A Study of the Opsono-Cytophagic Power of the Blood and Allergic Skin Reaction in Brucella Infection and Immunity in Man*

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IN a series of studies which the writers have been conducting for the past two years, in connection with the phenomenon of phagocytosis and allergic reactions in Brucella infection in man and animals, evidence has been obtained to the effect that one of the most important indications of infection with, and immunity to, Brucella in man is the phagocytic response of the blood based on measurements made *in vitro* in an opsono-cytophagic system and the existence of a state of allergy as determined by an allergic skin test.

Since the widespread occurrence of undulant fever in man in the United States was established, considerable attention has been given to the diagnosis of the disease, chiefly by cultural, serological and allergic methods. There are being accumulated data which indicate that the methods of diagnosis just mentioned are not always satisfactory in detecting many cases of the disease in man. Again, there are times when the results of the serological or allergic tests are misleading which in turn may result in an incorrect diagnosis.

Many physicians often observe the occurrence of a symptom-complex in patients not unlike that of acute or chronic undulant fever. One may be confused in making a diagnosis because, if Brucella infection is suspected, it might easily be ruled out on the basis of a negative blood, stool and urine culture, and negative agglutination test. If in addition to these examinations an intradermal test is performed with a suitable agent and a positive reaction is obtained, this evidence alone is not sufficient to warrant a diagnosis of undulant fever. The diagnosis remains uncertain because all individuals who have been infected with Brucella as well as those who are actively infected will show an allergic skin reaction to a satisfactory Brucella allergin.

Confusion may again arise when individuals who are ill or who are apparently healthy are found to have Brucella agglutinins in their blood serum. Those comprising this group are chiefly laboratory workers, practising veterinarians, farmers and packing house employees. By way of illustration let us consider the status of practising veterinarians toward the disease. Those who are engaged in cattle practice come in contact with *Br.*

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abortus to a greater extent than any other single group of people. It has already been shown by serological and intradermal tests made on veterinarians in Europe and this country, that a considerable percentage have Brucella agglutinins in varying degrees in their blood and give a positive intradermal test. While very few of them give a history of having a clinical course of the disease, many report the occurrence of skin eruptions and malaise after removing retained placentas from aborting cows. The malaise is usually characterized by dullness, head-ache, sweating, aching in the muscles and joints. There may occur an elevation of the temperature. These symptoms may also occur from the ingestion or inhalation of the organism alive or dead, or of sterile broth filtrates on which the organism has grown. It is necessary, of course, that these materials pass through the epithelium of the skin, or the respiratory or digestive tract. Individuals in the general population as well as those in particular groups may show the symptoms just mentioned when exposed to Brucella.

We have conclusive evidence that all of those individuals who recover from Brucella infection, be it clinical or subclinical, are left sensitive and in some cases hypersensitive to Brucella, perhaps for several years. Such individuals show not only a local reaction to the intradermal test when made, but may show symptoms exactly like those seen in typical undulant fever as well. The differentiation of such cases from active infection with Brucella, or from many other diseases characterized by similar symptoms, has been a difficult problem to the clinician.

In attempting to arrive at a satisfactory method of detecting Brucella infection in those who do not show agglutinins in their blood and of clarifying the status of those individuals who show symptoms characteristic of the disease when exposed to Brucella or to protein fractions of the organism, it was thought that a study of the phagocytic power of the blood in conjunction with an allergic skin test might possibly furnish considerable information on these problems and clarify the diagnosis of Brucella infection in many cases.

The literature is rather extensive on the phenomenon of phagocytosis and its meaning with respect to infection and immunity in other bacterial diseases. The application of allergic skin tests in detecting Brucella infection has also been given considerable study by many workers. Space does not permit at this time a review of the literature on all the important accomplishments in these directions.

Definite evidence was obtained during the course of these studies to support the view held by many others that a more accurate determination of an individual's actual phagocytic response to bacterial invasion may be gained by the use of whole blood, rather than by using the serum plus washed leucocytes of another individual or species of animal. In view of this fact the whole blood method was adopted for determining phagocytic response in Brucella infection and immunity in man in preference to the commonly used Wright and Douglas [1] opsonic index method.

Shortly after beginning this study we were surprised to find that the polymorphonuclear cells in whole defibrinated blood of many adults, regardless of their history as respects Brucella infection, show a decidedly marked ingestion of any of the three species of Brucella *in vitro* in a phagocytic system. Later it was found that when a certain amount of sodium citrate is added to a given specimen of whole blood, phagocytosis of Brucella is either inhibited, retarded or unaffected. The degree of phagocytosis that obtains

depends upon the history of the individual as respects Brucella infection. Hektoen and Ruediger [2] many years ago found that a great many other salts as well as sodium citrate, when added to whole blood in low concentrations, will retard or inhibit phagocytosis of certain bacteria. They succeeded in showing that the salts affected the opsonic property of the serum rather than the activity of the leucocytes.

We have found that the concentration of sodium citrate that is necessary to inhibit phagocytosis in whole blood varies with different species of animals. That is, a certain concentration of sodium citrate in the blood from one species of animal may prevent phagocytosis of Brucella altogether, but may not have the same degree of inhibiting action when added to the blood of another species of animal. By taking advantage of the inhibiting action of sodium citrate on phagocytosis, and applying it to a study of the opsono-cytophagic power of the blood of humans as respects Brucella, we find a marked difference in the opsono-cytophagic activity of the blood from the susceptible, from the infected and from the immune. In other words, one can measure an individual's status with respect to Brucella infection by determining the opsono-cytophagic power of the whole citrated blood *in vitro* for Brucella.

The concentration of sodium citrate in whole blood which has thus far given the most favorable results in determining gradations or degrees of phagocytic response toward Brucella sufficient for differentiating between the susceptible, the infected, and the immune is 0.8 per cent. If the concentration of sodium citrate in the blood is varied from the stated amount, differentiation between the three groups is not obtained.

In determining the phagocytic activity of whole blood, Glynn and Cox [3] coined the word "opsono-cytophagic" to indicate the phagocytic activity of blood in the presence of serum opsonins and homologous leucocytes. This expression has been adopted to describe the phenomenon with which this study deals. A description of the technic that has been employed and the results of the study follow.

METHODS OF STUDY

Determination of Opsono-Cytophagic Power of Blood. The method which we have adopted for determining the opsono-cytophagic power of blood for Brucella is a modification of the Leishman [4]-Veitch [5] technic. The modified technic consists of mixing equal quantities of a citrated (0.8 per cent) fresh blood and a heavy bacterial suspension of living organisms in small Wassermann tubes, incubating at 37° C. for 30 minutes, and subsequently making spreads and staining with Hastings stain. The addition of a definite amount of sodium citrate prevents clotting of the blood and inhibits the action of Brucella opsonins which are present in the serum of many normal individuals.

Preparation of Blood Specimen. The blood specimens on which the test is to be made are collected in 5 c.c. amounts in glass vials in which has been placed 0.2 c.c. of a 20 per cent solution of sodium citrate in physiological salt solution. The final dilution of sodium citrate that obtains in the blood is 0.8 per cent. The test should be conducted on the specimens within six hours after collection provided they are kept in a cool place. The polymorphonuclear cells in blood disintegrate very rapidly when it is kept warm for two or three hours. The specimens should be thoroughly shaken directly before mixing with the bacterial suspension.

Bacterial Suspension. The bacterial suspension is prepared fresh each day by suspending several loopfuls of the

growth from a 48 hour liver agar slant culture in sterile physiological salt solution of pH 7. The turbidity of the suspension should give a reading of approximately 2 cm. when measured with the Gates apparatus.[6] Suspensions of any of the three species of Brucella are suitable regardless of virulence.

The Test. Into clean small glass vials, such as are used for the agglutination or Kahn test, are placed 0.1 c.c. of the whole blood and 0.1 c.c. of the bacterial suspension. After mixing thoroughly, the vials are placed in an incubator for 30 minutes at 37° C. Certain strains of Brucella tend to become " fast " to ingestion by the cells after being transferred daily for many weeks. If a culture is being used daily for the test, it should be checked frequently for " fastness " to phagocytosis against whole citrated blood from a known immune person. Continuous agitation during the period of incubation tends to inhibit phagocytosis. Considerable sedimentation of the blood cells takes place during the incubation period. The cells should not be resuspended by shaking after the period of incubation. Directly after removing the tubes from the incubator, a small amount of the sedimented cells is removed by means of a finely drawn capillary pipette to which is attached a small rubber bulb. A drop of the cells is placed at one end of a thoroughly cleaned and polished glass slide, and drawn across the slide by placing the end edge of another slide at such an angle that the spread thins out and terminates at or near the middle. In a spread of this type, most of the leucocytes may be found near the terminating edge of the spread. The blood film should be dried as rapidly as possible to prevent shrinking of the leucocytes. Rapid drying may be obtained by placing the slides in front of a small electric fan. A small heating unit from an electric heater, if attached to the front of the fan and operated simultaneously, will greatly increase the speed of drying.

Staining Spreads. The slides are placed face-upward on a suitable rack and the spreads covered with 0.5 c.c. of Hastings stain. After an exposure of 15 seconds, 1 c.c. of distilled water is added to the stain on the slide. At the end of 10 minutes, the spread is gently washed free from stain with distilled water and dried in front of an electric fan.

Estimation of the Degree of Opsonocytophagic Activity. The size of the organism in question and the marked degree of phagocytosis which occurs in cells from immunes has necessitated the employment of a different system of recording phagocytic activity from that which is commonly used in studies of this nature. In routine work, a total of 25 cells is counted in different sections of the spread and each cell is recorded as negative when no phagocytosis occurs, as slight when from 1 to 20 bacteria are seen in the cell, as moderate when from 21 to 40 bacteria are found in a cell, and marked when the number of bacteria in the cell is above 40. The bacteria are so numerous in those cells showing marked phagocytosis that it is impossible to count all of them. Examples of different degrees of phagocytosis of Brucella are shown in Plate I.

It is realized that the foregoing method of measuring degrees of phagocytosis is only approximate. It was adopted after making thousands of examinations by different methods.

Brucella Allergic Skin Test. The agent used in making the allergic test was developed by Hershey in our laboratory during his study of the chemistry of Brucella. It is a soluble nucleo-protein fraction of the three species of Brucella in 1-1000 dilution in slightly alkaline physiological salt solution. The test is made by injecting

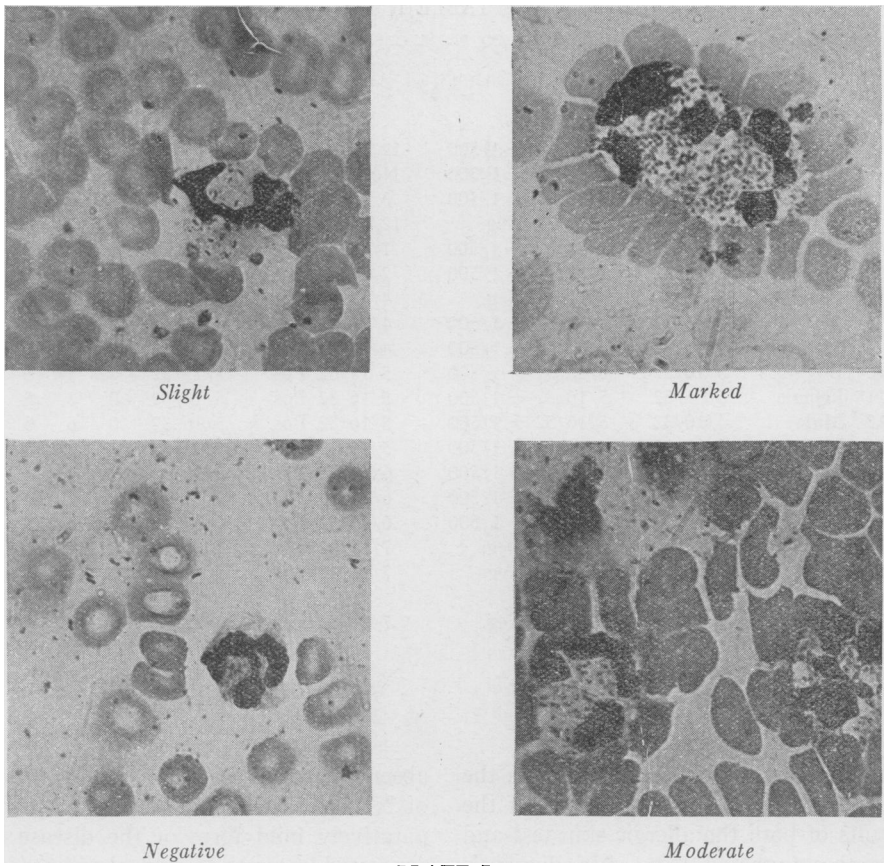

PLATE I

DEGREES OF PHAGOCYTOSIS OF BRUCELLA

about 0.1 c.c. of the fluid intracutaneously in the lateral surface of the forearm, using a 26 gauge needle. The size of the local reaction, which is characterized by a circumscribed erythema and slight edema, may vary from 1 to 2 inches in diameter and appears within 24 hours after injection. It may persist for 48 to 96 hours. There is no necrosis or sloughing of the tissue at the point of the local reaction. In the infected, the local reaction may be accompanied by a more marked manifestation of symptoms. Those that are hypersensitive will show a terrific symptomatic reaction along with the local reaction. Those that have not been sensitized to Brucella and who are probably susceptible to infection show no systemic reaction. Often one sees in certain individuals an erythema, about one-half inch in diameter with no edema around the point of the injection. It has the appearance of a nonspecific reaction.

RESULTS OF STUDY

Results on Blood Specimens from Cases Diagnosed as Undulant Fever. Each of the 19 cases presented in Table I has shown clinical symptoms similar to those seen in acute and chronic undulant fever before a laboratory diagnosis of the disease was made. The laboratory diagnosis was based on either a positive blood culture or on the combined results of the agglutination, allergic and opsono-cytophagic tests. In the absence of a positive blood cul-

TABLE I

OPSONO-CYTOPHAGIC POWER OF BLOOD FROM CASES DIAGNOSED AS UNDULANT FEVER

Case No.	Sex	Approximate Date of Onset	Aggl. Titer	Allergic Skin Test	Activity of Cells Number Date	Ma.	Mo.	S.	N.*
1	Male	12–'29	12/19/31 + 1/500	12/19/31 Pos.	12/19/31	0	8	10	7
2	Male	2–'31	4/13/31 + 1/500	None made	4/13/31	0	0	4	21
3	Female	3–'31	5/15/31 + 1/500	3/15/31 Pos.	5/15/31	0	0	10	15
4	Male	7–'31	12/ 4/31 Neg.	12/ 4/32 Pos.	12/ 4/32	0	0	0	25
5	Male	11/10/31	1/18/32 + 1/500	1/18/32 Pos.	1/18/32	0	0	5	20
6	Male	1/18/32	2/17/32 + 1/500	2/17/32 Pos.	2/17/32	6	4	15	0
7	Female	5–'30	4/ 7/32 Neg.	4/ 7/32 Pos.	4/ 7/32	0	0	0	25
8	Male	2/20/32	4/13/32 + 1/500	4/15/32 Pos.	4/15/32	0	2	3	20
9	Male	4/ 3/32	4/21/32 + 1/500	4/21/32 Pos.	4/21/32	0	0	21	4
10	Boy	3/10/32	5/15/32 + 1/500	5/15/32 Pos.	5/15/32	0	4	8	13
11	Female	5/ 1/32	5/16/32 + 1/500	5/16/32 Pos.	5/16/32	0	3	5	17
12	Male	4/10/32	5/16/32 + 1/500	5/16/32 Pos.	5/16/32	0	6	6	13
13	Male	3/20/32	5/16/32 + 1/500	5/16/32 Pos.	5/16/32	1	2	4	18
14	Male	6/ 7/32	6/ 9/32 + 1/500	6/ 9/32 Pos.	6/19/32	3	4	14	4
15	Female	6/10/32	6/24/32 + 1/500	6/24/32 Pos.	6/24/32	0	2	19	4
16	Male	6/ 1/32	6/30/32 + 1/500	6/30/32 Pos.	6/30/32	0	3	22	0
17	Female	2–'30	7/ 7/32 Neg.	7/ 1/32 Pos.	7/ 7/32	3	4	15	3
18	Girl (3 yrs.)	6/30/32	7/11/32 Neg.	7/11/32 Pos.	7/11/32	0	0	0	25
19	Infant (17 mo.)	7/19/32	7/22/32 Neg.	7/22/32 Pos.	7/22/32	0	0	4	21

* Ma.=Marked phagocytosis
Mo.=Moderate phagocytosis
+=Complete agglutination
S.=Slight phagocytosis
N.=No phagocytosis

ture and positive agglutination test, the diagnosis was made on the basis of the results of both the allergic skin test and opsono-cytophagic test. No diagnosis was based on these two tests until sufficient data were accumulated from previous studies to warrant their application to the diagnosis of undulant fever.

The laboratory tests and allergic skin test were used in the diagnosis of the disease within a few days after the onset of symptoms in a few cases, while in others the interval varied from six weeks to approximately two years.

Brucella melitensis was isolated from the blood of cases Nos. 3, 14 and 15. These were laboratory infections. *Brucella abortus* was isolated from the blood of cases Nos. 5, 8, 9, and 13. Blood cultures from the remaining 12 cases were negative.

It may be noted from the data presented in Table I that the opsono-cytophagic power of the blood is low or absent in all cases with the exception of No. 6. This individual had a comparatively mild form of the disease as expressed in terms of clinical symptoms. Experience with this test has shown that the phagocytic power rises and falls during the course of the disease. If the invading organism is very active in the body, the phagocytic activity of the cells is likely to be low. On the other hand, if it is not very active, as indicated by clinical symptoms, the phagocytic activity of the cells will be found higher.

In view of the fact that many individuals, either healthy or ill, will show an allergic skin reaction to Brucella due to previous infection, one might question a diagnosis of undulant fever based on a positive allergic skin test and low or negative opsono-cytophagic test in a patient in the absence of a positive blood culture and negative agglutination test. We have given considerable attention to this question in our studies.

The contention that a diagnosis of undulant fever can be made on the basis of the two tests in question is supported by the data which are to follow concerning the degree of ingestion of the organism on the part of the cells in blood examined shortly after and long after recovery from the disease, as compared to that which takes place in the blood from patients during the course of the disease. Furthermore, it is supported by the fact that those cases in question respond to specific treatment with either Brucellin or Pyronin, or both, when administered simultaneously and the cells in whole citrated blood show marked phagocytosis of Brucella.

Comparisons of the Results of Blood Examinations Made on Patients During and After Recovery from Undulant Fever. The comparative results are illustrated in Table II on eight known cases of undulant fever. The opsono-cytophagic test was made during the disease from two days to two months after the onset. The time of making the test on each case after recovery, varied from two days to approximately one month. The evidence on which recovery from the disease is determined is the complete disappearance of all those clinical symptoms by which it is characterized.

The data clearly show that the opsono-cytophagic power of the blood is low during the disease and becomes very marked after recovery.

Results of the Examination of Blood from Individuals Known to Have Had Undulant Fever. In Table III are illustrated the results of the opsono-cytophagic test and agglutination test on 15 individuals after recovery from undulant fever. These cases are not included in the group in Table II. The tests were made at intervals varying from 36 days to four years after recovery from the disease.

The opsono-cytophagic power of the blood for Brucella is very marked in all cases, regardless of the length of the interval between recovery and the phagocytic examination. It is interesting to note that there is no relation between Brucella agglutination titer of the individual's serum and the ingestion capacity for Brucella of the leucocytes suspended in the serum.

All individuals whose blood shows a marked opsono-cytophagic power for Brucella *in vitro* similar to that in those cases illustrated in Table III, will also show an allergic skin reaction to the same degree as those that are infected with Brucella when tested by a satisfactory Brucella allergin. The blood of the infected, however, will not show

TABLE II

OPSONO-CYTOPHAGIC POWER OF BLOOD OF CASES DURING AND AFTER RECOVERY FROM UNDULANT FEVER

		Activity of Cells During Infection					*Activity of Cells After Recovery*				
			Number					*Number*			
Case No.	*Period of Disease*	*Date*	*Ma.*	*Mo.*	*S.*	*N.**	*Date*	*Ma.*	*Mo.*	*S.*	*N.**
1	11/10/31 to 1/30/32	1/18/32	0	0	5	20	2/ 5/32	25	0	0	0
2	2–'31 to 4/20/32	4/13/32	0	0	4	21	4/23/32	23	0	2	0
3	2/20/32 to 4/26/32	4/15/32	0	2	3	20	5/15/32	23	2	0	0
4	3/20/32 to 5/18/32	5/15/32	0	4	8	13	6/11/32	25	0	0	0
5	4/10/32 to 7/15/32	5/16/32	0	6	6	13	7/23/32	25	0	0	0
6	3/20/32 to 5/26/32	5/16/32	1	2	4	18	6/11/32	19	6	0	0
7	6/ 7/32 to 6/20/32	6/ 9/32	3	4	14	4	7/26/32	25	0	0	0
8	6/10/32 to 7/ 5/32	6/24/32	0	2	19	4	7/ 7/32	25	0	0	0

* Ma.=Marked phagocytosis
Mo.=Moderate phagocytosis
S.=Slight phagocytosis
N.=No phagocytosis

TABLE III

OPSONO-CYTOPHAGIC POWER OF BLOOD OF INDIVIDUALS AFTER RECOVERY FROM UNDULANT FEVER

Case No.	Sex	Period of Disease	Activity of Cells Date	Number Ma.	Mo.	S.	N.*	Agglutination Test Date	Titer
1	Male	1–'30 to 4–'30	2/ 4/31	21	0	0	4	2/14/31	+ 1/25
2	Male	12–'26 to 4–'27	4/ 1/31	25	0	0	0	4/ 1/32	+ 1/25
3	Male	1–'26 to 12–'26	4/17/31	22	1	2	0	4/17/31	—
4	Male	7/ 3/30 to 7/15/30	4/17/31	22	2	0	1	4/17/31	—
5	Male	1–'28 to 4–'28	4/17/31	21	2	1	1	4/17/31	+ 1/50
6	Female	5/ 5/30 to 5/25/30	4/20/31	22	0	3	0	4/20/31	+ 1/25
7	Male	5–'30 to 6–'30	4/29/31	24	0	1	0	4/29/31	+ 1/50
8	Female	5/ 1/32 to 5/31/32	5/31/32	25	0	0	0	7/23/32	+ 1/500
9	Female	3–'31 to 5/25/31	5/31/31	25	0	0	0	5/31/31	+ 1/500
10	Male	12/ 6/29 to 1/15/30	4/17/31	25	0	0	0	4/17/31	+ 1/50
11	Male	4/15/30 to 6/30/30	2/ 8/32	23	2	0	0	2/ 8/32	—
12	Male	2–'29 to 4–'29	3/31/32	25	0	0	0	3/31/32	+ 1/25
13	Male	1–'31 to 3–'31	7/ 7/32	25	0	0	0	7/ 7/32	+ 1/25
14	Male	3–'30 to 5–'30	7/ 7/32	25	0	0	0	7/ 7/32	—
15	Male	9–'30 to 12–'30	7/ 7/32	25	0	0	0	7/ 7/32	P 1/25

* Ma.=Marked phagocytosis
Mo.=Moderate phagocytosis
+=Complete agglutination
S.=Slight phagocytosis
N.=No phagocytosis
P =Incomplete agglutination

marked phagocytic power for Brucella *in vitro*.

Results of the Examination of Specimens of Blood from Bacteriology Laboratory Workers. In Table IV are presented the results of the opsono-cytophagic and agglutination test in three groups of laboratory workers; namely, those who have been working with infective material for several years, those who have never knowingly handled infective materials, and a group before and after working with infective materials. Cases No. 1 and 2 represent the latter named group. The blood of these two individuals showed no phagocytic activity for Brucella before working with infective materials or infected animals. At some period within four months after beginning their laboratory work, both were infected with Brucella, as indicated by the results of the blood examinations. There was no clinical evidence of the disease in either case.

Those that have never knowingly worked with infective materials or infected animals are Nos. 6, 10, 12, 18, 19, and 20. The remainder of the group are known to have worked with cultures of the organism or infected animals. The blood of each of the latter, twelve in all, with the exception of one, shows a very marked phagocytic activity for Brucella.

In Table V are presented data on 17 packing house employees, to support the contention that those who are exposed constantly to Brucella infective materials or to Brucella infected animals will sooner or later become infected. The blood of these men was examined on November 11, 1931, with negative results to both the agglutination and opsono-cytophagic tests. One of the men in this particular group, engaged in the manufacture of sausages, developed clinical undulant fever in June, 1932. An examination of the blood of each, including the one, No. 17, who was showing symptoms typical of the disease on July 22, 1932, revealed valuable information pertaining to Brucella infection and immunity. The results of the agglutination test show that eleven had been exposed to infection; that is, the organism had passed beyond the epithelial barrier of the skin or mucous membranes. The opsono-

TABLE IV

OPSONO-CYTOPHAGIC POWER OF BLOOD OF LABORATORY WORKERS—NO HISTORY OF DISEASE

	Activity of Cells					*Agglutination Test*		
		Number						
Case No.	*Date*	*Ma.*	*Mo.*	*S.*	*N.**	*Date*	*Titer*	*History*
1	11/28/30	0	0	0	25	11/28/30	—	Veterinary student. 12/30
	4/30/31	19	2	4	0	4/30/31	+ 1/25	began testing blood specimens for Bang's disease
2	2/ 2/31	0	0	0	25	2/ 2/31	—	Graduate student. 10/31
	6/ 1/32	21	3	1	0	6/ 1/32	+ 1/50	began study of bovine mastitis
3	4/17/31	17	4	3	1	4/17/31	—	Veterinarian. Works with Bang's disease
4	10/ 7/30	25	0	0	0	10/ 7/30	+1/50	Has been in veterinary practice
5	3/31/32	11	8	6	0	3/31/32	+ 1/25	Dairy bacteriologist
6	2/ 2/31	0	0	0	25	2/ 2/31	—	Bacteriologist — never worked with infective material
7	2/ 4/32	25	0	0	0	2/ 4/32	—	Bacteriologist — worked with infective material 15 years ago
8	4/17/31	22	2	1	0	4/17/31	+ 1/50	Veterinarian. Works with infective material
9	12/31/31	25	0	0	0	12/31/31	—	Worked with infective
	1/14/31	25	0	0	0	1/14/31	—	material and cultures for
	9/10/31	25	0	0	0	9/10/31	—	17 years
	6/15/32	25	0	0	0	6/15/32	—	
10	11/14/30	0	0	0	25	11/14/30	—	Graduate student in bacteriology
11	9/25/30	25	0	0	0	9/25/30	—	Bacteriologist — works
	4/29/31	25	0	0	0	4/29/31	—	with infective material
	11/ 9/31	22	2	1	0	11/ 9/31	—	
	3/31/32	25	0	0	0	3/31/32	—	
12	2/ 2/31	0	0	2	23	2/ 2/31	—	Bacteriologist — never worked with infective material
13	9/25/30	25	0	0	0	9/25/30	+ 1/25	Veterinary bacteriologist.
	2/ 2/31	25	0	0	0	2/ 2/31	—	Works with infected cattle
14	4/17/31	25	0	0	0	4/17/31	—	Bacteriologist — works with infective material
15	4/29/31	0	0	1	24	4/29/31	—	Bacteriologist
16	4/17/31	22	1	2	0	4/17/31	—	Cares for infected experimental animals
17	3/31/32	17	6	2	0	3/31/32	—	Washes glassware containing cultures
18	5/11/32	0	0	0	25	5/11/32	—	Laboratory assistant — never worked with infective material
19	5/11/32	0	0	0	25	5/11/32	—	Laboratory assistant — never worked with infective material
20	5/11/32	0	0	0	25	5/11/32	—	Laboratory assistant — never worked with infective material

* See footnote to Table III.

TABLE V

OPSONO-CYTOPHAGIC POWER OF BLOOD OF A GROUP OF PACKING HOUSE EMPLOYEES BEFORE AND AFTER EXPOSURE TO BRUCELLA

	Examinations 11/12/31					*Examinations 7/28/32*					
		Activity of Cells Number					*Activity of Cells Number*				
Number of Employee	*Aggl. Titer*	*Ma.*	*Mo.*	*S.*	*N.**	*Aggl. Titer*	*Ma.*	*Mo.*	*S.*	*N.**	*Clinical Evidence of Infection*
1	Neg.	All cells neg.				+ 1/25	22	2	1	0	No
2	"	"	"	"		+ 1/500	4	4	17	0	Yes
3	"	"	"	"		Neg.	23	2	0	0	No
4	"	"	"	"		+ 1/100	18	7	0	0	No
5	"	"	"	"		Neg.	23	2	0	0	No
6	"	"	"	"		Neg.	0	0	0	25	No.
7	"	"	"	"		Neg.	0	0	6	19	No
8	"	"	"	"		+ 1/500	6	11	7	1	Yes
9	"	"	"	"		+ 1/500	0	3	8	14	Yes
10	"	"	"	"		+ 1/50	24	1	0	0	No
11	"	"	"	"		+ 1/500	2	11	9	3	Yes
12	"	"	"	"		+ 1/500	None made				Yes
13	"	"	"	"		Neg.	25	0	0	0	No
14	"	"	"	"		+ 1/50	22	3	0	0	No
15	"	"	"	"		Neg.	0	0	2	23	No
16	"	"	"	"		+ 1/500	None made				No
17	"	"	"	"		+ 1/500	0	16	9	0	Yes

* Ma.=Marked phagocytosis
Mo.=Moderate phagocytosis
S.=Slight phagocytosis
N.=No phagocytosis

cytophagic test was conducted on all except two. The results of this test showed that all those reacting to the agglutination test and two others that were negative had been exposed to infection. The blood of those, Nos. 2, 8, 9, 11, and 17, which were showing symptoms of the disease, had low phagocytic power for Brucella. The phagocytic power of the cells was either marked or negative in those showing no symptoms of the disease. *Br. suis* was isolated from the blood of No. 11.

The Results of the Examination of Blood Specimens from Large Groups of Individuals. The groups in question were veterinarians, packing house employees, college students enrolled in bacteriology courses, hospital patients and inmates of a State Prison.

The gross results of the opsono-cytophagic test and agglutination test for Brucella are presented in Table VI. Most of the 20 veterinarians examined had been engaged in cattle practice. The opsono-cytophagic power of the blood from 19 was very marked for Brucella. None gave a history of having clinical symptoms characteristic of undulant fever. Many of them, however, report the appearance of an erythema on their arms after removing retained placentas from aborting cows. This sign indicates hypersensitiveness to Brucella. The skin test is always positive in such cases.

Blood specimens were taken from 176 men and women in three packing houses and one stock yard in Michigan. Most of the men were engaged in work which necessitated the handling of fresh pork or beef. The women were employed in wrapping cured bacon for the market. The stock yard employees came in contact with live animals only. The results of the opsono-cytophagic test alone indicate that 40, or 22.7 per cent, of the employees have at some period in the past been infected with Brucella. Of the total examined, three were showing clinical symptoms of

TABLE VI

OPSONO-CYTOPHAGIC POWER OF BLOOD OF GROUPS OF INDIVIDUALS

Group	Number Examined	Date of List	Opsono-Cytophagic Test			Agglutination Test	Estimated Per Cent Exposed to Brucella Infection
			Number Showing				
			All Cells Marked	Few Cells Slight	All Cells Negative		
Veterinarians	20	6/20/31	19	0	1	Titers, varied from negative to + 1/500	95
Packing House Employees	176	11/12/31	40	47	89	Titers, varied from negative to + 1/500	22.7
College Students	29	5/10/31	5	9	15	Titers, negative to incomplete 1/25	17.2
Hospital Patients	240	During 1931–32	30	45	165	Titers, varied from negative to + 1/50	12.0
Men in State Prison	133	7/22/32	14	26	97	Titers, all negative except one + 1/500	10.5

undulant fever at the time the blood specimens were collected.

The 29 college students were in two separate groups, one of which was examined about one year after the other. Of the total number examined, the blood of 5, or 17.2 per cent, showed the degree of phagocytosis observed in those who have at one time been infected with Brucella.

The hospital patients from whom blood specimens were examined constituted a rather miscellaneous group of both males and females. They might be grouped as cases of infectious and organic disease, injuries, and blood donors from whom blood is taken for transfusion. Of the total number examined, the blood of 30, or 12 per cent, showed the degree of phagocytosis observed in those who have at one time been infected with Brucella.

TABLE VII

A PROPOSED SYSTEM FOR THE DIAGNOSIS OF UNDULANT FEVER ACCORDING TO RESULTS OF THE AGGLUTINATION, ALLERGIC AND OPSONO-CYTOPHAGIC TESTS

Agglutination Test	*Allergic Skin Test*	*Opsonophagic Power of Blood*	*Status Toward Brucella*
—	—	Cells negative to 20 per cent slight	Susceptible
—	+	Cells negative to 40 per cent marked	Infected
—	+	Cells 60 to 100 per cent marked	Immune
+	+	Cells 60 to 100 per cent marked	Immune
+	+	Cells negative to 40 per cent marked	Infected

The prison inmates examined were of two groups, namely, kitchen personnel and patients in the tuberculosis hospital. The latter group may be divided into diagnosed cases and suspects. Of those examined, the blood of 14 or 10.5 per cent, showed a marked degree of phagocytosis for Brucella. These men came from all walks of life and should represent a cross section of what one would expect to find from an examination of the blood of the general population. Patients examined in the hospitals and students also represent a cross section of the general population.

DISCUSSION

We present data on an opsonocytophagic study of the whole citrated blood of humans with respect to Brucella infections. A technic for testing the opsono-phagocytic power of the whole blood and a reagent and procedure for making a satisfactory allergic skin test are described. The two tests should be made simultaneously in determining the status of an individual as respects Brucella infection. The data have furnished a basis for measuring susceptibility, infection and immunity to Brucella infection.

The advantage of using whole blood in such studies has been pointed out by Shattock and Dudgeon,[7] Hektoen [8] and many others. The addition of sodium citrate to the blood serves a twofold purpose, namely, to prevent clotting and partially or totally to reduce the opsonic property of the serum in order to ascertain variations in the phagocytic power of the blood.

The basis for the assertion that the measurement *in vitro* of the opsonocytophagic power of whole citrate blood of humans in conjunction with the determination of skin allergy for Brucella, will determine susceptibility and immunity to, as well as infection with Brucella, rests upon the data which we have gathered. Although our data with respect to phagocytic activity of the blood in Brucella infection in man is new, it might be said here that many investigators have collected similar data from the study of other infectious diseases of man and animals. The interpretations which they have given to their results differ somewhat from those placed on our own. For example, a continuous high phagocytic power of the blood for an organism has been interpreted as meaning a "carrier state," by certain workers. Ledingham,[9] Gaehtgens,[10] Hamilton,[11] and others have observed that sera of typhoid carriers invariably show a high opsonic index when compared with the normal individual. Such findings have been considered useful in detecting the typhoid carrier. If the high phagocytic power of the blood seen in those who recover from undulant fever as well as many other individuals who give no history of the clinical manifestations of the disease, is taken as evidence of a carrier state as it is in typhoid, then there is a large group of individuals in this country who are constantly carrying Brucella in some part of their body. The data that we have accumulated on blood specimens from humans do not indicate that a continuous high phagocytosis for Brucella signifies a carrier state.

The observations which we have made over a period of years on the value of the intradermal test for detecting Brucella infection in humans convince us that a positive reaction does not necessarily mean active infection. A positive reaction signifies sensitization to Brucella. If a positive skin reaction is obtained in doubtful cases of undulant fever, the positive reaction does not have specific significance until a determination of the opsono-cytophagic power of the whole blood is made. The latter test should be made at the same time or before the skin test is performed.

In order that the interpretation of the combined agglutination, opsono-cytophagic and allergic tests may be clarified as respects the status of a given individual toward Brucella infection, we are proposing a system of diagnosis according to the combined results of the three tests. The proposed system is arranged in Table VII.

In any study which concerns the phenomenon of phagocytosis there always appear many problems and questions that need solving and answering. We have studied at considerable length the factors involved in bringing about the phagocytosis of Brucella in human blood and blood from other species of animals as well. We have also made some very interesting observations on the opsono-cytophagic power of the cord blood from infants and blood from new born calves before and after the ingestion of colostrum. These studies together with a considerable number of other observations will appear in a forthcoming paper.

SUMMARY

The studies which we have conducted on citrated blood of humans who were known to have had undulant fever in past years and shortly after recovery, who are actively infected, or who have no history of the disease, show that the *in vitro* activity of the polymorphonuclear cells in whole citrate blood for Brucella is an expression of immunity to Brucella and an indication of the progress toward recovery in active infection. The absence of or a low phagocytic activity obtained in conjunction with a negative allergic skin test is evidence of susceptibility to Brucella infection. Infection in an individual is indicated by a positive allergic skin test obtained with Brucella nucleo-protein in conjunction with negative or low opsono-cytophagic activity of the whole citrated blood for Brucella.

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