BRUCELLOSIS: ADVANCES IN DIAGNOSIS AND TREATMENT*

By HAROLD J. HARRIS, M.D., F.A.C.P.

Those of us who have come these long distances to attend this conference undoubtedly all are motivated similarly—by our interest in brucellosis and by admiration and respect for our neighbors to the South who have contributed so much to the knowledge of the subject. Ever since the First Inter-American Congress in Mexico in 1946 I have eagerly anticipated the opportunity to renew the acquaintance of those whom I met there and to learn what further advances have been made in the study of brucellosis.

ADVANCES IN DIAGNOSIS

We still must depend upon the same battery of tests—the blood agglutination reaction, the opsonocytophagic reaction, the intradermal reaction and culture—any of which individually often is inadequate and together by no means always adequate. Of course, positive cultural findings, rare in the chronic illness, are adequate. What apparently is needed is perfection of an antigen and of a technique for the performance of the blood complement fixation reaction for brucellosis. The present viewpoint toward this reaction ranges from a negative attitude to great enthusiasm. In its present form the procedure may serve to fill in some of the gaps that exist in our diagnostic armamentarium. It is not at all unusual to find negative blood agglutination reaction, non-informative opsonocytophagic reaction, negative skin test and negative culture in the presence of symptoms which strongly suggests brucellosis, later to find, through pure good fortune that the organism can be recovered perhaps during an acute exacerbation. Even with existing techniques, a positive blood complement fixation reaction may occur in the absence of other laboratory evidence of brucellosis. Although it may not be definite diagnostic evidence, it may along with clinical history, serve to stimulate additional study which may lead to an ultimate diagnosis.

Through the use of the existing battery of tests, the diagnosis of brucellosis can be established with reasonable degree of certainty in from 75 to 90 per cent of cases. The tests must be performed with meticulous technique and must be interpreted with greater accuracy and acumen than often is used. It is essential that antigens employed, whether for the blood agglutination reaction, opsonocytophagic reaction or skin testing, be standardized.

Although there is controversy concerning the need for polyvalent antigen in the performance of the blood agglutination reaction, the experience

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of Evans (1) and the Division of Laboratories and Research of the New York State Department of Health (2) seems to have been amply confirmed; a monovalent Brucella abortus alcohol treated antigen is used with accuracy whether the infecting organism be abortus, suis or melitensis.

In the performance of the opsonocytographic reaction, the more uniform adoption of the original precepts of Huddleson (3) is desirable. Various modifications have been made but have not proved helpful. I do not believe that increasing the citrate content of the blood, as suggested by Tovar (4), results in more accurate readings; rather I believe that it inhibits significant phagocytosis. We should be a bit less dogmatic in the interpretation of the reaction and more ready to evaluate it in the light of not only the intradermal reaction, but also of the blood agglutination reaction, history and clinical findings—and of the other laboratory data such as the erythrocyte sedimentation rate, red and white blood count and differential count. It is a bit embarrassing to state that a patient has not an active Brucella infection because there are no blood agglutinins, negative skin test and so-called negative opsonocytophagic reaction, only to find positive blood or tissue culture.

As for culture itself, the advances are not spectacular but nevertheless impressive. Castañeda, in 1946, demonstrated to some of us his method of culturing blood in a closed flask containing a tryptose broth agar slant along the vertical side of the bottle, with citrate and tryptose broth in the bottom of the bottle. When inoculated with fresh blood, in the presence of 10 per cent CO₂, we have had the good fortune to isolate Brucella in two patients, both of the abortus species. It is of special interest that in none of these patients was Brucella isolated by the more conventional methods in other laboratories.

As for skin testing, the recent advances can be summed up quickly. Hoffman (5) has prepared chemically precipitated fractions of each separate species of Brucella which he has designated Bacterial Antigen Complexes (B.A.C.). They have proved useful in skin testing in that they may indicate the specific skin allergy to the homologous species of Brucella, in patients never previously skin tested nor treated with Brucella antigens and may therefore serve as a guide to therapy. It is still necessary to use heat-killed whole Brucella organisms simultaneously to determine the patient's skin allergy to the genus Brucella since the Bacterial Antigen Complexes are not reliable in this category.

Also unreliable in my experience and that of others is Brucellergen as the sole skin testing agent. Angle and his coworkers (6) skin tested 163 inmates of an institution where raw milk from an infected herd was being served. The skin tests were carried out with heat-killed Brucella organisms in one arm and Brucellergen in the opposite arm, simultaneously. They noted 89 reactors to heat-killed Brucella organisms (54 per cent)
and only 44 reactors to Brucellergen (27 per cent). My own observations were carried out in 48 consecutive patients who had been referred because of the suspicion of brucellosis. There were 35 reactors to heat-killed Brucella abortus organisms (72.9 per cent) and 17 reactors to Brucellergen (35.4 per cent). It is particularly notable that in both series reactions occurred to Brucellergen in almost exactly half as many patients as to whole Brucella organisms. A classic example of the inadequacy of Brucellergen as a skin testing agent is the following: (7) A patient with a positive blood agglutination reaction with Brucella in titer of 1:2560 was skin tested with heat-killed Brucella abortus organisms and with Brucellergen, simultaneously. The reaction to heat-killed Brucella was definitely positive and to Brucellergen completely negative. Blood culture yielded Brucella suis.

**ADVANCES IN TREATMENT**

There has been a most unfortunate defeatist attitude toward therapy, especially in the United States. It was almost as if physicians, having failed to recognize the innumerable cases of brucellosis which occurred in their practices, attempted to assuage their qualms of conscience by such statements as: "It makes little difference whether or not brucellosis is diagnosed—there is no treatment for it in any event." There has been treatment, steadily gaining in effectiveness, ever since Wright (8) pioneered the idea of vaccine therapy 43 years ago. It is astonishing that, in the presence of visual and statistical evidence of the favorable effect of active immuno-therapy (when properly administered), there have been so many articles in reputable scientific journals re-echoing the archaic theory that treatment is of no avail. To further justify this laissez-faire attitude the concept that the disease "burns itself out in not more than 18 months," was perpetuated in the face of overwhelming evidence that the infection may persist or flare-up repeatedly over a period of time limited only by the life-span of the patient.

It is true that vaccine is of little or no value if used in careless fashion, without regard to the state of the patient's sensitivity or of localized pathology. In fact it can increase Brucella allergy in the hypersensitive and can be destructive in its effect on central nervous system or intraocular lesions if not given in subtolerance doses. However, careless use of any therapeutic agent cannot be justified nor the value of such an agent, when properly used, ignored.

There are some who insist that any improvement following specific vaccine therapy is ascribable to incidental psychotherapy. This view cannot be supported in patients who are carefully observed for long periods prior to and following vaccine therapy. A temporary or permanent remission of signs and symptoms in patients ill for years prior to treatment cannot be doubted by any open-minded observer who really observes.
Others have reported "cure" following various kinds of foreign protein therapy, including the intravenous use of typhoid vaccine. These remissions usually are but temporary and the method has gradually been abandoned. Some change in the patient's immunity and allergic status may be brought about by foreign proteins of various types. Favorable changes seem to have occurred in but a small proportion of patients and to have lasted but for short periods of time. Simpson (9) showed, many years ago, the futility of non-specific foreign protein therapy and the actual specificity of killed Brucella vaccine.

Brucella antigens will continue to be useful therapeutic agents. Even in those patients in whom the organism may be completely eradicated by the action of antibiotics, alone or combined with the sulfonamide compounds, a good immune reaction and desensitization may be of importance to guard against re-infection, (10) especially in those occupational groups wherein repeated exposure is unavoidable. It will only be when Brucella infection in animals, as the source of human infection, is eradicated, if ever that time arrives, that Brucella antigen can be completely discarded.

Castañeda's method (11) of the preparation of a solution of parts of the bacterial bodies of all 3 strains of Brucella has given splendid results, even in gravely ill patients. The use of bacterial antigen complexes made from each of the 3 separate species of Brucella is new. I have published little on these antigens (5, 12, 13) but I have been using them both in skin testing and in treatment for about 18 months, in a total of approximately 200 patients. These antigens are chemical precipitates of polysaccharide and protein fractions of each of the three separate species of Brucella after a method described by Josef Hoffmann. They are not available commercially. Their advantage lies in their relative freedom from marked local, focal or systemic reaction, the usually excellent immune response and the concomitant desensitization which is achieved. Like Foshay's (14) nitrous oxide treated (detoxified) Brucella abortus and suis vaccine, these antigens may be used in some hypersensitive patients in whom other antigens are contra-indicated. They are used intradermally, in any dilution that is necessary to avoid more than very slight and fleeting focal or systemic reactions, and in relatively short courses of from six to twenty injections at intervals of 4 to 7 days. No truly curative effect is claimed although, as from the use of any other successful antigenic therapy, a continued state of immunity and desensitization may result in prolonged or permanent freedom from symptoms. In some instances it is probable that the natural defenses of the body thus engendered results in ultimate eradication of the organism.

There is still controversy concerning the relative merits of chemically killed versus heat-killed Brucella organisms, as to mixed Brucella strains
versus a single strain, and as to use of the whole organism versus fractions of the bacterial bodies. Without intending to be dogmatic it may be said that experience in large numbers of patients observed over periods varying from 18 months to 15 years, has offered convincing evidence of the superior results obtained from three of the many available therapeutic antigens. These are: 1) heat-killed Brucella abortus vaccine, 2) a solution of the ground bacterial bodies of all three species of Brucella (M.B.P.—Castañeda) and 3) chemical precipitates of the three separate species of Brucella (bacterial antigen complexes B.A.C.—Hoffmann).

The route of administration—whether it is intramuscular, subcutaneous, intracutaneous or intravenous—is of great importance but is not to be considered as standardized any more than is the quantity of antigen or dilution of antigen. All factors must be adapted to the needs of the individual patient, depending upon degree of sensitivity, localization of infection or allergy, acuteness or chronicity of the infection and response to therapy.

The growing acceptance of subtolerance dosage of any antigen is to be considered as an evidence of advance in the therapy of brucellosis. There are still many who pay lip service to the idea of subtolerance dosage but who, in practice, ignore all reactions except perhaps the most violent ones. It must be admitted that such reaction-producing dosage may benefit a small proportion of patients. In a larger proportion they seem to engender greater sensitivity, lesser immunity and clinical evidence of harm to the patient.

Anti-Brucella serum therapy (Foshay) and transfusion of immune blood have continued to deserve a place among our therapeutic agents, particularly in tiding the patient over the acute illness. After either method, active immunization with a Brucella antigen may be important to prevent relapse. It will be of interest to learn more about the method advocated by Huddleson in the use of sulfadiazine or sulfamerazine orally and transfusion of non-immune blood. The method is predicated on the theory that the sulfonamide compound, the normal antibodies and the complement in the blood of the patient, form a complex which is bactericidal for Brucella organisms. Of particular interest will be the results of treatment after a period of years of observation. The impression gained from casual knowledge of the method is that its action may lie in (a) the well known effect of sulfonamides in some cases of brucellosis, or (b) the fortuitous presence of specific immune bodies in the supposedly non-immune blood, or (c) in a combination of both.

Although the miracle-working sulfonamides are being supplanted in some degree by the even greater miracle-producing antibiotics, we must not lose sight of the value of the sulfonamide compounds, alone or in combination with the antibiotics. At the present moment there are some patients so susceptible to the toxic effect of the antibiotics that
they cannot be used. There are others who fail to respond even to the most potent of the antibiotics. A sensible view is not to discard the old completely until it has been proved to be completely superseded by the new.

**ANTIBIOTIC THERAPY**

Whereas enthusiasm for the antibiotics is justifiable, the tendency to ignore the toxic effects of some of them is fraught with danger. It becomes a matter of very nice judgment when to use the more conservative approach which risks nothing except permanency of recovery in favor of an antibiotic such as streptomycin which very possibly will bring about actual cure but at the expense of more or less permanent damage to eighth nerve function. It seems impossible to formulate definite criteria for the use of potentially dangerous methods. Rather, it seems rational to decide each case on its merits—and not to allow the patient to stampede the physician into a decision which both may regret.

An example is the following: A 40 year old male had a history indicating that his chronic brucellosis had existed for several years. He had a most annoying and disfiguring papulo-pustular generalized eruption and involvement of one hip joint in an infectious arthritic process. Following administration of *Brucella* abortus vaccine and later on *Brucella* B.A.C., this skin eruption of long standing almost completely disappeared, his fatigue lessened and his general health became infinitely better. However, later on the hip joint condition flared up and was accompanied by slight recrudescence of the skin eruption. His physician was most anxious that streptomycin and sulfadiazine be administered. I agreed that it was indicated but advised great caution in its use, with dosage to begin with between 2 and 3 grams daily for about 5 days, then to be reduced to between 1 to 2 grams daily for the balance of the course of therapy. It seemed safest to advise that the streptomycin be stopped after a total of ten or twelve days and that sulfadiazine be continued for the full period of 21 days. It was urged that streptomycin be stopped at the first sign of any toxic reaction of any nature. The patient returned to his home for this course of treatment. In the meantime his physician had read an article written sometime before by Heyl and Howe (15) in which it was stated that the only cure of *Brucella* infection had been brought about through large doses of streptomycin. Therefore the family physician decided to ignore the advice as to starting with a moderate dose and decreasing and used exactly the opposite principle. He initiated treatment with 3 grams of streptomycin daily and, after 5 days, increased the dose to 6 grams daily, giving sulfadiazine concomitantly. Clinically the patient had a splendid result so far as his *Brucella* infection was concerned,
including the localized manifestations in the hip joint. However, marked damage to the eighth nerve was noted; in fact it had developed during the course of treatment—and was still present in a severe degree when I again saw the patient—months later. The patient had had to relearn to walk and recently had again learned to dance. However, he had simply accommodated himself to partial loss of his sense of equilibrium which presumably will be permanent. Approximately ten other examples of streptomycin damage to the eighth nerve have come to my attention, in all, save one, the result of too large dosage or too continued use of the drug after the onset of frank evidences of toxicity.

The definite indications for the use of streptomycin and sulfadiazine seem to me to be the following:

(1) Patients in whom there is inadequate response to Brucella antigenic or other conservative forms of therapy.
(2) Patients who persistently relapse in spite of temporary effectiveness of conservative therapy.
(3) Patients with localization of infection where tissue destruction is likely to follow uncontrolled infection or allergic reaction (e.g., ocular involvement, central nervous system involvement, endocarditis).
(4) Patients in the septicaemic phase of the illness wherein actual eradication of the organism might be accomplished, with prevention of its localization in granulomatous tissues and establishment of intracellular growth.
(5) Other special indications which cannot be readily envisioned.

As for dosage and duration of administration of streptomycin, I favor a middle of the road policy—neither very large doses nor the very small doses advocated by some. For a patient weighing in excess of 150 pounds initiation of treatment with 2½ to 3 grams daily seems justified, to be continued for approximately 3 to 5 days. For the next 3 to 4 days dosage of 1.5 to 2 grams daily is advisable and for the remainder of the course of treatment, 1 gram daily is probably safest. The total course may need to be as long as 21 days but my present concept is that a course of from 10 to 14 days should be adequate in the majority of patients, the sulfadiazine to be continued for 21 days.

As for other antibiotics, the one giving greatest promise of effectiveness without toxicity at the present time seems to be aureomycin (Duo-mycin-Lederle). Chloromycetin has not yet been evaluated in human brucellosis. Polymyxin (aerosporin A) gives some promise of effectiveness but its nephro-toxicity should preclude its use unless the nephro-toxic factor can be removed. The British have produced aerosporin B from a separate strain of bacillus polymyxa which does lack the nephro-toxic factor. However, it has not yet been evaluated in human Brucella infection. A less toxic (perhaps non-toxic form of streptomycin is now becoming available for clinical trial and may prove to be safe and effec-
tive. However, it is probable that it will be effective only by the parenteral route.

My own experience with aureomycin has been limited, as indeed has been that of others with the possible exception of Spink who has recently been to Mexico for the purpose of experimental use of this antibiotic. We may be fortunate enough to hear from him during this Congress. The reason for the limitation in numbers of cases is that aureomycin has not been produced in sufficient quantities to allow its employment in other than urgent cases in which the diagnosis has been confirmed by cultural findings. It is hoped that the stringency of supply will be lessened in the near future. The obvious advantages of aureomycin over streptomycin are: (1) Aureomycin is effective orally whereas streptomycin must be given parenterally. (2) Aureomycin seems to be free of toxic effects whereas the possible toxicity of streptomycin is only too well known. (3) Because of the lack of toxicity and the effectiveness of aureomycin when given orally, the patient is spared the rather prolonged and expensive stay in hospital.

It must be emphasized that it has not yet been proved in human Brucella infection that actual eradication of the organisms can be accomplished by use of aureomycin. Laboratory evidence and the effectiveness of this antibiotic in other infections, including typhus, Tsutsutamagushi fever and Rocky Mountain spotted fever, furnishes grounds for optimism, however. On the other hand, as with streptomycin, we may find either failure of cure in a small to large percentage of patients or that the aureomycin must be given concomitantly with a sulfonamide. To date I have treated but 8 patients with aureomycin orally. The first patient received 1.8 grams daily for 7 days, the others 4 grams daily for 3 days and 2 grams daily for the balance of 7 days.

The gastrointestinal symptoms were variable. One patient vomited from 1 to 2 hours after almost every dose and had to discontinue the medication on the fifth day; diarrhea had also been severe. Another patient had rather severe diarrhea but with only occasional vomiting. A third patient had rather marked vomiting for the first 2 days but it lessened immediately on reducing dosage to 2 grams daily. All three of these patients noted intensification of previously existing weakness and malaise. In a fourth patient, fever subsided within 3 days and there were virtually no unpleasant side-effects. Clinical recovery was rapid. The other patients are still under treatment as this is written. Three patients whose sedimentation rates were elevated prior to treatment had subsidence to normal rates at the end of the course of treatment or within a week thereafter. Leucocytosis in one disappeared by the end of the 7 days of treatment. Anemia developed in none. An eighth patient, treated at a Veterans Administration Hospital had profuse vomiting and diarrhea when the drug was administered orally and also when it was given parenterally.
SUMMARY

An attempt has been made to describe actual improvement in diagnostic and treatment measures in brucellosis. Warnings concerning the toxicity of some antibiotics have been repeated. Apparently favorable effects of aureomycin have been reported, in a preliminary way to call attention to the probable value of this new antibiotic when administered orally.

BIBLIOGRAPHY

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Diagnóstico: El diagnóstico de la brucelosis depende todavía de las mismas pruebas: seroaglutinación, índice obsonocitofágico, reacción intradérmica y cultivo, los que individualmente son con frecuencia inadecuados y juntos tampoco resultan definitivos, como lo comprueba la variedad de los resultados obtenidos. Lo que aparentemente es necesario, es el perfeccionamiento de un antígeno y una técnica para realizar la prueba de fijación del complemento, pues se ha demostrado que aun con la técnica empleada hoy día se logran reacciones positivas en ausencia de otros resultados de laboratorio. Se estima que con las pruebas de laboratorio enumeradas se puede lograr del 75 al 90% de diagnósticos, siempre que se realicen las técnicas cuidadosamente y su interpretación se haga con buen criterio. Al discutirse individualmente las ventajas y desventajas de cada una de las pruebas mencionadas, se confirma la necesidad de no guiarse por una sola, sino emplear las que se puedan practicar correctamente, repitiéndolas si es necesario.

Tratamiento: Desafortunadamente prevalece una actitud pesimista sobre el tratamiento de la brucelosis, especialmente en los Estados Unidos. Sin embargo, desde que Wright concibió la vacunoterapia hace 43 años, se han realizado grandes progresos sobre el tratamiento de esta afección. Sorprende que, a pesar de los datos estadísticos favorables publicados sobre inmunoterapia, aparezcan tantos trabajos en publicaciones científicas de reputación haciendo eco a la teoría arcaica de que el tratamiento de esta afección no tiene valor. Es cierto que la vacuna es de poco o ningún valor si no se emplea con cuidado, y puede aumentar la hipersensibilidad del enfermo a la Brucella y producir reacciones indeseables. Los resultados obtenidos con reacciones debidas a una proteína extraña, incluso el uso intravenoso de vacuna contra la tifoidea, no han dado resultados satisfactorios y continúan los antígenos de Brucella siendo los agentes terapéuticos de mayor utilidad. El método de Castañeda en la preparación de estos antígenos ha dado espléndidos resultados, introduciendo el A. el uso de complejos antígenicos de tres especies diferentes de Brucella, los que ya se han experimentado en más de 200 casos. Estos antígenos son precipitados químicos de las fracciones proteicas y de los polisacáridos de la Brucella. Existen controversias sobre el valor relativo de los microorganismos inactivados químicamente y aquellos muertos por el calor, así como el uso de una sola o varias cepas de Brucella. La seroterapia antibrucelosa y la transfusión de sangre inmune, han continuado mereciendo atención entre otros agentes terapéuticos. Huddleson recomienda el empleo de sulfadiazina y sulfamerazina por vía oral en combinación con sangre no inmune, basándose en la teoría de que el compuesto sulfonamido, los anticuerpos normales, y el complemento en la sangre del enfermo, forman un complejo que es bactericida para la Brucella. Con referencia al empleo de antibióticos debe tenerse cuidado de no ignorar sus posibles efectos tóxicos. Un tratamiento combinado de estreptomicina y sulfadiazina parece indicado para: aquellos enfermos en los que la respuesta antígenica es inadecuada; aquellos con recáldas persistentes; aquellos con localización de la infección en que es posible la destrucción de tejidos; y aquellos en la fase septiémica de la enfermedad. Con referencia a otros antibióticos, la aureomicina parece ser la más prometedora, no habiéndose avaluado aun la cloromicetina. Las ventajas de la aureomicina sobre la estreptomicina son: la aureomicina es eficaz oralmente, mientras que la estreptomicina debe administrarse por la vía parentérica; la aureomicina está libre de efectos tóxicos, mientras que la toxicidad de la estreptomicina es bien conocida; la falta de toxicidad en su administración oral, evita una hospitalización prolongada. La experiencia con aureomicina es aún limitada: los 8 enfermos tratados por vía oral por el A. obtuvieron una mejoría clínica.