351	-	-	-	++	-	-	-	5	V
14071	-	±	-	-	++	-	-	6	III-II
14694	-	-	-	-	-	+	±	7	II-III
14688	-	±	±	-	-	±	+	8	?

* All antisera were absorbed with homologous boiled antigen.
** The slide agglutination test was used for all tests.
*** The Marsh and Firehammer serotyping system, 1953.
\+ = Good agglutination at a titer of 1/10.
++ = Exceptional agglutination at a titer of 1/10.
± = Doubtful agglutination at a titer of 1/10.
? = Little or no reaction with any of the serotyping antisera.

TABLE X

The heat-labile antigens of 55 *Vibrio fetus* isolates determined by the slide agglutination test

	Antisera[a]							
	14544	4440	13823	12351	14071	14694	14688	
Absorbed with whole-cell antigens	none	14544 13823 12351 14071 14694 14688	14544 4440 12351 14071 14694 14688	14544 4440 13823 14071 14694 14688	14544 4440 13823 12351 14694 14688	14544 4440 13823 12351 14071 14688	14544 4440 13823 12351 14071 14694	Heat-labile antigen number
Antigen								
14544[b]	+	–[d]	–	–	–	–	–	1
4440	–	+	–	–	–	–	–	2
13823	–	–	+	–	–	–	–	3
12351	–	–	–	+	–	–	–	5
14071	–	–	–	–	+	–	–	6
14694	–	–	–	–	–	+	–	7
14688	–	–	–	–	–	–	+	8
14864	+	+	–	–	–	+	+	2-7-8-1[e]
14863	+	–	+	–	–	–	–	3-1
13925	–	–	+	–	–	–	–	3
14903	–	+	+	–	–	–	–	2-3
13797	–	+	+	–	+	–	+	6-2-8-3
13924	–	–	+	–	–	+	–	3-7
15103	–	–	–	–	–	–	+	8
15109	–	–	+	–	–	–	+	3-8
15122	–	–	–	–	–	+	–	7
14904	–	–	+	+	–	–	–	3-5
15108	–	–	+	–	–	–	–	3
13831	–	+	+	–	+	–	–	3-6-2
15121	–	–	+	–	+	–	–	3-6
15120	–	–	–	–	–	+	–	7
2315	–	+	+	–	+	–	–	3-6-2
15107	–	–	–	+	–	+	–	5-7
13930	–	–	–	+	–	–	–	5
13161	+	–	–	+	–	–	–	1-5
13316	–	–	–	+	–	–	–	5
13930	–	–	–	+	–	–	–	5
14901	–	–	+	–	–	+	–	7-3

continued

TABLE X
(continued)

	Antisera[a]							
	14544	4440	13823	12351	14071	14694	14688	
Absorbed with whole-cell antigens	none	14544[b] 13823 12351 14071 14694 14688	14544 4440 12351 14071 14694 14688	14544 4440 13823 14071 14694 14688	14544 4440 13823 12351 14694 14688	14544 4440 13823 12351 14071 14688	14544 4440 13823 12351 14071 14694	Heat-labile antigen number
Antigen								
13841[b]	-[d]	+[c]	+	-	+	-	-	3-6-2
14940	-	-	-	-	+	+	-	6-7
14664	-	-	-	-	-	-	-	?[f]
13011	-	-	-	+	-	-	-	5
14902	+	+	+	-	-	+	-	1-2-3-7[e]
15126	-	+	-	-	-	+	+	2-8-7
14907	-	+	-	-	+	+	+	2-7-8-6
7904	-	-	-	+	-	-	-	5
7418	-	-	-	+	-	-	-	5
14905	-	-	-	+	-	-	-	5
15123	+	-	-	-	-	-	-	2-1
15124	-	-	-	-	-	-	-	?
14908	-	+	-	+	-	-	-	2-5
15110	-	-	-	-	-	+	-	7
15113	-	-	-	-	-	-	-	?
15112	-	+	-	-	-	-	-	2
14866	-	-	-	-	-	-	-	?
14865	-	-	-	-	-	-	-	?
15111	-	+	-	-	-	-	-	2
14906	-	+	-	-	+	+	+	2-8-7-6
14539	-	+	+	-	+	+	+	2-3-6-7-8
15125	+	-	-	-	-	-	-	1
14634	+	-	-	-	-	-	-	1
14586	+	-	-	-	-	-	-	1
9437	+	-	-	-	-	-	-	1
13136	+	-	-	-	-	-	-	1

continued

TABLE X
(continued)

[a] All antisera were absorbed with their homologous boiled antigen.
[b] Isolate numbers were also used to identify antigens.
[c] Symbols: + = good or exceptional agglutinations at a titer of 1/40 for all antisera except antiserum 14544, which was tested at a titer of 1/10.
[d] Symbols: - = no or doubtful agglutination at a titer of 1/40 for all antisera except antiserum 14544, which was tested at a titer of 1/10.
[e] The heat-labile antigen numbers were written according to the observed agglutination titer, beginning with the strongest and ending with the weakest.
[f] ? = little or no agglutination with any of the antisera.

TABLE XI

Isolates grouped according to their heat-labile antigens

Isolate number									Heat-labile antigens	Heat-labile group
9437,	15125,	14634,	14544,	14586,	.	.	.	.	1	1
4440,	15111,	15112,	.	.	.	.	.	.	2	2
15108,	13823,	13925,	.	.	.	.	.	.	3	3
13039,	14905,	7904,	7418,	13011,	12351,	13930,	13140,	13316	5	4
14071,	.	.	.	.	.	.	.	.	6	5
14694,	15110,	15120,	15122,	.	.	.	.	.	7	6
15103,	14688,	.	.	.	.	.	.	.	8	7
15123,	.	.	.	.	.	.	.	.	1,2	8
14863,	.	.	.	.	.	.	.	.	1,3	9
13161,	.	.	.	.	.	.	.	.	1,5	10
14902,	.	.	.	.	.	.	.	.	1,2,3,7	11
14864,	.	.	.	.	.	.	.	.	1,2,7,8	12
14903,	.	.	.	.	.	.	.	.	2,3	13
14908,	.	.	.	.	.	.	.	.	2,5	14
13841,	13831,	2315	.	.	.	.	.	.	2,3,6	15
15126,	.	.	.	.	.	.	.	.	2,7,8	16
13797,	.	.	.	.	.	.	.	.	2,3,6,8	17
14907,	14906,	.	.	.	.	.	.	.	2,6,7,8	18
14539,	.	.	.	.	.	.	.	.	2,3,6,7,8	19
14904,	.	.	.	.	.	.	.	.	3,5	20
15121,	.	.	.	.	.	.	.	.	3,6	21
14901,	13924,	.	.	.	.	.	.	.	3,7	22
15109,	.	.	.	.	.	.	.	.	3,8	23
15107,	.	.	.	.	.	.	.	.	5,7	24
14840,	.	.	.	.	.	.	.	.	6,7	25

These seven antisera were used to determine the heat-labile antigens of 55 isolates. For this purpose, each of the antisera except the antisera produced against heat-labile antigen number 1 was absorbed with the whole-cell antigens used to produce all six of the other antisera, including whole-cell antigen number 1 (table X). Individual isolates agglutinated with from one to five of the specific antisera. When the isolates were grouped according to the heat-labile antigens they possessed, 25 different groups were formed (table XI). Heat-labile antigen number 5 occurred alone in more isolates than any other heat-labile antigen (table XIII). This may be due to the fact that serotype V (MF) was found by itself in more isolates than any other Marsh and Firehammer serotype. (Table XIII).

Temperature tolerance groups of *V. fetus* compared with other properties.

Three major temperature tolerance groups were revealed and designated as the 37 C group, the 42 C group, and the 45 C group (table XII). The 37 C group contained 27 isolates which grew well at 37 C, but not at 42 or 45 C. The minimum temperature at which good growth could be obtained varied from 17 C for six isolates, 20 C for 5 isolates, 25 C for 13 isolates, and 37 C for two isolates. On the basis of the glycine tolerance test, this group was divided into two subgroups. One subgroup (37 C_v) contained the *V. fetus* var. *venerealis* (glycine intolerant) isolates, and the other subgroup (37 C_i) contained the *V. fetus* var. *intestinalis* (glycine tolerant) isolates.

All 19 isolates in subgroup 37 C_v were isolated from cattle (table XIII). Fifteen were classified as type 1 (B), and four were classified

as subtype 1 (B). Type 1 (B) isolates were not found in either the 42 C or the 45 C temperature tolerance groups; subtype 1 (B) isolates were found in all three temperature tolerance groups.

Eighteen of the 19 isolates in the 37 C_v temperature tolerance subgroup were agglutinated by serotype III (MF) antiserum, and the other isolate was not serotyped. All 13 isolates for which the somatic antigens were determined, contained somatic antigen A. One of these isolates (14863) appeared to contain somatic antigens B and C in addition to A. Five (14854, 14863, 15109, 15108, and 2315) of the seven isolates representing Mitscherlich and Liess' somatic serotype 1, and two (14903 and 14904) of the five isolates representing Morgan's somatic serotype A were found in this subgroup (table XIII). All these isolates (14863, 14864, 15108, 15109, 2315, 14903, and 14904) contained somatic antigen A (tables IV and V). All seven of the heat-labile antigens were found in this subgroup. However, heat-labile antigen number 5 was only found in one isolate, which also contained heat-labile antigen number 3. Two isolates contained heat-labile antigen number 1 in addition to other heat-labile antigens. Three isolates contained only heat-labile antigen number 7. One isolate contained only heat-labile antigen number 6 and another only heat-labile antigen number 8. It is significant to note that 12 of the 18 isolates serotyped with respect to heat-labile antigens contained heat-labile antigen number 3. Heat-labile antigen number 3 was found in only three isolates from other temperature tolerance groups. From these data, it appears that a relationship exists between temperature tolerance subgroup 37 C_v, type 1 (B) serotype III (MF), and heat-labile antigen number 3.

TABLE XII

Temperature tolerance groups and subgroups

Isolate	10-13 C	17 C	20 C	25 C	37 C	42 C	45 C	Temp. group or sub group
14864	nd	nd	-	T	++	-	-	37 C_v
14863	nd	nd	-	++	+++	-	-	37 C_v
13925	nd	nd	-	++	++	T	-	37 C_v
14903	nd	nd	T	T	++	-	-	37 C_v
13797	-	-	T	++	+++	-	-	37 C_v
13924	-	-	T	++	+++	-	-	37 C_v
15103	-	-	T	++	+++	T	-	37 C_v
15550	-	T	T	++	+++	-	-	37 C_v
15109	-	T	T	++	+++	-	-	37 C_v
14694	-	T	T	++	+++	T	-	37 C_v
15122	-	T	T	++	++++	-	-	37 C_v
14904	nd	nd	++	+++	+++	-	-	37 C_v
15108	-	T	++	+++	++++	-	-	37 C_v
14071	-	T	++	++++	++++	-	-	37 C_v
13823	-	++	++	+++	++++	-	-	37 C_v
13831	-	++++	+++	++++	++++	-	-	37 C_v
15121	T	+++	++	++++	++++	-	-	37 C_v
15120	-	++	++	++++	++++	T	-	37 C_v
2315	T	++	+	++	+++	T	T	37 C_v
14185	nd	nd	-	T	+++	T	-	37 C_i
15107	nd	nd	-	++	++++	-	-	37 C_i
13930	nd	nd	-	++	++++	T	-	37 C_i
13161	nd	nd	-	++	+++	T	-	37 C_i
13140	nd	nd	-	+++	+++	T	-	37 C_i
13316	nd	nd	++	++++	++++	T	-	37 C_i
13039	T	T	+++	++++	++++	T	-	37 C_i
14901	-	++++	++++	++++	++++	T	T	37 C_i
13841*	-	T	+++	+++	++++	++	-	42 C_v
14840*	-	+++	++	+++	+++	++	-	42 C_v
14664*	T	+++	++	+++	+++	++	-	42 C_v
13011	-	T	++	++++	+++	+++	T	42 C_i
13014	-	T	+++	+++	++++	+++	-	42 C_i

continued

TABLE XII
(continued)

Isolate	10-13C	17 C	20 C	25 C	37 C	42 C	45 C	Temp. group or sub group
7904	-	++	++	+++	+++	++	-	42 C_i
7418	T	++++	+	+++	++++	+++	-	42 C_i
13641	-	++++	+++	++++	++++	++++	-	42 C_i
14905	T	+++	++	++++	++++	+++	-	42 C_i
14902	-	T	+++	+++	+++	++	T	42 C_i
15113	-	+++	+++	+++	++++	++	-	42 C_i
15112	T	++++	++	+++	+++	++	-	42 C_i
15124	T	+++	+	+++	+++	+++	-	42 C_i
14866	-	+++	++	+++	+++	+++	-	42 C_i
14865	T	++++	+++	++++	++++	+++	-	42 C_i
15111	T	++++	+++	+++	++++	+++	-	42 C_i
15110	T	++++	+++	+++	+++	+++	-	42 C_i
15123	T	+++	+++	++++	++++	+++	-	42 C_i
14908	-	+++	+	++++	++++	++	-	42 C_i
14906	T	++++	+++	++++	++++	+++	T	42 C_i
14907	-	++++	+	+++	++	++	-	42 C_i
14539	-	+++	+	+++	+++	+++	-	42 C_i
15126	-	T	+++	++++	++++	++++	T	42 C_i
14688	-	+++	++	+++	+++	+++	-	42 C_i
4440	-	+++	++	+++	+++	++	-	42 C_i
13350	-	-	T	T	++++	++++	++++	45 C_i
14544	-	-	T	T	++++	+++	+++	45 C_i
15125	nd	nd	-	-	+++	++++	++++	45 C **
14634	nd	nd	-	-	+++	++++	++++	45 C_i
14586	nd	nd	-	-	+++	+++	++	45 C **
8916	nd	nd	-	-	+++	++++	++++	45 C_i
9437	nd	nd	-	-	+++	++++	++++	45 C_i
14698	nd	nd	-	-	+++	+++	++++	45 C_i
13262	nd	nd	-	-	+++	+++	+++	45 C_i
13136	nd	nd	-	-	+++	++++	++++	45 C_i

continued

TABLE XII
(continued)

nd = not done
T = Trace or 1 + reading.
37 C_v = The designation given to V. fetus var. venerealis (v) isolates belonging in subgroup v of the 37 temperature tolerance group.
37 C_i = The designation given to V. fetus var. intestinalis (1) belonging in subgroup i of the 37 C temperature tolerance group.
* = Herd histories indicate that isolates 13841 and 14840 were capable of causing infertility and, therefore, are designated as V. fetus var. venerealis in spite of the fact that they are found in the 42 C temperature tolerance group, a temperature tolerance group composed primarily of V. fetus var. intestinalis isolates. No herd history is available for isolate 14664.
** = Subtype 1 isolates (Bryner et al., 1962) Although these two isolates had the same metabolic properties as the subtype 1 isolates found in the 37 and 42 C temperature tolerance groups, they were not designated as V. fetus var. venerealis because there is no evidence that the subtype 1 isolates found in the 45 C temperature tolerance group are capable of causing infertility.

TABLE XIII

Heat-labile antigens compared with somatic antigens, the serotypes of Marsh and Firehammer, 1953 (MF) Mitscherlich and Liess, 1958 (Mit) and Morgan (1959) (M) and with the physiologic types of Bryner et al., 1962 (B).

Isolate	Temp. group or sub group	Type (B)	Serotype (MF)	Heat labile antigens	Serotype (Mit)	Serotype (M)	Somatic antigens	Host	Source	Site of isolation
14864	37 C_v	1	III-II	2-7-8-1	1		A	Bovine	Germany	Mucus
14863	37 C_v	1	III	3-1	1		A-B-C	Bovine	Germany	Mucus
13925	37 C_v	1	III-V	3			A	Bovine	Montana	Fetus
14903	37 C_v	1	III	2-3		A	A	Bovine	Britain	
13797	37 C_v	1	II-III-V	6-2-8-3			A	Bovine	Montana	Fetus
13924	37 C_v	1	III-II	3-7	1		nd	Bovine	Montana	Fetus
15103	37 C_v	1	II I-II	8			nd	Bovine	Nebraska	Fetus
15550	37 C_v	1	nd	nd			nd	Bovine	Montana	Fetus
15109	37 C_v	1	III	3-8	1		A	Bovine	Germany	Mucus
14694	37 C_v	1	III-II	7			A	Bovine	Montana	Fetus
15122	37 C_v	1	V-II I-II	7			nd	Bovine	N.A.D.L.	
14904	37 C_v	1	III-V	3-5		A	A	Bovine	Britain	
15108	37 C_v	1	III	3	1		A	Bovine	Germany	Mucus
14071	37 C_v	Sub-1	III-II	6			A	Bovine	Montana	Fetus
13823	37 C_v	1	III	3			A	Bovine	Montana	Fetus
13831	37 C_v	Sub-1	III	3-6-2			A	Bovine	Montana	Fetus
15121	37 C_v	Sub-1	III-II	3-6			nd	Bovine	N.A.D.L.	
15120	37 C_v	Sub-1	III	7			nd	Bovine	N.A.D.L.	Mucus
2315	37 C_v	1	III-V	3-6-2	1		A	Bovine	N.A.D.L.	Fetus
14185	37 C_i	2	V-III	nd			nd	Ovine	Utah	Bile
15107	37 C_i	2	?	5-7	1		A		Britain	
13930	37 C_i	2	V	5			A	Ovine	Britain	Fetus
13161	37 C_i	2	V	5-1			A-B	Ovine	Montana	Fetus
13140	37 C_i	2	V	5	1		A	Ovine	Montana	Fetus
13316	37 C_i	2	II-V	5			A	Ovine	Montana	Pla.
13039	37 C_i	2	V	5			A	Ovine	Montana	Fetus
14901	37 C_i	2	III	7-3		A	A	Bovine	Britain	
13841	42 C_v	Sub-1	II-III	3-6-2			A	Bovine	Montana	Fetus
14840	42 C_v	Sub-1	III-V	6-7			nd	Bovine	Montana	Mucus
14664	42 C_v	Sub-1	V-II	?			i	Bovine	New Zea.	
13011	42 C_i	2	V	5			A	Ovine	Montana	Fetus

continued

TABLE XIII
(continued)

Isolate	Temp. group or sub group	Type (B)	Serotype (MF)	Heat labile antigens	Serotype (Mit)	Serotype (M)	Somatic antigens	Host	Source	Site of isolation
13014	37 Ci	2	V	nd			nd	Ovine	Montana	Fetus
7904	42 Ci	2	?	5			A	Ovine	Montana	Bile
7418	42 Ci	2	V	5			A	Ovine	Montana	Bile
13641	42 Ci	2	V	nd			nd	Ovine	Utah	
14905	42 Ci	2	V-II	5		A	nd	Ovine	Britain	
14902	42 Ci	2	II-V	2-3-7-1	1	A	A-B	Bovine	Britain	
15113	42 Ci	2	?	?	2		B-A	Human	Germany	Fetus
15112	42 Ci	2	?	2	2		B-A	Bovine	Germany	Semen
15124	42 Ci	2	?	?			nd	Bovine	N.A.D.L.	Bile
14866	42 Ci	2	II	?	2		B	Bovine	Germany	Fetus
14865	42 Ci	2	II	?	2		B	Bovine	Germany	Fetus
15111	42 Ci	2	II	2	2		B	Bovine	Germany	Fetus
15110	42 Ci	2	?	7	2		B	Bovine	Germany	Fetus
15123	42 Ci	2	?	2-1			B	Bovine	N.A.D.L.	Bile
14908	42 Ci	2	II-V	2-5	2	B	B	Ovine	Britain	
14906	42 Ci	2	II-II	2-8-7-6		B	B	Bovine	Britain	
14907	42 Ci	2	I-III	2-7-8-6		B	B	Bovine	Britain	
14539	42 Ci	2	II	2-3-6-7-8	2		B	Ovine	Britain	Fetus
15126	42 Ci	2	II	2-8-7			B	Bovine	N.A.D.L.	Bile
14688	42 Ci	2	?	8			B	Bovine	Montana	Fetus
4440	42 Ci	2	II	2			B	Ovine	N.A.D.L.	Fetus
13350	45 Ci	2	I	nd			nd	Human	C.D.C.	
14544	45 Ci	2	I	1			C	Ovine	Utah	Pla.
15125	45 C	Sub-1	I	1			nd	Bovine	N.A.D.L.	Bile
14634	45 Ci	2	I	1			C	Bovine	Montana	Feces
14586	45 C	Sub-1	I	1			C	Bovine	Montana	Fetus
8916	45 Ci	2	I	nd			C	Ovine	Montana	Bile
9437	45 Ci	2	I	1			C	Ovine	Montana	Bile
14698	45 Ci	2	I	nd			nd	Ovine	Idaho	Fetus
13262	45 Ci	2	I	nd			nd	Ovine	Idaho	Fetus
13136	45 Ci	2	I	1	13		nd	Ovine	Montana	Fetus

continued

TABLE XIII
(continued)

nd = Not done

37 C_v = The designation given to V. fetus var. venerealis (v) isolates belonging in subgroup v of the 37 C temperature tolerance group.

Mucus = Either cervical or vaginal mucus.

N.A.D.L. = National Animal Disease Laboratory, Ames, Iowa.

? = Little, or no reaction with any of the serotyping sera.

Sub-1 = Subtype 1 (B). These isolates have metabolic properties intermediate between V. fetus var. venerealis and V. fetus var. intestinalis. Subtype 1 (B) isolates closely associated with type 1 (B) isolates are regarded as V. fetus var. venerealis (Bryner et al., 1964.)

37 C_i = The designation given to V. fetus var. intestinalis. (i) isolates belonging in subgroup i of the 37 C temperature tolerance group.

Bile = Mucus membrane lining the gall bladder or liquid bile.

Pla. = Placenta.

C.D.C. = Communicable Disease Control, Atlanta, Georgia.

** = In spite of the fact that the subtype 1 (B) isolates found in the 45 C temperature tolerance group had the same metabolic properties as those found in the 37 and 42 C temperature tolerance groups, they were not designated as V. fetus var. venerealis because there is no evidence that the subtype 1 (B) isolates found in the 45 C temperature tolerance group are capable of causing infertility.

All eight isolates in subgroup 37 C_i were V. fetus var. intestinalis and were therefore classified as type 2 (B) (table XIII). The somatic antigen of one isolate was not determined. The other seven isolates contained somatic antigen A. One of these isolates also contained somatic antigen B. An isolate (15107) representing Mitscherlich and Liess' somatic serotype 1 and another (14901) representing Morgan's somatic serotype A were found in this subgroup. These two isolates contained somatic antigen A (table IV). Six of the 37 C_i isolates were isolated from sheep, one from a cow, and the source of the other isolate was not known. This isolate did not agglutinate with any of the Marsh and Firehammer antisera. The isolate from the cow agglutinated with serotype III (MF) antiserum, and all six of the isolates from sheep agglutinated strongly with serotype V (MF) antiserum. Of the seven isolates examined for heat-labile antigens, six contained antigen number 5. The other isolate contained heat-labile antigens number 7 and 3. Therefore, it appears that considerable relationship exists between serotype V of Marsh and Firehammer and heat-labile antigen number 5.

The 25 isolates in the 42 C temperature tolerance group grew well at 42 C, but failed to grow well at 45 C (table XII). The minimum temperature at which good growth occurred was 17 C for 20 isolates and 20 C for five isolates. This temperature tolerance group was also divided into two subgroups. Subgroup 42 C_v contained the V. fetus var. venerealis isolates, and subgroup 42 C_i contained the intestinalis variety isolates.

Only three isolates were found in the 42 C_v subgroup. Herd histories indicated that isolates 13841 and 14840 were capable of causing infertility.

No information was available concerning the herd from which isolate 14664 was obtained. All three isolates were classified as subtype 1 (B) (table XIII). Isolate 13841 was agglutinated by both serotype II and serotype III (MF) antisera. This isolate contained heat-labile antigens 3, 6, and 2. It also contained somatic antigen A. Isolate 14840 was agglutinated by serotype III and serotype V (MF) antisera and contained heat-labile antigens 6 and 7. Its somatic serotype was not determined. The third isolate (14664) was agglutinated by serotype V and serotype II (MF) antisera, but not by any of the specific heat-labile antisera. The results of the somatic serotyping of this isolate were inconclusive. None of the isolates representing either Mitscherlich and Liess' or Morgan's somatic serotypes were found in this subgroup composed of subtype 1 (B) isolates. This is in agreement with the finding that none of the 19 isolates received from Europe were classified as subtype 1 (B) (table XIII).

Ten of the 22 isolates in subgroup 42 C_1 either were not serotyped with respect to somatic antigens, contained both somatic antigens A and B, or contained only somatic antigen A (table XIII). Of the ten, four were not serotyped, three contained both antigens A and B, and another three contained only somatic antigen A. The three serotype A isolates contained heat-labile antigen number 5, and two of these isolates agglutinated with serotype V (MF) antiserum. In all, six of the ten isolates agglutinated with serotype V (MF) antiserum. Two of these six were also agglutinated by serotype II (MF) antiserum. One of these two isolates was agglutinated by serotype V (MF) antiserum only at the low titer of 1-25. This isolate contained heat-labile antigens 2, 3, 7, and 1, but not heat-labile antigen

number 5. The five other isolates which agglutinated with serotype V (MF) antiserum and for which the heat-labile antigens were determined, contained heat-labile antigen number 5. Four of the ten isolates did not agglutinate with any of the antisera in the Marsh and Firehammer serotyping system. One isolate (14905) representing Morgan's somatic serotype A and another isolate (14902) representing both somatic serotype 1 and A of Mitscherlich and Liess and Morgan, respectively, were found among these ten isolates. Two isolates (15113 and 15112) representing somatic serotype 2 of Mitscherlich and Liess and containing both somatic antigens A and B (tables V and XIII) were also found among these ten isolates. The somatic serotype of isolate 14905 was not determined; isolate 14902 contained both somatic antigens A and B (tables IV and XIII). These results strengthen the conclusion that a relationship exists between serotype V (MF) and heat-labile antigen number 5. Also, these results together with the data from the 37 C temperature tolerance group, indicate that both Marsh and Firehammer serotype III and serotype V are associated with somatic antigen A, and that there is a relationship between somatic serotype 1 (Mit), somatic serotype A (M) and somatic antigen A (tables III, IV, and V).

Twelve isolates in subgroup 42 C_1 contained only somatic antigen B (table XIII). Three of these 12 isolates failed to agglutinate with any of the Marsh and Firehammer antisera. The other nine serotype B isolates agglutinated predominantly with serotype II (MF) antiserum. One of these also agglutinated with serotype III (MF) antiserum, and one agglutinated with serotype V (MF) antiserum in addition to serotype II (MF) antiserum.

One of the somatic serotype B isolates contained heat-labile antigen number 8, and another contained heat-labile antigen number 7. Eight isolates, seven of which had agglutinated with serotype II (MF) antiserum, contained heat-labile antigen number 2. Two of these eight isolates contained only heat-labile antigen number 2, while the remaining six contained one or more other heat-labile antigens. Two of the isolates which agglutinated with serotype II (MF) antiserum, failed to agglutinate with any of the specific heat-labile antisera. Nevertheless, it appears that a relationship exists between serotype II (MF), heat-labile antigen number 2, and somatic antigen B. Five (14865, 14866, 14908, 15110, and 15111) of the seven isolates representing somatic serotype 2 of Mitscherlich and Liess and all three (14906, 14907, and 14908) of the isolates representing Morgan's somatic serotype B were found among these 12 serotype B isolates. As indicated, all of these isolates contained somatic antigen B (table IV).

The 45 C temperature tolerance group appeared to be homogeneous with respect to temperature tolerance and contained 10 isolates which gave 2 + growth readings or better at 37, 42, and 45 C, but failed to grow well at temperatures below 37 C (table XII). Six of the isolates in this group were isolated from sheep, three from cattle, and one from a human being (table XIII). All the isolates in this group were serotype I (MF), none of which was found in the other temperature tolerance groups. All six isolates for which the heat-labile antigens were determined contained only heat-labile antigen number 1. Five isolates contained somatic antigen C and the somatic antigen was not determined for the other five isolates. Eight of the isolates were classified as V. fetus var. intestinalis

(type 2 (B)), and two were classified as subtype 1 (B). These two subtype 1 (B) isolates were not classified as V. fetus var. venerealis because there was no evidence that they could cause infertility. However, the herd history was available for only one of the two herds from which these isolates were obtained. None of the isolates representing Mitscherlich and Liess' or Morgan's somatic serotypes were found in this 45 C temperature tolerance group. It is understandable that Morgan in England, did not encounter isolates with the characteristics of the isolates in this temperature tolerance group because most of the isolates in this group were obtained from sheep, and Morgan worked with a limited number of isolates from sheep. Mitscherlich, in Germany, did not encounter these isolates because he worked with a limited number of isolates from sheep, and furthermore, there is no evidence that isolates with these characteristics have ever been isolated in Germany (Winkenwerder, 1967).

CHAPTER V

DISCUSSION

The results of this paper strongly indicate that Morgan's somatic antigens, A and B, are identical to the first two somatic antigens in tables III and IV and also that these two antigens are identical to somatic antigens 1 and 2 of Mitscherlich and Liess, respectively. The somatic serotype of one of the isolates (14905) representing Morgan's somatic serotype A was not determined. The other four isolates (14901, 14902, 14903, and 14904) representing somatic serotype A of Morgan and all seven of the isolates (2315, 14864, 14902, 15107, 15108, and 15109) representing somatic serotype 1 of Mitscherlich and Liess contained somatic antigen A (tables IV, V, and XIII). All three of the isolates (14906, 14907, and 14908) representing somatic serotype B of Morgan and all seven of the isolates (14865, 14866, 14908, 15110, 15111, 15112, and 15113) representing somatic serotype 2 of Mitscherlich and Liess contained somatic antigen B (tables IV, V, and XIII).

Therefore, it was concluded that somatic antigens A and B (tables III, IV, and V) are identical to the somatic antigens A and B of Morgan. It was also concluded that somatic serotypes 1 and 2 of Mitscherlich and Liess correlate, within the accuracy of the serotyping systems[24], with somatic serotypes A and B of Morgan respectively.

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[24]Inaccuracy could result from the fact that both Morgan, and Mitscherlich and Liess apparently placed isolates which contained two somatic antigens in one or the other of their two somatic serotypes, depending upon the amount of agglutination with specific antisera of their two serotypes. They apparantly never encountered any isolates containing somatic antigen C.

These conclusions are verified by the results of Söderlind (1961) and the findings of Winkenwerder (1967). Winkenwerder used the complement fixation test to examine the serology of two somatic serotype A isolates (13140 and 13924, table XIII) and one somatic serotype B isolate (14539, table XIII). These isolates were sent to him by the Montana Veterinary Research Laboratory. He found that the somatic serotype A isolates contained somatic antigen 1 (Mit) and that the somatic serotype B isolate contained somatic antigen 2 (Mit).

Although the somatic serotyping system of Mitscherlich and Liess (1958) has historical priority over the system of Morgan (1959), the somatic antigens (tables III, IV, and V) were named according to the system of Morgan for two reasons. First, the somatic antigens were prepared (with slight modifications) according to the method of Morgan, and the agglutination test, rather than the complement fixation test was used. Secondly, the somatic serotyping system of Mitscherlich and Liess now contains 12 somatic serotypes of Vibrio, of which only three[25] (including somatic serotype 7) are concerned with V. fetus (Winkenwerder, 1966). Because a somatic serotyping system concerned only with V. fetus was desired, the system of Morgan was used and extended to include somatic serotype C.

Winter and Dunne (1962) found two somatic serotypes which they

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[25]Isolates containing somatic antigen C have been sent to Dr. Winkenwerder by the Montana Veterinary Research Laboratory. Using the complement fixation test, he found them unrelated to serotypes 1 and 2 (Mit) and will include them in the somatic serotyping system of Mitscherlich and Liess as somatic serotype 13.

designated the major O group and the minor O group. A serotype V (MF) (somatic antigen A)[26] isolate (5090) was found in the major O group and a serotype II (MF) (somatic antigen B)[27] isolate (2221) was found in the minor O group. Extensive analyses of the antigens of many isolates of V. fetus (Winter and Dunne, 1962, Winter, 1963, Winter, 1965, and Winter, 1966) reveal two somatic antigens. In all probability the two somatic serotypes of the serotyping systems of Mitscherlich and Liess (1958)[28], Morgan (1959) and Winter and Dunne (1962) are based on the same two somatic antigens (A and B, tables III, IV, and V).

Isolates containing somatic antigen C (serotype I (MF) are commonly isolated from sheep. As far as can be determined, these isolates have never been isolated from the genital organs of cattle and only once from a bovine fetus (isolate 14586, table XIII) in the United States. Since Mitscherlich and Liess (1958) and Winter and Dunne (1962) worked primarily with isolates from cattle, it is not surprising that they did not encounter isolates containing somatic antigen C. Morgan, who worked with isolates from both sheep and cattle, apparently either did not encounter this serotype or

[26]Isolate 5090 was not serotyped with respect to somatic antigens. However, since all the isolates tested which agglutinated predominantly with (MF) serotype V antiserum contained somatic antigen A (table XII) it is highly probable that isolate 5090, also, contains somatic antigen A.

[27]Isolate 2221 was not serotyped with respect to somatic antigens. However, since all the isolates which agglutinated only with (MF) serotype II antisera contained somatic antigen B, it is highly probable that isolate 2221, also, contains somatic antigen B.

[28]Reference is made only to somatic serotypes 1 and 2 of Mitscherlich and Liess as serotype 7 (Mit) appears to be a subtype of serotype 2, and serotype 13, the fourth V. fetus serotype in their system, is only now in the process of being included.

failed to recognize it due to non-specific agglutinations and lack of specific antisera for somatic antigen C.

The existence of somatic antigen C was verified by Winkenwerder (1967). Using the complement fixation test, he found that somatic serotype C isolates, which were sent to him by the Montana Veterinary Research Laboratory, contained a somatic antigen which was unrelated to the somatic antigens found in serotypes 1, 2, or 7 (Mit). Price et al. (1955) found that one group of isolates from sheep contained a somatic antigen which was not found in isolates from cattle. Since isolates containing somatic antigen A and isolates containing somatic antigen B have been isolated from cattle (table XIII), it may be that the isolates which were not antigenically related to those from cattle (Price et al., 1955) were somatic serotype C isolates.[29]

In addition to having a specific somatic serotype, Marsh and Firehammer serotype, heat-labile antigen, and temperature tolerance group, the serotype C isolates have different growth requirements (Tritz and Ogg, 1967) and a different morphology (Firehammer and Lovelace, 1961) than all other V. fetus isolates.

When 62 V. fetus isolates were divided into three groups according to temperature tolerances, it was found that a relationship existed among temperature tolerance groups, Marsh and Firehammer serotypes, heat-labile antigens, and somatic antigens. The majority of isolates that agglutinated predominantly with (MF) serotype III antiserum contained heat-labile antigen number 3, somatic antigen A and were found in the 37 C temperature

[29]Isolates, antigens, and antisera representing the somatic serotypes of Price et al. are no longer available.

tolerance group (table XIII). Most isolates which agglutinated predominantly with (MF) serotype V antiserum contained heat-labile antigen number 5, somatic antigen A and were found in both the 37 C and the 42 C temperature tolerance groups. The majority of the isolates that agglutinated predominantly with (MF) serotype II antiserum contained heat-labile antigen number 2, somatic antigen B and were found in the 42 C temperature tolerance group. Most of the serotype I (MF) isolates contained heat-labile antigen number 1, somatic antigen C and were found in the 45 C temperature tolerance group (table XIII).

Type 1 (B) isolates were found only in the 37 C temperature tolerance group. Subtype 1 (B) isolates and type 2 (B) (V. fetus var. intestinalis) isolates were found in all three temperature tolerance groups (table XIII). These results indicate that subtype 1 (B) and type 2 (B) do not constitute homogeneous groupings. Some subtype 1 (B) isolates contained somatic antigen C, while others contained somatic antigen A. Of the 30 V. fetus var. intestinalis (type 2 (B)) isolates for which the somatic antigens were determined, five contained only somatic antigen C, 12 contained only somatic antigen B, four contained both somatic antigens A and B, and nine contained only somatic antigen A.

V. fetus var. venerealis (type 1 (B), 37 C temperature tolerance) are isolated from cattle, while V. fetus var. intestinalis (type 2 (B)) are more commonly isolated from sheep. The body temperature of sheep is slightly higher than that of cattle. Therefore, not only might pathogenic differences exist between V. fetus var. intestinalis isolates having different somatic antigens, but also between V. fetus var. intestinalis

isolates having different temperature tolerances. More specifically, serotype A V. fetus var. intestinalis isolates in the 42 C temperature tolerance group may have a different pathogenic potential than serotype A intestinalis isolates in the 37 C temperature tolerance group.

Indeed, the V. fetus var. intestinalis isolates that were venereally transferred (Park et al., 1962, and Florent, 1963) may have been V. fetus var. intestinalis isolates having an upper temperature tolerance of 37 C. These V. fetus var. intestinalis isolates have the same temperature tolerance as well as the same somatic antigen as the V. fetus var. venerealis isolates, which are transferred venereally without exception.

The conclusion that Marsh and Firehammer serotype III isolates contain somatic antigen A as do the (MF) serotype V isolates (table XIII) is supported by temperature tolerance data and clarifies the results of other workers. Five isolates which agglutinated predominantly with (MF) serotype V antiserum were found in the same temperature tolerance group with the majority of the serotype III (MF) isolates (table XIII).

Winter (1966), using somatic antigens, found a line of identity between a V. fetus var. venerealis isolate and a V. fetus var. intestinalis isolate in a double diffusion gel precipitation test. Since all V. fetus var. venerealis isolates contain somatic antigen A, this datum can be explained if the V. fetus var. intestinalis isolate was a serotype V (MF) isolate. Morgan (1959) and Mitscherlich (1961) found isolates obtained from sheep as well as isolates obtained from cattle in their somatic serotypes A and 1, respectively. These results can be explained since serotype V (MF) isolates are commonly isolated from sheep and contain the same

somatic antigen (somatic antigen A) as do the majority of the isolates from cattle. Mitscherlich (1961) found that 37% of the isolates in somatic serotype 1 (Mit) tolerated glycine, while 63% did not. Somatic antigen 1 (Mit) is identical to somatic antigen A. Therefore, these data can be explained by the fact that serotype V (MF) isolates (somatic antigen A) are glycine tolerant, while serotype III (MF) isolates (somatic antigen A) are not.

This conclusion that serotype III and serotype V (MF) isolates contain the same somatic antigen was verified by Winkenwerder (1967). He used the complement fixation test to examine the serology of a serotype III (MF) isolate (13924, table XIII), a serotype V (MF) isolate (13140, table XIII) and a serotype II (MF) isolate (14539, table XIII). These isolates were sent to Germany from the Montana Veterinary Research Laboratory. Winkenwerder found that both the serotype III and the serotype V (MF) isolates contained somatic antigen 1 (Mit), while the serotype II (MF) isolate contained somatic antigen 2 (Mit).

The results of experiments conducted without serologic knowledge apply only to one, undetermined, serologic group of V. fetus var. intestinalis, rather than to all three serologic groups.

The Marsh and Firehammer serotyping system distinguishes between V. fetus var. venerealis (serotype III (MF)) and V. fetus var. intestinalis and also between all three somatic serotypes of V. fetus var. intestinalis (serotype V (MF)-somatic serotype A, serotype II (MF)-somatic serotype B, serotype I (MF)-somatic serotype C). However, due to cross agglutinations, the results obtained with this system are not extremely reliable. In some experiments, the variety of the experimental isolates was determined, but

since it was not recognized that the intestinalis variety consists of three somatic serotypes, the serology was not investigated. This applies to most of the experiments conducted to determine the significance of V. fetus var. intestinalis in cattle vibriosis and may account for the variation in results (see "Review of Literature"). In other experiments the somatic serotypes of the experimental isolates were determined, but since it was not recognized that somatic serotype A consisted of both V. fetus var. venerealis and V. fetus var. intestinalis, the variety of the isolates was not determined.

Mitscherlich (1961) performed a statistical analysis between the presence of serotype 1 (Mit) (somatic serotype A) isolates in cattle herds and infertility and, also, the presence of serotype 2 (Mit) (somatic serotype B) isolates in cattle herds and infertility. Two hundred serotype 1 (Mit) isolates were isolated from herds where an infertility problem was present and 156 serotype 1 (Mit) isolates were isolated from herds where an infertility problem was not present. Seven serotype 2 (Mit) isolates were isolated where an infertility problem was present, and nine serotype 2 (Mit) isolates were obtained from herds where an infertility problem was not present. A correlation coefficient of 0.26 was obtained between the presence of serotype 1 (Mit) isolates and infertility, while a correlation coefficient of 0.3 was obtained between the presence of serotype 2 (Mit) isolates and infertility. These results indicate that serotype 2 (Mit) (V. fetus var. intestinalis, somatic serotype B, table XIII) isolates

are of little importance in cattle vibriosis[30]. The probability that V. fetus var. intestinalis isolates were present among the serotype 1 (Mit) isolates of this statistical study is indicated by the fact that 37% of the serotype 1 isolates of Mitscherlich and Liess tolerated 0.8% glycine (Mitscherlich, 1961). However, no information concerning the importance of these isolates (V. fetus var. intestinalis, somatic serotype A, table XIII) in cattle was obtained because neither the serotype 1 (Mit) isolates from herds suffering from infertility or those from herds where no infertility was observed were subjected to the glycine tolerance test.

That important pathogenic differences exist between glycine tolerant and glycine intolerant isolates belonging to the same somatic serotype is demonstrated by the fact that glycine intolerant somatic serotype A isolates have never been isolated from naturally infected sheep, whereas glycine tolerant somatic serotype A isolates are commonly isolated from sheep.

False-positive results have been noted with the use of serologic diagnostic tests employing somatic or whole-cell V. fetus antigens. This can be explained by Winter's finding (1965) that all cattle of breeding age have antibodies against the somatic antigens of V. fetus. However, this does not prove that serologic diagnostic tests employing purified heat-labile antigens would not be successful.

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[30] Twenty nine percent of the serotype 2 isolates of Mitscherlich and Liess were glycine intolerant. This may have been due to classifying some of the venerealis isolates which contained both somatic antigens 1 and 2 (isolate 14863, table XII) as somatic serotype 2 (Mit). It may be that if only glycine tolerant isolates had been included, the correlation coefficient between the presence of serotype 2 (Mit) isolates and infertility would have been even lower.

Morgan (1959) found eight specific heat-labile antigens, which he felt did not correlate with his somatic serotypes. In the present study only seven heat-labile antigens were found. However, considerable correlation was demonstrated between the Marsh and Firehammer serotypes and their corresponding heat-labile antigens (tables IX and XIII). Since serotype III (MF) and serotype V (MF) contain the same somatic antigen, heat-labile antigens must be responsible for differentiating these serotypes. Furthermore, some heat-labile substance must cover the antigenic site on the somatic antigen, or cross agglutination between serotype III (MF) and serotype V (MF) would occur in all cases. This is in agreement with the results of Wiidik and Hlidar (1955), Ristic et al. (1957), Keeler et al. (1966), and Ritchie et al. (1966).

Bacterins against vibriosis have been found effective in both sheep and cattle. However, it has not been determined whether the somatic antigens or the heat-labile antigens or both are the protective antigens. Hoerlein and Kramer (1964) compared bacterins prepared from cells killed by holding at 65 C for 30 minutes with bacterins prepared from formalin-killed cells. Ten per cent of the cattle became pregnant in the control herd, which received no treatment. Eighty percent became pregnant in the herd receiving the heat-killed bacterin, with three services required per pregnancy. All the cattle became pregnant in the herd which received the formalin-killed bacterin, and 1.8 services were required for pregnancy. Since Morgan (1959) found that all heat-labile antigens of V. fetus were not destroyed by boiling for 30 minutes, the bacterin prepared from cells killed by holding at 65 C for 30 minutes cannot be regarded as containing only

somatic antigens. Nevertheless, the results of this comparison indicate that somatic antigens provide protection and that protection is enhanced by heat-labile antigens.

An immunogenic difference between two groups of V. fetus was demonstrated during investigations with V. fetus bacterins for sheep. Miller et al. (1964) found that while bacterins prepared from either serotype I (MF) or serotype V (MF) isolates gave good protection against homologous challenges, no protection was afforded against heterologous challenges. A bacterin containing both serotypes provided protection against challenge with either serotype I (MF) or serotype V (MF) isolates.

In all probability other immunogenically different groups of V. fetus exist. Indeed, nine of 43 sheep vaccinated with a bacterin containing both serotype I (MF) and serotype V (MF) isolates aborted due to V. fetus var. intestinalis.[31] The isolates obtained from these sheep, in contrast to the majority of isolates obtained from sheep, agglutinated strongly with serotype III (MF) antiserum.[31]

In controlled experiments (Te Punga, 1962, Hoerlein and Kramer, 1963, and 1964, and Newhall, 1966), where challenge was made with homologous organisms, V. fetus bacterins for use in cattle have been demonstrated to provide a high degree of protection. However, in field trials (Te Punga et al., 1964, Hoerlein et al., 1965, and Newhall, 1966), where vaccinated cattle may have encountered immunogenically different groups of V. fetus, the pregnancy rates between herds fluctuated from 48 to 100 percent when bacterins containing only one isolate were used. When bacterins (Firehammer

[31]Montana Veterinary Research Laboratory progress report, 1964.

and Berg, 1966, and Newhall, 1966) containing three isolates of different antigenicity were used, the lowest pregnancy rate in the vaccinated cows of seven different herds was 86%. The pregnancy rates of control cattle from each of the above herds indicate that V. fetus was present in all experimental herds. In addition, cultural isolations of V. fetus were made from each of the experimental herds. These data indicate that vibriosis in cattle is caused by more than one immunogenic group of V. fetus.

Because the antigenic groups of V. fetus are not clearly defined, this theory cannot be adequately investigated, and the antigenic groups of V. fetus which must be represented in V. fetus bacterins for adequate protection cannot be determined. Also, diagnostic tests employing cattle serum cannot be used. A better understanding of the antigenic groups of V. fetus not only would help in preparing the most efficient bacterin, but might also lead to a practical means of diagnosing vibriosis.

CHAPTER VI

SUMMARY

Various serologic and physiologic characteristics of 62 *Vibrio fetus* isolates were compared.

The Marsh and Firehammer serotyping system divides *V. fetus* into four serotypes. Serotype III (MF) consists of *V. fetus* var. *venerealis* (Florent) isolates, which are glycine intolerant. Serotypes I, II, and V (MF) consist of *V. fetus* var. *intestinalis* (Florent) isolates, which are glycine tolerant. Due to cross agglutinations between these serotypes, they are not highly reliable. There is some evidence that these serotypes are based on heat-labile antigens.

There are three somatic serotypes of *V. fetus*. Somatic serotype A of Morgan is identical to somatic serotype 1 of Mitscherlich and Liess and contains both serotype III and V (MF) isolates. Somatic serotype B of Morgan is identical to somatic serotype 2 of Mitscherlich and Liess and consists of serotype II (MF) isolates. Somatic serotype C (not defined previously) consists of serotype I (MF) isolates. The glycine tolerance test is a reliable method for differentiating between somatic serotype A *V. fetus* var. *venerealis* isolates and somatic serotype A *V. fetus* var. *intestinalis* isolates. The three somatic serotypes of *V. fetus* var. *intestinalis* can be differentiated by an agglutination test employing boiled antigens and absorbed antisera.

Physiologic type 1 of Bryner et al. consists of serotype III (MF) isolates. Physiologic subtype 1 predominantly consists of serotype III (MF) isolates. Physiologic type 2 consists of serotype I, serotype II, and serotype V (MF) isolates. Some physiologic subtype 1 isolates do not

have the serologic or temperature tolerance characteristics of the majority of the V. fetus var. venerealis isolates, which indicates that all physiologic subtype 1 isolates may not be capable of causing venereal infections.

All serotype I (MF) isolates grew at 45 C, but were the only serologic group of isolates which grew at this high temperature. All serotype II (MF) isolates grew at 42 C. Some of the serotype V (MF) isolates grew at 42 C, while others failed to grow at 42 C, but grew at 37 C. Isolates which were classified both as serotype III (MF) and as physiologic type 1 grew at 37 C, but failed to grow at 42 C. All the physiologic type 1 isolates grew at 37 C, but failed to grow at 42 or 45 C. Physiologic subtype 1 and type 2 isolates grew at all three temperatures.

At least seven heat-labile antigens are present in V. fetus isolates. These antigens may be immunogenically important and may lead to a practical means of diagnosing vibriosis.

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