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Brucella Hypersensitivity

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UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

February 2 - February 7, 1948

No. 188

Monday, February 2

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; Interns' Quarters, U. H.

9:15 - Fracture Rounds; A. A. Zierold and Staff; Ward A, Minneapolis General Hospital.

10:00 - 12:00 Neurology Ward Rounds; A. B. Baker and Staff; Station 50, U. H.

11:00 - 11:50 Physical Medicine Conference; Subject to be Announced; Glenn Gullickson; E-101, U. H.

11:00 - 11:50 Roentgenology-Medicine Conference; Staff; Veterans' Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and D. State; Eustis Amphitheater, U. H.

12:15 - 1:20 Pediatric Seminar; Problems in Latent Virus Infection; Robert Good; 6th Floor Seminar Room, U. H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; M-435, U. H.

12:30 - 1:20 Pathology Seminar; Temporary Ischemia of Kidneys; Craig Freeman; 104 I. A.

12:30 - 1:30 Physiology Seminar; Interactions Among Iodine, Goitrogens and Thyrotropic Hormone; A. Albert, Mayo Clinic; 214 M. H.

12:30 - 1:50 Surgery Grand Rounds; A. A. Zierold, Clarence Dennis and Staff; Minneapolis General Hospital.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - 5:00 School of Public Health Seminar; Nutritional Defects of Indian Diets; N. Purshottam; 113 MeS.

Tuesday, February 3

8:30 - 10:20 Surgery Seminar; Lyle Hay; Small Conference Room, Bldg. I, Veterans' Hospital.

9:00 - 9:50 Roentgenology Pediatrics Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

10:30 - 11:50 Surgical Pathological Conference; Lyle Hay and Nathanial Lufkin; Veterans' Hospital.

12:30 - 1:20 Pathology Conference; Autopsies; Pathology Staff; 102 I. A.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III, Veterans' Hospital.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U. H.

3:30 - 4:20 Clinical Pathological Conference; Staff; Veterans' Hospital.

4:00 - 5:30 Surgery-Physiology Conference; O. H. Wangensteen and M. L. Visscher; Eustis Amphitheater, U. H.

5:00 - 5:50 Roentgenology Diagnosis Conference; Daniel L. Fink and Staff of Veterans' Hospital; M-515, U. H.

SPECIAL LECTURE: Tuesday, February 3, 1948

8:00 p.m. Annual Clarence M. Jackson Lectureship sponsored by Phi Beta Pi; The Mechanisms of Interepidemic Survival of Viruses; D. Jerome T. Syverton, Professor and Head of Department of Microbiology, Louisiana State University School of Medicine; Museum of Natural History Auditorium.

Wednesday, February 4

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-515, U. H.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker and Joe R. Brown; Veterans' Hospital.

11:00 - 11:50 Pathology-Medicine-Surgery Conference; Acute Exacervation of Chronic Cholecystitis and Lithiasis; E. T. Bell, O. H. Wangensteen, C. J. Watson and Staff; Todd Amphitheater, U. H.

4:00 - 5:00 Infectious Disease Routes; Todd Amphitheater, General Hospital, Veterans' Hospital.

Thursday, February 5

8:15 - 9:00 Roentgenology-Surgical-Pathology Conference; Walter Walker and H. M. Stauffer; M-515, U. H.

8:30 - 10:20 Surgery Grand Rounds; Lyle Hay and Staff; Veterans' Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Surgery-Radiology Conference; Daniel Fink and Lyle Hay; Veterans' Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and D. State; Eustis Amphitheater, U. H.

12:00 - 12:50 Physiological Chemistry Seminar; Further Studies on Oxidative Phosphorlyation; Marie Bohland; 214 M. H.

1:00 - 1:50 Fracture Conference; A. A. Zierold and Staff; Minneapolis General Hospital.

1:30 - 3:00 Pediatric Psychiatric Rounds; Reynold Jensen; 6th Floor West Wing, U. H.

4:00 - 4:50 Bacteriology Seminar; Little Pigs Disease; G. Young; 214 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 5:50 Roentgenology Seminar; Color Film: Gastroscopy and Roentgenology of the Stomach; Eustis Amphitheater.

7:00 - 8:00 Urology-Roentgenology Conference; H. M. Stauffer and George Eaves; M-515, U. H.

Friday, February 6

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:20 Medicine Grand Rounds; Staff; Veterans' Hospital.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:00 - 12:00 Surgery-Pediatric Conference; C. Dennis, A. V. Stoesser and Staffs; Minneapolis General Hospital.

11:30 - 12:50 University of Minnesota Hospitals General Staff Meeting; Carcinoma of the Tongue; Donald Peterson; New Powell Hall Amphitheater.

1:00 - 1:50 Dermatology and Syphilology; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

12:00 - 1:00 Surgery Literature Conference; Clarence Dennis and Staff; Minneapolis General Hospital; Small Class Room.

4:00 - 5:00 Pediatric-Surgery Conference; I. McQuarrie and O. H. Wangensteen and Staffs; 6th Floor, Child Psychiatry Clinic, U. H.

Saturday, February 7

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; Station 21, U. H.

8:00 - 9:30 Psychiatry and Neurology Grand Rounds; Staff Veterans' Hospital.

9:00 - 9:50 Surgery-Roentgenology Conference; O. H. Wangensteen, L. G. Rigler, and Staff; Todd Amphitheater, U. H.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-515, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; M-515, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - 12:20 Anatomy Seminar; Bone Marrow Biopsy in Laboratory Animals; R. Dorothy Sundberg; Congenital Hips; Donald R. Lannin; 226 I. A.

II. DERMAL HYPERSENSITIVITY IN HUMAN BRUCELLOSIS

Abraham I. Braude

Introduction

The demonstration by Koch of an accelerated inflammatory reaction to dead tubercle bacilli injected into the skin of infected guinea pigs led to the search for similar reactions produced by other organisms. The existence of a parallel phenomenon in Brucella infections was discovered early in the course of these studies, but only in the case of tuberculosis has the clinical application of this principle produced a properly employed adjunct to other diagnostic methods. The problem of brucellosis, on the other hand, appears to have suffered from the confusion arising from the misuse and misunderstanding of the principle of bacterial hypersensitivity. Perhaps the primary source of this confusion is the habit of many individuals to derive from dermal reactions to Brucella antigens inferences which have no justification by comparison with corresponding situations in tuberculosis skin testing. Because of this state of affairs, the following evaluation of our studies and of others has been made to help clarify matters.

Brucella Antigens

Numerous processes have been employed in the preparation of Brucella antigens. The earliest recorded use of such materials is that described in 1909 by McFadyean and Stockman[1] who exposed a broth culture to live steam, filtered it through porcelain and injected the preparation subcutaneously or intravenously into animals. The test was said to be positive if a fever resulted. This technique was utilized by others with modifications until 1918 when Fleischner and Meyer[2] repeated Koch's experiment with Br. abortus instead of Mycobacterium tuberculosis and found that a saline suspension of heat killed organisms produced positive intradermal tests in infected guinea pigs. During the next thirty years a variety of agents were produced by workers who treated Brucella cells with whichever method of preparing bacterial antigens was currently popular. The long list of antigens as noted in Table I includes most of those which have been reported. In general, they may be classified into the following three groups:

Group I - Bacterial Suspensions

- Heat killed cells
- Formalin killed cells

Group II - Filtrates of Broth Cultures

- Melitin
- Abortin
- Brucellin

Group III - Extracted Fractions

- Brucellergen
- Purified Protein derivative
- Sonic filtrate
- Polysaccharide derivative
- Fat-Free antigen

Such repeated attempts to introduce new materials for Brucella skin testing reflects, among other things, the failure of any of these to qualify fully as a satisfactory antigen. The desirable features, all of which are possessed by the tuberculoprotein, are few in number but difficult to obtain in combination. They characterize a true haptene, which by definition is a partial antigen capable only of reacting specifically in vivo or in vitro with corresponding antibodies without stimulating their production. It is because of their property of producing agglutinins and hypersensitivity, as well as their lack of specificity, that the Brucella antigens heretofore introduced have proved unsatisfactory.

With these desirable features in mind, four representative antigens were studied to compare their relative merits and shortcomings (Table II). These were applied simultaneously to 181 human subjects so that it was possible

Table I.

Chronological listing of Brucella antigens introduced by various investigators.

Antigen	Investigator	Date Introduced
Abortin (1)	McFadyean & Stockman	1909
Heat Killed Suspension (2)	Fleischner & Meyer	1918
Melitin (3)	Burnet	1922
Abortin (27)	Schoenholz & Meyer	1927
Fat-Free Antigen (23)	Levin	1930
Heat Killed Suspension (4)	Giordano	1929
Brucellor (12)	DuBois	1931
Bovine Ando Extract (5)	Leavell & Amoss	1931
Bovine Lancefield Extract (5)	Leavell & Amoss	1931
Saline Extract (5)	Leavell & Amoss	1931
Heat Killed Suspension (15)	Favorite & Culp	1934
Brucellergen (10)	Huddleson	1934
Alcoholic Precipitate (28)	Gwatkin	1935
Brucellolysate (A) (12)	Zdrodowski	1937
P.P.D. (8)	Morales-Otero	1938
Brucellolysate (B) (13)	Plum & Russeff	1939
Brucella Polysaccharide (9)	Lederle Corporation	1941
M.B.P. (31)	Castaneda & Cardenas	1941
Sonic Filtrate (14)	Stubbs & Lives	1942
Formalin Killed Suspension(6)	Leon & Sosa	1947
Phospholipid Complex (abortus) (29)	Paterson, Pirie, Stableforth	1947

Table II.

Antigens Employed and Dosage

1. Heat killed Brucella cells -- 20 million organisms
2. Purified protein fraction -- 0.01 mg.
3. Carbohydrate fraction -- 0.01 mg.
4. Protein nucleate fraction -- 0.01 mg.

to determine the incidence of hypersensitivity in a sample of the population. The use of four, instead of a single antigen, proved advantageous in this regard as exemplified by the fact that in 45 per cent of those in whom hypersensitivity was found, at least one of the four tests was negative.

From Table II it can be seen that a bacterial suspension and three extracted fractions* were selected as representative preparations. No use was made of broth culture filtrates as these had long ago been discarded because of their unreliability. They were introduced by Brunet[3] in 1922 as the first intradermal Brucella test in humans but their specificity was soon questioned by investigators both in Europe and in this country. Giordano[4] in 1929 and Leavell and Amoss[5] in 1931 found that so many false positive reactions were obtained with such a heterogeneous mixture that they abandoned its use early in their study of Brucella antigens.

The bacterial suspension is essentially the same as that described by Fleischner and Meyer in their basic work. It consists of a heat killed agar grown culture of Br. abortus and Br. suis suspended in normal saline solution. In preparation, it compares also with suspensions used by other workers with the exception of minor variations in the media, length of cultivation, and method of killing. Although heat killing is the means generally employed, some have used formalin[6]. Leavell and Amoss[5] prepared a saline extract by shaking a suspension of Brucella in a shaking machine for forty-eight hours and centrifuging it for one hour. No material differences from the ordinary suspensions were noted in their results, however. Although Yeckels and Chapman[7] noticed no difference between the results obtained with the melitensis and abortus antigens, very little information can be found to indicate whether infection produces complete cross-hypersensitivity between each of the three species of Brucella. Therefore, both suis and abortus cells were included in our suspension. The existence of a quantitative difference in cross-hypersensitivity has been reported by Leon and Sosa[6] who found that more than 70 per cent of their cases gave a stronger reaction to the homologous antigen.

The purified protein, furnished by Morales-Otero of San Juan, P. R., was

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* Several other Brucella extracts have been highly recommended by their discoverers but are not available. It is probable, however, that their properties are sufficiently similar to those in our series to warrant the conjecture that no additional information would have been obtained by their use in this study. One of these is a soluble protein described in a collective work published in Russian in 1937 on brucellosis in sheep[12]. It is called Brucellolysate by P. Zdrodowski who made it from living Brucella organisms that were dried, ground fine, and extracted with a slightly alkaline saline solution. According to the Russian Committee for investigations of brucellosis in sheep, it has been used for diagnosis with complete success in eradicating brucellosis after agglutination tests proved unsatisfactory in the differentiation of infected from non-infected animals.

Brucellolysate is the name also applied to an extract reported in 1939 from Copenhagen[13]. The authors spoke of it as a "Bacillary juice" obtained after destroying the membrane of the cells by alternately freezing (with liquid air) and thawing the powdered ground organisms and extracting it with alkaline distilled water. It is alleged to form no agglutinins after infection into animals although some skin hypersensitivity results.

Lives and Stubbs[14] used the sonic method for disintegrating Brucella. The treated material was centrifuged and the filtered supernatant used as an antigen. The active principle appeared to be a protein which did not sensitize control animals to subsequent skin tests but did stimulate agglutinin production.

"M.B.P."[31] is a ball-mill filtrate advocated for use by Castaneda and Cardenas. It is prepared inexpensively and is alleged to be free of agglutinin stimulating properties[32].

produced by a modification of Seibert's method for preparing purified protein from tuberculin[8]. The process involves extraction and purification of the protein fraction present in a solution of the cellular constituents of Brucella cells. The final product gives positive biuret and Millon's tests and a positive xanthoproteic reaction.

The polysaccharide fraction was developed from encapsulated Brucella at the Lederle Laboratories in Pearl River, New York, and when tested there in infected rabbits gave very strong reactions[9]. The molisch test is strongly positive with it, and the tests for protein, including the biuret, Millon's and trichloracetic precipitation, are negative.

Brucellergen is the Brucella protein nucleate popularized by Huddleson and distributed commercially by Sharp and Dohme. It is not to be confused with Brucellin[10], another Huddleson product. The latter is a culture filtrate similar to that described by Burnet and is used for treatment, whereas Brucellergen is intended by Huddleson to be a diagnostic agent only. Its activity depends almost entirely on the protein portion of the compound. This was shown by Stahl. Pennell and Huddleson[11] who separated the protein from the nucleic acid and found that the nucleic acid portion is virtually inactive in the dermal reaction.

Dosage, Administration, and Measurement of Reactions

When dosages for the antigens were selected, it was decided to use .01 mg. of brucellergen as recommended by Huddleson[10]. The purified protein derivative and polysaccharide fraction were used in the same amount, thus making it possible to compare them quantitatively. The number of heat killed Brucella cells was determined by testing a number of individuals with known hypersensitivity. It was found that twenty million cells most consistently produced a distinct but not violent reaction in these persons. This is not in accord with the recommendation of Leon and Sosa[6] who reported that one hundred thousand organisms were the optimal number for differentiating proved cases of brucellosis from controls. In their study, as few as one million organisms gave 26.1 per cent false positives, an incidence enormously larger than that obtained with the dosage used here. When more than twenty million organisms are injected a reaction frequently occurs due simply to the presence of foreign particles. Favorite and Culp[15] studied this problem in a large number of controls and concluded that eighty million Brucella cells produced a non-specific papule corresponding to that resulting from the injection of a mixed vaccine containing E. coli, pneumococcus, staphylococcus and streptococcus.

The four antigens were injected into the volar surfaces of both forearms with a 25 gauge needle, employing an inoculum of .1 cc. The reactions were measured at fifteen minutes, one hour, twenty-four hours and forty-eight hours. The following scale was devised for measuring their intensity:

Negative -- no erythema or induration.
Weak -- smallest diameter less than 1.5 cm. with either erythema or induration.
Moderate -- smallest diameter 1.5 - 2.5 cm. with both erythema and induration.
Strong -- smallest diameter greater than 2.5 cm. with both erythema and induration.
Violent -- necrosis.

Incidence of Hypersensitivity

In order to determine the incidence of hypersensitivity in various groups of individuals, the categories shown in Table III were studies and the results are listed in Table IV.

Table III

Classification of Subjects to Whom Skin Tests Were Applied

Persons tested	181
1. Agglutinins present	65
2. Agglutinins absent	69
3. Agglutination tests not done	40
4. Blood cultures positive	22
5. Agglutinins absent, no history of exposure	30

Table IV.

Per Cent of Individuals with Dermal Hypersensitivity Using 4 Antigens

With positive cultures	100%
With positive agglutination tests	94%
With negative agglutination tests	42%
With negative agglutination tests and no history of exposure	17%

The occurrence of dermal hypersensitivity in all cases of bacteriologically proved brucellosis by the four test method coincides with the observations of others[2,4,15]. A few authors with a large experience in the problem, however, have reported the absence of skin reactivity in varying percentages of so-called proved cases. Castaneda, Tovar, and Velez[16] found this situation in 11 per cent of cases with positive blood cultures. And Huddleson[10] states that 5.5 per cent of actively infected patients may be expected to have a negative brucellergen skin test. In both instances only one antigen was applied to each patient. As stated previously, in 45 per cent of those persons in whom hypersensitivity was ultimately demonstrated in this study, at least one of the four agents failed to elicit a postive reaction.

It seems unlikely that any single antigen can be so standardized that it will elicit positive reactions in all infected cases and at the same time produce only negative reactions in non-infected persons. Part of the accuracy in one instance must undoubtedly be sacrificed to provide maximum dependability in the other. This was convincingly shown by Leon and Sosa[6] when they found that the dosage necessary for one-hundred per cent positive reactions in proved cases was that amount which also caused false positives in twenty-six per cent of controls. At the same time, they noticed that the dosage which gave optimum differentiation between proved cases and controls, failed to detect skin sensitivity in 11.1 per cent of the former. The conclusion appears justified, therefore, that the failure* of some workers to demonstrate the existence of hypersensitivity in all infected cases is based on the inadequacy of any single antigen and that this problem can be solved by the use of several qualitatively different ones, simultaneously applied in their optimum dosage.

It can also be seen that hypersensitivity is almost universally present in the group with positive agglutination tests. In six per cent of these persons with serological evidence of brucellosis, however, there was no sign of skin reactivity to any of the four tests. An explanation for this is not difficult to provide as it is known that several organisms produce cross agglutinins for Brucella. This is especially well known in the cases of Pasteurella tularense[17] and Vibrio cholerae[18] and also occurs when agglutinins for Eberthella typhosa and Bacillus proteus x 19 are present[19]. On the other hand, a corresponding cross skin sensitization apparently does not result in at least the first two instances[17,20]. The skin test, therefore, possesses value in distinguishing between true and agglutinins, in some instances.

Among those persons with no demonstrable agglutinins in their serum, negative blood cultures, no history of exposure to

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* The occurrence of negative skin tests in very early cases with positive blood cultures must also be considered as a possible basis for this discrepancy.

the disease, and no symptoms or signs characteristic of brucellosis, there is a surprisingly large number who display skin sensitivity. It is not unreasonable to suspect that cross hypersensitivity may be responsible for the positive reactions in this control group in a manner similar to that by which cross-agglutinins are produced. In the previous paragraph it was stated that Pasteurella tularense and Vibrio cholerae, which possess common antigenic factors, do not stimulate cross-hypersensitivity. On the other hand, there has been some indication that both tuberculosis and syphilis may be responsible for this type of reaction. Stroem consistently obtained atypical positive reactions with a Brucella suspension in tuberculous guinea pigs and concluded that tuberculosis produced a non-specific sensitization for Brucella which is much less pronounced than for tuberculin. He maintained also that this is well known in humans but gives no supporting data. Giordano[4] found only one positive reaction in his control series and this occurred in a patient confined to a tuberculosis sanatorium. The possibility that syphilis may be a factor in this problem is suggested in the study by Yeckel and Chapman[7] who described reactions closely resembling true positives, in patients under intensive antisyphilitic treatment. In favor of the role of cross sensitivity, is the fact that only one gave a strongly positive reaction of the one hundred and three skin tests applied to our thirty controls. All other positives were of weak or moderate intensity, and could well correspond with the atypical non-specific sensitivity described by Stroem.

Perhaps the most satisfactory explanation for the high incidence of dermal sensitivity in this group is simply that exposure may occur so insidiously that the subject is unaware of it. Whatever the cause may be, it is quite apparent that a positive test by itself is insufficient evidence for making the diagnosis of active brucellosis.

By tabulating the results with the individual agents as shown in Table V,

Table V.

Apparent Reliability of Various Antigens

	Aggl. test negative	No symptoms Aggl. test neg. No exposure	Agglut. test positive
Number of persons	67	30	65
Skin tests applied	231	96	217
Number positive skin tests	71	13	181
% skin tests positive	31	13.5	83
% CHO positive	27(18/67)*	6.5(2/27)	87.5(56/64)
% saline Brucella suspension positive	27(16/67)	3.0(1/26)	82.0(49/60)
% protein positive	25(17/67)	6.5(2/27)	75.0(45/60)
% brucellergen positive	60(18/30)	50.0(8/16)	94.0(31/33)

*Numerator refers to number of positive reactions and denominator to total number of tests in the case of each antigen.

it was possible to evaluate their relative reliability. This was done by determining the incidence of hypersensitivity elicited by each of the three groups of subjects and then comparing the individual with the composite values. From this it can be seen that in each instance there is close agreement between the percentage of positive tests obtained by the carbohydrate, protein, and cell suspension antigens and that variation from the overall percentage is small. Brucellergen, however, is in striking disagreement with the others, particularly in the control group where fifty per cent of the tests performed with that material were positive. This is entirely out of proportion with not only the expected results but also those given for the other three materials. One can only conclude from this comparison that Brucellergen is unsatisfactory as a diagnostic material because of the production of numerous false positive reactions.

Stimulation of Antibody Production by Skin Testing

The problem of agglutinin production due to a single injection of these materials was studied by measuring the titre both at the time the skin test was performed and three to four weeks later. Only a four-fold rise in titre was considered significant. The results shown in Table VI indicate that agglutinin

Table VI.

Stimulation of Antibody Production by Skin Testing

	Rise in Titre	No Change
With 4 antigens	6	8
With CHO alone	5	5
With brucellergen alone	0	8
	11	21

production is stimulated in approximately 50% of individuals when the four antigens are applied simultaneously or when the polysaccharide is injected alone. Brucellergen gave rise to no agglutinins. This was expected from the statements of Huddleson[10] and of Kirby and Rantz[22] who have pointed out independently that a single injection of Brucellergen produces a low titre in only a small percentage of subjects. Huddleson also says that these agglutinins disappear in about sixty days. The agglutinin stimulating property of bacterial suspensions has already been well established so that it was unnecessary to repeat those observations. Giordano's[4] subjects with existing titres invariably demonstrated a rise following injection of heat killed Brucellae, and seventy per cent of those with no agglutinins before the skin test developed titres afterwards. Goldstein's[23] extensive study confirmed these facts and further demonstrated that extraction of fats by treatment with alcohol and ether greatly reduced this property without altering the reliability of the test.

Protein antigens have also been studied from this standpoint by both Lives and Stubbs[14] and Plum and Russeff[13] using sonic filtrate and Brucellolysate, respectively. These products are described elsewhere in this paper. Brucellolysate failed to produce agglutinins in guinea pigs but sonic filtrate did so effectively in all dilutions when rabbits were injected. It is not known whether Morales-Otero's purified protein derivative has been investigated in this regard.

The ability of antigens to sensitize animals to subsequent skin tests must also be determined. It was shown by Koch[24] that the injection of dead tubercle bacilli caused the same altered skin reactivity to later injections that resulted from inoculation with a living culture. Fleischner and Meyer[2], however, failed to duplicate this phenomenon in guinea pigs injected with millions of dead abortus organisms. Stroem[21] believed the difference arose from the ability of insoluble tubercle bacilli to establish a definite focus in the tissues, but he failed to produce any

sensitivity even when Brucella were introduced with a substance (kieselguhr) designed to develop such a focus artificially. By the intradermal vaccination of guinea pigs with heat killed Brucella suspensions at weekly intervals, we have produced hypersensitivity after failing to do so with a single injection. This question has not been investigated at the University of Minnesota with the extracted Brucella fractions. Huddleson[10] makes the statement, however, that brucellergen does not produce skin sensitiveness. The protein in Brucellolysate established slight sensitivity in the guinea pig experiments of Plum and Russeff, whereas the protein in sonic filtrate produced none in the animals used by Lives and Stubbs.

Carbohydrate Fraction

Special consideration is directed to the polysaccharide antigen which is immunologically unique when compared to any other Brucella skin testing material heretofore described (Table VII). Its

Table VII.

Properties of Polysaccharide Antigen

1. Immediate wheal and erythema
2. Suppressed by benadryl
3. Induces agglutinin production in 50% of individuals
4. Sensitivity can be passively transferred
5. Positive in subacute bacterial endocarditis due to Br. abortus
6. No violent reactions in .01 mg. skin test doses.

chemical properties and its ability to stimulate the production of agglutinins have already been mentioned. The polysaccharide differs primarily from the ordinary Brucella antigens in the character of its dermal reaction. This reaction, as in the case of the carbohydrate fractions of the pneumococcus and tubercle bacillus is of the anaphylactic immediate type in contrast to the tuberculin type of delayed reaction produced by other Brucella antigens. Immediately after injection, a wheal appears surrounded by erythema. After two hours it disappears and is followed by a delayed reaction which persists for about 18 hours and fades leaving a residual brown stain. The center of this delayed reaction is sometimes hemorrhagic.

The immediate, but not the delayed, phase of the reaction was reproduced by passive transfer. This was done by the intradermal injection of one cc. of serum from known positive reactors into the forearm of six persons with negative Brucella skin tests. After twelve hours a carbohydrate skin test was made in the prepared area and identical reactions to those in known positives occurred. A control site prepared with one cc. of saline on the opposite forearm was negative. This demonstration of circulation antibodies by passive transfer is in accord with positive precipitin reactions obtained at the Lederle Laboratory[9] with the same material. Sera of animals infected by all three species precipitated dilutions of this fraction containing only .001 mg. per cc.

The effects of this antigen compare with those of other anaphylactic agents, in their resemblance to the effects of histamine. The appearance of the dermal reaction is identical to the wheal and erythema produced by histamine. It is highly significant also that it was possible to suppress the immediate reaction with an anti-histamine substance. The procedure consisted of the addition of one mg. of benadryl to ten cc. of the polysaccharide solution following which the mixed reagent was injected and compared with the pure antigen. Repeated experiments resulted in consistent suppression of the immediate phase, although the delayed reaction was entirely uninfluenced.

Descriptions by Francis and others of the reaction to pneumococcus polysaccharide[25] indicate that it is identical to that produced by the corresponding Brucella fraction including the delayed phase. It is of interest, also,

that both reactions resemble closely that occurring in the Arthus phenomenon which is considered by Rich[24] to be an anaphylactic type of sensitivity. An Arthus type of reaction results experimentally from the repeated injection of antigen at frequent intervals. It is characterized by the development first of an immediate transitory wheal and erythema, followed after a longer period of injections by a reaction in which the prompt stage is succeeded by a delayed one described as hemorrhagic and even necrotic. Rich gives strong evidence to support his view that the Arthus reaction simply represents a higher degree of anaphylactic sensitivity. There is a temptation to classify the delayed carbohydrate reaction in the same category with the Arthus phenomenon as a naturally occurring counterpart because of their similar features. If the premise offered by Rich is correct, however, the data in Table VIII speaks against such an association, as it demonstrates no constant relationship between the intensity of delayed and the intensity of immediate reactions. In other words, there is nothing to indicate that a high degree of anaphylactic sensitivity is necessary for the occurrence of the delayed reaction with the Brucella antigen. A distinct delayed reaction may follow a negative or weak immediate reaction and a weak delayed may follow a strong immediate.

Table VIII.

Relationship of intensity of immediate to delayed reactions produced by carbohydrate antigen in 22 consecutive subjects

Total Number	Immediate Reactions	Delayed Reactions		
		Weak	Mod.	Strong
5	Negative	3	2	0
4	Weak	2	1	1
8	Moderate	3	2	3
4	Strong	2	1	1

Another observation which implies a difference in the significance of the two stages of reaction has been made in two patients with Brucella endocarditis. In this condition it was found at first that only the immediate reaction to the carbohydrate was positive and that no delayed reaction took place with any of the four antigens. Upon retesting after specific therapy, which eradicated the infection, the delayed phase of the carbohydrate appeared at the same time that the other three tests became positive. In a third patient without endocarditis the same sequence developed. These data, though limited by the scarcity of such infections, strongly suggest that the delayed reaction is related more closely to the tuberculin than to the anaphylactic type of sensitivity. This point of view, which is compatible with the facts that the delayed reaction is not altered by benadryl, and cannot be passively transferred, leads to the possibility that some Brucella protein fraction, not detectable by the usual tests, may be active in the polysaccharide solution.

Relation of Intensity of Reaction to Agglutinin Titre

Agglutination tests were obtained in one hundred thirty four individuals to whom a total of four hundred and forty-six skin tests were applied. Adequate figures were available, therefore, to correlate the intensity of these tests with the titre of agglutinins. Although no absolute correlation was possible, certain definite trends were noticed especially with regard to the negative tests and strongly positive ones. In Table IX, it can be seen, as one might expect, that in the very low titres the negative and weak reactions predominate. In titres from 320 to 1280, however, by far the greatest percentage of reactions were quite marked. The latter part of the table, which includes titres of 2560 and 5120, indicate a surprising frequency of weak reactions. This can be explained in part by the fact that several of the tests in these brackets

Table IX.

Correlation of intensity of 446 skin reactions with agglutinin titre. Figures represent percentage of negative, weak, moderate, and strong reactions at each titre.

Titre	0	40	80	160	320	640	1280	2560	5120
Negative	70	38	18	14	0	10	0	15	0
Weak	18	18	38	30	8	18	10	55	48
Moderate	8	32	35	28	28	14	20	8	26
Strong	4	10	9	28	64	58	70	22	26

were applied to patients with Brucella endocarditis, a condition in which the delayed type of skin sensitivity is absent.

Discussion

None of the information afforded by this study justifies the designation of a satisfactory skin testing agent from the group of substances whose properties have been reviewed. Each of those scrutinized in our experiments possess disqualifying deficiences, some of which are included in the summary in Table X. Brucellergen elicits too many false positive and violent reactions. The polysaccharide and vaccine produce agglutinins which would confuse the diagnostic picture. The purified protein derivative of Morales-Otero may prove to be the antigen of

Table X.

Comparison of Antigens

	Type of Reaction	Reliability in Detecting Hypersensitivity	Stimulation of Agglutinins	Reactions in S.B.E.	Violent Reactions
CHO	Immediate	Satisfactory	50%	+	0%
Vaccine	Delayed	Satisfactory	70% ±	0	1%
Protein	Delayed	Satisfactory	?	0	4%
Brucellergen	Delayed	Too sensitive	±	0	9%

choice if it is found to have no antibody stimulating effect. It appears to be highly specific and provokes only infrequent severe local reactions. Other protein extracts, however, have been found to possess marked agglutinin stimulating properties so that the protein fraction studied here cannot be exonerated until its innocence is demonstrated. The same must be said regarding the possibility that it may sensitize the skin to subsequent tests.

More important than considering the specifications of a satisfactory antigen is evaluating its practical significance as a diagnostic device. It is immediately apparent from the high incidence of dermal sensitivity in those persons with no signs of brucellosis that a positive test is not peculiar to active infection. Nor can the intensity of the reaction be used as a guide, for it was learned that in active infections there is little difference in

the incidence of weak or strong reactions*. A positive test implies only the acquisition of hypersensitivity through previous exposure to the organism and no inference can be made relative to the status of infection on the basis of the test alone. In endemic areas where the problem is of most importance, and where it is difficult to escape exposure, a positive test, therefore, has no special significance. It has been observed without exception in large numbers of patients that when the agglutination tests and blood cultures have been negative, the positive skin test has failed to influence the diagnosis. Simpson and Fraizer[26], Giordano[4], and others have advocated the use of skin tests to detect those active cases alleged to have absent agglutinins. Reports by Castaneda[16] as well as Boak and Carpenter[4] describe the absence of agglutinins in six to twelve per cent of proved cases. It is felt that such instances represent exceedingly early infections and that agglutinins undoubtedly occurred eventually within the expected period of time. The authors have never encountered a bacteriologically proved case of brucellosis which failed to develop a positive agglutination test.

It is probable that the chief value of the skin test is in those patients in whom it is negative. The incidence of negative reactions in proved cases in this series and in those of others is appreciable. Hence, it would be dangerous to rule out the diagnosis of brucellosis without the benefit of other procedures. But in those instances in which there exists the possibility of cross agglutinins from another infection, a negative skin test might serve as significant evidence against active Brucella infection.

Finally, the question of detecting "Brucella allergy" arises. Huddleson[10], who is a veterinarian, and others, believe that this entity is responsible for many human cases with symptoms of malaise, dulness, headache, sweating, and aching in the muscles and joints. These symptoms are said to occur in patients seldom giving a history of clinical infection but having repeated exposure to dead or living Brucella through ingestion, inhalation or direct skin contact. Frequently a low titre of agglutinins is present. In the impressive statistical study of Darley and Gordon[30], there was a higher incidence of positive skin tests with Brucellergen in chronically ill patients than in asymptomatic controls. Complaints similar to those above were present in the sensitized patients as a group but not necessarily as individuals. They felt, therefore, that "indolent brucellosis" was a distinct clinical condition but that its characteristics were too indefinite to be of practical value in the consideration of the individual patient. If this syndrome is some day verified, a negative skin test will be of value in ruling it out.

* Of ninety-three tests applied to twenty-four cases with positive cultures, nine were negative, thirty-three were of weak intensity, seventeen moderate and thirty-four strong.

Summary:

1. An evaluation of Brucella skin testing agents has been made and none found satisfactory.

2. The significance of hypersensitivity in brucellosis is discussed.

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