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Proceedings of

Fourth National ANAPLASMOSIS Conference

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General Background

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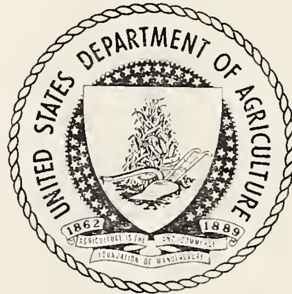

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Program Committee

Glenn C. Holm
Dean of Veterinary Medicine
Oklahoma State University
(Chairman)

E. E. Saulmon
Associate Director
Animal Disease Eradication Branch
USDA
Washington, D. C.

M. N. Riemenschneider
Chairman, Anaplasmosis Section
U. S. Livestock Association
Oklahoma City, Oklahoma

K. L. Kuttler
Chairman, Department of Veterinary Science
University of Nevada

W. E. Brock
Professor, Veterinary Science
Oklahoma State University

W. T. Oglesby
Head, Department of Veterinary Science
Louisiana State University

The Fourth National Anaplasmosis Conference was sponsored jointly by the Animal Disease and Parasite Research Division, USDA; the Animal Disease Eradication Division, USDA; and the various State Agricultural Experiment Stations.

Persons Attending Fourth National Anaplasmosis Conference

Reno, Nevada, April 26 and 27, 1962

Dr. Charles R. Adams
1107 Riverside Drive
Reno, Nevada

D. W. Anthony
USDA, ARS
Beltsville, Maryland

R. J. Avery
P. O. Box 640
Letabridge — Alta
Canada

Dr. A. G. Beagle
1008 Greenwood Drive
Salem, Oregon

Dr. Paul Becton
USDA, ARS, ADE
Little Rock, Arkansas

Mr. David M. Bedell
Box 8192
Baton Rouge 3, Louisiana

Dr. W. L. Bendix
State Veterinarian
Virginia Dept. of Agriculture
Richmond, Virginia.

C. B. Bills
1220 North Street
Sacramento, California

Harold Breen
P. O. Box 960
Laramie, Wyoming

Dr. W. E. Brock
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma

Ross Brown
American Cyanamid Company
Levittown, Pennsylvania

W. G. Bruce
USDA, ADE
Washington 9, D. C.

Edward C. Burns
Louisiana State University
Baton Rouge, Louisiana

Dr. C. L. Campbell
State Veterinarian
Department of Agriculture
Tallahassee, Florida

Dudley T. Campbell
801 East 17th Avenue
Denver 18, Colorado

Dr. R. W. Carter, Director
USDA, ARS, ADE
Clemson College
Columbia, South Carolina

E. F. Chastain
4217 Berrendo Drive
Sacramento, California

Dr. E. S. Cox
7355 East 22nd
Denver, Colorado

Dr. Edmund F. Cushing
P. O. Box 827
Sparks, Nevada

Dr. A. A. Cuthbertson
Box 708
Elko, Nevada

Dr. W. D. Daugherty
1018A
Sparks, Nevada

Dr. George T. Dimopoullos
Department of Veterinary Science
Louisiana State University
Baton Rouge, Louisiana

Dr. R. L. Elsea
103 April Drive
Camp Hill, Pennsylvania

Dr. Carlos Espana
Inst. DE Inv. Pecuarias,
Palo Alto
Mexico City, Mexico

Mrs. Ethel M. Espana
Inst. de Inv. Pecuarias,
Palo Alto
Mexico City, Mexico

Dr. W. F. Fisher
1306 North Sierra Street
Reno, Nevada

Dr. J. G. Flint
1761 Gramsie Road
St. Paul 12, Minnesota

Dr. Lon E. Foote
Department of Veterinary Science
Louisiana State University
Baton Rouge, Louisiana

R. G. Fowler
18 2nd Avenue
San Mateo, California

Dr. T. E. Franklin
School of Veterinary Medicine
Texas A & M College
College Station, Texas

J. H. Gainer
32 Harris Boulevard
Kissimmee, Florida

Dr. D. W. Gates
USDA
Washington, D. C.

C. L. Gooding
3842 Berrendo Drive
Sacramento 25, California

Dr. William W. Green
P. O. Box 301
Winnemucca, Nevada

Dr. O. J. Halverson
1102 Hauser
Helena, Montana

Dr. F. W. Hanson, Jr.
804 East Eubanks
Oklahoma City, Oklahoma

R. H. Hartman
Box 561
Victoria, Texas

Dr. John Healy
Box 4129
Jacksonville, Florida

Dr. Fred C. Heck
School of Veterinary Medicine
Texas A & M College
College Station, Texas

R. A. Hoffman
USDA, ARS
Kerrville, Texas

Dean Glenn C. Holm
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma

Dr. J. A. Howarth
College of Veterinary Medicine
University of California
Davis, California

Dr. D. E. Howell
Department of Entomology
Oklahoma State University
Stillwater, Oklahoma

Dr. Harvey H. Hoyt
University of Minnesota
St. Paul, Minnesota

Dr. O. J. Hummon
5601-42nd Avenue
Hyattsville, Maryland

Dr. David Ibson
State Veterinarian
State House
Little Rock, Arkansas

Dr. M. Inverso
3309 Hawthorne
Boise, Idaho

Dr. E. W. Jones
College of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma

Dr. E. M. Joneschild
1000 Butler
Reno, Nevada

Mr. Olin Kliewer
Box 792
Pawhuska, Oklahoma

Dr. L. M. Koger
1893 Sunset Drive
Ontario, Oregon

Dr. Julius P. Kreier
906 East Michigan
Urbana, Illinois

Dr. Kenneth L. Kuttler
Department of Veterinary Science
University of Nevada
Reno, Nevada

Dr. J. L. Lancaster, Jr.
Department of Entomology
University of Arkansas
Fayetteville, Arkansas

Dr. Paul D. Langham
1515 Holcomb Lane
Reno, Nevada

Dr. G. N. Lukas
Division of Animal Industry
Department of Agriculture
Fresno, California

Dr. R. G. Lynch
Box 11
Minden, Nevada

Dr. E. E. Maas
95 Rancho Manor Drive
Reno, Nevada

Philip A. Madden
2002 Roanoke Street
Hyattsville, Maryland

Dr. Walter H. Martin
1911 Longmead Road
Silver Spring, Maryland

Dr. Nyle J. Matthews
University of Nevada
Reno, Nevada

Dr. B. R. McCrory
Assistant Director, ADP Division
USDA
Beltsville, Maryland

Dr. Harry F. McEwan
Assistant to the Director
Idaho Bureau of Animal Industry
Boise, Idaho

Dr. Norvan Meyer
605 North Mansfield
Alexandria, Virginia

Dr. C. J. Mikel
2338 Adina Drive, Northeast
Atlanta 5, Georgia

Dr. Donald Miller
6710 Jansen Court
Springfield, Virginia

Dr. James G. Miller
Agriculture Experiment Station
University of Georgia
Tifton, Georgia

Ralph W. Mitchell
5536 Marconi
Carmichael, California

Dr. Frank Neville
Box 849
Winnemucca, Nevada

Dr. W. T. Oglesby, Head
Department of Veterinary Science
Louisiana State University
Baton Rouge, Louisiana

Dr. John L. O'Harra
940 Pine Ridge Drive
Reno, Nevada

Dr. Richard E. Omohunoro
2008 N. Kensington Street
Arlington 5, Virginia

Dr. John W. Osebold
College of Veterinary Medicine
University of California
Davis, California

Dr. Albin G. Pass
2027 Lee Drive
Baton Rouge, Louisiana

Dr. Lewis J. Pate
Animal Disease Eradication Division
Jackson, Mississippi

Dr. C. C. Pearson
Box 987
Pawhuska, Oklahoma

Dr. Willet J. Price
11-J Street
McGill, Nevada

Dr. Bill Pritchard
School of Veterinary Medicine
Iowa State University
Ames, Iowa

Dr. Jack R. Pitcher
Box 84
Elko, Nevada

George M. Ramsey
Box 55
Fallen, Nevada

Dr. J. E. Rasmussen
1754 East 3051 South
Salt Lake City, Utah

Dr. Glenn B. Rea
State Veterinarian
245 Sonora Way
Salem, Oregon

Dr. M. N. Riemenschneider
State Veterinarian
Oklahoma City, Oklahoma

Dr. M. Ristic
College of Veterinary Medicine
University of Illinois
Urbana, Illinois

Dr. R. H. Roberts
Belts Branch Experiment Station
Stoneville, Mississippi

Dr. T. O. Roby
USDA, ARS
Beltsville, Maryland

Dr. E. E. Saulmon
USDA, ADE
Washington 25, D. C.

Dr. J. W. Scales, Head
Department of Veterinary Science
Mississippi State University
State College, Mississippi

Dr. S. F. Scheidy
College of Veterinary Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Dr. A. P. Schneider
2025 North 23rd
Boise, Idaho

Dr. C. L. Seger
Department of Veterinary Science
Louisiana State University
Baton Rouge, Louisiana

Dr. Robert S. Sharman
USDA, ADE
Washington 25, D. C.

Dr. Paul Silva
1170 Airport Road
Reno, Nevada

Dr. William L. Sippel
Box 847
Kissimmee, Florida

Dr. Dean H. Smith
Diagnostic Laboratory
Oregon State University
Corvallis, Oregon

Dr. Louis H. Smith
1116 East 23rd
Cheyenne, Wyoming

Dr. W. A. Summers
Medical School
Indiana University
Indianapolis, Indiana

Dr. George M. Thomas
1420 Sanders
Laramie, Wyoming

Dr. W. M. Thompson
State Veterinarian
344 Capitol Building
Phoenix, Arizona

Dr. M. J. Twiehaus
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas

Dr. Vaughn A. Seaton
Iowa State University
Ames, Iowa

Dr. N. B. Tyler
1707 Van Dyke Avenue
Raleigh, North Carolina

Mr. Charles N. Voyles
Public Information
Oklahoma State University
Stillwater, Oklahoma

Dr. C. Joseph Welter
Diamond Laboratories
Des Moines, Iowa

Dr. F. H. White
University of Florida
Gainesville, Florida

Dr. John W. Whiteley
State Veterinarian
Department of Agriculture
Salt Lake City, Utah

Dr. J. L. Wilbur
10303 Walnut Bend Drive
Austin, Texas

Dr. H. G. Wixom
1220 North Street
Sacramento, California

Dr. W. W. Worcester
Route 2, Box 2022
Elk Grove, California

Dr. M. R. Woulfe
3734 Felton Street South
Salem, Oregon

Dr. F. E. Ziegenbein
State Veterinarian
State Capitol Building
Lincoln, Nebraska

Dr. James A. Zimmerman, Jr.
504 East "V" Street
Tumwater, Washington

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Proceedings of

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General Background

Financial Losses From Anaplasmosis

W. T. Oglesby

Identical queries concerning financial losses from anaplasmosis were sent to three persons in each of the fifty states and Puerto Rico. The three persons were: the State Veterinarian, the Federal Veterinarian in Charge and the Dean of the Veterinary School where a school exists, or to the Department of Veterinary Science or Animal Pathology where there is not a degree granting college (in a couple of instances, both). In a number of cases one person answered for all. Where there were multiple answers they were generally in pretty close agreement though in a few instances all of the reporters did not peek through the same telescope.

What is anaplasmosis costing the cattle producers of the United States? Two points should be mentioned in connection with this question. First, the charge to anaplasmosis, which is very substantial, is not spread over the entire country. A few states bear the brunt of the cost—and to them it is a *very major* problem. You can well bet they didn't ask for this—it is their lot because the ecological conditions which prevail enable the disease to be perpetuated. Second, 18 states indicated that anaplasmosis is of *major* consequence to them. All states did not use the same measuring stick because the problems reported varied from some feed lot cases, a small segment of a state, or to an entire state where the disease is considered enzootic over the entire area. Almost all of the states indicated that there is no uniform occurrence and referred specifically to the fact that after a bad outbreak the number of clinical cases in years immediately following was usually reduced.

Two comments impressed me because of the regularity with which they appeared. States where the disease was *not* reported to be a *major* problem, but where breaks occur now and then, almost without exception stated that the disease had been imported to them, usually with feeder animals, resulting in cases breaking out in the feed lots. Several states which indicated anaplasmosis as a *major* problem stated that the occurrence of clinical cases had been about the same for the past 3 or 4 years, while some stated specifically that there were fewer clinical cases. This trend in reduction in rate of clinical cases was attributed by the reporters to the heavy exposure of calves—pasture premunization, if you please.

Few who answered had any clear cut idea about the morbidity rate and costs chargeable to the animals which weather an attack. This statement is not a criticism, just a fact. Morbidity refers to the percentage of the population which suffers from the disease. We are advised that in some years in some places there might be one animal die out of every 20 recognized sick and on other occasions in the same area, only one out of every 4 which is recognized sick dies. The number which die is deducted from the number originally sick and the morbidity charges (loss of weight, loss in milk, extra feed costs, veterinary service, drugs, etc.) are generally attached to those which live. This isn't the whole story, however, as there are bound to be charges (treatment, care, etc.) beyond the value of the animal which *must* be assessed against the animal which died. Generally speaking, those who attempted to answer the question concerning morbidity costs felt that this charge was 2 to 4 times whatever figure was put on the death loss that particular season.

Some referred to the *rate of infection* based on serologic tests. Some stated they intend to do some testing *"to find the incidence of anaplasmosis"* in their state or in a given area of their state. These statements seem to pose a question because positive

serology cannot be equated with the clinical syndrome. The word anaplasmosis, like tuberculosis and brucellosis, refers to the disease process. *What* is the interpretation of the premunized calves? Do they have the disease anaplasmosis? Or, what about Brucella calfhood vaccinates? Do they have the disease brucellosis? In both cases these young animals react and in neither case are they sick. Admittedly, there is one distinct difference between the two diseases, namely, the fact that the anaplasmosis premunized calf is a carrier of the infective agent.

Granting the complement-fixation test to be fairly reliable in detecting the carrier status of an animal *does not suggest* it to be of value in determining morbidity, i.e., (*sickness*) rate. We repeat, the term morbidity refers to sickness (the disease anaplasmosis) and calves exposed in the field generally do not become clinically sick, many become immune from the initial exposure or from repeated exposures. Admittedly, they are carriers, but if they do not become sick, *can* they be charged to morbidity rate? I am not belaboring the point, however, I am definitely suggesting that all of us *should be very careful of how we interpret and use the test.* Let us not abuse it.

All of us realize the difficulty in attempting a survey when a condition is not acute. Responses are poor and we learn little. In 1958 we had the most severe outbreak of clinical anaplasmosis ever experienced in our state. We surveyed the veterinary practitioners and the county agents. Since all parishes (counties) in Louisiana do not have resident veterinary service and since many practitioners work in as many as 3 to 5 parishes, it was very difficult to get a picture from the veterinarians even though the responses to our inquiries were good. On the other hand, the parish agents know the number of cattle in their parishes. They know the type of cattle and they work closely with livestock owners and veterinarians.

Sixty-two of the 64 parishes answered our queries and they even estimated costs on the basis of the types of animals in their areas. As an example, one parish which did not have an extremely high death loss in numbers did have a high cost because animals lost were bulls and imported dairy cows. Another parish had a high death loss, but in what might be called stocker cattle which had an entirely different dollar sign on the animals. The next year the disease was not nearly so bad and the responses which we got to our inquiry were not nearly as good. Please do not misunderstand me, I would not wish anyone an experience like ours for the sake of statistics.

None of the factors mentioned are new to those (veterinarians or livestock owners) who have been close to this complex disease. These points and others are just part of the complex and must be considered in any control program, and in planning and projecting research.

From the standpoint of cost then, what did I learn?

1) Little has been done the country over in an effort to get a true picture of the incidence, though most states having the problem know where it exists and know much about its behavior.

2) Some 15 or 16 states gave enough information, in addition to indicating the disease of importance, to suggest that they are watching it closely.

3) Nine states carry the brunt of the cost of this disease. The attack rate in these states (measured by clinical disease) varies from state to state and year to year.

4) Losses in 1958 were most extensive—in area, volume, and cost—of any year reported. This is likely the year of heaviest losses ever recorded. Death losses that year were on the order of 50,000 animals and the monetary value of these animals something over $12,000,000. Taking the morbidity charge at 3 times *death loss cost* (rather than 2 or 4 as referred to previously) we have a figure of $36,000,000. Thus, in 1958 anaplasmosis cost our livestock producers $48-$50,000,000.

5) Information is not nearly so complete for years before or after 1958, but estimates presented for 1959, 60 and 61 suggest losses were one-half to two-thirds of what they were in 1958.

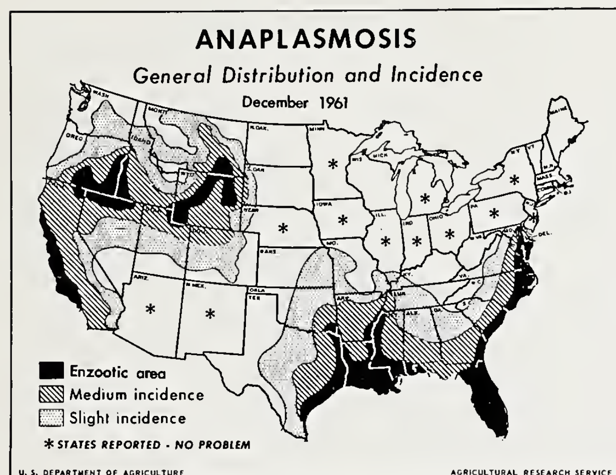
Finally, it would appear that we are well in the range of reality in stating that anaplasmosis, in all its complexity, (*and this is important*) is costing the cattle owners of our country at least $34 to $35 million every year.

Changes In The Anaplasmosis Map

E. E. Saulmon

A limited amount of complement-fixation testing for anaplasmosis had been accomplished throughout the country when the map depicting the general distribution and incidence of the disease was developed for the Third National Conference on Anaplasmosis, held in 1957.

It was necessary to rely on the disease reporting systems of the different states, the knowledge gained over the years by workers with anaplasmosis, discussions with regulatory officials and cattle owners, and the results of the C/F testing that had been done. It was recognized that the distribution and incidence



reflected in specific areas could be debated by individuals who were intimately acquainted with the anaplasmosis situation in that area. It has been extremely encouraging to have discussions with research and regulatory workers throughout the country and be informed of their agreement with the information reflected on the map, as they are acquainted with it in their state or area.

There have been some minor changes indicated by surveys, utilizing the C/F test. Dr. Kenneth Kuttler, University of Nevada, performed a comprehensive survey of cattle from each county in Nevada, which indicated agreement with the endemic and medium incidence areas but did indicate that the slight incidence area should be extended southward. The extension of this line was verified by testing performed in Utah.

C/F testing performed in Texas indicated that the slight incidence area should be extended farther westward than was shown on the original map in that state.

You will recall we stated at the meeting in Manhattan, Kansas, that the terms *slight* and *medium* incidence are relative and difficult to reflect. If sufficient anaplasmosis testing could be accomplished in the Dakotas and Nebraska, I am inclined to expect that the slight incidence line, at least, would be extended farther into the states from the west. Sufficient testing has not been performed for us to be in a position to make an accurate determination as to the true incidence in other areas. From the limited information available and the limited testing that has been done, it certainly would appear that the shading on the map to indicate slight incidence should be extended farther up the Mississippi River valley to near the Iowa state line. Minor modifications such as this may be indicated in other areas at a later time.

The primary change indicated since the last conference is the starring of New York State. Soon after the last meeting, anaplasmosis was diagnosed in New York State for the first time as far as our records are concerned. Since then, additional infection has been disclosed, but of a minor nature and usually associated with recent movements from an enzootic area.

As mentioned earlier, it has been extremely encouraging to have individuals voice agreement with the distribution and incidence of anaplasmosis in the United States, as indicated on the map that was developed. What has been even more encouraging is the correlation of the information depicted on the map and the information that this has been garnered from the many, many thousand C/F tests that have been performed and the results made known to us during the past five years. This, too, has indicated that the map is still extremely accurate in the form first published.

As additional reliable information is gained, the map will be corrected to reflect that information.

Bovine Anaplasmosis
The Disease, Its Clinical Diagnosis and Prognosis

E. Wynn Jones and Ben B. Norman

Introduction—The clinical findings of anaplasmosis are related to variations in susceptibility due to age, the communicable nature of the disease, the occurrence of fever, anemia and complications of the anemia. The cardinal features of anaplasmosis are said to be anemia, weakness, fever, normal urine, and constipation (1, 4). These are usually accompanied by icterus, inappetence, depression, dehydration, labored respiration and irrational behavior (1, 4). The clinical syndrome of anaplasmosis is discussed in relation to clinical-pathologic findings in the experimentally induced disease.

Age susceptibility—Animals of all ages are susceptible to anaplasmosis. The severity of the resulting disease is directly related to age. The syndrome in animals less than 1-year old is usually mild, often inapparent, in yearlings and 2-year olds of moderate intensity and is severe, frequently fatal, in older cattle

(1, 2, 5). Fatalities are more frequent in aged animals (2). The nature of the resistance of the young animal is unknown, although it can be overcome by splenectomy.

Other factors modifying susceptibility are undetermined. A less acute syndrome is reported in cattle native to endemic areas (4) and fatalities are said to be more common in fat animals (2).

Transmissibility—Since transmission occurs by biting flies and ticks, anaplasmosis occurs more frequently in the summer (1, 5). Seasonal incidence is less apparent in southern regions. Accidental transmission usually accounts for disease episodes out of season.

Fever—Although it is usually overlooked, pyrexia is the first sign of anaplasmosis. Yearlings infected with 1 ml. of carrier blood developed a fever 17-45 days (average 31 days) after infection (6). The relation between the onset of fever and the appearance of marginal bodies in the erythrocytes was not reported. The febrile period varied from 2-18 days (6). The temperature usually remained elevated throughout this period. Marginal bodies were usually present in the red cells when pyrexia occurred. In mature Jersey cows the febrile period ranged from 1-5 days (average 2.4) (6). Subnormal temperatures were recorded before death in 11 of 21 mature cows (6). A subnormal temperature was observed in only 1 of 11 comparable animals which recovered. The temperature was commonly greater than 105° F in young animals but rarely exceeded this in mature cattle (6). Temperatures did not exceed 104° F in fatal cases (6).

In 15 two-year-old Herefords infected with 5 ml. of carrier blood we recorded temperatures as high as 107° F. Pyrexia occurred prior to infection of 1 percent of the red blood cells and was still apparent during early convalescence. In a second group of 12 heifers temperatures were never higher than 104.5° F, although they were likewise elevated prior to infection of 1 percent of the erythrocytes. When red blood cells are destroyed rapidly, pyrexia frequently occurs. This is attributed to result from excessive breakdown products (7). Since in anaplasmosis the fever exists prior to severe erythrocyte loss, it is apparently caused by other factors.

Anemia—The onset of symptoms is determined by the degree and rate of red cell loss and therefore the oxygen carrying capacity of the blood. The red cell count per cu. m.m. does not necessarily determine the severity of the symptoms. If anemia occurs slowly, the body is able to adjust to the reduced oxygen in the blood and can therefore tolerate greater red cell loss without obvious embarrassment (7).

Anemia is indicated by pallor of the skin, nose, mucous membranes of the eye and vulva of the female, by signs of physiologic adjustment of the circulatory and respiratory systems and by weakness and depression (7).

Correlation of symptoms with the degree of anemia was attempted in 15 two-year-old Hereford cattle. The animals were restrained for examination in a chute. Daily clinical observations of visible membranes, the circulatory and respiratory systems, the rumen motility, the feces and the general attitude were made. Color photographs of the muzzle and conjunctiva were made every other day. Consistent recognition of pallor was not possible until 40-50 percent of the red cell mass was depleted (figure 1). Examination of the conjunctiva provided the most reliable indication of pallor. Color changes of the vulval mucosa were often obscured by the cyclic variations of estrus. A pale muzzle was only a reliable guide in severe anemia. Dryness of the muzzle was not a common finding.

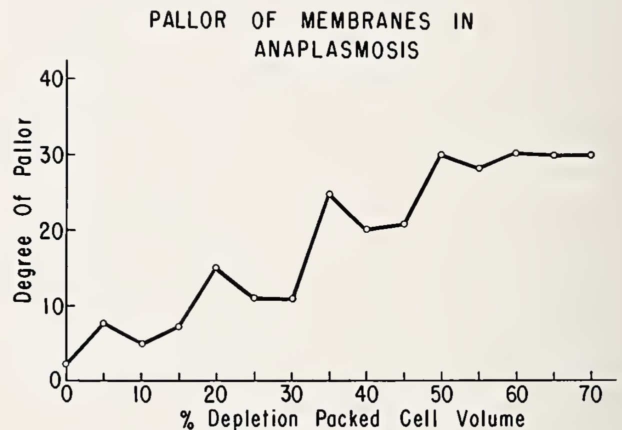


Figure 1. Pallor of the membranes in Anaplasmosis—The pallor was evaluated by observation of the conjunctiva, the muzzle, and the vulva. The degree was rated from 0 to 40. The graph depicts the mean values of 15 animals.

Physiologic adjustments of the circulatory and respiratory systems coincident with anemia include an increased cardiac rate and output, reduced circulation time and increased pulmonary ventilation (7). The heart was accelerated when as few as 20-25 percent of the erythrocytes were lost (figure 2). Marked acceleration did not occur until more than 50 percent of the red cells had been destroyed. Cardiac auscultation indicated an increased incidence of systolic murmurs and greater intensity of the heart sounds. Apart from increased fullness, no obvious changes were observed in the character of the pulse. Respirations were accelerated and the intensity of bronchial sounds was increased. These changes were detected when 25-30 percent of the red cells were lost and were maximal

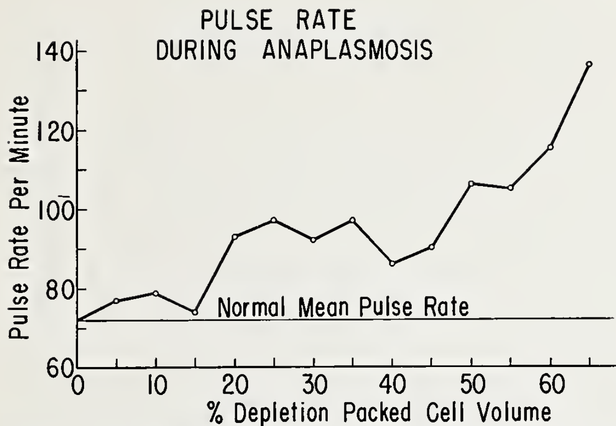


Figure 2. Pulse rate during Anaplasmosis—The graph represents mean values from 15 animals during the patent phase of the disease.

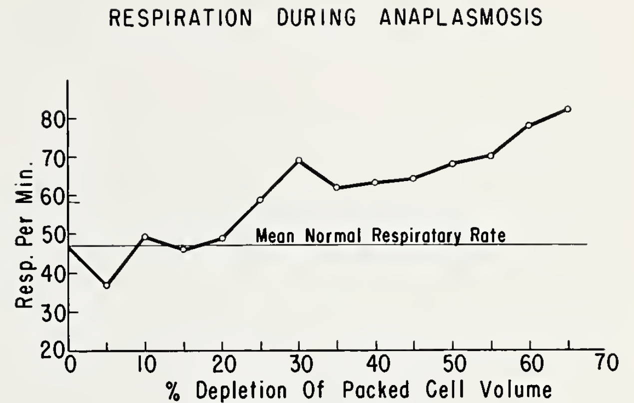


Figure 3. Respiration during Anaplasmosis—The graph represents mean values from 15 animals during the patent phase of the disease.

when the anemia was the most severe (figure 3). In the lot or pasture, diseased animals were most easily detected by observation of the respirations.

Other symptoms associated with the fever and anemia were supressed rumen motility and appetite, and weakness. The latter was only apparent when anemia was severe and the red blood cell count approached 1 million per cu. m.m. Dehydration, when observed, is probably secondary to weakness and reduced water intake. Muscle tremors are frequently observed in weak animals. Although constipation is reported to be common in anaplasmosis, (4) it was not seen in the animals studied. During the initial fever the stool was loose; subsequently it was formed and often mucus covered. Blood was not apparent. Orange-yellow tinged feces were seen in some cases during the severe phase of the anemia. This was believed to reflect increased excretion of fecal urobilinogen. Hemoglobinuria is not observed in anplasmosis, (1, 4, 5) a fact which probably reflects the absence of intravascular hemolysis and elevation of plasma hemoglobin values. It is not surprising that weight loss also occurs in anaplasmosis. A maximum loss of 7.5 percent was observed 10-12 days after anemia was most severe. As in all serious infections, animals in advanced pregnancy frequently abort during anaplasmosis. Male bovines and non-pregnant females may exhibit signs of suppressed function of the gonads.

Complications of the anemia—If physiologic adjustments of the circulatory and respiratory systems fail to provide adequate oxygenation of the tissues or if an additional stress is imposed upon the heart (exercise, obesity and treatment for example) cardiac decompensation and ultimate failure are probable. Myocardial anoxemia due to defective oxygen transport predisposes the heat to failure (7). The onset of cardiac failure is indicated by weakness, muscle tremors, an accelerated irregular heart, a weak pulse, a subnormal temperature and pulmonary rales.

Anoxia consequent to severe anemia causes liver damage, defective elimination of bile pigments and therefore icterus. Serial needle biopsies of the liver during anaplasmosis indicate that liver damage is not detected until anemia is severe, (red cell count < 2 million per cu. m.m.) (3), (figure 4). Icterus is therefore a late symptom, is usually only observed in the convalescent animal and probably reflects concomitant myocardial anoxemia. It is important to appreciate that although such animals are convalescent, they are most susceptible to cardiac failure.

Cases of anaplasmosis which are severely anemic are often restless or even vicious. The cause of this irrational behavior is undetermined but probably results from cerebral anoxia. Nervous irritability is reported to occur during anemia of the human (7).

Diagnosis—This is aided by a history indicating an age and seasonal incidence and the existence of disease in more than one animal. Differential diagnosis requires consideration of other causes of acute anemia in cattle. These include leptospirosis, bacillary and idiopathic hemoglobinuria, and poisoning due to rape, certain crucifera and bracken. Although bovine babesiasis does not occur in this country, it should not be ignored. The absence of hemoglobinuria or hematuria in anaplasmosis should exclude these conditions. In any case hematologic confirmation is essential for prognosis and for control. Convalescent cases of anaplasmosis with icterus, anemia, leucocytosis reflecting bone marrow hyperplasia and but few infected erythrocytes, can be mistaken for leptospirosis. Unlike anaplasmosis the leucocytic response occurs early in the patent phase of leptospirosis. Serologic tests should differentiate these syndromes when doubt exists.

Prognosis—Prognosis is modified by age, the degree of cardiac embarrassment, the stage at which disease is detected, and the bone marrow response.

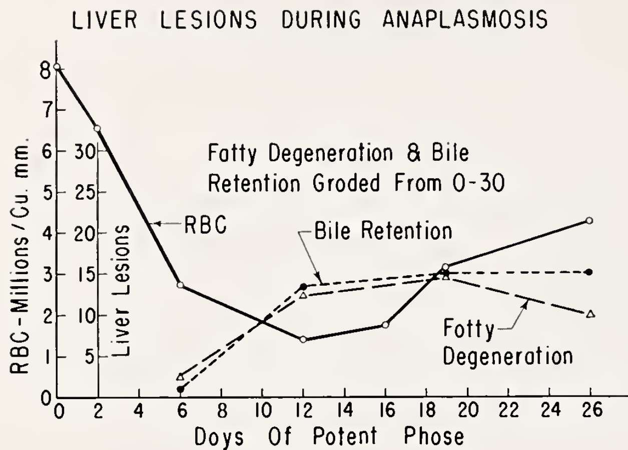


Figure 4. Liver lesions during Anaplasmosis—The graph represents mean values obtained from 4 animals.

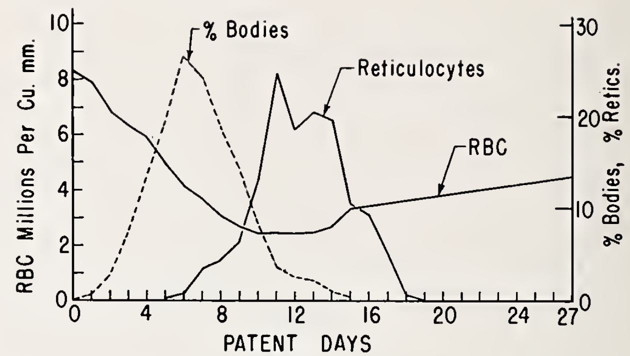


Figure 5. Graph illustrates the relationship of red cell loss, percent red cells infected with anaplasma bodies, and the bone marrow response. Data comprises mean values from 5 animals.

The significance of age has been discussed. The degree of cardiac embarrassment depends upon the extent of physiologic adjustment required and upon other burdens imposed upon the heart. Severe cases of anemia are characterized by red cell counts below 2 million per cu. m.m. and frequently by icterus. It is essential to realize that an icteric animal is usually convalescent and that treatment should therefore be conservative.

Treatment is more effective and less hazardous when performed early prior to the onset of complications due to the anemia and while numerous infected red cells can still be seen in the circulation (figure 5). The chance of survival of cases detected and treated early is much greater than those treated during the peak of the anemia.

The ability of the bovine to survive the anemia depends upon the rate and degree of red cell loss in relation to the response of the bone marrow. Since the red cell loss is closely reflected by the number of infected cells, animals with a high percent of infected erythrocytes will become very anemic unless treated. Rapid blood loss without evidence of bone marrow response (presence of reticulocytes, increased mean corpuscular volume, polychromasia, and leucocytosis) is invariably fatal (3). The appearance of reticulocytes in the peripheral circulation usually indicates a convalescent animal despite the fact that the red cell count may continue to decline (figure 5) (2).

Conclusion—Pyrexia provides initial clinical evidence of the disease. Anemia is not apparent until several days later. Recognition of anemia prior to the loss of 40-50 percent of the red cell mass requires careful clinical observation. Detection of pallor is unreliable except in advanced cases. Accelerated respirations, especially after exertion, appear to provide more reliable evidence of the early phases of anaplasmosis. Severe cases are recognized by weakness, cardiac embarrassment and icterus. The latter sign does not usually occur until early convalescence.

The history and fever, and the anemia without hemoglobinuria make a tentative diagnosis simple. Hematologic and if necessary serologic confirmation is essential for a positive diagnosis. Since prognosis is more favorable when cases are treated as soon as possible, early diagnosis is essential. It is very probable that many mild cases of anaplasmosis go unrecognized.

Prognosis is grave in mature and especially old cattle, when the red cell count is less than 2 million per cu. m.m., and when weakness is observed. Recovery is heralded by an active bone marrow response and return of appetence. Icterus reflects the early convalescence of a severely anemic animal.

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References

1. Blood, D. C. and Henderson, J. A.: Text Book of Veterinary Medicine, (1960): 682 - 5. Williams & Wilkins Co., Baltimore, Maryland.

2. Brock, W. E.: Personal communication.

3. Brock, W. E.: The Pathogenesis of the Anemia of Bovine Anaplasmosis, Ph.D. Thesis, Oklahoma University, (1958).

4. Carricaburu, J. B.: The Clinical Diagnosis of Anaplasmosis, 3rd National Research Conference on Anaplasmosis in Cattle, (1957): 19 - 22.

5. Ristic, M.: Anaplasmosis. Advances in Veterinary Science. 6 (1960): 112 - 192.

6. Schmidt, H.: Manifestations and Diagnosis of Anaplasmosis. Ann. N. Y. Acad. Sci. *64*, (1956): 27 - 30.

7. Wintrobe, M. M.: Clinical Hematology, 3rd Edition, (1951): 358 - 363.

Anaplasmosis–How I Handle It In My Practice

L. M. Koger

The title might be interpreted to imply the veterinarians of the Snake River Valley presume to a disease control program that is effective in the control of anaplasmosis. Quite the contrary is the case. As a matter of fact, the best we can hope for at the present are modifications of ranch management that permit us to live with the disease. Malheur County, Oregon and adjacent southwest Idaho is an enzootic area. Dr. A. G. Moore of Ontario, Oregon first obtained laboratory confirmation of anaplasmosis about 1920, when heavy losses were occurring. I have been unable to determine when anaplasmosis was first introduced in the Snake River Valley area but apparently losses have occurred with variation since 1920.

It is the consensus of local opinion that the best hope of satisfactory control of anaplasmosis outbreaks lies in effective immunizing techniques.

Although rumor has it that premunization from known carriers is practiced surreptitiously in some localities, for many reasons I cannot subscribe to the idea, for the present at least.

In our area the majority of clinical cases fall in a rather well defined pattern paralleling the presence of the tick (Dermacentor andersonii). The disease does not occur naturally in cattle raised on irrigated pastures where *D. andersonii* is absent. This fact suggests tick control as a means of controlling anaplasmosis. However, since recovery rates are better in younger animals than in older animals, it appears desirable that yearlings and two-year-olds contact the disease at a season of the year when feed, water, and weather is favorable. A somewhat confusing anemic syndrome encountered occasionally in cattle on irrigated pastures may be caused by Hemobartonella, but generally this condition is clinically distinct. A survey conducted in 1954 and 1955, of range cows on sagebrush pastures in Malheur County, revealed an incidence from 0 to 99 percent with an average of about 60 percent reaction to the complement-fixation test for anaplasmosis.

Clinical cases among the mature native cattle are infrequent and are treated symptomatically. The use of tranquilizers in moderate dosage permits safer restraint for treatment, and I believe the consequent reduction of stress improves the animal's chance of recovery. Some veterinarians contend that blood transfusion is inconsequential therapeutically and often it is true that the subject dies despite the transfusion or even during the operation. It is my opinion that a blood transfusion given properly and in adequate amounts may tip the balance toward survival. So, if feasible, a large blood transfusion (½ - 2 gallons) is given followed by quinolene diphosphate Aralen, in a dextrose solution and oxytetracycline at the rate of 3 mg. per pound of body weight. Also an arsenical hematinic, disodium salt of acetarsonic acid (Aricyl) is administered. We have also injected an iron and B vitamin compound which apparently stimulated hematopoiesis. Proper nutrition and comfort are very important considerations for recovery, and often are about all we can hope to accomplish.

More numerous but less severe are the cases among the 2- and 3-year-old heifers. Considering the gravity of the anemia, the recuperative capacity of these animals is remarkable. Generally a veterinarian is not consulted for these cases, as most cattlemen expect recoveries if feed and water are adequate. The prevention of transfer of the disease by dehorning or similar surgical procedures is a positive recommendation. The validity of these prevention measures are occasionally proven by an outbreak where such sanitation was disregarded. We preach sanitation at every opportunity.

Greater financial loss per head among imported fitted breeding bulls is an unfortunate feature of the disease. One of my clients says he buys two bulls for each one he needs—"a bull to use and a bull to lose" —from anaplasmosis. Consequently this man buys his bulls for as low a figure as possible. As a result the local breeders meet a buyer's resistance which keeps top prices comparatively lower throughout the area of anaplasmosis losses. This loss prompts the cattlemen to urgently request a practical prophylaxis, inasmuch as the feeding of antibiotics under range conditions is not feasible, nor is the eradication of the tick vector in sight. To illustrate the problem, a client put 300 mature replacement cows, purchased from an adjoining county, in an infested pasture in the spring of 1961. The death loss was an even 30 or 10 percent. He suffered an even greater loss from abortions, loss of condition in recovered cows, orphaned calves, and lowered weaning weight. On April 1, 1962, he turned 200 two-year-old heifers in the same pasture. As of April 21 no clinical cases had been observed. The heifers were purchased from an infested ranch and the client hopes for results similar to what he could expect from heifers of his own raising. Needless to say, this man would be a prime customer for a vaccine if one were available.

But to return to the 30 sick cows, I must report a discouraging picture. When a cow was sick enough to be diagnosed, she was unable to survive the stress of being driven to a corral or restrained in any manner. To rope such a cow was to kill her in most cases, because they were easily agitated. Marginal cases, undisturbed, apparently usually recovered uneventfully, but had they been stressed by handling might have collapsed and died. This frustrating prospect deters

a practitioner from attempting therapy except under ideal conditions.

Occasionally under favorable conditions a single case will appear in mature cows which we would have otherwise considered resistant to clinical symptoms. No apparent reason for stress or lowered resistance was evident. These are usually during the feeding season, and if practicable, the foregoing treatments are used. In addition, an alternative mixture containing arsenic trioxide, organic iodine, sulfur and willow bark is given in feed for its hematinic effect, which is surprisingly effective. An attempt is made to provide an abundant mineral supplement, and an optimum diet during convalescence.

Concrete advice for prevention of anaplasmosis may be summarized in two classifications which are generally accepted by our cattlemen as valid, but often violated when opportunities seem to outweigh caution.

1—Do not add imported mature cattle from non-infected herds to herds where anaplasmosis is known to exist.

2—Wherever possible, introduce additions to the infected herd as immature cattle and permit them to acquire anaplasmosis at an early age and at a season when they will have the most favorable circumstances for survival.

To reiterate, few of our clients would take issue with the foregoing conclusions, but they often disregard the advice.

Report on Incidence of Anaplasmosis in Minnesota

Harvey H. Hoyt

Anaplasmosis has been recognized in Minnesota since November 1949. A number of cases have been reported and investigated since that time. The histories on all cases except one, in 1953 in a native dairy herd, indicated importation from one of several western states. The native dairy herd was pastured adjacent to western feeder cattle that were overlooked on the original investigation. The method of transmission has been associated with bleeding for Brucellosis tests, dehorning and apparent insect vectors. Cases have been relatively sporadic but a continuing problem since 1949. In 1959, 1960 and 1961 the disease has been of greater significance as a result of increased numbers of infected herds and larger numbers of cattle being involved. The increased incidence stimulated added concern and a survey of beef herds in one county (Clay) in the northwestern part of the state was undertaken by A.R.S. and the Minnesota Livestock Sanitary Board in connection with an area Brucellosis test in the fall of 1961.

The following tabulation summarized the cases of anaplasmosis brought to the attention of the Livestock Sanitary Board in Minnesota since 1949.

Most of the cases were investigated by members of the staff of the Veterinary Clinic, College of Veterinary Medicine, University of Minnesota. The diagnoses were all confirmed by the demonstration of anaplasma in smears, inoculation of splenectomized calves or positive complement-fixation tests run in the A.R.S. laboratories in Washington, D. C., or Beltsville. In many instances all three criteria were employed.

Results of the Clay County survey recently reported by the Minnesota Livestock Sanitary Board and A.R.S. follow:

217 herds negative	3619 neg. cattle	
18 herds (suspect)	351 neg. cattle	(23 suspects)
5 herds (reactor)	230 neg. cattle	(5 suspects 5 reactors)

Date of Case	Affected Herd	Origin	% Mortality	Assumed Method of Transmission
Nov. 49	400 cows & steers*	Western	6	Brucellosis test
June 53	49 cows*	Oklahoma	4	Dehorning
Aug. 53	21 cows**	Native	4	Pasture exposure (insect)
Sept. 53	18 cows*	So. Dakota	6	Pasture exposure (insect)
Sept. 55	32 cows*	So. St. Paul	30	Pasture exposure (insect)
Apr. 59	28 cows*	Wyoming	10	Brucellosis test
May 59	48 cows*	Nebraska	6	Brucellosis test
May 59	18 cows*	Nebraska	--	Brucellosis test
Dec. 59	1 bull*	So. Dakota	--	No history
Dec. 60	43 steers*	-- --	2	25 Dehorned
Sept. 61	79 cows**	-- --	--	No history
Oct. 61	270 cows†	Western & Native	35	Pasture exposure (insect)
Nov. 61	50 cows*	-- --	--	No history
Jan. 62	88 cows††	Wyoming	2	Brucellosis test Dehorning

*Hereford
**Holstein
†Mixed breed
††Shorthorn

All herds found to be infected in Minnesota have been quarantined to avoid spread of infection. In most instances the entire herd has been slaughtered after confirmation of the diagnosis. Feedlot animals have been fed out and slaughtered preceding the insect season. In one case, involving one imported bull, no herd spread was shown on CF and only the bull was slaughtered. The one dairy herd was tested every 60 days until all reactors were detected and the balance of the herd negative to inoculation of splenectomized calves, using composite blood from ten cows for each calf.

The survey results indicate endemic infection in one county with fairly low cattle population of predominantly beef breeds. This confirms past suspicion that although the incidence is low in Minnesota there are undetected cases providing a potential for more serious losses in the future. An effort now to detect these carriers and to avoid further introductions deserves serious consideration.

Questions, Answers and Comments

QUESTION—Dr. Wolfe, Salem, Oregon

I would like to ask Dr. Jones if a microscopic examination of blood slides is made on the rising temperature case and if so what proportion of these cases are positive?

ANSWER—Dr. Jones, Stillwater, Oklahoma

The majority of them have bodies present when there is a temperature rise. There may be less than 1 percent of the red cells infected but there are some bodies in the blood. If you're not experienced in looking for them, you may miss them at this stage.

QUESTION—Dr. Tyler, Raleigh, North Carolina

Dr. Koger, upon what did you base your diagnosis of Hemobartonellosis?

ANSWER—Dr. Koger, Ontario, Oregon.

Slides were submitted to Dr. L. R. Vater at Corvallis and then to Dr. Roby. They did not find evidence of anaplasmosis and they believe it to be due to Hemobartonellosis. The clinical symptoms parallel cases described in dogs and are differentiated from anaplasma bodies by lack of icterus. They are quite distinct. It's a little difficult to describe the two. In our instance they occurred in cattle, in the farming areas, in irrigated pastures, in places which we do not expect to find anaplasmosis and in immature animals, yearlings and large calves.

QUESTION—Dr. Silva, Reno, Nevada

Dr. Jones, in regard to bulls infected with anaplasmosis, what is the duration of subsequent infertility?

ANSWER—Dr. Jones, Stillwater, Oklahoma

This is similar to any other cause of testicular degeneration. My experience with cases of this nature and the ability to follow them subsequently is based on two animals in which normal semen did not recur for four to five months. The severity of the degeneration would depend upon the degree of the anemia. We have observed similar effects in leptospirosis.

COMMENT—Dr. Koger, Ontario, Oregon.

In the spring of 1944-45, cattlemen in the area were concerned about bulls which had been affected with yellow fever, to use their term. As I recall there were six or seven bulls. Samples of semen were taken in the spring, the bulls having been infected at least the summer before or the year before that. All of the bulls were fertile.

QUESTION—Dr. Breen, Laramie, Wyoming.

I'd like to ask Dr. Saulmon this question. Dr. Saulmon, in view of the fact that most of this work is based on research or the evaluation of clinical data, do you believe that there is proper justification for specific regulatory action to include anaplasmosis as a specific disease entity for trying to control interstate movement, such has been done by your division. I want to expand that a little further, inasmuch as we have so many different disease entities which we realize go interstate, for which we have no specific diagnostic test and which we therefore do not recognize in compliance with regulations. This has been done with anaplasmosis. Do you believe this is justified when you do not look for the entity and do not diagnose it, you automatically assume that the animals do not have it and you play ostrich insofar as the disease is concerned.

ANSWER—Dr. Saulmon, Washington, D. C.

I won't try to repeat everything Dr. Breen said. In the first place, as I will mention in my talk tomorrow morning, the basic authority under which the Federal regulatory service has operated since 1884 excludes the interstate movement of animals having infectious contagious disease. It's a law. It's not a regulation. We have no choice in the matter. An individual who knowingly moves an animal that has an infectious contagious disease interstate, is in violation of basic law. So, in answer to your question, Dr. Breen, we have no choice.

Probably shouldn't refer to this, I forget what your expression was, but you implied that there are disease animals that move interstate in which no action is taken. We are aware that this happens. That does not mean, if we get the evidence that an individual knowingly moves diseased animals interstate, that we have the authority to ignore it. If they knowingly move diseased animals interstate, and the evi-

dence is available, we have no choice but to wrap the case up.

This too, I will refer to in the morning, but in relation to anaplasmosis, it has been recognized since the diagnostic test has been developed that it would be desirable to have authority to move those reactor animals, with proper controls, across state lines. This has been requested and an Act of Congress, a law, has been introduced in this session. Action has not, as yet, been taken on it. We would hope that we can have it. The sooner the better, because we think it will give moderate relief to the owner who has reactor animals in his herd, and it will give him a chance to find a market for these animals.

QUESTION—Mr. Dudley Campbell, American National Cattlemen's Association

There is some difference in opinion as to whether an animal once recuperated from disease is immune or a low level carrier. Would this mean if regulations were drafted animals once affected would be subject to regulations governing interstate movement of cattle?

ANSWER—Dr. Saulmon, Washington, D. C.

I believe this will be verified by those who are doing research on anaplasmosis. The animal which had an acute case and recovered is a carrier animal and is capable of transmitting the disease if the proper situations prevail. (vectors, mechanical transmission of the blood to susceptible animals) So it is not an immune animal, it is a carrier animal. Therefore, it is a threat to other susceptible cattle. Now, certain animals clear up, I don't know why, spontaneously, but they will recover and are no longer carrier animals. I don't know whether anyone knows the incidence of recovery from the carrier state. With the hope that the antibiotic treatment can be successful, it is possible that a carrier animal can be cleared up. Certainly if that were done, and it could be demonstrated that the animal was no longer a threat to susceptible animals, I can see no reason why it can't be allowed interstate movement at a later date.

Etiology

Recent Research on The Characteristics of The Etiologic Agent of Anaplasmosis

W. E. Brock

Newer and more refined research techniques have led to increasingly productive work on the etiologic agent of anaplasmosis. This work in the recent past has resulted in more detailed information on the structure of the anaplasma body, its minimum infectious size, the oxygen it requires and the ability of its infectious form to survive changes in environment.

Morphology As Shown By Light Microscopic Techniques

The classic descriptions of Anaplasma marginale were made with a light microscope and various blood stains. The organism thus described was a basophilic stained spherical body, 0.2 u. to 1.0 u. in diameter, situated within and near the margin of the erythrocyte. Many other shapes including rod, comma, ring, triangular, rough, smooth and sporoid were described by workers using light microscopy and different methods of preparation. (1, 2)

Recently, Franklin and Redmond (3) have presented evidence of projections from the anaplasma body with Giemsa stained material. Definite tail-like structures projecting from anaplasma bodies toward the interior of infected erythrocytes, or in some cases extending outside the erythrocyte were evident in their pictures.

Other workers in the past 3 years have shown anaplasma with distinct tail-like appendages. By lysing the erythrocytes with saponin and examining the wet preparations with phase contrast microscopy, Espana et al. (4) have demonstrated anaplasma with both ring and tail-like projections from the head. Occasionally, 2 anaplasma appeared to be joined by the tail structures to form a dumbbell shape.

Similar forms have been reported by Pilcher *et al.* (5) in anaplasma infected erythrocytes lysed by freezing and thawing and examined by phase contrast. In addition, Pilcher stated that heads were either single or divided into a number of spherical or elongated segments.

The presence in lysed erythrocytes of the forms described by Espana and Pilcher were confirmed last year by Madden (6), using a flourescent antibody specific for anaplasma. Espana, Pilcher, and Madden each reported the observation of classically shaped anaplasma in addition to the variant forms. Anaplasma showing only the classic morphology were first demonstrated with the anaplasma specific fluorescent antibody technique by Ristic in 1957 (7).

Morphology As Shown By The Electron Microscope

Evidence concerning the morphology of Anaplasma marginale derived from electron microscopy appears to confirm many of the characteristics observed in light microscopy. In the earliest report of anaplasma observed with the electron microscope, De Robertis *et al.* (8) indicated that the anaplasma body may be composed of a homogeneous mass, of a central mass surrounded by elementary bodies 0.17 to 0.22 u. in diameter, or of only the elementary bodies. The elementary bodies also were seen singly, not associated with an anaplasma body. Except for the anaplasma body with elementary particles surrounding a central mass, these descriptions agree quite closely with the smooth anaplasma composed of a single homogeneous mass, the rough form containing 8 "sporoids" and the single "sporoids" as seen by Lotze and Yiengst (2) with light microscopy.

Later workers, Espana in 1957 (9) and Ristic in 1960 (10) using the electron microscope, tended to confirm and extend these observations. Espana (9), in his preliminary report, was unable to show the single elementary body in erythrocytes. Ristic (10) was able to show single initial bodies which he tentatively identified with De Robertis' elementary bodies. He also identified a smaller particle which he named a polyhedral body. Ristic thought the initial bodies were the basic infective unit of anaplasmosis. From 1 to 8 of the initial bodies were found to form an anaplasma body. The polyhedral body appeared to be a subunit of the initial body, but, while seen as single units, their function was not determined. Ristic did not show anaplasma bodies as composed of a single homogeneous mass, only those made up of a number of initial bodies around an undifferentiated mass.

Foote *et al.* in 1958 and 1961 (11, 12) and Ristic in 1960 and 1961 (10, 13) described the anaplasma body as seen in ultra-thin sections by electron microscopy. Essential agreement was found in these reports; in cross section, the anaplasma body was composed of 1 to 8 subunits and enclosed by a membrane. Each subunit was composed of irregular masses of dense material, some containing a central dense mass, and limited by a double membrane. The subunits were identified by Ristic as the initial bodies seen on whole mounts.

From these descriptions and photographs it is possible to find general areas of agreement. The classic anaplasma body is composed of 1 to 8 subunits called

sporoids by Lotze, elementary bodies by De Robertis, subunits by Foote, and initial bodies by Ristic. The subunit may exist as a separate entity not associated with the anaplasma body.

While the above reports of electron microscopic studies of the anaplasma body tended to add submicroscopic detail, they did not change the basic concept of the shape of the classic body. The electron microscopic studies of Espana in 1959 (4), however, agreed with the many light microscopic studies showing the anaplasma body with variously shaped appendages. The appendages in this study were shown as rings, tails, and dumbbell shapes.

At present, the morphologic description of Anaplasma marginale is undecided or at least incomplete. Until additional work resolves the differences between those whose work indicates a spherical or oval body composed of subunits and those who show a tailed or flagellated body, the morphology and classification will remain doubtful.

Filtration and Size

Recently Ristic confirmed and refined the earlier work of Foote (16) that the etiologic agent of anaplasmosis may be separated from the erythrocyte, passed through a filter and retain the ability to produce anaplasmosis. Ristic in 1960 (14) demonstrated that the infective agent could be filtered through membrane filters, 0.65 u. average pore size, after disintegration of acutely infected erythrocytes by either mechanical homogenization or serologic techniques. A substrate of fresh whole blood was used to accept and preserve the organism. The entire procedure was accomplished within 1 hour.

Allbritton (15) later was able to filter the anaplasma organism through membrane filters 0.30 u., but not 0.22 u. average pore size after disintegration of infected erythrocytes by sonic oscillation. In these experiments, no substrate was used and the organism remained infective for 8 hours at 25° C.

These filtration experiments indicate that some infective form of the anaplasma organism is less than 0.30 u. in size and that it will remain infective in a cell-free medium for at least 8 hours at 25° C.

Oxygen Consumption

Oxygen consumption of anaplasma infected erythrocytes, when studied by Pilcher *et al.* (5), was found to be approximately twice that of uninfected erythrocytes. Pilcher, in his literature review, stated that erythrocytes of monkeys infected with malaria show 25 to 100 times greater than normal oxygen consumption, while virus infected tissues show no increased oxygen consumption. The amount of oxygen required by anaplasma, therefore, lies between that required by protozoa and that required by virus.

Viability Under Various Physical Changes in Environment

Whole anaplasma infected blood was found by Bedell (17) to remain infective after being subjected to temperatures of 60° C. for 30 minutes, 45° C. for 4 hours, 38° C. for 3 days, 25° C. for 9 days, 4° C. for 7 days, —66° C. for 56 days.

Bedell also subjected the infected whole blood to sonic oscillation at temperatures of 22° C for 210 minutes and 35° C. for 15 minutes without destruction of infectivity.

Summary

Although the individual descriptions of the variations from the classic anaplasma body continue to differ, there appears to be two broad areas of agreement between the descriptions of those working on morphology. One area includes the descriptions of the organism as composed of 8 or less subunits in a more or less spherical arrangement without projections. The subunits may be single in the erythrocyte or plasma. The other area includes those descriptions which show variously shaped delicate projections from the anaplasma body.

An infective form of anaplasma may be filtered and kept viable for 8 hours at room temperature in a cell-free medium. Electron microscopy and filtration indicate that the infective filterable form may be less than 0.30 u. in diameter.

Oxygen requirements of the anaplasma organism are much less than the protozoan parasite of malaria, but higher than requirements for virus.

The anaplasma organism remains viable in whole blood for 15 minutes at 60° C. and for 56 days at —66° C. with the time generally, but variably, increasing as the temperature is lowered from 60° C. to —66° C.

References

1. Dikmans, G.: Anaplasmosis. VI. The morphology of anaplasmosis. J. A. V. M. A., 83:203-213. 1933.
2. Lotze, J. C. and Yiengst, M. J.: Studies on the nature of anaplasma. Am. J. Vet. Res., 3:312-320, 1942.
3. Franklin, T. E. and Redmond, H. E.: Observations on the morphology of *Anaplasma Marginale* with reference to projections or tails. Am. J. Vet. Res., 19:251-253. 1958.
4. Espana, C., Espana, E. M. and Gonzales, D.: *Anaplasma marginale*. I. Studies with phase contrast and electron microscopy. Am. J. Vet. Res., 20:795-805. 1959.
5. Pilcher, K. S., Wu, W. G., and Muth, O. H.: Studies on the morphology and respiration of *Anaplasma Marginale*. Am. J. Vet. Res., 22:298-307. 1961.
6. Madden, P. A.: Structures of *Anaplasma Marginale* observed in acute infections by using fluorescent antibody techniques. Accepted for publication by Am. J. Vet. Res. Used by permission of the author.

7. Ristic, M., White, F. H. and Sanders, D. A.: Detection of *Anaplasma Marginale* by means of fluorescein labeled antibody. Am. J. Vet. Res. 18:924-928. 1957.
8. De Robertis, E. and Epstein, B.: Electron microscope study of anaplasmosis in bovine red blood cells. Proc. Soc. Expl. Biol. and Med. 77:254-258. 1951.
9. Espana, C.: Electron microscopy in anaplasmosis. Proc. 3rd Nat. Res. Conf. Anapl. 72-78. 1957.
10. Ristic, M.: Structural characterization of *Anaplasma Marginale* in acute and carrier infections. J. A. V. M. A. 136:417-425. 1960.
11. Foote, L. E., Geer, J. C. and Stick, Y. E.: Electron microscopy of the anaplasma body: ultra-thin sections of bovine erythrocytes. Sci., 128:147-148. 1958.
12. Scot, W. L., Geer, J. C. and Foote, L. E.: Electron microscopy of *Anaplasma Marginale* in the bovine erythrocyte. Am. J. Vet. Res. 22:877-881. 1961.
13. Ristic, M. and Watrach, A. M.: Studies in Anaplasmosis II. Electron microscopy of *Anaplasma Marginale* in deer. Am. J. Vet. Res. 22:109-116. 1961.
14. Ristic, M.: Studies of anaplasmosis I. Filtration of the causative agent. Am. J. Vet. Res., 21:890-894. 1960.
15. Allbritton, A. R. and Parker, L. T.: Filtration of the infective agent of bovine anaplasmosis. Accepted for publication. Am. J. Vet. Res. Used by permission of the senior author.
16. Foote, L. E.: New information on anaplasmosis. No. Am. Vet., 35:19-21. 1954.
17. Bedell, D. M.: Studies on some biologic properties of Anaplasma marginale. M. S. Thesis, La. State Univ. 1962.

Recent Observations on the Biologic Nature of Anaplasma marginale*

David M. Bedell

For the past three years our research group at Louisiana State University has been studying the biologic nature of *Anaplasma marginale,* as affected by changes in its environment. This interest in studying the biologic nature of the organism was prompted by the lack of basic information on *A. marginale.* These data are becoming increasingly important to a systematic approach to the problem of bovine anaplasmosis. This paper presents a general discussion of results obtained in studies on the effects of temperature and sonic energy on infectivity of the etiologic agent Infectivity of the various treated preparations was tested in splenectomized calves.

I. **Effects of temperature on infectivity.**—The infectious property of *A. marginale* in whole blood was destroyed by exposure at 60 C. for 50 minutes but not at 15 and 30 minutes; at 45 C. for 8 hours but not at 4 hours; at 38 C. for 86 hours but not at 72 hours; at 25 C. for 288 hours but not at 216 hours; at 4 C. for 21 days but not at 7 days; at —20 C. for 9 days; and at —66 C. for 112 days but not at 56 days (Figure 1). Details of materials and methods and results have been reported[1]

As the time of exposure to the various temperatures was increased there was a lengthening of the incubation time for the disease. However, as the

[1]Raytheon Magnetostriction Oscillator Model S 102A, 50-watt, 9-kc.

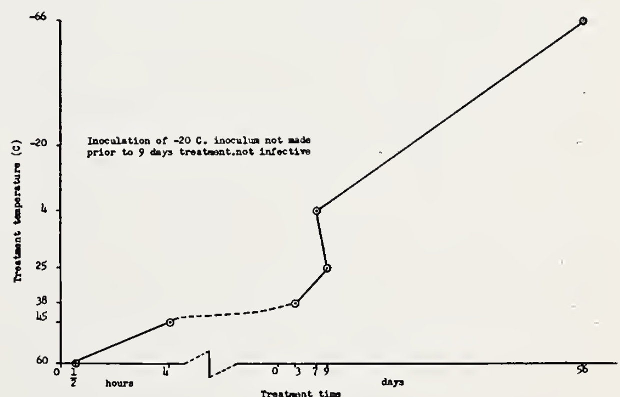

Figure 1. Composite results of the effect of temperature on minimal infections viability of **A. marginale** in whole blood as influenced by time of treatment.

*The data obtained in these investigations have been the result of a cooperative research program in the Department of Veterinary Science, Louisiana State University involving the authors, Dr. G. T. Dimopoullos, Mr. G. T. Schrader, Mr. T. E. Rogers, and Mr. R. J. Lousteau.

Funds for support of this research project were obtained in part, from the Louisiana Agricultural Experiment Station and the National Institutes of Health, National Institute of Allergy and Infectious Diseases under grant E2250.

temperature increased from —66 to 60 C. there was a decrease in the time that *A. marginale* in whole blood retained its infectious property.

At the treatment temperatures of 45, 38, 25, and —66 C., there was some evidence that the peaks in the number of infected erythrocytes that developed in the infected calf decreased with the increase in treatment of the inoculum used. This observation is best demonstrated by the inoculums treated at 38 C. (Figure 2), but the course of the disease was not altered in that the calf with the peak infection of 23 percent developed the same degree of anemia as the calf with the peak infection of 91.5 percent.

The calves that failed to develop the disease, as the result of inoculation with the treated preparations, were re-inoculated after a 75 day incubation period. There was no immune response shown as the syndrome of anaplasmosis was observed in all of these calves.

II. **Effects of sonic energy on infectivity.**—The infectious property of *A. marginale* in whole blood was destroyed when exposed to sonic energy treatments, conducted at 33 to 35 C., for 90 minutes but not at 75 minutes (Figure 3). Exposure to treatments, conducted at 19 to 22 C. and 17 to 18 C., did not destroy infectivity after 90 and 210 minutes, respectively (Figures 4, 5). Details of materials and methods, and results have been reported (2).

An increase in the incubation time was observed as the time of treatments was lengthened, but the course of the disease was not altered after the appearance of the marginal bodies in the erythrocytes at the diagnostic level of one percent. Preliminary observations indicate that the calves infected by the sonically treated inoculum developed higher peaks of erythrocytes containing the marginal body of anaplasmosis.

As a general summation, these studies indicate that *Anaplasma marginale* is not the sensitive or fragile

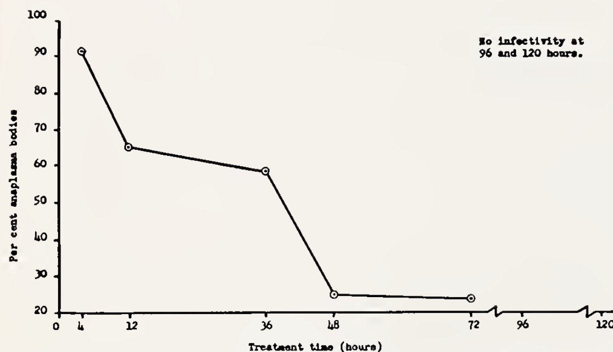

Figure 2. Effect of temperature (38C) on development peak anaplasma body counts in splenectomized calves.

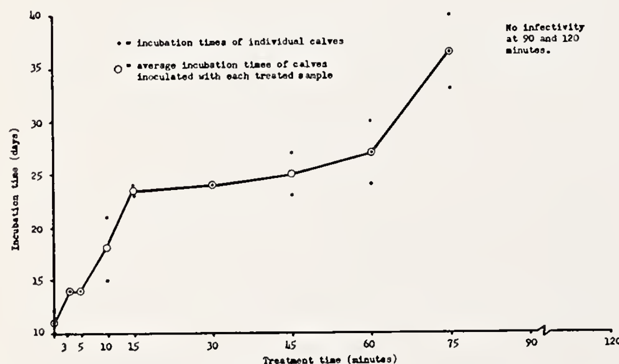

Figure 3. Effect of sonic oscillation on incubation time of anaplasmosis with oscillator cup temperature at 33 to 35 C.

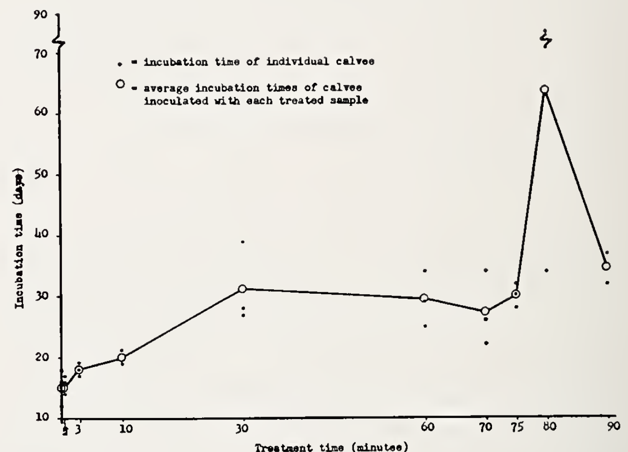

Figure 4. Effect of sonic oscillation on incubation time of anaplasmosis with oscillator cup temperature at 19 to 22 C.

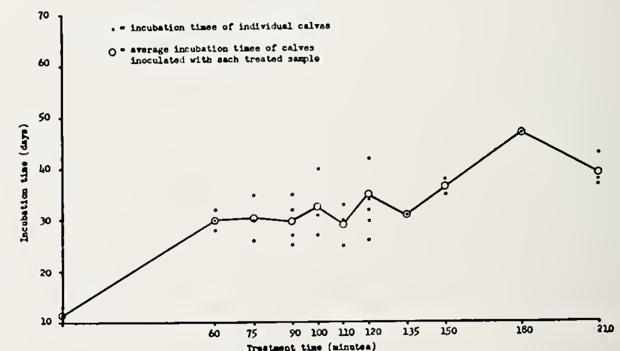

Figure 5. Effect of sonic oscillation on incubation time of anaplasmosis with oscillator cup temperature at 17 to 18 C.

organism that it has been thought to be (3). It was further observed that the course of the disease was not altered after the appearance of the marginal bodies at the one percent diagnostic level and the treated preparations that failed to produce the disease likewise failed to stimulate a protective immune response in the splenectomized calf. Observations made during the studies further indicated the need for the determination and evaluation of the minimal infective unit of *A. marginale*. This information is necessary before a critical analysis of the organism can be made.

References

1. Bedell, D. M. and Dimopoullos, G. T.: Biologic Properties and Characteristics of *Anaplasma marginale*. 1. Effects of Temperature on the Infectivity of Whole Blood Preparations. Am. J. Vet. Res., *23*, (May 1962): 618-625.
2. Bedell, D. M.: Studies on Some Biologic Properties of *Anaplasma marginale*. M. S. Thesis. Louisiana State University. (January 1960): 1-60.
3. Mott, L. O.: The Nature of Anaplasmosis. Proc. 3rd. Natl. Res. Conf. on Anaplasmosis, Manhattan, Kansas. (June 1957): 1-9.

Studies on Anaplasma marginale Using Direct Fluorescent Antibody Methods

P. A. Madden

The direct fluorescent antibody technique, developed by Coons, (1) has become increasingly useful in the diagnosis of many diseases. Ristic (2) was the first to use this technique to demonstrate *Anaplasma marginale* in bovine erythrocytes.

Briefly, the direct fluorescent antibody technique may be described as follows: When a fluorochrome dye is conjugated to a specific antibody protein in immune serum and then applied to its specific antigen, an antigen-antibody-dye complex is formed. This complex when examined microscopically with ultraviolet light can be seen fluorescing a yellow-green.

Recently, Madden (3) reported his studies on the use of this technique to demonstrate structures of *A. marginale* in acute infections. This report deals with some of the more recent developments.

The methods used for conjugation, and for the preparation, fixation, and staining of blood films are the same as those previously reported (3) with one exception. Instead of conjugating the unfractionated serum to fluorescein isothiocyanate, only the globulin portion is used. The globulin is extracted from the serum with Rivanol as described by Saifer and Lipkin (4).

One conjugated globulin made from calf 4665, gave consistently good reactions. It was used routinely to study all blood preparations and therefore served as a standard with which to compare all new conjugated globulins. During routine examinations of infected blood with this conjugate, projections were observed on *Anaplasma* bodies from an infected calf, No. 5040. However, with a new conjugate subsequently derived from this animal, projections on the anaplasmata were not observed. Since both conjugates were prepared in the same manner, it became necessary to determine why these projections were not present with the new conjugate. Therefore, it was decided to conjugate serum from calf 5040 at approximately weekly intervals, to determine if these projections could be detected with all conjugates from this calf. Calf 5040 had a recrudescence of the parasitemia enabling us to react a series of conjugated globulins from this animal with its anaplasmata.

The reactions between the conjugated globulin of calf 4665 and the anaplasmata from calf 5040 are shown in Table 1. It should be noted that projections were demonstrable at all bleedings when calf 5040 was showing *Anaplasma* bodies, column No. 4.

The reactions between the conjugated globulins from calf 5040 drawn at weekly intervals, and the anaplasmata from 5040, blood drawn on 3-14-62, are shown in Table 2. The interesting point here is that although the anaplasmata from calf 5040 were showing projections as demonstrated with conjugated globulins from 4665, the globulins from 5040 were not always capable of detecting these projections when reacted with the blood from 5040. Column No. 4 shows the variable results obtained.

Table 1. Fluorescent Antibody Reactions to Anaplasmata from Calf 5040 with Conjugates from Blood Serum of Calf 4665.

Date Blood Collected	Percent RBC's Infected (Giemsa)	Complement-Fixation Titer of Serums	Presence of Projections
2-13-62	46	1:640	+
2-21-62	1	1:640	+
2-28-62	—	1:640	—
3-7-62	3	1:160	+
3-12-62	20	1:80	+
3-19-62	3	1:80	+

Figures 1, 2, and 3 give a pictorial representation of the reactions in Tables 1, and 2. Figure 1 shows the typical reaction between conjugated globulin from 4665 and anaplasmata from 5040. Figure 2 shows the typical reactions between conjugated globulin from 5040 and anaplasmata from 5040 on those dates (2-13, 2-21, 3-7, and 3-12-62) when the conjugated globulin from 5040 was unable to detect the projections.

Figure 3 shows the typical reactions between conjugated globulin from 5040 and anaplasmata from 5040 on those dates (2-28 and 3-19-62) when the conjugated globulin from 5040 was able to detect the projections.

Table 2. Reactions Between Fluorescein-Conjugated Globulins from Weekly Blood Samples of Calf 5040 and Anaplasmata from Calf 5040.

Blood from 5040 Date Drawn for Globulins	Complement-Fixation Titer of Serums	Blood from 5040 Date Drawn for Anaplasmata*	Presence of Projections
2-13-62	1:640	3-14-62	—
2-21-62	1:640	3-14-62	—
2-28-62	1:640	3-14-62	+
3-7-62	1:160	3-14-62	—
3-12-62	1:80	3-14-62	—
3-19-62	1:80	3-14-62	7

*21% RBC's Infected (Giemsa)

These findings indicate that the serum from an infected calf from which anaplasmata show projections may not at all times during the acute stage of anaplasmosis contain an antibody for these projections, at least in sufficiently high titer to be detected by this technique. Therefore, there must be an optimal time during acute anaplasmosis at which to draw serum from conjugation. To be able to determine this time would greatly help the research worker engaged in fluorescent antibody studies of *A. marginale.*

References

1. Coons, A. H., Creech, H. J., and Jones, R. N.: Immunological Properties of an Antibody Containing a Fluorescent Group. Proc. Soc. Exper. Biol. and Med., *47* (1941):200-202.
2. Ristic, M., White, F. H., and Sanders, D. A. Detection of *Anaplasma marginale* by Means of Fluorescein-Labeled Antibody. Am. J. Vet. Res., *18,* (1957):924-928.
3. Madden, P. A. In Press. Am. J. Vet. Res.
4. Saifer, Abraham, and Lewis E. Lipkin. Electrophoretic and Immunologic Studies of Rivanol-Fractionated Serum Proteins. Proc. Soc. Exper. Biol. and Med. *102*:1959.

Figure 1. Anaplasmata from 5040 reacted with conjugated globulin from 4665. This reaction shows the Anaplasma bodies with projections.

Figure 2. Anaplasmata from 5040 reacted with conjugated globulin from 5040. (3-12-62). This reaction is typical of those where the projections were not demonstrable.

Figure 3. Anaplasmata from 5040 reacted with conjugated globulins from 5040. (3-19-62). This reaction is typical of those where the projections were demonstrable.

Further Studies on The Morphology of Anaplasma marginale With Phase Contrast And Light Microscopy

Carlos Espana and Ethel M. Espana[1]

Introduction

In spite of intensive work by a number of investigators during the last few years, the controversy as to the nature of *Anaplasma marginale* still persists. To summarize briefly some of the conflicting views, most early workers agreed with Theiler's classification of *A. marginale* as a protozoa (1); however, Seiber (2) believed the organism to be more virus-like in nature. Several recent investigators have presented data supporting the virus theory of the etiology of anaplasmosis (3, 4, 5), but the more commonly accepted view at present, based mainly on studies of ultra-thin sections with electron microscopy and of the respiratory rate of infected erythrocytes, is that *A. marginale* belongs to the *Rickettsiales* (6, 7, 8). There are others, including Roby (9) and the authors, who believe that attempts to classify the parasite on the basis of existing information are premature.

Filamentous structures have been described attached to the punctiform Anaplasma bodies seen in stained smears (10, 11). The finding of these structures in fresh preparations of hemolyzed blood observed with phase contrast microscopy (12) has been confirmed by Pilcher *et al.* (8) and by Summers (13). Because of the lack of uniformity in the finding of such structures by various investigators, it has been suggested that they might be artifacts produced by hemolysis and actually represent the matrix of the Anaplasma as seen in ultra-thin sections of deer erythrocytes (7) or that they might be the reticulum of immature erythrocytes (6). It has also been postulated that the investigators who have encountered filaments were dealing with mixed infections or that they were working with different Anaplasma strains or species (4).

The present study is an attempt to clarify the relationship between the two morphological types of Anaplasma, those with and those without filaments.

Materials and Methods

Blood samples from 11 field cases, representing 7 distinct isolates of *A. marginale* from different parts of Mexico were observed with phase contrast and light microscopy. Samples from 15 animals experimentally infected with 3 different Anaplasma "strains" were studied during the course of the disease. Twelve of the animals were calves, splenectomized before inoculation to render them more susceptible, and 3 were adult, intact cows. The cattle were inoculated either intravenously or subcutaneously with infectious blood from acutely ill or carrier animals. Two calves received first and second sheep passage blood. Heparin-Sodium, 100 units per mg.[2], was used as an anticoagulant for the blood samples, which were taken daily during the course of the disease in the experimental animals.

Smears were stained with Wright-Giemsa and preparations for study with phase contrast microscopy were made with blood which was hemolyzed by treatment with specific hemolysin or with water. With the smears, counts were made of (1) the number of infected erythrocytes and (2) the number of infected erythrocytes with two or more Anaplasma bodies. With the phase contrast preparations, in addition to these counts, the number of Anaplasma having filaments and the number with a given type of filamentous structure was counted. Depending upon the stage and the severity of the disease, from 500 to 2500 erythrocytes and at least 100 Anaplasmas were counted.

Results

In smears stained with Wright-Giemsa *A. marginale* appears as compact, deeply stained, punctiform bodies of from 0.2 to 1.2 u. in diameter, generally located in a marginal position in the erythrocyte. Early in the disease there is seldom more than one Anaplasma per infected erythrocyte while at the peak the majority of the infected cells contain two or more parasites (fig. 1). When viewed by phase microscopy in hemolyzed erythrocytes the organism appears either as: (1) round, oval or slightly irregularly shaped bodies, similar to those seen in stained smears, the larger ones often seeming to consist of several compact globular units or (2) apparently identical structures to which are attached filaments. When the Anaplasmas turn, some of the filaments may appear less compact and resemble comets, suggesting that the structure might be a membrane instead of a filament, as mentioned by Pilcher *et al.* (8). Parasites in which the filament was in the form of a ring were found in two of the field cases studied and only in the experimental animals inoculated with blood from these. They usually represented from 5 to 15 percent of the

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[1]This work was carried out under a joint program of the School of Veterinary Medicine of the University of Pennsylvania and the Secretaria de Agricultura y Ganaderia de Mexico, at the Instituto de Investigaciones de Pecuarias, Palo Alto, D. F., Mexico.

Supported in part by grant E-3390 from the National Institutes of Health, National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

[2]National Biochemicals Corporation.

filamented forms, at some stages of the disease reaching as high as 25 percent. In addition to these two forms, Anaplasmas resembling dumbbells were encountered in all of the animals. There were few early in the disease; their number increased gradually, reaching a maximum around the peak of the disease when one-half of all the filamented forms might be dumb-bell-like parasites, then decreased as the infection subsided. Bizarre forms, consisting of several round or irregularly shaped bodies, joined by filaments, were seen in acute infections when many of the infected erythrocytes contained multiple parasites (fig. 2).

These morphological findings have been confirmed, as reported previously (9), by studies with the electron microscope. The filamentous structures have also been demonstrated in preparations stained with modifications of Fontana's silver impregnation and Noland's stain for ciliates (fig. 3, 4), (14).

In order to determine whether the round bodies seen in smears stained with Wright-Giemsa were identical to the organisms, both with and without filaments, observed in hemolyzed erythrocytes with phase contrast microscopy, duplicate counts were made on blood samples taken daily during the course of the infection in 13 animals. In general there was close agreement between the two counts as shown in the representative graph (fig. 5). Discrepancies were most often encountered early in the disease when the percentage of infected cells found with phase contrast was higher than with light microscopy. This, we believe, was because the Anaplasmas were more easily recognized, especially the filamented forms. In 5 animals comparative counts were also made of the number of infected erythrocytes with two and with multiple parasites. Again the two curves agree quite well, considering that such observations are subject to considerable error (fig. 6, 7). It would appear that the bodies seen in infected cells by the two methods are the same organism and that we are not dealing with a mixed infection.

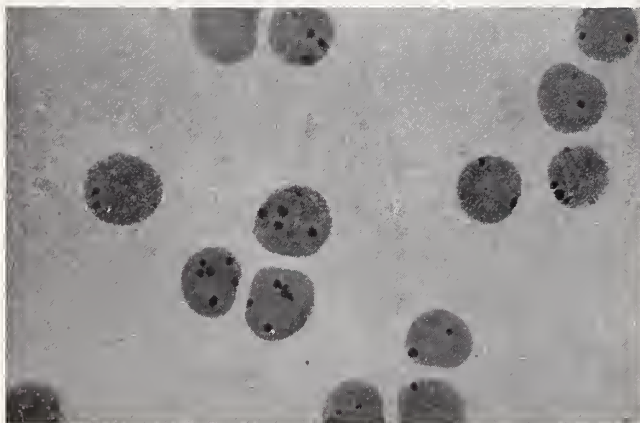


Figure 1. Smear of blood from an acute case of anaplasmosis. Stained with Wright-Giemsa.

When the various forms of *A. marginale* were first described, we believed that all of the parasites in most cases had a filamentous structure. It has now been found that the percentage of these forms varies, apparently with the stage of the infection, with the individual animal and with the "strain" of *A. marginale* and possibly with other factors. In the blood samples from all except 2 of the 11 field cases a significant number, from 20 to 100 percent, of the Anaplasmas had filamentous structures. In the 2 animals in which the Anaplasma were devoid of filaments 3 and 7 samples of blood were examined. In the 15 experimental animals counts done daily during the course of the infection showed fluctuations in the relative numbers of Anaplasmas with and without filaments. Unfortunately, there was not sufficient uniformity in the variations so that they could be correlated with

Figure 2. Hemolyzed infected erythrocytes as seen with phase contrast microscopy.

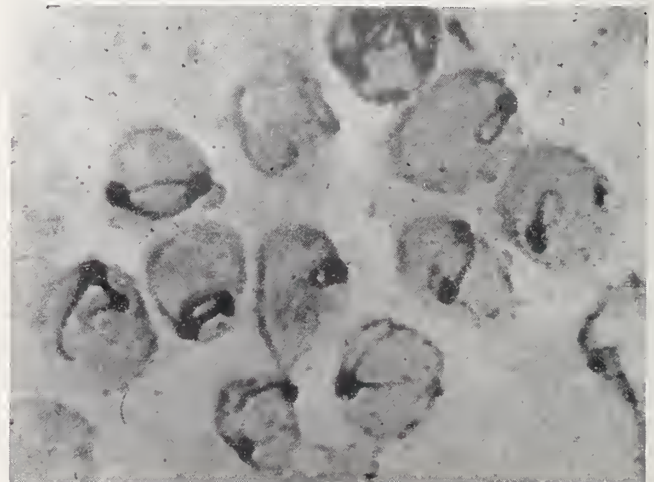


Figure 3. Smear of blood from an acute case of anaplasmosis stained by the silver impregnation method of Fontana.

common factors among the animals. In 6, inoculated with 3 different samples of infectious blood, the percentage of filamented forms was high early in the disease with a marked drop occurring as the percentage of infected erythrocytes increased (fig. 8).

This was observed in 4 splenectomized calves during primary infections, 1 splenectomized calf during a relapse and in 1 intact adult cow. All of these animals had very severe infections and 4 of them died. In 4 other animals the percentage of Anaplasmas with filaments remained high during the whole course of the disease (fig. 9). Two were calves which received sheep blood; one was an intact cow, which was inoculated with a different "strain" of *A. marginale.* The three had relatively mild infections. The fourth animal was an adult cow which suffered a fatal relapse after splenectomy. There appeared to be little in common between the latter and the first 3 animals to explain the similarities in the counts. In 2 calves which received the same infectious blood the percentage of infected erythrocytes remained below 20 and the percentage of filamented forms below 10 during the course of the disease (fig. 10). One of these animals had a severe infection and died, while the other, which was treated wtih Amphoteracin-B[2] early in the disease, had a milder infection and survived. In 1 intact adult cow the percentage of infected erythrocytes and the percentage of Anaplasma with filaments increased gradually at similar rates (fig. 11).

[2]E. R. Squibb and Sons

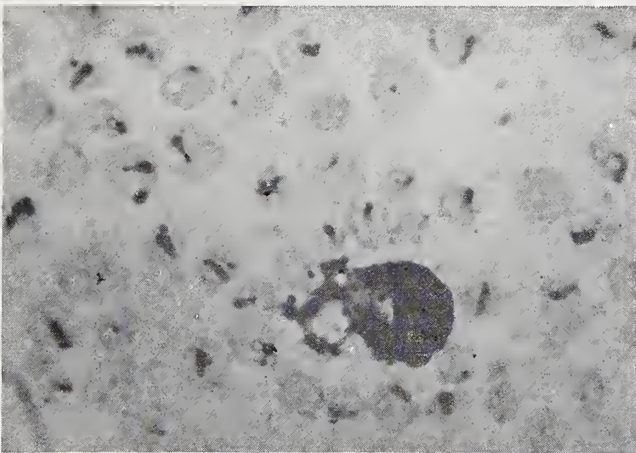
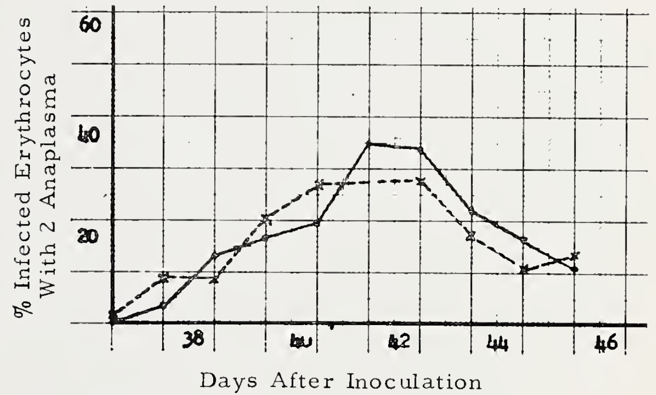


Figure 4. Smear of blood from an acute case of anaplasmosis stained by the method of Noland. Shows Anaplasma without filaments and the various filamented forms described in the text.



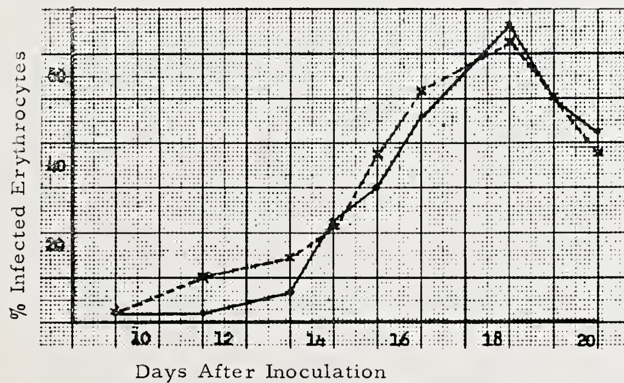
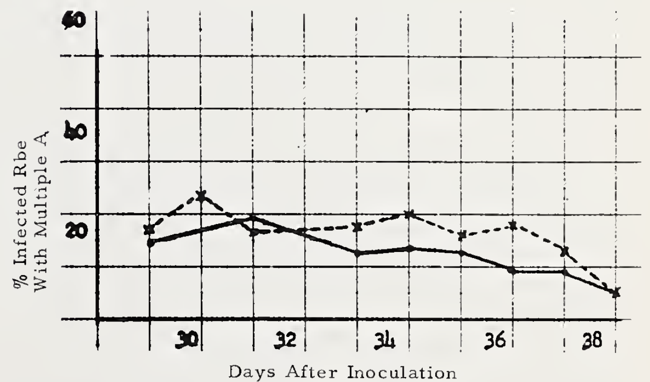


Figure 5. Comparsion of the percentage of infected erythrocytes as counted in smears stained with Wright-Giemsa (continuous line) and in fresh preparations of hemolyzed erythrocytes observed by phase contrast microscopy (broken line).



Figures 6, 7. Comparison of percentage of infected erythrocytes with 2 Anaplasmas (fig. 6) and those with 2 or more as observed with light (smear) and phase contrast microscopy (fresh preparation).

Discussion and Summary

In view of the present study and, since several other investigators have confirmed our earlier findings, the existence of *A. marginale* with filamentous structures can no longer be ignored nor can such forms be assumed to be artifacts. Both Pilcher *et al.* (8) and the authors have presented data indicating that the marginal bodies in stained smears are the same as the several forms seen with phase contrast microscopy. Further confirmation is found in the fact that, while the filamentous structures do not stain with the Romanowsky stains, they can be stained by other methods.

There is not agreement among the workers who have observed *A. marginale* with the phase contrast microscope as to the exact morphology of the parasite. Pilcher *et al.* did not find ring forms while Summers (13) did. The former investigators did not encounter samples in which all, or nearly all, of the Anaplasmas were devoid of filaments. In fact, they reported only occasional spherical bodies without "tails" in samples taken at intervals throughout the disease. On the other hand, it was shown repeatedly in this study that there was great variation in the numbers of filamented and non-filamented forms in different animals and at different stages of the disease. Ristic (4) has not found Anaplasma with filamentous structures either with the phase contrast or the electron microscope.

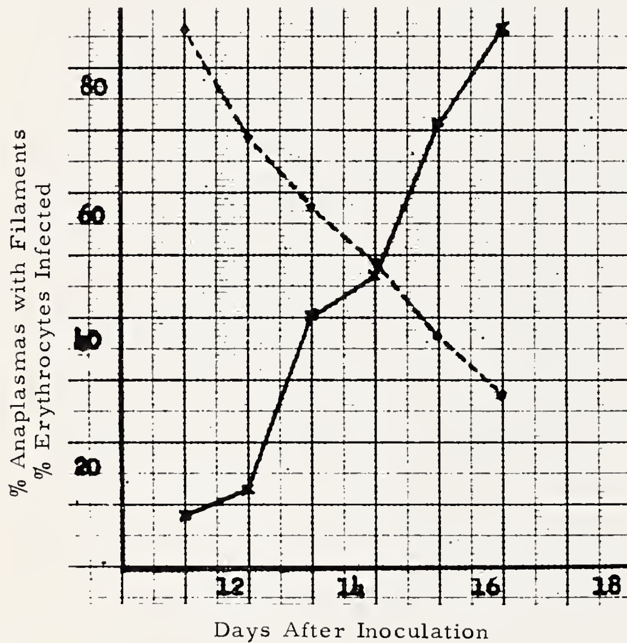


Figure 8. Percentage of infected erythrocytes (continuous line) and percentage of Anaplasmas with filaments from a severe, acute fatal case of anaplasmosis.

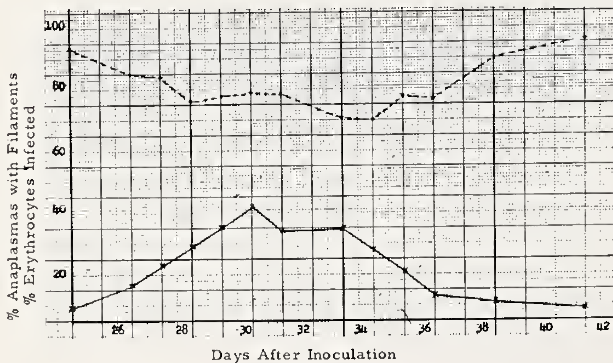


Figure 9. Same as in Fig. 8 but from an acute case of anaplasmosis, relatively mild.

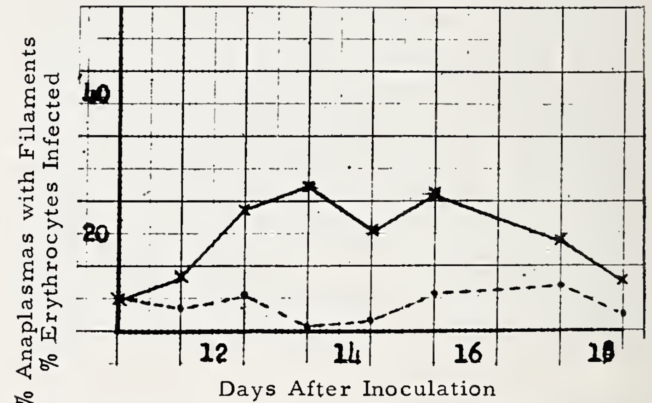


Figure 10. Same as Fig. 8 but from a splenectomized cow which survived an infection.

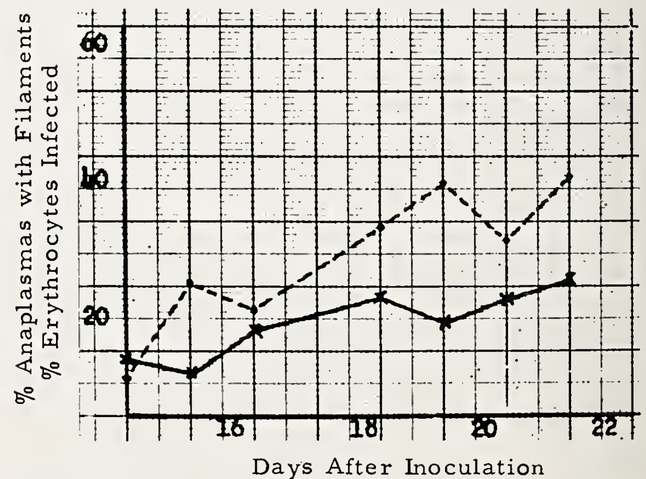


Figure 11. Same as Fig. 8 but from an intact cow which had a fatal infection.

These differences suggest that the various workers have been studying morphologically distinct "strains" of *A. marginale*.

It was hoped that from the counts of the several Anaplasma forms done daily during the infection a pattern would evolve giving evidence of a developmental cycle. Although the percentage of filamented forms in a given animal seemed to vary from day to day with a certain regularity, there was not uniformity in the findings in different animals, even in those inoculated with the same "strains" of *A. marginale*. However, the appearance in all of the experimental animals studied of several distinct forms of the organism, the relative numbers of which changed as the disease progressed, suggested a growth cycle. Although several animals showed similar variations in the percentage of filamented Anaplasma during the course of the infection, these could not be correlated with common factors among the animals, nor could irregular fluctuations be explained. It is obvious that further studies are needed to elucidate the nature of *A. marginale* and every attempt should be made to correlate and evaluate conflicting findings in order to arrive at a better understanding of this controversial parasite rather than to insist upon classifying it.

References

1. Theiler, A.: *Anaplasma marginale* (Genus et spec. nov.). The Marginal Points in the Blood of Cattle Suffering from a Specific Disease. Rept. Govt. Vet. Bacteriol. Transvall. S. Africa, (1908-09): 7-64.
2. Seiber, H.: *Anaplasma marginale* (Theileri). Govt. Rept. Vet. Bacteriol. Union S. Africa, (1909-1910): 104-116.
3. De Robertis, E., and Epstein, B.: Electron Microscope Study of Anaplasmosis in Bovine Red Blood Cells. Proc. Soc. Exptl. Biol. & Med., 77, (1951): 254-258.
4. Ristic, M.: Structural Characterization of *Anaplasma marginale* in Acute and Carrier Infections. J. A. V. M. A., 136, (1960): 417-428.
5. Foote, L. E., Gen, J. C., and Stich, Y. E.: Electron Microscopy of the Anaplasma Body: Ultra-thin Sections of Bovine Erythrocytes. Science, 128, (1958): 147-148.
6. Scott, W. L., Geer, J. C., and Foote, L. E.: Electron Microscopy of *Anaplasma marginale* in Bovine Erythrocytes. Am. J. Vet. Res., 22, (1961): 877-881.
7. Ristic, M., and Watrach, A. M.: Studies in Anaplasmosis. II. Electron Microscopy of *Anaplasma marginale* in Deer. Am. J. Vet. Res., 22, (1961): 109-116.
8. Pilcher, K. S., Wu, W. G., and Muth, O. M.: Studies in the Morphology and Respiration of *Anaplasma marginale*. Am. J. Vet. Res., 22, (1961): 298-307.
9. Roby, T. O.: Studies on the Biological Nature of *Anaplasma marginale*. Proc. 64th Ann. Meeting U. S. Livestock Sanitary Assoc., (1960): 88-94.
10. Boynton, W. H.: Further Observations on Anaplasmosis. Cornell Vet., 22, (1932): 10-28.
11. Franklin, T. E., and Redmond, H. E.: Observations on the Morphology of *Anaplasma marginale* with Reference to Projections or Tails. Am. J. Vet. Res., 19, (1958): 252-253.
12. Espana, C., Espana, E., and Gonzales, D.: *Anaplasma marginale*. I. Studies with Phase Contrast and Electron Microscopy. Am. J. Vet. Res., 20, (1959): 795-805.
13. Summers, W. A.: 1960 Personal Communication.
14. Espana, C.: Unpublished data.

Forms of Anaplasma marginale Theileri as Observed by Electron Microscopy

D. W. Gates and A. E. Ritchie[1]

The density of the intact erythrocyte prevents direct electron optical examination for the presence of *Anaplasma marginale*. Several methods may be employed to reduce the density of the preparation and permit the penetration of the electron beam. These methods may be divided into two groups: (1) Those involving thin sectioning of the red cells; and (2) those producing partial or complete hemolysis of the erythrocytes.

The present study deals with hemolysis of the erythrocytes and the recognition of the parasites within them.

Danon, Nevo and Marikovsky (1) dialyzed a suspension of erythrocytes against a solution of volatile salt producing ghost cells with thin walls and granular surfaces relatively free from rents and craters. Ritchie (2) applied this method to bovine erythrocytes with similar results.

Blood from splenectomized bovine animals was collected into sodium citrate, added to veronal buffered saline, pH 7.3, transferred to a dialyzing casing and suspended in 0.33 percent ammonium acetate. Dialysis continued for 30 to 60 minutes at room temperature. The stromata were lightly packed by centrifugation and the supernatant fluid discarded. The sediment was resuspended in a quantity of distilled water equal to the amount of veronal buffered saline used for dialysis. Fixation with osmium tetroxide as recommended by Danon *et al.* (1) was used in some cases to enhance the contrast. Additional handling of the stromata served only to promote clumping of the erythrocyte membranes. The Formvar-covered grid was clamped firmly in the jaws of forceps. A drop of suspension containing stromata was applied to the grid by means of a syringe and allowed to stand for 3 to 5 minutes. The excess fluid was removed by touching a pointed piece of filter paper to the surface of the droplet. The thin residual film was removed at the grid margin by tilting the grid; care was taken to remove any fluid trapped between the forcep jaws. Specimens were shadow-cast with germanium at approximately a 20° angle and a film of carbon applied to minimize beam damage. Micrographs were taken with an RCA - EMU - 3C electron microscope at magnifications ranging from 4,000 to 30,000 diameters.

Several forms of *Anaplasma* were observed. Those found within the stromata were distinctly outlined against the cell membrane and some internal structures could be seen.

The most prominent feature of the intact parasite was a terminal, round, electron-dense structure, and an associated sac-like projection (Fig. 1). These round structures were frequently subdivided into a variable number of smaller units. Some organisms possessed round structures on each end of the sac (Fig. 2). Double-ended forms were generally not

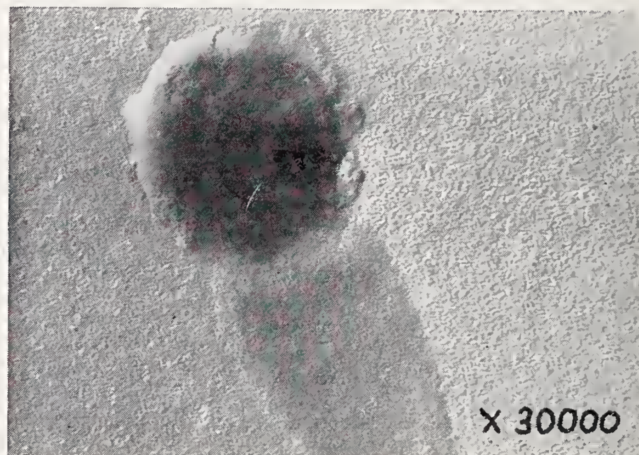


Figure 1. Terminal, round, electron-dense structure and associated sac-like projection.

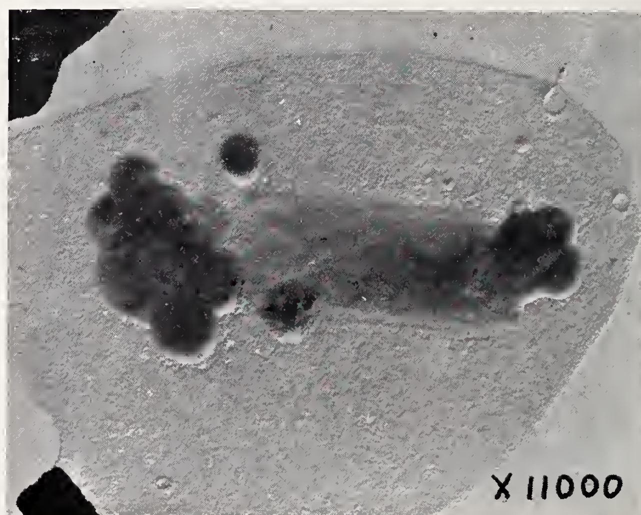


Figure 2. Sac-like projection with a round structure on each end.

[1]National Animal Disease Laboratory, Animal Disease and Parasite Research Division, ARS, USDA, Ames, Iowa.

symmetrical; one end was frequently differentiated into granules, the other was not. Occasionally, one end of the sac was sharply tapered. Although most of the parasites were retained within the cell membrane, occasionally one was observed outside the cell. In a few instances, small rectangular structures (Fig. 3) were observed at one end of the sac. These structures were found to be associated with the terminal round bodies. Other forms as shown in Figures 4 to 7 inclusive were also observed. Figure 8 represents a stroma from an anaplasmosis-free animal.

The foregoing is a simple and rapid method of preparing specimens of *A. marginale* for electron microscopy. Although some may be damaged as a result of handling, this technique results in most of the parasites being recovered intact. With the excep-

Figure 5.

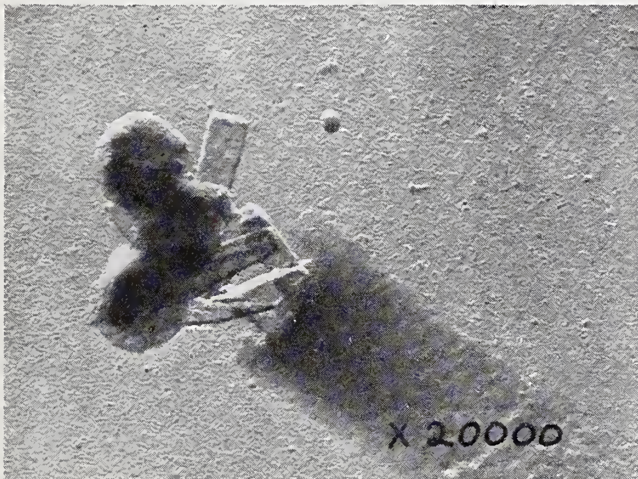


Figure 3. Small rectangular structure associated with the terminal round structure.

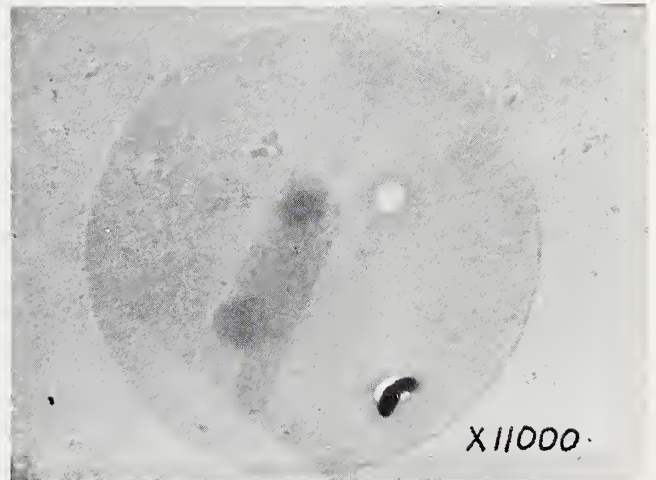
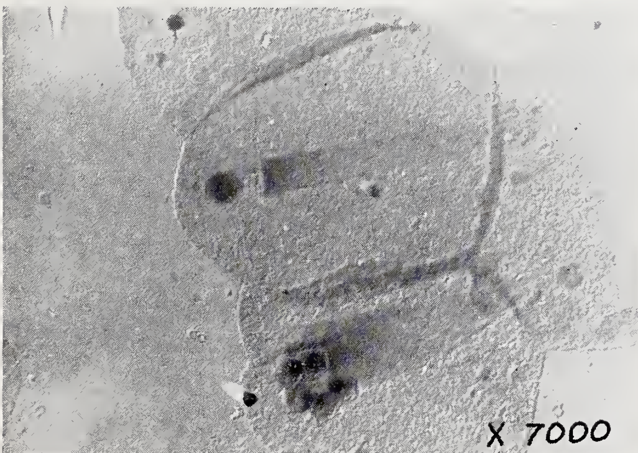


Figure 6.



Figures 4, 5, 6, 7. Other forms of the parasite observed by electron microscopy.

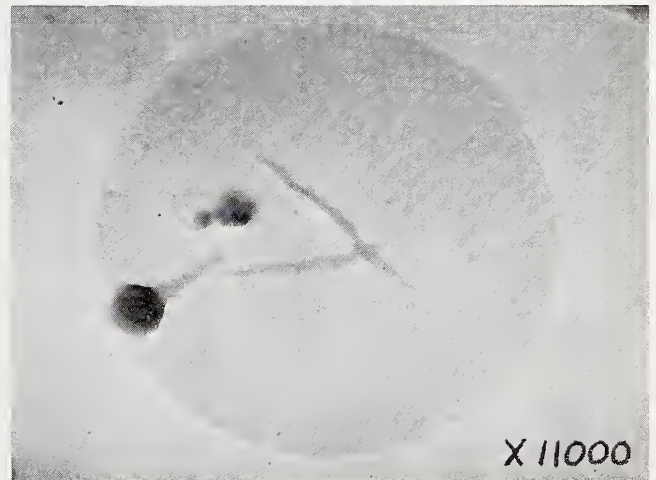


Figure 7.

Figure 8. Normal stroma from an anaplasmosis-free animal.

tion of the rectangular bodies, the forms we have seen are similar to those observed by other workers. Sieber (1911) (3), Boynton (1932) (4), Lotze and Yiengst (1942) (5) all reported projections on *A. marginale.* More recently, Franklin and Redmond (1958) (6), Espana *et al.* (1959) (7), Summers (1959) (8), and Pilcher *et al.* (1961) (9), observed similar structures and discussed in detail the complex nature of these projections.

The present study has provided additional morphological detail of at least one form of the anaplasmata by disclosing rectangular forms between the terminal body and the sac-like projections. This suggests a close relationship between the rectangular forms and the small, round, electron-dense bodies.

Summary

A rapid and simple method is described for preparing specimens of *Anaplasma marginale* for examination under the electron microscope. The technique includes, in part, dehemoglobinizing the erythrocytes by dialysis against a hypotonic solution of a volatile salt. The complex structure of *A. marginale* as reported by previous workers is confirmed—Sieber (1911) (3), Boynton (1912) (4), Lotze and Yiengst (1942) (5), Franklin and Redmond (1958) (6), Espana *et al.* (1959) (7), Summers (1959) (8), and Pilcher *et al.* (1961) (9).

The authors gratefully acknowledge the advice and assistance of Mr. D. W. Anthony, of the Entomology Research Division, in connection with the photographic problems.

References

1. Danon, D., Nevo, A., Marikovsky, Y. Bull. Res. Council of Israel, 1956, 6E:36.
2. Ritchie, A. E. Abstract, Biophysical Society Sixth Annual Meeting, Feb. 14-16, 1962.
3. Sieber, H. Report of the Government Veterinary Bacteriologist, 1909-1910, Union of South Africa: 104-116.
4. Boynton, H. W., Cornell Vet. 22(1932):10-28.
5. Lotze, J. C., Yiengst, M. J. Am. J. Vet. Res. 3(8), July 1942:312-320.
6. Franklin, T. E., Redmond, H. E. Am. J. Res. 19(70), Jan. 1958:252-253.
7. Espana, C., Espana, E. M., Gonzales, D. Am. J. Vet. Res. 20, 1959:795.
8. Summers, W. A. Personal communication. 1959.
9. Pilcher, K. S., Wu, W. G., Muth, O. H. Am. J. Res. 22(87), March 1961:298-307.

Tailed Forms In Anaplasmosis And Their Relationship To Anaplasma marginale

Julius P. Kreier

In recent veterinary literature several papers have been published which described a tailed organism as the causative agent of anaplasmosis. In our work with anaplasmosis at Urbana we had never been able to detect tailed forms. We formulated three hypotheses to guide our work in explaining the difference between the morphology of the organisms with which we were working and the tailed organism described by the other workers. (1) The tailed organism is present in our anaplasma but we did not see it. (2) There is a strain difference in *Anaplasma marginale*; some strains have tails. (3) The tailed organism is some new hitherto undescribed form occurring in mixed infection with *Anaplasma marginale.*

The first hypothesis was eliminated when we obtained an inoculum of the tailed parasite from Oregon. We promptly found the tailed structures, so we may conclude that we used the proper methods for demonstrating tails; they just were not present in our Florida strain of *Anaplasma marginale.* The decision between hypothesis 2 and 3 is the subject of the present talk. The evidence which we have gathered supports hypothesis 3 that the tailed organism is a new parasite occurring in mixed infection with *Anaplasma marginale.*

The evidence that the tailed organism is a new parasite occurring in mixed infection with *Anaplasma*

marginale is varied. It is based on serology, immunology, morphology, and host range.

The first evidence that the tailed organism was distinct from anaplasma came from morphologic studies. While it is true that in Giemsa stained blood films there is no readily detectable difference between the tailed organism and the anaplasma marginal body, by other techniques of study the tailed organism is different from anaplasma. The reason that the tailed parasite in the Giemsa stained preparation resembles the anaplasma body is that only the nucleus stains, the other structures do not. The apparent similarity of the two parasites in Giemsa stained preparations and the fact that all animals injected with the tailed parasite give a positive CF test for anaplasmosis, caused by the coincident anaplasma infection, resulted in the assumption that the tailed organism was anaplasma. The morphologic differences can be seen by phase contrast study of lysed infected cells, and study with the fluorescent antibody technique. All animals infected with the tailed parasite have had a variable proportion of morphologically typical anaplasma present in their blood cells at the same time.

The serologic evidence for the distinctness of the tailed parasite from anaplasma is based on fluorescent antibody studies. Animals which are convalescent from anaplasmosis have in their serum antibody which can be labeled with fluorescein dye, and which will specifically attach to anaplasma marginal bodies. In infected blood films stained with these labeled antibodies the marginal bodies fluoresce when observed with the ultraviolet microscope. With Florida strain anaplasma infections no tailed structures can be detected. If labeled blood serum for Florida strain anaplasma infections is applied to blood films for animals infected with a strain containing the tailed organism, marginal bodies can also be seen, but no tailed organisms are visible. Labeled blood serum from animals infected with an *anaplasma* strain containing the tailed organisms when applied to blood films for animals infected with an anaplasma strain containing the tailed organism causes specific fluorescence of tailed parasites as well as fluorescence of typical marginal bodies. If this labeled blood serum for animals injected with the tailed organism is applied to blood films from animals injected with Florida strain, anaplasma only typical marginal bodies can be observed to fluoresce.

If labeled serum from ainmals infected with an *anaplasma* strain containing the tailed organism is absorbed with a purified preparation of *anaplasma marginal* bodies before it is applied to blood films from animals infected with tailed organisms and blood films from pure *anaplasma marginale* infections than, only the tailed organisms are labeled and marginale bodies are no longer labeled. This indicates that serums from animals infected with anplasma strains containing tailed organisms contain two distinct antigenically different antibodies, one which attaches specifically to anaplasma marginal bodies and another that attaches to tailed organisms.

Taxonomically related organisms have identical or closely related antigens. A clear-cut separation of antibodies by absorption indicates that the antigens responsible for the antibody response were not identical and probably not closely related. This indicates that the organisms containing the antigens are not closely related either. The serological reactions described for convalescent serums can be duplicated with serums produced in rabbits by injection of infected cells from cattle with Florida strain anaplasma or a strain of anaplasma containing tailed parasites.

Animals in good health which have recovered from an attack of anaplasmosis are immune to subsequent challenge because of the permanent state of premunition which develops. When Florida strain anaplasma carrier is challenged with blood from an animal with the tailed infection, it goes through a mild but definite clinical infection. Only tailed organisms are present in the blood. After recovery from this infection, subsequent challenge with tailed organisms does not produce clinical infection again.

It has been established that the white-tailed deer is susceptible to *Anaplasma marginale* infection. If deer are infected with blood from the Oregon strain of *Anaplasma marginale* containing tailed organisms the deer develop clinical anaplasmosis. The tailed parasite does not grow. This indicates that the tailed parasite is a distinct parasite with a different host range from that of anaplasma.

A last point of difference between infection with the tailed parasite and anaplasmosis is seen in the clinical pictures of the two conditions. Clinical anaplasmosis has an incubation period which varies according to the infective dose, then marginale bodies begin to appear and increase rapidly in numbers until a crisis occurs when there is rapid destruction of erythrocytes and marginale bodies disappear from the blood. If the animal survives, there may be one or two secondary courses of infection similar to the first but less severe. Animals infected with the tailed parasite develop a crisis associated with the presence of marginale bodies, but the tailed parasites may persist in the blood at a relatively constant level for weeks.

In conclusion our studies indicate that the Oregon strain of Anaplasma is a mixed infection. It contains typical *Anaplasma marginale* and some other parasite.

Pathology

The Histopathology of Anaplasmosis

C. L. Seger and Donna White

The purpose of this study was to investigate the involvement of the reticulo-endothelial system of the various tissues in the removal of disintegrated or devitalized erythrocytes from the circulation during the development of anemia in anaplasmosis.

Tissues were selected from 23 splenectomized calves infected with anaplasmosis in various experiments conducted by Foote, Allbritton and Bedell in the Veterinary Science Department of Louisiana State University. Hematocrit readings for the calves prior to necropsy ranged from 20% to 75% of pre-infection values. Nineteen of the calves were killed for necropsy and four died during the acute phase of the anemia.

Gross alterations observed at necropsy included dehydration, emaciation, watery blood, enlarged livers some of which were mottled yellow to brown, distended gall bladders, serous atrophy of renal and cardiac fat, and enlarged visceral lymph nodes. Diffuse, brown coloration was observed in the medulla of the hepatic and mediastinal lymph nodes in the most acute cases of anemia. Irregular brown areas were observed in the cortex of these nodes as well as in the cortex and medulla of the bronchial and mesenteric nodes. A bronze hue was noted in the otherwise normal appearing lungs of the acute cases necropsied near maximal anemia.

Tissues were fixed in 10 percent formol-saline solution and subjected to routine histological processes. Paraffin sections were stained with hematoxylin and eosin for routine examination for microscopic lesions. Duplicate sections were treated with potassium ferrocyanide and 0.2 hydochloric acid for the detection of hemosiderin by the Prussian blue reaction.

Alterations from normal were observed in all liver sections. In sections from calves with hematocrit values near 75 percent of normal the principal change was an increase in size of Kupffer cells. The cytoplasm was noticeably enlarged and tended to be basophilic. A few hemosiderin granules were observed in a minority of the Kupffer cells at this stage. Erythrophagia could be detected only by very thorough examination. An increased number of lymphocytes and monocytes were seen in the interlobular connective tissue.

Erythrophagia was more readily observed in the more advanced cases of anemia. The more marked the anemia the greater was the accumulation of hemosiderin in Kupffer cells. In cases where hematocrit values fell to 25 percent of normal, Kupffer cells were so distended that many parenchymal cells were distorted or displaced. In such livers, macrophages laden with hemosiderin accumulated in central veins. In those livers, fatty degeneration of parenchymal cells was common but frank centrolobular necrosis was infrequent.

Erythrophagia and hemosiderosis were infrequently observed in lymph node sections from calves in the early stages of the anemia. However, it was observed

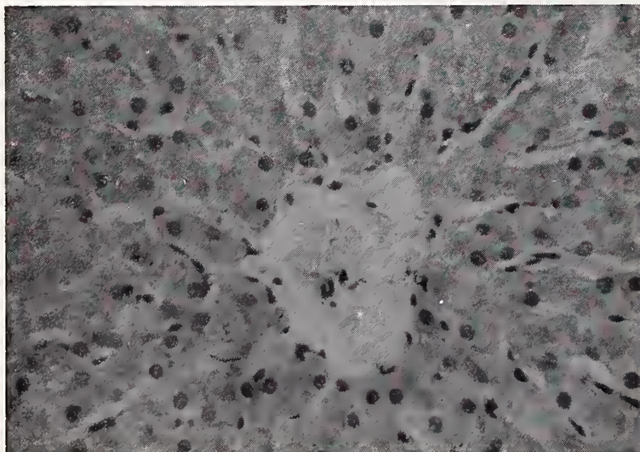


Figure 1. Liver section from a normal calf. H & E stain; X323.

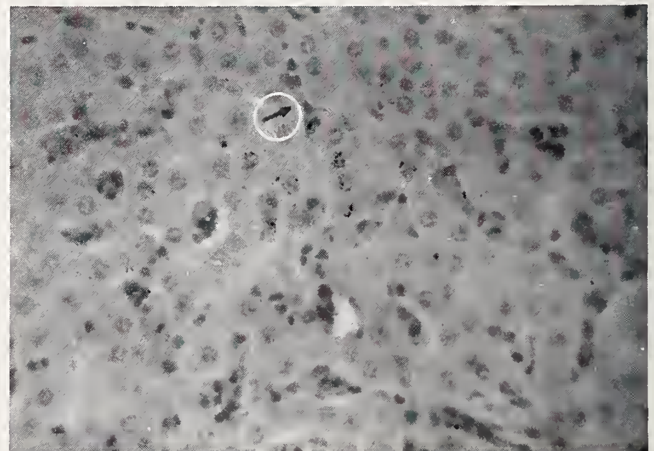


Figure 2. Kupffer cells (arrow) laden with hemosiderin occupy most of space in sinusoids in liver section from splenectomized calf killed during anemic crisis of anaplasmosis. H & E stain; X323.

that reticulo-endothelial elements appeared more prominent than normal, particularly in the medullary sinuses of hepatic mediastinal, bronchial and mesenteric nodes. Plasma cell types also seemed more numerous.

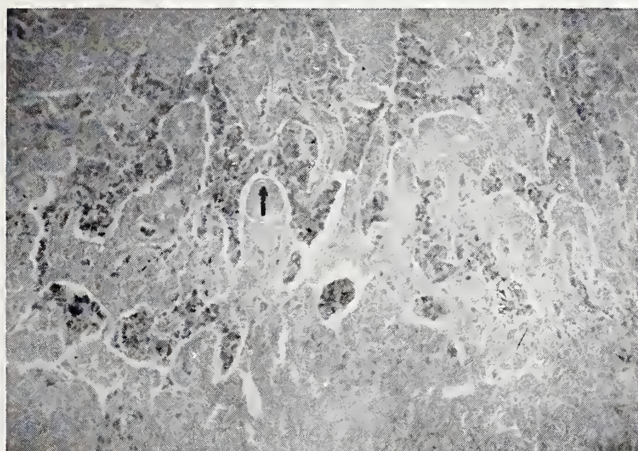


Figure 3. Hepatic lymph node of calf killed at anemic crisis of anaplasmosis. The section was treated with potassium ferrocyanide. The dark stain is the Prussian blue reaction indicating the presence of hemosiderin. No counter stains were used. Note that the heaviest reaction has occurred in the medullary sinuses (arrow). Lymph cords and follicles did not react. X75.

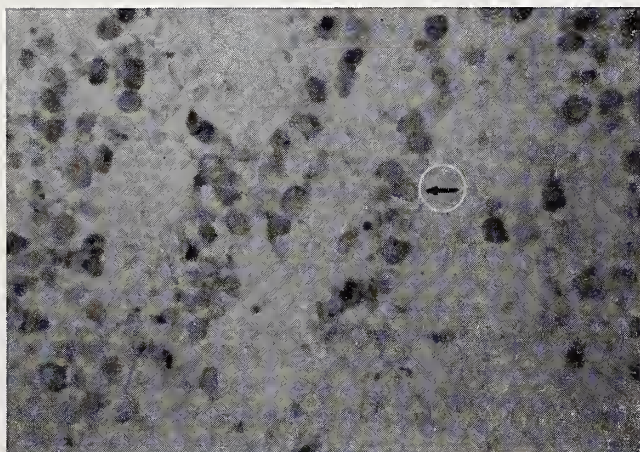


Figure 4. Same section and treatment as Fig. 3. Note that the reaction occurs in the cytoplasm of macrophages (arrow). The large unstained areas in the macrophages are the nuclei. The smaller unstained areas in the macrophages are the nuclei. The smaller unstained globules are partially digested erythrocytes with hemosiderin appearing at the periphery of the erythrocytes. X323.

Massive, diffuse erythrophagia and hemosiderosis was observed only in the medullary sinuses of the hepatic and mediastinal nodes from calves which had developed a maximal anemia with hematocrit values down to 25 percent of normal. This phenomenon was also observed in discrete areas of the cortical sinusoids of these nodes. A similar pattern with less

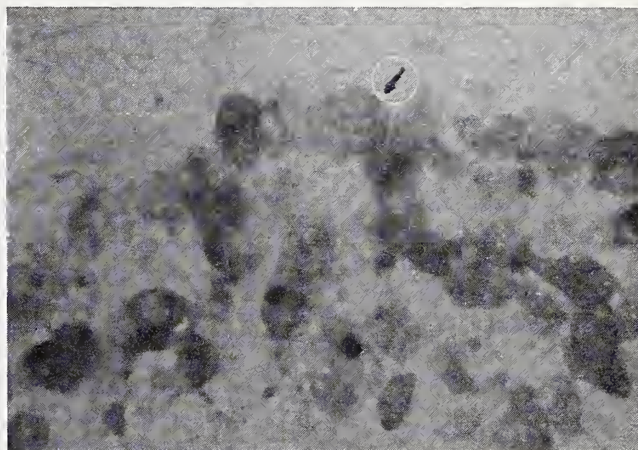


Figure 5. Oil emersion photomicrograph of same field seen in Fig. 4. Note the number of erythrocytes in one macrophage (arrow). The dense staining masses are heavy accumulation of hemosiderin within the cytoplasm of macrophages. No counter stain was used so nuclei are unstained. X728.

Figure 6. Lung of splenectomized calf killed at anemic crisis of anaplasmosis. Section was treated with potassium ferrocyanide. No counter stain was used. Prussian blue reaction occurred in the interalveolar septa and in macrophages in vessels (arrow) surrounding the bronchiole. X75.

extensive involvement was seen in bronchial and mesenteric nodes with some sections of the latter nodes exhibiting little or no hemosiderosis even in the medullary sinuses. Most sections of hemal nodes from acute cases showed some hemosiderosis.

As in the lymph nodes, extensive hemosiderosis was observed in the interalveolar septa of lung sections from calves which had developed a severe anemia. The pigment laden macrophages were confined within the septa and were seldom observed free in the alveoli. Accumulations of macrophages with hemosiderin were observed in veins and lymph vessels of interlobular septa.

Erythrophagia was not detected in bone marrow sections nor was hemosiderin observed in any substantial amounts. The sections were from red marrow collected at the proximal end of the femur. Centers of hemopoiesis appeared relatively numerous and active particularly in the more advanced cases of anemia.

Other tissues examined but much less extensively included muscle, skin, thyroid, thymus, testicle adrenal, kidney, intestine and brain. In some severe cases of anemia a slight amount of hemosiderosis was observed in reticulo-endothelial elements of the intestine and the adrenal gland. In several severe cases of anemia rather extensive hemosiderosis was observed in the vascular, interstitial tissue between the tubules of the renal cortex. Some tubular degeneration was also seen in those cases.

Tissues were also examined from two cows that died with acute anaplasmosis. Heavy deposits were observed in the spleen. Hemosiderin in the liver was much less extensive than that which was seen in splenectomized calves but involvement of the lung of the cows was as extensive as was seen in calves.

Retention of bile was detected in bile canaliculi of one of the cows which happened to be in advanced pregnancy. The bile retention was associated with areas of necrosis radiating from the central veins. Evidence of fatty degeneration was more pronounced than was observed in the other livers examined.

Liver, lung, lymph node, and spleen were also examined from a horse dead from infectious equine anemia. Erythrophagia was observed and hemosiderosis was extensive in all of the tissues examined. Hemosiderosis was less extensive in the equine spleen than in the bovine spleen. Hemosiderosis was much greater in the equine liver than in the adult bovine liver and was quite similar in extent to that seen in the calves with the most severe anemia.

Summary

In the splenectomized calf the reticulo-endothelial elements of the liver, lung and associated lymph nodes appear to have the capacity to remove from the circulation the erythrocytes devitalized or disintegrated during the anemic crisis of anaplasmosis. Lesions involving parenchymal cell degeneration and necrosis were observed in livers and kidneys of splenectomized calves only when hematocrit values dropped to 25 percent of normal.

References

1. Histochemical Technique, W. G. Bruce Casselman, page 158-159.

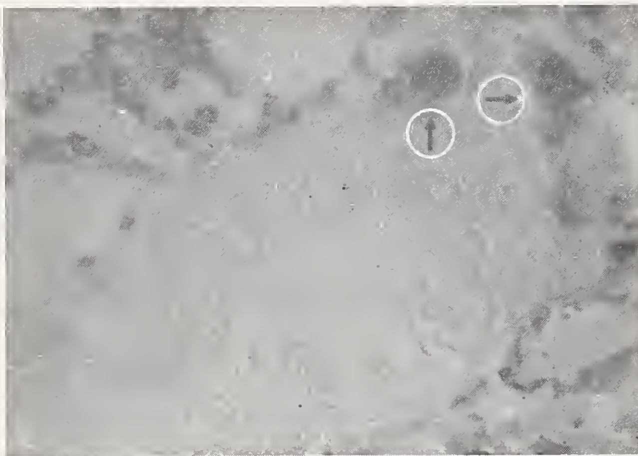


Figure 7. High power view of same field as Fig. 6. Note hemosiderin in cytoplasm of macrophages in lumen of small vessel (arrow) adjacent to bronchiole. X323.

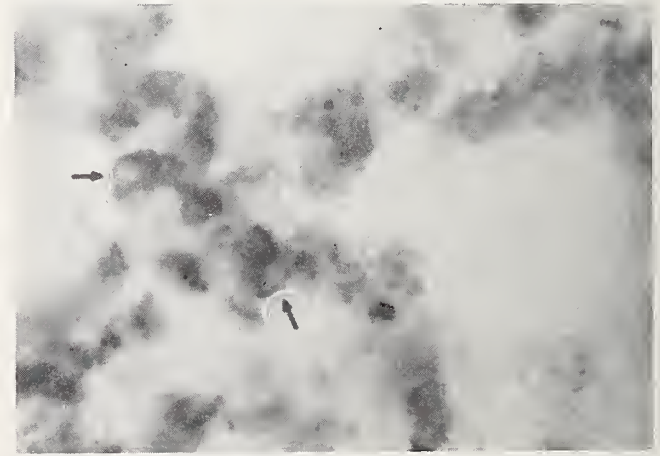


Figure 8. Oil immersion view of same field as Fig. 7 showing hemosiderin in cytoplasm of cells interalveolar septa. Note that nuclei (arrows) are not stained. X728.

Biochemical, Immunochemical, and Biophysical Studies in Anaplasmosis*

George T. Dimopoullos

For about the past five years our research group at Louisiana State University has oriented its investigations on anaplasmosis in three main categories; specifically, studies of the biological properties of the infectious entity, studies of the chemical and physical alterations which occur in the erythrocytes and other blood components, and immunochemical investigations on the complement-fixing antibodies.

The discussion on the biological properties of the infectious agent has been given by Dr. D. M. Bedell. This narration involves discussions on data obtained in studies dealing with alterations in the erythrocytes and plasma and characterization of the complement-fixing antibodies.

Our interests in studying the chemical and physical alterations which occur in the erythrocytes during anaplasmosis have led us to develop hypotheses on the mechanisms which may be responsible for the removal of the cells from the peripheral circulation, specifically the initial changes in the cells which promote the anemia. Most of these studies have involved analyses on the chemical composition of the erythrocytic membrane with particular reference to the structural phospholipid components. Other corollary data such as erythrocytic fragility, numbers of erythrocytes containing marginal bodies, phospholipid concentration of plasma, and surface properties of erythrocytic stromata have also been obtained.

Let us now consider the changes that occur in the erythrocytes as a result of experimental infections of *Anaplasma marginale* in splenectomized calves.

When erythrocytic fragility, numbers of erythrocytes and marginal body counts are compared (Fig. 1, 2) it is observed that an inverse relationship exists between fragility values and erythrocytic counts. Maximum numbers of marginal bodies appear at a time when the fragility of the erythrocytes is also at a maximum value. As the numbers of marginal bodies decrease and new erythrocytes appear in the peripheral circulation the fragility of these erythrocytes returns to normal.

The changes in the fragility of the erythrocytes give an indication that structural alterations occur during the disease. Since the phospholipids are major structural components of the erythrocytic membrane a study of these substances would be expected to give direct evidence for chemical changes in the erythrocytes. When the phospholipid concentration of these cells is studied it is observed that a decrease begins with the onset of the anemia and it reaches a low value when the erythrocytic count is also low (Fig. 3). When new cells are produced after the anemic crisis the phospholipid concentration of these cells is high even at a time when there are relatively few new

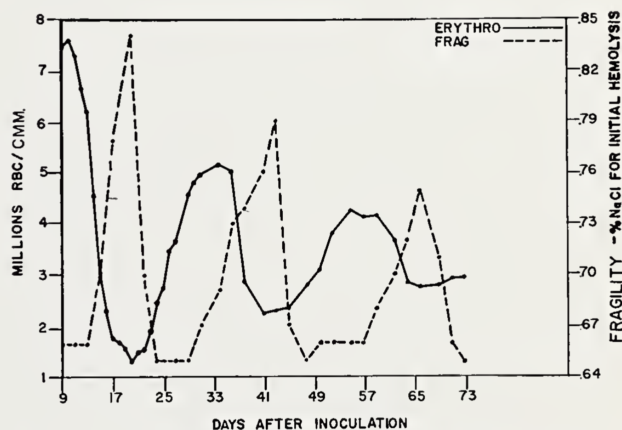


Figure 1. Relationship of erythrocyte count and osmotic fragility of erythrocytes, in calves infected with **A. marginale.**

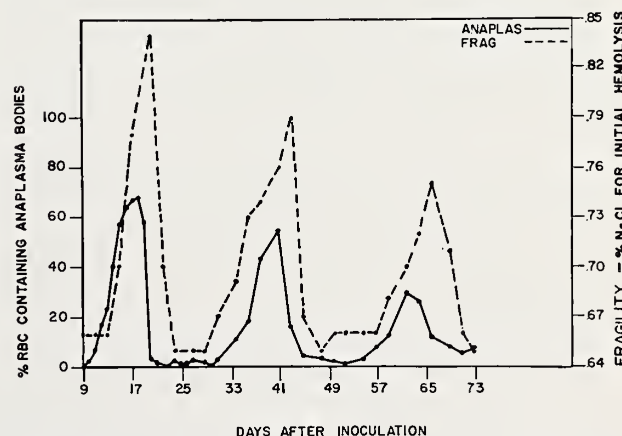


Figure 2. Relationship of osmotic fragility of erythrocytes and marginal body count in calves infected with **A. marginale.**

*The data reported herein resulted from a cooperative research program in the Department of Veterinary science, Louisiana State University involving the author, Dr. D. M. Bedell, Mr. G. T. Schrader, Mr. T. E. Rogers, and Mr. R J. Lousteau.

Funds for this research project were obtained, in part, from the Louisiana Agricultural Experiment Station and the National Institutes of Health, National Institute of Allergy and Infectious Diseases under grant E-2250.

cells in the peripheral circulation. It is further observed that once the marginal body count reaches approximately two-thirds of its peak value the concentration of erythrocytic phospholipid decreases rapidly.

Our thoughts on these particular changes have been that, in some manner which is unexplained at the present time, the organism utilizes the active phosphate of the erythrocytic phospholipids as an energy source for the synthesis of nucleic acids and for subsequent multiplication.

The phospholipid concentration of the plasma is altered drastically during the disease (Fig. 4). Once the initial changes occur and the phospholipids of the plasma decrease in concentration their level does not return to pre-infection values at any time throughout the disease. We have thought that the precursors of phospholipids are utilized to a greater extent for the biosynthesis of erythrocytic phospholipids rather than for plasma. In other words, synthesis of phospholipids for the structural integrity of new cells takes preference over the synthesis of plasma phospholipids.

To show a further relationship of physical change to chemical alterations as indicated by erythrocytic fragility and total phospholipid concentration of the erythrocyte we observe an inverse relationship, that is, as the concentration of phospholipid of the cell membrane decreases there is a corresponding increase in fragility values (Fig. 5).

All of these data give substantial evidence for both chemical and physical alterations in the erythrocytes during infections of *A. marginale* in the splenectomized calf. We believe that these cellular modifications promote the removal of the erythrocytes by the reticulo-endothelial system. The cells are either detected directly after the changes occur or an antibody is produced against the altered membrane which sensitizes it and is subsequently removed by the reticulo-endothelial system (Fig. 6). Dimopoullos and Bedell, (2).

Although we initially studied the entire phospholipid fraction of the erythrocytic membrane, further investigations on the partition of the individual fractions demonstrated both qualitative and quantitative changes. In erythrocytes obtained during the anemic crises the lecithins had practically disappeared and there were decreases in the concentrations of cephalins and sphingomyelins (Fig. 7). The values of these phospholipid fractions returned to normal when new cells were regenerated. Schrader and Dimpoullos, (5).

Chemical changes in the erythrocytic membrane which are related to the decrease in the concentration

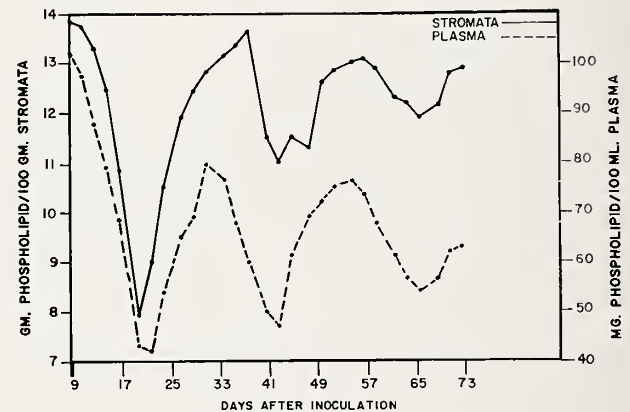


Figure 4. Relationship of total phospholipid concentrations of erythrocytic stromata and plasma in calves infected with **A. marignale.**

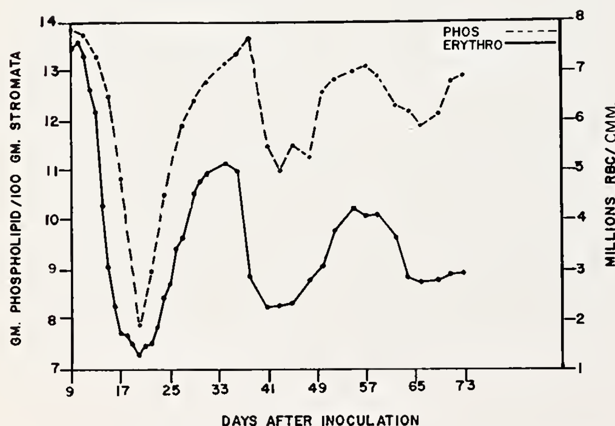


Figure 3. Relationship of total phospholipid concentration of erythrocytic stromata and erythrocyte count in calves infected with **A. marginale.**

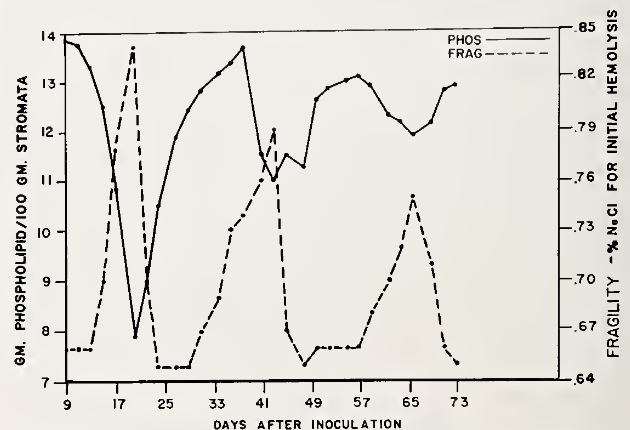


Figure 5. Relationship of total phospholipid concentration of erythrocytic stromata and osmotic fragility of erythrocytes in calves infected with **A. marginale.**

of phospholipids can also be demonstrated with the naked eye. Dimopoullos and Bedell, (1). The flocculating properties of stromata are changed during the course of the disease. As the phospholipid concentration decreases the stromata tend to remain in suspension for relatively long periods of time. The differences observed in these flocculating properties are due to a change in the isoelectric point of the erythrocytic membrane. Since the phospholipids are acidic in nature the isoelectric point of the cell is shifted toward a more alkaline pH value because of the decrease in phospholipid concentration.

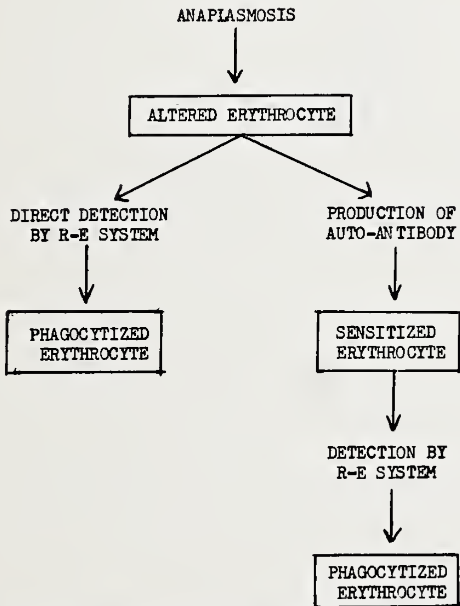


Figure 6. Hypothetical schemes in the initiation of the anemia in anaplasmosis.

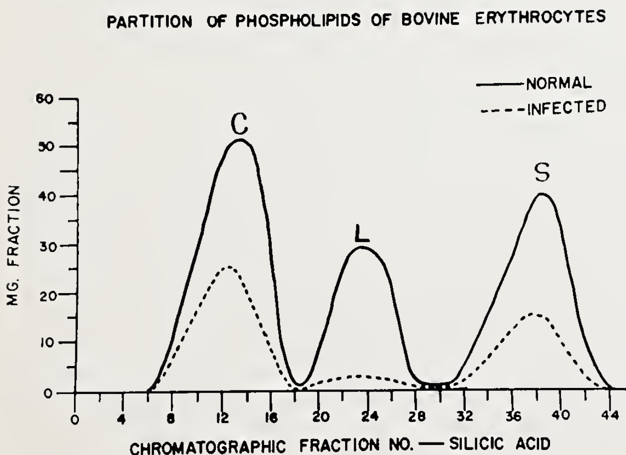


Figure 7. Partition of cephalins, lecithins, and sphingomyelins in the phospholipid fractions of erythrocytic stromata in bovine anaplasmosis.

It has been established that the phospholipids of the erythrocytic membrane dominate the surface properties of the erythrocyte. Due to the fact that there is a change in the isoelectric point of the cell as a result of the decrease in the phospholipid concentration it would be expected that alterations in the buffering capacity of the cell would also occur. Preliminary studies on the buffering capacities of erythrocytes from normal and infected calves demonstrate that infected cells have a significantly lower buffering capacity than normal cells.

Our studies on the alterations occurring in the serum proteins in anaplasmosis were initiated to determine if qualitative and quantitative changes could be observed which could serve as diagnostic aids. Dimopoullos, Schrader, and Foote, (3). Although the changes did not appear to be specific for the disease certain quantitative changes occurred which were of interest in subsequent immunochemical investigations on complement-fixing antibodies. During the acute stages of anaplasmosis the serum *alpha*- and *beta*-globulins increased in concentration, whereas the concentration of *gamma*-globulin decreased. As the animal recovered from the anemia and progressed into the convalescent stages the concentrations of *alpha*- and *beta*-globulins returned to normal levels and the *gamma*-globulin concentration increased and remained elevated for a relatively long period of time. Due to these serum protein changes it was suggested that the complement-fixing antibodies may reside in the *alpha*- and *beta*-globulins during the acute disease and during the convalescent period the complement-fixing antibody activity would be associated with the *gamma*-globulin (Table 1).

Further studies of these changes and separation of the individual serum proteins into multiple fractions showed the following results. In the acute stages of the disease the complement-fixing antibody activity was associated with the *alpha*- and *beta*-globulins of lower mobilities and the *gamma*-globulins of higher mobility. In convalescent sera the antibody activity

Table 1—Absolute and relative concentrations of serum protein fractions in acute and convalescent stages of anaplasmosis

Serum fraction	Protein conc. (acute)		Protein conc. (convalescent)	
	absolute (g/100ml.)	relative (percent)	absolute (g/100ml.)	relative (percent)
gamma-glob.	1.70	27.0	3.18	45.0
beta-glob.	1.21	19.3	0.76	10.5
alpha-glob.	1.17	18.7	0.92	13.0
albumin	2.22	35.0	2.26	31.5
T. S. P.*	6.30	---	7.12	---

*Total serum protein concentration

resided in the *beta*-globulins of lower mobility and the *gamma*-globulins of higher and intermediate mobilities. Complement-fixing antibody activity was not detected in the alpha-globulin fraction during convalescence. Rogers and Dimopoullos, (4), (Fig. 8 and 9).

Our plans for future investigations in these phases of research on anaplasmosis call for studies on the chemical composition of the various lipid classes of the erythrocytic and plasma lipids, kinetics of phospholipid turnover, studies on the electrical properties and permeability of erythrocytes, and characterization of the antigenic principle which is active in the complement-fixation test for anaplasmosis.

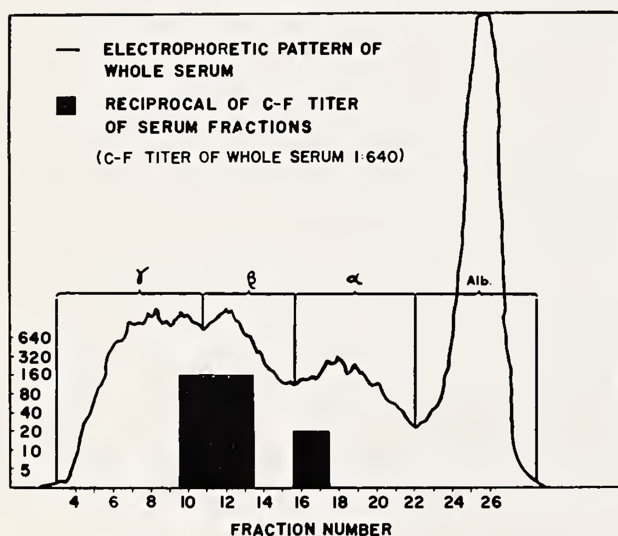


Figure 8. Complement-fixing antibody activity in acute phase sera in anaplasmosis.

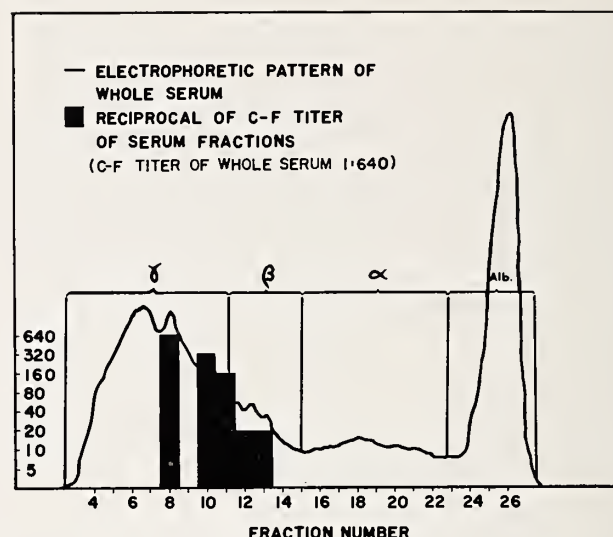


Figure 9. Complement-fixing antibody activity in convalescent phase sera in anaplasmosis.

References

1. Dimopoullos, G. T., and Bedell, D. M. (1960). Studies of bovine erythrocytes in anaplasmosis. II. Role of chem-properties of stromata in water. Proc. Soc. Exptl. Biol. & Med., *105*: 463-466.
2. Dimopoullos, G. T., and Bedell, D. M. (1962). Studies of bovine erythrocytes in anaplasmosis. II. Role of chemical and physical changes in erythrocytes in the mechanism of anemia in splenectomized calves. Am. J. Vet. Res., in press.
3. Dimopoullos, G. T., Schrader, G. T., and Foote, L. E. (1960). Electrophoretic studies of bovine serum. III. Serum protein changes in anaplasmosis. Am. J. Vet. Res. *21*, 222-225.
4. Rogers, T. E., and Dimopoullos, G. T. (1962). Fractionation and activity of complement fixing antibodies in bovine anaplasmosis. Bact. Proc., *62*: 142.
5. Schrader, G. T., and Dimopoullos, G. T. (1962). Partition of erythrocytic phospholipids in bovine anaplasmosis. Bact. Proc., *62*: 143.

Radioiron and Erythropoietic-Marrow Studies of Young Calves Administered Serial Doses of Filtered Serums From Anaplasma Infected Calves

Lon E. Foote, Sam L. Hansard and Joann Parker

Summary

The erythropoietic suppression syndrome of anaplasmosis anemia was investigated by concomitant radioiron, erythropoietic bone marrow and hematologic studies using 8 infected and 3 non-infected donor calves and 4 normal recipient calves. Initial suppression of erythropoiesis was demonstrated in serums of infected animals soon after the appearance of erythrocytes containing anaplasma bodies and up to the time of peak marginal body count. Erythrophagocytosis or hemolysis were evidenced during this same period, but were not observed in calves receiving normal serums or in calves receiving serums from calves killed during the recovery period of the disease.

Introduction

Previous radiochemical and hematologic studies concerned with the mechanics and pathogenesis of anemia associated with induced anaplasmosis in more than 70 non-splenectomized calves have been reported (14, 15, 16). These data indicated an initial supression of the erythropoietic system with reduced iron utilization, total red blood cell (RBC) count, hemoglobin, and decreased red cell life span early in the course of the disease. Studies by Baker *et al.* (2) indicated that total anemia was associated with RBC destruction with no suppression of erythropoiesis. It is self-evident, however, that erythropoietic suppression is not demonstrated by chromium-51 tagged cells.

It has been pointed out that this initial suppression was transient in nature (15, 16) and was followed by a marked compensatory response of erythropoiesis in animals during the recovery period of the disease. This response resulted in increased hemoglobin, then in total erythrocyte count 2 to 4 days following the occurrence of peak anaplasma bodies. However, when the initial suppression persisted, individuals died with the disease. Evidence of definite erythrocyte destruction was verified by subsequent ferrokinetic studies and cross transfusion of chromium-51 labeled RBC into normal and infected calves (16). The absence of a definite correlation between the number of RBC containing bodies and severity of the disease also has been reported (16, 23).

The purpose of this study was to investigate further the nature of the erythropoietic suppression syndrome and its importance in anaplasmosis anemia. Concomitant radiochemical, erythropoietic bone marrow and hematologic procedures were employed.

Materials and Methods

Four grade recipent calves 13, 44, 8 and 39, 1 to 4 months of age, were administered intravenously processed serums from 3 normal and 8 anaplasma induced donor calves, 2 to 5 months old. All calves were fed a 16 percent protein maintenance ration during the experiment. None of the calves were splenectomized, and all were negative to the complement-fixation test prior to the experiments. Each donor calf was infected with 100 ml. of blood taken from one splenectomized anaplasmosis-carrier. Recipient and donor calves maintained in metabolism units (17) were administered intravenously single tracer doses (30-50 micro-curies) of iron-59 citrate to provide criteria for response measurements. The infected donor calves were administered the isotope 3 to 6 days after inoculation with infective blood. At specific times after the onset of anaplasmosis, as indicated by iron-59 uptake and marginal body count, the 8 infected donor calves were sacrificed by exsanguination from the jugular veins or carotid arteries. The non-infected donor calves were also killed by exsanguination. Serums were harvested from all donor calves, checked for radioactivity, passed through a Seitz S3 filter of 0.1 micron porosity, and stored at —65° F until used. Selected tissue samples from the donor calves were collected for histologic studies and radiochemical measurements.

Filtered serums from the non-infected calves, 6, 14, and 16, were administered to calf 13; those from the infected calves, 33, 34, 37 and 4, 5 and 26, 28 and 29, were administered to the respective recipient calves 44, 8 and 39. Only calf 39 was administered individual separate serums from his respective donor calves. Recipient calves 13, 44 and 8 were inoculated with the mixed serums from their respective donor calves. When RBC iron-59 incorporation approximated 8 to 25 percent of the total dose injected, each recipient calf was quantitatively administered the initial serial aliquot (4.8 ml./lb. body wt.) of donor serums via the jugular vein. The time intervals between the first and second, second and third, and third and fourth doses of the serums to all recipient calves were approximately 19, 9 and 13 hours, respectively. The time interval between the remaining doses of the serums was 12 hours.

Erythropoietic bone marrow samples were procured with a trephine 5 mm. in diameter, from the dorsal ends of the right and left eighth to twelfth ribs, inclusive, before and after the donor and recipi-

ent calves were administered the infective blood and filtered serums, respectively. Specimens obtained from the dorsal ends of the eighth to twelfth ribs inclusive of a normal 3-month-old calf indicated the erythropoietic cellular composition of these bones to be similar. Ribs were chosen as bone marrow sources to provide for both marrow sections and marrow cell impression smear count studies. Prior to taking each biopsy the area was closely clipped, washed and tincture of Zephiran[1] was applied. Ten ml. of 2 percent procaine hydrochloride was injected into the skin, fascia, muscles and on the periosteum of the chosen rib. An incision, one and one-half inches long, was made through the skin, muscles and fascia to the rib. A specimen of the bone marrow from each selected rib was then obtained by means of the trephine and the incision was closed with chromic catgut, size 0. Erythropoietic bone marrow smears were made on clean glass slides and permitted to dry. Specimens were then immediately placed in buffered formalin, weighed and radioiron activity determined. Subsequently, marrows were decalcified, embedded, sectioned and stained with hemotoxylin-eosin for histologic studies.

The marrow impression smears were stained with Wright's or May Greenwald's and counter-stained with Giemsa's. Sum totals of 500 myelocytic and erythrocytic cells were counted on each impression smear and classified as myelocytic, prenormoblastic and normoblastic cells. The prenormoblasts include rubriblasts, prorubricytes and rubricytes. The marrow normoblasts included only metarubricytes.

Blood samples from donor and recipient calves were drawn routinely for total RBC count, percentage of erythrocytes containing bodies, hemoglobin, percent packed cell volume (PPCV) bilirubin and radioactivity values. Erythrocyte counts were made with a Haemoscope.[2] Hemoglobin was measured by the cyanmethemoglobin method (6), and bilirubin was determined by the Van den Bergh procedure as modified by Malloy and Evelyn (12, 20).

The recipient calves, 13, 44, 8 and 39, were subsequently splenectomized 13, 3, 16, and 7 weeks, respectively, after the last serums were administered in order that the absence of anaplasma infections could be verified and residual radioactivity storage measured.

Results

Effects upon RBS iron-59 Incorporation. — The effects of anaplasmosis on RBC iron-59 uptake in the infected donor calves are illustrated in Figures 2, 3, 4 and the RBC iron uptake of non-infected calves is shown in Figure 1. The effects of serially administered filtered serums from non-infected and infected calves on recipient calves are also shown on the graphs.

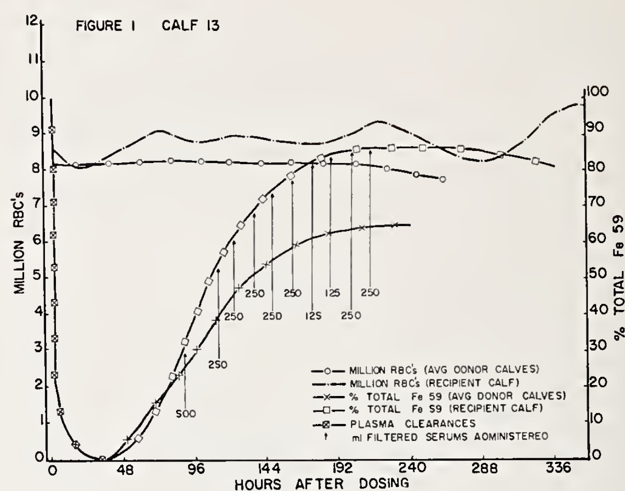


Figure 1.

These data indicate that when serums are harvested from non-infected calves and serially injected into a normal calf (Fig. 1) the RBC iron-59 incorporation is stimulated somewhat and uptake values exceed that evidenced in the donor calves. The apparent erythropoietic factor (erythropoitin) was not demonstrated in serums from anaplasmosis infected, nonsplenectomized calves in previous experiments.[3] However, the absence of suppressive or erythrophagocytic factors in these normal calves was significant.

When a normal calf was serially administered serums harvested from calves sacrificed during the initial stages of experimental anaplasmosis (Fig. 2) RBC iron-59 uptake was markedly reduced. Values indicated not only the delayed radioiron incorporation by RBC, but also the prevalence of erythrophagocytosis or hemolysis of cells tagged *in vivo* with iron-59. These same effects were further evidenced when a normal calf was serially administered serums from animals sacrified 8 days after anaplasma bodies appeared (Fig. 3) indicating that the factor(s) were still present at approximate peak body count.

However, when serums harvested from calves killed 3 to 6 days after peak marginal body counts were serially injected into a normal calf (Fig. 4), neither depression *in vivo* of RBC iron-59 uptake was observed, nor was there indication of erythrophagocytosis. This was indicated by the apparent normal iron incorporation rate and by the iron utilization values. These findings were substantiated by results of concomitant marrow and hematologic studies.

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[1]Winthrop Laboratories, New York 18, New York

[2]Haemoscope Corporation, New York 17, New York.

[3]Gurny, C. W.: Personal Communication. University of Chicago Medical School, Chicago, (1960).

Erythropoietic Bone Marrow and Hematologic Effects. — Results of the erythropoietic bone marrow studies, tissue sections and impression smears, and the concomitant hematologic values substantiated the iron-59 findings. Sections of the marrows of calves 8 and 44 showed suppression of erythropoiesis. The number of erythroblastic cells in the erythroblastic cell islands were decreased after administration of the second serum dose; however, this characteristic began to disappear 8 to 10 days following the last dose of serums administered to calves 44 and 8. The suppression was reflected in marrow erythroblastic cell counts of impression smears (Tables 2, 3) and indicates a decrease in development of normoblasts (metarubricytes) as compared to counts made previous to the administration of serums. These findings were not observed for calves 13 and 39 (Tables 1, 4).

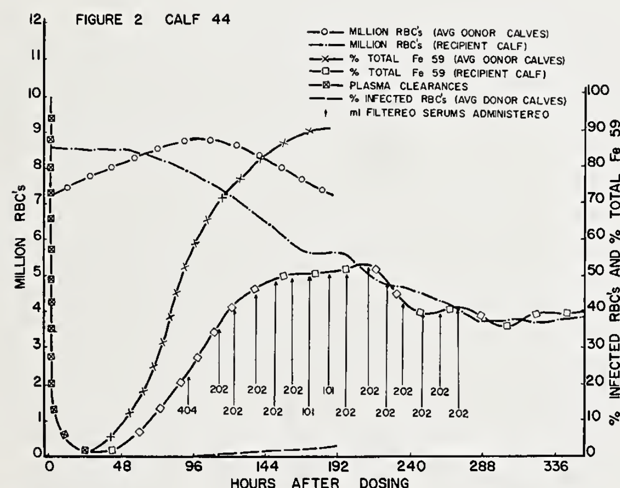

Figure 2.

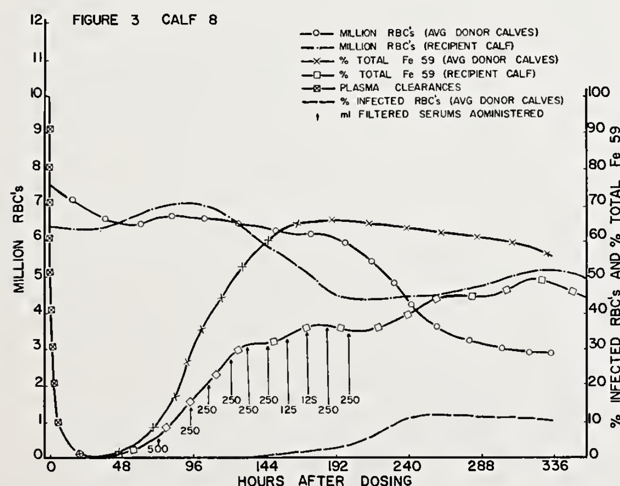

Figure 3.

The depreciation of the total RBC and subsequent decline of the iron-59 curve of calves 8 and 44, after the administration of serums, indicated the presence of erythrophagocytosis. Serums of calves 4 and 5 and of calves 33, 34 and 37 did not contain isolysins against the erythrocytes of calves 8 and 44, respectively[4]. Although the serums of calves 6, 14 and 16 and those of 26, 28 and 29 were not tested for isolysins against erythrocytes of calves 13 and 39, iron utilization curves indicate the absence of isolysins in these calves.

Hemoglobin values of calves 8 and 44 depreciated from average pre-dose normals of 10.8 and 11.0 gm. per 100 ml. of blood to 7.5 and 6.4 gm., respectively. The PPCV values of these calves were reduced from average pre-dose values of 27.4 and 33.5 to low values of 19.3 and 19.8, respectively. The low values for hemoglobin and PPCV for both calf 8 and 44 were reached one day after the last serums were administered.

The total RBC values of calf 13 increased from pre-dose averages of 8.7×10^6 to an excess of 9.8×10^6/mm.^{3} Hemoglobin and PPCV increased from

Table 1—Erythropoietic Marrow Impression Smear Counts of Recipient Calf No. 13

Date	% Myelocytic Cells	% Prenormoblastic Cells	% Normoblastic Cells	Rib
11-16-61	40	26	34	8th left
11-16-61	40	30	30	12th right
11-17-61	38	32	30	8th right
11-19-61	39	33	28	9th left
11-20-61	37	33	30	9th right
11-21-61	40	31	29	10th left
11-22-61	37	33	30	11th left
11-25-61	38	32	30	12th left

Recipient calf 13 was administered the first dose of mixed filtered serums from donor calves 6, 14 and 16 on November 17; 10:15 A.M., and the last dose on November 22; 3:00 A.M.

Table 2—Erythropoietic Marrow Impression Smear Counts of Recipient Calf No. 44

Date	% Myelocytic Cells	% Prenormoblastic Cells	% Normoblastic Cells	Rib
1-24-62	41	26	33	10th right
1-26-62	35	32	33	9th left
1-29-62	38	29	33	10th left
1-31-62	40	37	23	11th left
2-1-62	39	39	22	12th left
2-2-62	40	37	23	8th left
2-3-62	31	44	25	11th right
2-5-62	38	42	20	11th right
2-6-62	35	47	18	12th right
2-8-62		No slide		9th right
2-11-62	30	46	24	12th right
2-14-62	38	40	22	8th right

Recipient calf 44 was administered the first dose of mixed filtered serums from donor calves 33, 34 and 37 on January 29; 9:45 A.M., and the last dose on February 5; 3:00 P.M.

[4]Stormont, C.: Personal Communication. School of Veterinary Medicine, University of California, Davis, (1962).

12.55 to 14.9 gm. percent and 35.0 to 44.0 PPCV, respectively, 21 days after the last serums were administered. This further substantiated the iron-59 utilization values illustrated in Figure 1.

The total RBC count, hemoglobin and PPCV values did not vary appreciably in calf 39 during this study, and none of the recipient calves showed immature erythrocytic cells, e.g., basophylic, basophylic punctated or nucleated cells in peripheral bloods.

Of the 4 recipient calves, only one, calf 44, showed significant increases in bilirubin values during the period the serums were administered. Five days after the first dose of serums the bilirubin values of calf 44 were as follows: Direct, 0.45, indirect, 0.85 and total 1.31 mg. per 100 ml. of plasma. Dacie (8) states that "bilirubin concentration is an unreliable measure of hemolysis, as it depends not only on the amount of pigment produced, but also on the efficiency of the liver in excreting it".

Complement-fixation tests of recipient calves 8, 39 and 44 indicated they became passive reactors and later became negative to the complement-fixation test before splenectomy. Recipient calf 13, however, did not show positive reaction to the test at any time.

None of the recipient calves have developed anaplasmosis to date indicating the absence of the infective agent.

Table 3—Erythropoietic Marrow Impression Smear Counts of Recipient Calf No. 8

Date	% Myelocytic Cells	% Prenormoblastic Cells	% Normoblastic Cells	Rib
10-26-61	38	32	30	8th right
10-27-61	37	32	31	8th left
10-28-61	41	30	29	12th right
10-30-61	36	36	28	11th left
11-1-61	44	36	20	11th right
11-3-61	47	37	16	9th right
11-6-61	50	32	18	9th left
11-8-61	48	27	25	10th right
11-10-61	42	31	27	10th left
11-13-61	40	29	31	12th left
11-15-61	41	23	36	12th right
11-16-61	43	26	31	8th left
11-24-61	44	25	31	12th left

Recipient calf 8 was administered the first dose of mixed filtered serums from donor calves 4 and 5 on October 27; 2:40 P.M., and the last dose on November 1; 8:45 P.M.

Table 4—Erythropoietic Marrow Impression Smear Counts of Recipient Calf No. 39

Date	% Myelocytic Cells	% Prenormoblastic Cells	% Normoblastic Cells	Rib
1-6-62	38	33	29	9th right
1-8-62	37	34	29	11th left
1-9-62	39	32	29	12th right
1-10-62	36	34	30	8th right
1-12-62	39	33	28	9th left
1-13-62	37	34	29	10th right
1-15-62	38	32	30	10th left
1-17-62	36	32	32	8th left

Recipient calf 39 was administered the first dose of separate filtered serums from donor calves 26, 28 and 29 on January 6; 1:40 P.M., and the last dose on January 11; 7:00 P.M.

Discussion

These studies indicate the anemia of anaplasmosis to be complex in its mechanism. Erythrocyte iron-59 utilization curves show the apparent presence of two factors in the serums of young, non-splenctomized, infected donor calves killed either before or at approximate peak anaplasma body count. One of these factors suppresses erythropoeisis and the other appeared to be either hemolytic or erythrophagocytic or both. The suppression factor was also evidenced in bone marrow studies and by the rapid decline of total erythrocyte count, hemoglobin and PPCV values observed in recipient calves 8 and 44. Neither of these factors were shown by radioiron, bone marrow or hematologic studies of recipient calf 13 receiving serums from non-infected donor calves (Fig. 1), nor in like studies of recipient calf 39 receiving serums from infected donor calves (Fig. 4) killed 3 to 6 days following peak anaplasma body counts.

Suppressive Factors(s)—The suppressive factor(s) were reflected in studies of erythropoietic marrow sections and erythroblastic cell counts of marrow impression smears. The erythroblastic cells of the erythroblastic cell islands were reduced in numbers and the numbers of normoblasts were decreased. Similar observations were found in a previous study (13) of adult cows administered large amounts of infected packed RBC for the production of CF antigen. However, the number of erythroblastic cell islands and cells of the islands present were reduced, and the reduction in bone marrow normoblasts (metarubricytes) and increase in myelocytic cells were more accentuated. It has been reported (4) that bone marrow of infected cattle produce only 60 percent of the normal number of erythrocytes during the last 15 days of the prepatent period. Depression of erythropoietic bone marrow in anaplasmosis affected calves also has been reported by Ristic (21). Boynton (3) observed a

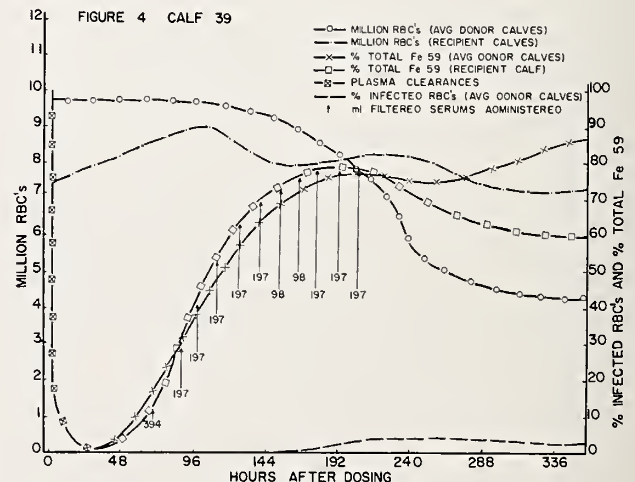

Figure 4.

swift recovery of anaplasmosis affected animals when rapid regeneration of their RBC was evident. This was detected by the appearance of megalocytes in the peripheral blood, followed in order by chromatophilic, punctate basophilic and finally by large numbers of nucleated red cells.

Studies of marrow sections reveal structural relationship of the cells while this is lost in marrow smears or puncture biopsies. In this experiment, studies of sections permitted a clearer concept of erythropoietic suppression than did cellular counts of the marrow impression smears which are somewhat similar to puncture biopsies. Dameshek, *et al.,* (10) Custer (7) and Brock (4) have indicated that puncture biopsies may be inaccurate. Diggs *et al.,* (11) however, do not indicate favor for or against puncture biopsies. Dameshek (9) states that marrow smears could not be excelled for exact cytological study. In an extensive study of erythropoietic marrow in bovine embryos, calves and adult cattle cellular composition of marrow from various bones were found to be quite similar (25). Calhoun (5) suggests bone marrow studies should be used more in veterinary medicine, and in such studies of adult cattle, ribs anterior to the twelfth be used.

It appears that serial studies of impression smears give a general trend of the activity of the bone marrow, and it is evident that suppression of bone marrow is of utmost importance in peracute and severe acute cases of anaplasmosis.

In comparative radioiron and hematologic procedures Hansard and Foote demonstrated the progressive and variable nature of anaplasmosis anemia. Results implied a more direct relationship to erythrocyte production than to iron utilization. During the onset of the disease there was a compensatory production of short lived erythrocytes (possibly malformed) released some 2 weeks before acute clinical signs were observed. Erythrocyte iron utilization, and plasma iron clearance were decreased and total iron excretion increased from 5 days before and until peak red cell marginal bodies were evidenced. These findings were reflected in the low iron-59 content found in spleen, liver and kidneys of calves killed an estimated 6 days before peak marginal body count.

Suppression of erythropoiesis may be responsible for the observed wide seasonal variations in disease severity. It is a known fact that the only treatment effective in assuring the recovery of some acute cases of anaplasmosis is the administration of multiple blood transfusions.

Erythrophagocytic Factor(s) — The presence of autoantibody (hemolysins) has been recently demonstrated in calves following the decline of PPCV (22) and persisted, although it declined in concentration, for some 12 days after the lowest recorded PPCV. The hemolytic phenomenon in anaplasmosis was reported as improbable because of the low content of free complement in bovine serum and the absence of free hemoglobin in the plasma during the period of progressive anemia. It was suggested that the anemia is probably brought about by the phagocytosis of the sensitized erythrocytes. Geer[5], Seger[6] and Foote (13) have reported erythrophagocytosis in anaplasmosis of splenectomized and non-splenectomized cattle.

However, it has been observed in man and other species that several plasma proteins (1, 18, 19, 24) (haptoglobins) have the property of combining with the hemoglobin liberated into plasma which transports it as a hemoglobin-haptoglobin complex. The marked increase in plasma iron and the decrease in total hemoglobin and plasma iron turnover rate in anaplasmosis induced calves observed 3 days before peak RBC anaplasma bodies were evidenced (15) may reflect the presence of haptoglobin levels in bovine plasma. The possibility of a hemoglobin-haptoglobin complex has been suggested (13) in cattle affected with acute or peracute anaplasmosis. Hemoglobinemia and hemoglobinuria in most cases of anaplasmosis therefore is prevented.

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[5]Geer, Jack: Personal Communication. Medical School, Louisiana State University, New Orleans, (1958).

[6]Seger, C. L.: Personal Communication. Department of Veterinary Science, Louisiana State University, Baton Rouge, (1960).

References

1. Aber, G. M., Neale, F. C., and Northam, B. E.: Binding of Haemoglobin. Brit. Med. J., 2, (1957): 1368.
2. Baker, N. F., Osebold, J. W., and Christensen, J. F.: Erythrocyte Survival in Experimental Anaplasmosis. Am. J. Vet. Res., 22, (1961): 590-596.
3. Boynton, W. H.: Further Observations on Anaplasmosis. Cornell Vet., 22, (1932): 10-28.
4. Brock, W. E.: A Study of the Pathogenesis of the Anemia in Acute Anaplasmosis. Doctor of Philosophy Dissertation, Oklahoma University, (1958): 1-93.
5. Calhoun, M. L.: A Cytological Study of Costal Marrow. II. The Adult Cow. Am. J. Vet. Res., 15, (1954): 395-404.
6. Crosby, W. H., Munn, J. I., and Furth, F. W.: Standardizing a Method for Clinical Hemoglobinometry. U. S. Armed Forces Medical Journal, 5, (1954): 693-703.
7. Custer, R. P.: Studies on the Structure and Function of Bone Marrow. I. Variability of the Hemopoietic Pattern and Consideration of Method of Examination. J. Lab. Clin. Med., 17, (1932): 951-959.
8. Dacie, J. V.: The Haemolytic Anaemias, Congenital and Acquired. Part I — The Congenital Anaemias. 2nd Ed. Grune and Stratton, New York, (1960): 6.
9. Dameshek, W: Biopsy of the Sternal Bone Marrow. Am. J. M. Sci., 190, (1935): 617-640.
10. Dameshek, W., Henstell, H. H., and Valentine, E. H.: The Comparative Value and the Limitations of the Trephine and Puncture Methods for Biopsy of the Sternal Marrow. Ann. Int. Med., 11, (1937): 801-818.

11. Diggs, L. W., Sturm, D., and Bell, A.: The Morphology of Human Blood Cells. W. B. Saunders Co., Philadelphia and London (1956).

12. Ducci, H., and Watson, C. S.: The Quantitative Determination of Serum Bilirubin with Special Reference to the Prompt-Reacting and the Chloroform Soluble Types. J. Lab. Clin. Med., 30, (1945): 293-300.

13. Foote, L. E.: The Pathogenesis of the Anemia of Anaplasmosis. Master of Science Thesis, Texas A & M College (1961): 1-37.

14. Hansard, S. L., and Foote, L. E.: Effects of Anaplasmosis on Physiological Behavior of Chromium 51 and Iron 59 in Young Calves. Fed. Proc., 17, (1958): 1875.

15. Hansard, S. L., and Foote, L. E.: Anemia of Induced Anaplasmosis in the Calf. Am. J. Phys., 197, (1959): 711-716.

16. Hansard, S. L., and Foote, L. E.: Absorbtion and Physiological Movement of Iron in Calves Induced with Anaplasmosis. J. Animal Sc., 20, (1961): 395.

17. Hansard, S. L., Comar, C. L., and Plumblee, M. P.: Radioisotope Procedures with Farm Animals. Nucleonics, 9, (1951): 13, 38.

18. Laurell, C-B., and Nyman M.: Studies on the Serum Haptogloblin Level in Hemoglobinemia and Its Influence on Renal Excretion of Hemoglobin. Blood, 12, (1957): 493-506.

19. Liang, C.: The Formation of Complexes Between Haemoglobins and Plasma Proteins in a Variety of Animals. Biochem. J., 66, (1957): 552-558.

20. Malloy, H. T., and Evelyn, K. A.: Bilirubin, "Standard Methods of Clinical Chemistry", Vol. 1, Academic Press Inc., New York, (1953): 11-15.

21. Ristic, M., and Sippel, W. L.: Effect of Cortisone on the Mechanism of Anaplasma Immunity in Experimentally Infected Calves. II. Studies of Pathological Changes. Am. J. Vet. Res., 19, (1958): 44-50.

22. Ristic, M.: Studies in Anaplasmosis. III. An Autoantibody and Symptomatic Macrocytic Anemia. Am. J. Vet. Res., 22, (1961): 871-876.

23. Schmidt, H., and Franklin, T. E.: Experimental Bovine Anaplasmosis and its Treatment with Trifloryl. Proc. U. S. Livestk. San. Assn., 55, (1951): 260-270.

24. Smithies, O.: Grouped Variations in the Occurrence of New Protein Components in Normal Human Serum. Nature, 175, (1955): 307-308.

25. Winqvist, G.: Morphology of the Blood and the Hemopoietic Organs in Cattle Under Normal and Some Experimental Conditions. Acta. Anat. Suppl. 21 = 1 ad. 22, 1954.

Experiences With Anaplasmosis In Species Other Than Cattle

John W. Osebold[1]

The infection of cattle by *Anaplasma marginale* and its many interesting manifestations have been the principal concern of investigators. However, the relationship of this parasite to other hosts has recently become the subject of increasing interest. This report is intended to record some observations and interpretations on anaplasmosis in species other than cattle which have been made in California during the past few years.

Black-tailed Deer Studies

Collections of blood from wild Columbian black-tailed deer (*Odocoileus hemionus columbianus*) for serological study were begun in 1957 in Mendocino County. Serum samples were stored in a freezer and then tested for complement-fixing antibodies after a group had accumulated. Preservatives were not added to the first 32 serum samples. One serum had a 2+ reaction, 7 samples gave ± readings, and 25 gave no reaction. The necessity for adding a preservative to avoid bacterial contamination of the samples during transit to the laboratory became apparent and 0.5 percent phenol was added to the next 18 samples. Fixation of complement was then much greater with three 4+ fixations at the 1:5 serum dilution, 7 suspect reactions, and only 8 negative tests. This erratic behavior in serological results was disturbing and it was decided to avoid the uncertain effect of phenol on the test system. Henceforth, samples were preserved with 1:1,000 merthiolate. As testing continued it became apparent that definitive information would not be obtained by the serological test and whole blood injection into splenectomized calves was begun. Transmission was effected in 29 of 40 calves injected with either individual or pooled blood samples obtained from 71 deer. These studies are fully reported elsewhere (1,2). Table 1 shows that 55 percent of the transmissions were made with deer blood that contained no demonstrable complement-fixing antibodies. The significance of weak serological reactions was uncertain. Among 11 calves which did not develop anaplasmosis following the injection of deer blood, 6 of

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[1]From the Department of Microbiology, School of Veterinary Medicine, University of California, Davis, California.

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Table 1—Relationship of Antibody Titer to Anaplasmosis Carrier Status of Columbian Black-tailed Deer

Anaplasmosis Transmissions			No Transmission		
CF Titer of Deer Blood Inoculum	Number of Calves	Percent of Calves	CF Titer of Deer Blood Inoculum	Number of Calves	Percent of Calves
0	16	55%	0	4	36%
±	10	34%	±	6	54%
1+ to 2+	3	10%	4+	1	9%
Total	29	100%	Total	11	100%

the inocula contained ± titers at the 1:5 serum dilution and one had a 4+ titer as determined on phenolized serum. Ticks obtained from this species of wild deer have been shown to be capable of transmitting anaplasmosis to calves (3).

The findings on black-tailed deer were in contrast to anaplasmosis in cattle wherein carrier status is usually associated with demonstrable complement-fixing antibodies. Observations made on natural infections in deer were found to correlate with experimental findings. When 4 adult non-splenectomized males were inoculated with blood from a bovine carrier, all produced antibodies, just as would be expected in bovine infections (Fig. 1). However, the titers were not high and by 56 days after infection the geometric mean titer for the group had fallen to 1:9.5. When tested 112 days after infection, none of the sera were in the reactor category for cattle (4). This same situation prevailed 11 months after infection. At that time 3 of the animals were tested for carrier status by inoculating their blood into splenectomized calves, and all 3 were still carriers (5). Splenectomized fawns also demonstrated antibody response to infection, but the titers rapidly receded below a detectable level in surviving animals (Fig. 2). Therefore, the black-tailed deer appears to represent a species which is naturally infected by this microorganism, but the animal's infectious status cannot be determined by the complement-fixation test.

Studies with other Vertebrates

Some attempts have been made to transmit anaplasmosis by inoculating calves with blood from other members of the family *Cervidae* which conceivably could be carriers. These included blood samples from 11 tule elk, 2 blesbok antelope, and a Grant gazelle (Table 2). Three of the sera had titers that would classify the animals as suspects, while the remaining 11 samples were negative. No transmissions were obtained.

In a study on elk in Wyoming, Post and Thomas (6) tested 11 animals on 3 occasions. Three animals gave 4+ fixations at the 1:5 serum dilution on all tests. The other 8 elk varied among 2+, 3+, or 4+ reactions. The blood from these animals did not

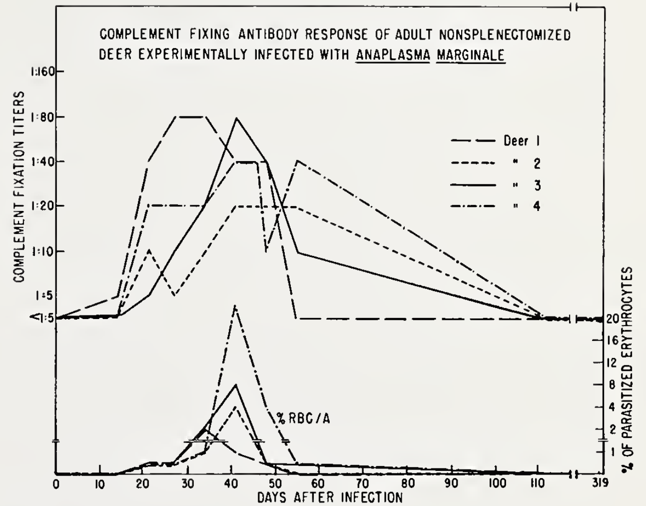

Figure 1.

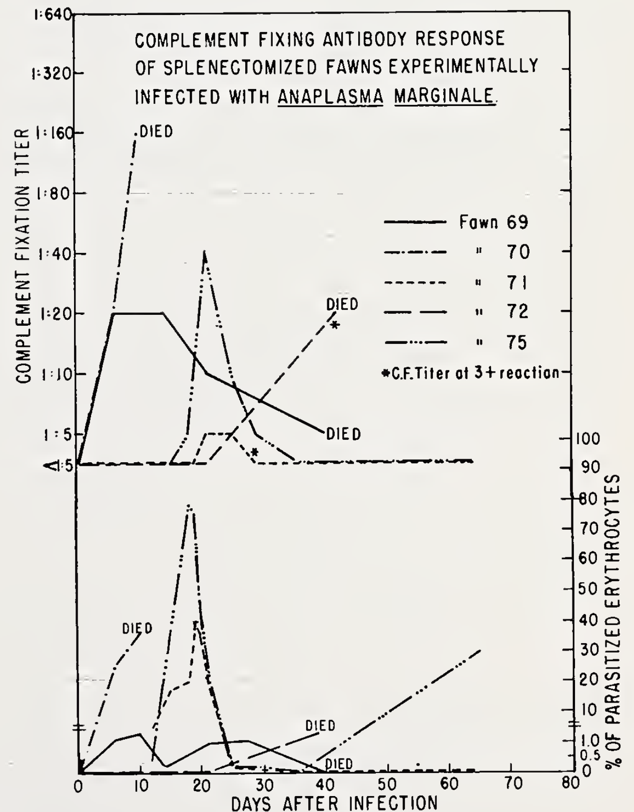

Figure 2.

transmit analpasmosis when inoculated into 2 calves. It could be inferred from the report that the complement-fixation test is prone to give false positive reactions in species other than the bovine animal.

Our experience has been quite the opposite when using merthiolate-preserved serum samples. To test the assertion that a high rate of non-specific fixation occurs among ruminants other than cattle, a wide range of zoological species was tested. Many of the samples were obtained at the San Diego Zoo while a lesser number were obtained from animals in their natural habitat. Table 3 summarizes results in the family *Bovidae*. No reactions were obtained among 6 species of antelope nor among the goat and buffalo species represented. Some reaction was obtained in Karakul sheep, and, unfortunately, we do not have experiments to demonstrate the possibility of *A. ovis* infection in those animals. Tests performed on animals in the families *Cervidae* and *Camelidae* are reported on Table 4. No 4+ fixations were obtained although Columbian black-tailed deer samples were obtained from many proven carriers. Three samples from American elk were negative. This is in contrast to the results of Post and Thomas with elk which were presumably of this species. Table 5 summarizes complement-fixation test results on sera obtained from a variety of non-ruminant mammals, birds, reptiles, and fish. Here again the negative results were of interest.

Table 2—Attempted Transmission of Anaplasmosis by Calf Inoculation with Blood from Various Species

Species	Habitat	CF Titer of Inoculum (1:5 Dil.)	Volume of Inoculum (ml.)	Splenectomized Calf No.	Result
Tule Elk	Kern	0			
" "	County,	0			
" "	Elk	0	128	366	No
" "	Reserve	0			Transmission
" "		0			
" "		0			
" "		2			
Tule Elk	Inyo	±			
" "	County,	1	200	364	No
" "	Wild	0			Transmission
" "		0			
Blesbok Antelope	San				
" "	Diego	0			No
	Zoo	0	20	840	Transmission
Roosevelt Grant	San				
Gazelle	Diego	0	10	839	No
	Zoo				Transmission

Table 3—Complement-fixation Reactions with (Anaplasma) Antigen by Various Sera from the Family (Bovidae)

Family Bovidae Species	Number of Animals Tested	Range of CF Reactions (1:5 Dil.)	Percent of Represented Reactions
Blesbok Antelope	5	0	
Eland	4	0	0=94.6%
Springbok Antelope	2	0	±= 1.8%
Blackbuck Antelope	1	0	1= 1.8%
Coke Hartebeest	1	0	2= 1.8%
Roosevelt Grant Gazelle	1	0	
Karakul Sheep	10	0-2	
Aoudad	4	0	
Nelson Bighorn	3	0	
Rocky Mountain Bighorn	2	0	
Himalayan Tahr	11	0	
Domestic Goat	6	0	
Water Buffalo	2	0	
Bison	2	0	
Cape Buffalo	1	0	
Anoa	1	0	
Total	56		

Table 4—Complement-fixation Reactions with (Anaplasma) Antigen by Various Sera from the Families (Cervidae) and (Camelidae)

Family Cervidae Species	Number of Animals Tested	Range of CF Reactions (1:5 Dil.)	Percent of Represented Reactions
Columbian black-tailed deer	160	0-3	
Mule deer	6	0	0=70.0%
Axis deer	1	0	±=14.8%
Muntjac	1	0	1= 8.7%
Tule elk	11	0-2	2= 6.0%
American elk (Wapiti)	3	0	3= 0.5%
Moose	1	0	
Subtotal	183		
Family Camelidae			
Lama	5	0-1	
Alpaca	5	0	0=93.3%
Guanaco	4	0	1= 6.7%
Bactrian camel	1	0	
Subtotal	15		
Total	198		

Table 5—Complement-fixation Reactions with (Anaplasma) Antigen by Various Vertebrate Sera

Source of Sera	Number of Animals Tested	Range of CF Reactions (1:5 Dil.)	Percent of Represented Reactions
Miscellaneous Non-ruminant Mammals (17 Species Represented)	22	0-±	0=95.5% ±=4.5%
Birds (26 Species Represented)	46	0	0=100%
Reptiles			
Snakes (14 Species Represented)	27	0	0=100%
Lizards	1	0	
Fish (3 Species Represented)	10	0	0=100%
Total	106		

Discussion and Summary

Anaplasmosis transmission studies have included inoculation of splenectomized calves with blood from Columbian black-tailed deer, tule elk, blesbok antelope, and a Roosevelt Grant gazelle. A high percentage of the deer have been shown to be infected, whereas blood from the other species did not transmit the disease.

Complement-fixation test results on sera obtained from 360 vertebrates, not including domestic cattle, gave no fixations as strong as 4+ at the 1:5 serum dilution. This is of interest for three reasons. First, it has been shown that the level of antibody in Columbian black- tailed deer recedes to a range beyond the sensitivity of this test. Therefore, present serological means cannot be expected to identify infected deer. Second, these merthiolate-preserved samples did not cause confusing non-specific fixation reported by others where phenol was presumably used as a preservative. Third, the numerous species represented in this study were affected by a great variety of apparent and inapparent parasitisms. Consequently, their sera could be expected to carry numerous antibody populations. The fact that the samples were so free of activity for *Anaplasma marginale* is a significant observation suggesting that the antigens of the organism have a higher order of immunological specificity.

References

1. Osebold, J. W., Christensen, J. F., Longhurst, W. M., and Rosen, M. N.: Latent *Anaplasma marginale* Infection in Wild Deer Demonstrated by Calf Inoculation. Cornell Vet., *49* (1959):97-115.
2. Christensen, J. F., Osebold, J. W., and Rosen, M. N.: The Incidence of Latent *Anaplasma marginale* Infection in Wild Deer in an Area where Anaplasmosis is Enzootic in Cattle. Proc. 62nd Ann. Meet. U. S. Livestock San. A., Nov. 4-7, 1958, (1959):59-65.
3. Osebold, J. W., Douglas, J. R., and Christensen, J. F.: Transmission of Anaplasmosis to Cattle by Ticks Obtained from Deer. Am. J. Vet. Res., *23*:21-23, 1962.
4. Christensen, J. F., Osebold, J. W., and Rosen, M. N.: Infection and Antibody Response in Deer Experimentally Infected with *Anaplasma marginale* from Bovine Carriers. J.A.V.M.A., *132* (April 1, 1958):289-292.
5. Christensen, J. F., Osebold, J. W., Harrold, J. B., and Rosen, M. N.: Persistence of Latent *Anaplasma marginale* Infection in Deer. J.A.V.M.A. *136,* (May 1, 1960):426-427.
6. Post, G., and Thomas, G. M.: A Study of Anaplasmosis in Elk. J.A.V.M.A. *139,* (August 1, 1961): 357-358.

Bone Marrow Studies In Anaplasmosis

C. C. Pearson, W. E. Brock and I. O. Kliewer

That the bone marrow plays a role in anaplasmosis infection has been suspected for some time. In 1954, Foote hypothesised, from indirect evidence, that the anemia of anaplasmosis was caused by a partial or a complete paralysis of the hemopoietic system (1). Ristic and Sipple (2) later showed that there was hypoplasia of the bone marrow in anaplasmosis infected calves which died following splenectomy. This paper reports studies of bone marrow aspirations started during the incubation period and continued through the early stage of convalescence in a group of splenectomized calves and in a group of 2-year-old Hereford cattle.

The splenectomized group consists of nine calves splenectomized before infection. The 2-year-old group consists of ten intact animals. No treatment was given to either group during or before the course of the disease.

Bone marrow aspirations were taken from each animal at two to four day intervals. Hemoglobin, hematocrit, total red blood cell, and total white blood cell determinations were made at the same time. After the appearance of anaplasma bodies, reticulocyte percentages were determined.

The results of these determinations for the splenectomized calves are shown in Figure 1. The data for individual calves are grouped by adjusting the results to the day 1 percent of the erythrocytes were infected with anaplasma bodies. This is shown on the figures as day zero. The percentage of erythroblasts on the bone marrow slides is determined by counting the number of erythroblasts found in a total of 500 nucleated cells.

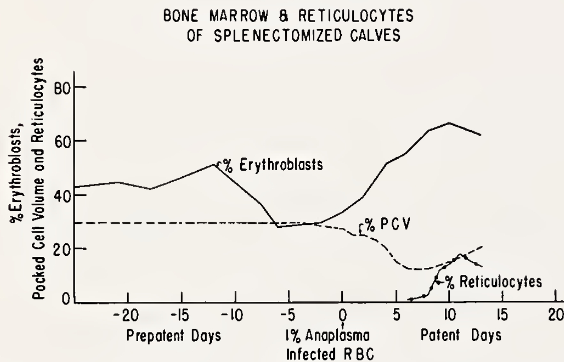

Figure 1. The average values for the percentage of erythroblasts in the bone marrow, the packed cell volumes and the percentage of reticulocytes found in the splenectomized calves.

The average pre-infection percentage of erythroblasts is 41 percent. From the ninth day before the day zero, to the third day after day zero, a period of 12 days, the percentage of erythroblasts in the bone marrow averages approximately 10 percent below the normal value. This is followed by a rapid rise in the percentage of erythroblasts over the next eight days to a peak of 66 percent.

The pre-infection hematocrit value is 29.5 percent. The hematocrit continues at this level until about the fifth day before day zero. During the next six days and before the marked decrease due to hemolysis is apparent, the hematocrit decreases 2 percent.

The apparent decrease in erythropoeisis, and the hematocrit values concurrent with this decrease, indicate a possible influence of the bone marrow on the hematocrit. Baker and Douglas (3), working with calves, have indicated the life span of the red blood cell to be 55 days. Hansard and Foote (4) show a mean red blood cell life span of 47.4 days. These data would indicate that there is, therefore, a normal turnover of approximately 2 percent of the red blood cells per day.

The calves in this experiment had a mean count of 7.50 million RBC per cmm. This would be a turnover of approximately 150,000 RBC per cmm. per day. The apparent decrease in erythropoietic activity is 10 percent. This would indicate a negative balance in red cell replacement of 15,000 RBC per cmm. per day. The decrease in erythropoeisis continues for approximately 8 days, which would produce a total estimated loss of 120,000 RBC per cmm.

An estimate of the actual loss of red blood cells during this period may be made by determining the number of red blood cells per cmm. that is represented by 1 percent of the hematocrit. In these calves 1 percent hematocrit is equivalent to approximately 254,000 RBC per cmm. Since there is a decrease during this period of 2 percent in the hematocrit values, the number lost may be estimated at 508,000 RBC per cmm.

The predicted loss estimated from the decrease in apparent erythropoiesis is 120,000 RBC per cmm., while the actual loss is estimated to be 508,000 RBC per cmm. If these estimates are valid, many more red blood cells were lost than can be accounted for by the apparent depressed bone marrow erythropoiesis. It may be possible to account for the additional red blood cell loss by assuming that it is an early reflection of hemolysis of anaplasma infected red blood cells.

The increase in the erythropoiesis in the bone marrow is reflected in the peripheral circulation by the presence of reticulocytes. These animals first show reticulocytes on the seventh patent day when the erythroblast percentage has risen to about 55 percent or slightly more than 10 percent above normal. The percentage of reticulocytes rose rapidly to a peak of 18 percent on the tenth (patent) day. By the thirteenth day, when observations were terminated, the reticulocytes had decreased to 10 percent. Concurrent with the appearance and rapid increase in the percentage of reticulocytes and the increase above normal of the erythroid elements of the bone marrow, the hematocrit ceases to drop, and at the peak of reticulocytes and erythropoiesis, starts upward.

Since four of these splenectomized calves died, it was felt that a comparison of the bone marrow of those that died with those that lived might provide some information relative to the cause of death. Figure 2 presents the data on the four calves that died and the five calves which lived. While the percentages of erythroblasts in those calves that died does not appear to decrease as low as in those that lived, the actual mean percentage decrease from the pre-infection levels is about 12.5 percent in both cases. The only apparent difference between the two groups that might have some effect upon the livability is the two day earlier rise above normal in the group that lived.

While the reticulocytes appeared in the peripheral circulation of the animals that died earlier by one day than in those that lived, the animals died too quickly for the percentage of reticulocytes to rise above 1 percent. Whether the animals died because new red blood cells were not produced rapidly, as evidenced by

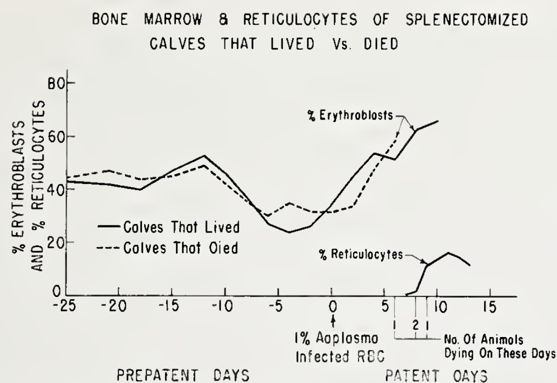

Figure 2. The average values of the percentage of erythroblasts in the bone marrow and the percentage of reticulocytes found in the splenectomized calves that lived as compared with the same values found in the splenectomized calves that died.

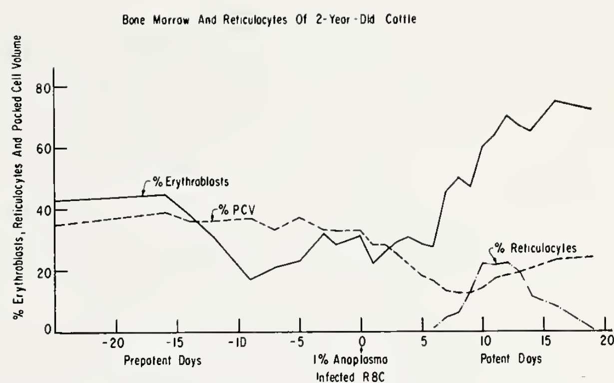

Figure 3. The average values for the percentage of erythroblasts in the bone marrow, the packed cell volume and the percentage of reticulocytes found in the 2-year-old cattle.

the lack of reticulocyte response, or the animals died too quickly for there to be any reticulocyte response cannot be determined from these data. It does appear, however, that a high percentage of reticulocytes may be indicative of a favorable prognosis.

Figure 3 presents the same determinations made from the group of older Hereford cattle. The results from the older cattle are essentially similar to those of the splenectomized calves. The percentage of erythroblasts is below normal in the older animals for twelve days, decreasing two days earlier and increasing two days later than in the group of calves. The average below normal value of the erythroblasts in the bone marrow is 16.7 percent for these twelve days.

Calculations similar to those made with the data of the splenectomized group show that there was an actual loss in the circulation of 580,000 RBC per cmm., while the loss estimated from the decrease in the percentage of erythroblasts in the bone marrow is 333,000 RBC per cmm. Although the estimated loss in this group more closely approximates the actual decrease in red blood cells, there are some 250,000 RBC which must be accounted for in some other way.

In summary, these results indicate that there is a decrease in erythroblasts in the bone marrow of some 10 to 20 percent during the incubation period in anaplasmosis, and extending 2 to 6 days into the patent period of the disease. While this apparent decrease in erythropoiesis may cause some decrease in the number of circulating red blood cells there appears to be a greater number of erythrocytes lost in some other manner. It seems possible that this additional decrease in erythrocytes may be due to hemolysis of some of the cells infected early with anaplasma.

References

1. Foote, L. E., New Information on Anaplasmosis. No. Am. Vet. 35: 19-21. 1954.
2. Ri tic, M. and Sipple, W. L., Effect of Cortisone on the Mechanism of Anaplasma Immunity in Experimentally Infected Calves. II. Studies of Pathological Changes. Am. J. Vet. Res. 19: 44-50. 1958.
3. Baker, N. J. and Douglas, J. R., The Pathogenesis of Trichostrongyloid Parasites. II. Ferrokinetic Studies in Ruminants. Am. J. Vet. Res. 18: 295-302. 1957.
4. Hansard, S. L. and Foote, L. E., Anemia of Induced Anaplasmosis in the Calf. Am. J. Physiol. 197: 711-716. 1959.

Development of Anaplasma marginale In The Living Host

Miodrag Ristic and A. M. Watrach[1]

This study presents observations made by means of electron microscopic and fluorescent antibody techniques on the development of *Anaplasma* in the bovine host.

An acutely infected splenectomized bull calf served as the source of blood used in this study. Blood specimens for fluorescent antibody studies and electron microscopy were made daily during the infection.

Based upon the frequency of occurrence of *Anaplasma* and its localization in the infected erythrocytes, four developmental stages of the organism were observed with the fluorescent antibody technique: a) early stage of initial body; b) stage of mixed population of marginal and initial bodies; c) vigorous growth and transfer stage and d) massive multiplication stage characterized by predominantly marginal bodies. During the period of vigorous growth an apparent transfer of initial and marginal bodies between adjacent erythrocytes was observed. Occasionally free forms were seen in the plasma which according to their size were either initial or marginal bodies.

Sequential studies of normal and infected erythrocytes by means of electron microscopy revealed that erythrocytes were first invaded by initial *Anaplasma* bodies on the 5th day following infection. The first marginal *Anaplasma* bodies, composed of 2 to 3 initial bodies, were observed on the 10th day post infection. Marginal bodies occurring later in the course of infection consisted of 8 to 10 initial bodies.

There was also an increase in size of some of the initial bodies. Those observed early in infection and before the first appearance of marginal bodies were generally smaller and measured approximately 0.2 μ in diameter. After the appearance of marginal bodies and during the later stages of infection initial bodies were larger, being on the average of 0.3-0.4 μ in diameter.

The invasive mechanisms of *Anaplasma* as revealed by electron microscopy involves the penetration of the erythrocytic membrane by initial bodies. The evidence of such a penetration where the initial body either enters or leaves the erythrocyte is shown in figure 1.

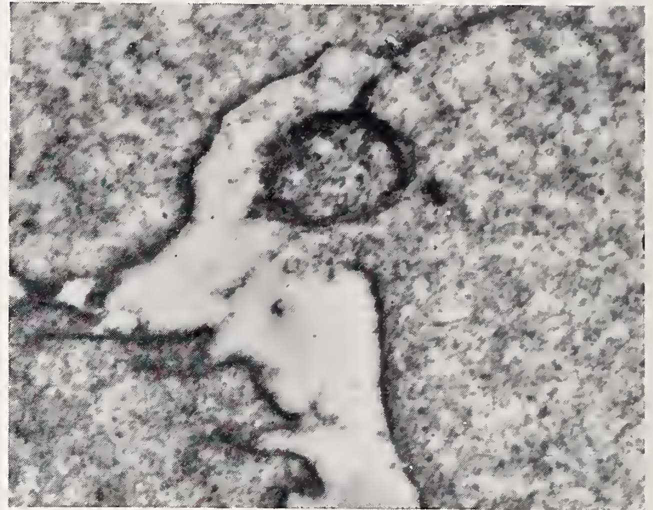

Figure 1. Ultra-thin section of **Anaplasma** infected erythrocyte showing an initial **Anaplasma** body in process of penetration of erythrocytic membrane. 60,000 X.

Static electron microscopic evidence presented in figure 2 indicates the ability of the initial body to reproduce by binary fission. Figure 2-a (arrow) shows an early stage of reproduction. This is represented by elongation of an initial body and slight constriction of its double membrane. The next stage is characterized by further constriction of the membrane (Fig. 2-b). At this stage, high electron density material (probably nucleo-proteins) is present in each of the two daughter organisms being formed. Those daughter forms, however, still remained connected by a narrow band of "plasma." The terminal stage of reproduction is illustrated in figure 2-c; the two daughter organisms are completely separated by their double membranes, but remain attached to each other. Complete separation of the daughter forms evidently follows as the final step of reproduction (Fig. 2-d). The initial bodies now appear as single subunits within the matrix of the marginal body.

Analysis of the information given here concerning the development of the causative agent of anaplasmosis in the course of an acute infection has aided our efforts to formulate and graphically illustrate a hypothesis concerning such a cycle (Fig. 3). This hypothesis which provides for inter-relationship of the various growth stages of the *Anaplasma marginale* considers the initial *Anaplasma* body to be the agent for initiation of the infectious cycle.

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[1]From the Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana.

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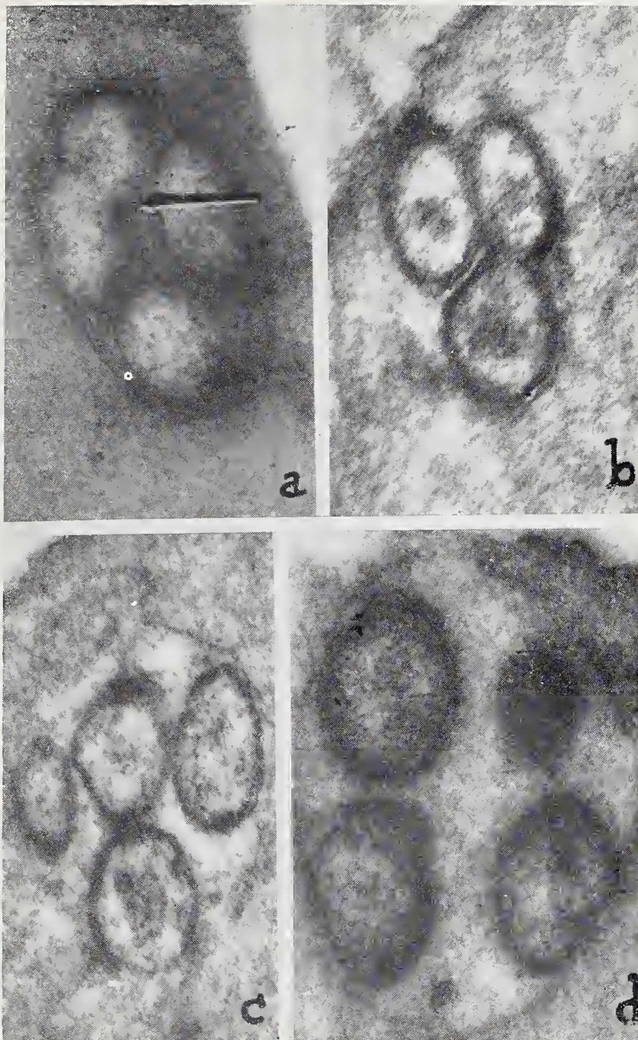

Figure 2. Ultra-thin sections of **Anaplasma** infected erythrocytes showing reproduction of initial bodies by binary fission. Early stage represented by slight constriction of the double membrane (a); further constriction of the membranes (b); formation of two daughter forms (c) and newly formed completely separated initial bodies (d). 50,000 X.

The hypothesis further proposes that the complete developmental cycle of the organism occurs in the mature erythrocytes. It is also proposed that binary fission of the initial body and its direct transfer between erythrocytes fully explains the mode of development of this agent.

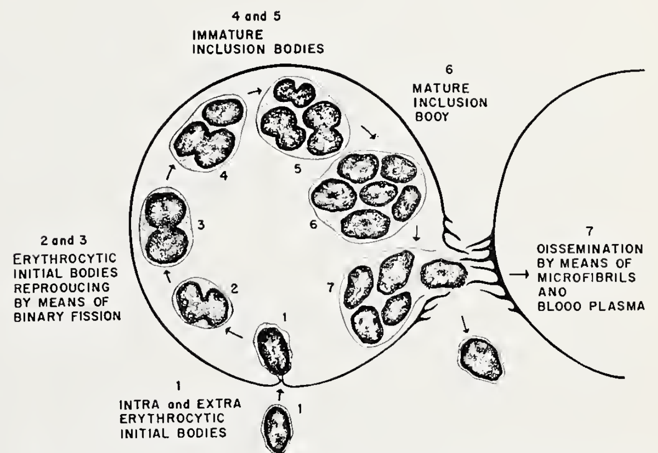

Figure 3. Hypothetical depiction of interrelationships of the various growth stages of **Anaplasma marginale** in perpetuation of this organism in a bovine erythrocyte.

Treatment

Control of Anaplasmosis by Low Level Feeding of Aureomycin During The Vector Season

J. W. Scales, J. C. Collins, R. A. Hoffman, R. H. Roberts

It has been demonstrated that anaplasmosis is transmitted during the periods when the insect vectors are prevalent. This has been shown in the Mississippi Delta area to be from approximately April 15 through November 15—the grazing season. Therefore, a method to provide protection from anaplasmosis during these months each year would prevent losses from the disease in this area.

There is evidence from experimental trials to indicate that chlortetracycline (aureomycin) had anaplasma inhibitory effect and that it is of value at varying dose levels in the prevention and treatment of anaplasmosis (1) (2) (3) (4) (5). Since protection is needed during the vector or pasture season to prevent much of the loss from anaplasmosis, the authors felt that it should be determined what protection is afforded by continuous low level feeding of chlortetracycline on pasture.

Experimental Procedure

The low level feeding of aureomycin was carried out through the vector seasons of 1959, 1960 and 1961 as follows: In 1959, 29 yearling steers complement-fixation negative and 25 complement-fixation reactors were divided into two lots and placed on separate pastures. Lot 1 was composed of 15 negatives and 12 reactors. The steers were fed 0.5 mg/lb/day of aureomycin—both negative and positive cattle—from June 23, 1959, until November 21, 1959. Lot 2 had 14 negatives and 13 reactors and served as untreated controls. In 1960 two lots of 40 complement-fixation negative adult cattle were divided so that Lot 1 contained 21 reactors and 20 negative cattle. The negatives and reactors were fed 0.5 mg/lb/day aureomycin from April 27, 1960, through September 30, 1960. Lot 2 consisted of 21 reactors and 19 negative cattle which served as untreated controls.

In 1961, 20 yearling steers and 4 grade cows which were complement-fixation negative for anaplasmosis were placed in 2 lots of 12 animals each (10 steers 2 cows). Lot No. 1 was fed 0.5 mg/lb/day of aureomycin from March 20, 1961, through August 31, 1961. Two acutely infected anaplasma animals were placed in each group on April 19, 1961. A new acutely infected animal was added to each group at monthly intervals during the vector season. These infected animals did not receive daily low level feeding of aureomycin.

Each group of cattle was weighed monthly and the formulation of aureomycin adjusted accordingly. They were fed in open troughs in 1959 and 1961. In 1960 the aureomycin was incorporated in range pellets and fed on pasture.

Blood was collected monthly from each animal and was subjected to complement-fixation tests, hematocrit reading and blood smears studies.

Results

There was no transmission of anaplasmosis during 1959 and 1960 vector season in the test lots or their controls. The anaplasmosis reactors that were fed 0.5 mg/lb/day of aureomycin during the 1959-1960 season showed a decrease in complement-fixation titer after 60 to 90 days of feeding. In 1959, nine of twelve reactors became negative, but slowly returned to a reactor status after aureomycin feeding was discontinued, Table 1. The unfed controls continued to show four plus reactor titers, Table 2. In 1960, twelve of twenty-one reactors fed aureomycin became negative, Table 3, while the unfed controls remained reactors, Table 4.

During the 1961 reactor season, three of the negative treated animals, 2 steers and 1 cow, developed anaplasmosis, Table 5. All twelve of the unfed control animals became infected with anaplasmosis by August 31, 1961, Table 6.

Table 1—Anaplasmosis Reactors Fed 0.5 mg/lb/da Aureomycin on Pasture—1959

Animal Number	Date 6-23	7-14	8-12	9-10	10-6	10-21	11-20	12-15	1-1-60
159	NT	4+	--	1+	1+	N	N	2+	1+
161	4+	4+	4+	2+	4+	N	N	1+	4+
165	4+	4+	4+	4+	1+	4+	N	3+	4+
167	4+	4+	1+	N	N	N	N	1+	--
171	4+	4+	4+	4+	4+	4+	4+	4+	4+
172	4+	4+	NT	3+	1+	1+	--	2+	1+
173	4+	4+	4+	4+	4+	4+	4+	4+	2+
175	4+	4+	4+	4+	4+	4+	4+	4+	4+
176	4+	4+	4+	N	N	N	N	1+	4+
178	4+	4+	2+	N	N	N	N	N	N
183	4+	4+	4+	1+	1+	1+	N	4+	4+
184	4+	4+	4+	1+	2+	2+	N	2+	4+

Summary

The feeding of 0.5 mg/lb/day of aureomycin slowly reduces the blood titers of anaplasmosis reactors to reduce their infectivity.

The development of 3 cases of anaplasmosis in animals being fed 0.5 mg/lb/day of aureomycin would indicate that this is the minimum amount that can be fed to prevent the spread of anaplasmosis under stress infectivity.

Discussion

These results show that it is possible to prevent heavy losses from anaplasmosis by feeding 0.5 mg. of chlortetracycline per pound of body weight during the insect vector season. During the 1959 and 1960 trials, no cases of anaplasmosis developed in the test animals or their controls. This we are at a loss to explain, as there were transmissions in other projects not related to antibiotic feeding.

Table 2—Anaplasmosis Reactors Not Fed Aureomycin 1959

Animal Number	Date 6-23	7-14	8-12	9-10	10-6	10-21	11-20	12-15	1-1-60
160	4+	4+	4+	4+	4+	4+	4+	4+	4+
162	4+	4+	4+	4+	4+	4+	4+	4+	4+
163	4+	4+	4+	4+	4+	4+	4+	4+	4+
164	4+	4+	4+	4+	4+	4+	4+	4+	4+
166	4+	4+	4+	4+	4+	4+	4+	4+	4+
168	4+	4+	4+	4+	4+	4+	4+	4+	4+
169	4+	4+	4+	4+	4+	4+	4+	4+	4+
170	4+	4+	4+	4+	4+	4+	4+	4+	4+
174	4+	4+	4+	4+	4+	4+	4+	4+	4+
177	4+	4+	4+	4+	4+	4+	4+	4+	4+
180	4+	4+	4+	4+	4+	4+	NT	4+	4+
182	4+	4+	4+	4+	4+	4+	4+	4+	4+
226	4+	4+	4+	4+	4+	4+	4+	4+	4+

Table 3—Anaplasmosis Reactors Fed 0.5 mg/lb/da Aureomycin on Pasture — 1960

Animal Number	Date 3-20	4-26	6-1	6-28	7-28	9-1	9-30
378	4+	4+	4+	1+	N	N	N
380	4+	4+	1+	2+	N	4+	N
385	4+	4+	3+	N	N	N	N
386	4+	2+	2+	N	1+	4+	N
390	4+	4+	4+	2+	1+	N	N
391	4+	NT	2+	N	N	N	N
394	4+	4+	4+	4+	4+	4+	3+
402	4+	4+	4+	4+	4+	NT	4+
403	4+	4+	3+	1+	4+	4+	4+
411	4+	4+	4+	2+	N	N	N
422	4+	4+	4+	3+	4+	4+	2+
423	4+	4+	4+	4+	4+	4+	4+
433	4+	4+	4+	4+	4+	4+	3+
445	4+	4+	N	N	N	N	N
447	4+	4+	N	N	N	N	N
452	4+	4+	N	N	N	N	N
454	4+	4+	4+	4+	4+	4+	4+
455	4+	4+	N	N	4+	4+	N
459	N	1+	1+	1+	4+	1+	2+
465	1+	4+	N	N	N	N	N
502	4+	4+	4+	3+	4+	4+	4+

Table 4—Anaplasmosis Reactors Not Fed Aureomycin — 1960

Animal Number							
376	--	4+	4+	4+	4+	4+	2+
379	4+	4+	4+	4+	4+	2+	4+
381	4+	NT	4+	4+	4+	4+	2+
382	4+	4+	4+	4+	4+	NT	4+
383	4+	4+	4+	4+	4+	4+	4+
384	4+	4+	4+	3+	4+	4+	4+
387	2+	3+	3+	4+	4+	1+	4+
389	4+	NT	4+	4+	4+	4+	2+
392	2+	4+	4+	4+	4+	4+	3+
395	4+	4+	4+	4+	4+	4+	4+
399	4+	4+	3+	3+	1+	4+	3+
401	4+	4+	4+	4+	4+	4+	4+
406	4+	4+	4+	4+	4+	1+	4+
407	4+	4+	4+	4+	4+	4+	3+
408	4+	2+	1+	N	4+	1+	4+
415	4+	4+	4+	4+	4+	4+	4+
425	4+	4+	4+	4+	4+	4+	4+
427	4+	3+	4+	4+	4+	4+	4+
428	4+	4+	4+	3+	4+	4+	4+
429	4+	3+	3+	1+	--	3+	2+
431	4+	4+	4+	4+	4+	4+	4+

Table 5—Anaplasmosis Reactors Fed 0.5 mg/lb/da Aureomycin on Pasture

Animal Number		Date 1-20	3-7	4-5	5-3	6-7	7-7	8-2	8-31
5		N	N	N	N	N	N	N	N
9		N	N	N	N	N	N	N	N
21		N	N	N	N	N	N	N	N
48		N	N	N	N	N	N	N	2+
51		N	N	N	N	N	N	N	N
66		N	N	N	N	N	N	N	4+
86		N	N	N	N	N	N	N	N
99		N	N	N	N	N	N	3+	N
107		N	N	N	N	N	N	N	N
110		N	N	N	N	N	N	N	N
8		N	N	N	N	N	N	N	N
31		N	N	N	N	N	N	N	4+
Added Infected Animals									
64	Transfused (4-19)				1-60	N	1-20	1-20	1-20
79	Transfused (4-19)				1-180	1-160	1-80	N	1-80
56	Transfused (5-15)					1-80	2+	N	1-5
265	Transfused (6-22)						1-320	1-80	--
276	Transfused (7-13)							1-60	

Table 6—Controls Not Fed Aureomycin—1961

Animal Number								
2	N	N	N	N	N	N	4+	4+
32	N	N	N	N	N	N	N	4+
108	N	N	N	N	N	N	4+	4+
105	N	N	N	N	N	N	4+	4+
121	N	N	N	N	N	N	N	4+
122	N	N	N	N	N	4+	4+	4+
123	N	N	N	N	N	4+	4+	
125	N	N	N	4+	4+	4+	4+	3+
127	N	N	N	N	N	N	4+	4+
128	N	N	N	N	N	N	N	4+
21	N	N	N	N	N	N	N	N[1]
24	N	N	N	N	N	N	N	4+

[1]Died 9-21

Added Infected Animals

	1-20	3-7	4-5	5-3	6-7	7-7	8-2	8-31
46	Transfused 4-19			1-40	1-40	1-5	1-20	1-40
118	Transfused 4-19			1-320	1-60	1-80	1-40	1-20
120	Transfused 5-15			1-640	1-60	1-40	1-40	1-40
47	Transfused 6-22					1-40	1-5	
277	Transfused 7-13						1-320	1-320

The results of the 1961 trials show that three of twelve animals fed 0.5 mg/lb/day aureomycin developed anaplasmosis. All 12 of the untreated controls developed anaplasmosis when stress was placed on them by the addition of new acutely infected animals each month during the vector season. This would indicate that feeding 0.5 mg/lb/day is the minimum border line amount of aureomycin that will prevent the spread of anaplasmosis.

References

1. Foote, L. E., Farley, H. and Gallalier, B.: The Use of Aureomycin in Anaplasmosis. North Am. Vet. 32 (1951): 547-549.
2. Foote, L. E. and Wulf, M.: Anaplasmosis Carrier Infection Destroyed with Aureomycin. North Am. Vet. 33 (1952): 406-408.
3. Splitter, E. J. and Miller, J. G.: The Apparent Eradication of the Anaplasmosis Carrier State with Antibiotics. Vet. Med. 48 (1953): 486-488.
4. Brock, W. E., Pearson, C. C., Staley, E. E., Kliewer, I. O.: The Prevention of Anaplasmosis by Feeding Chlortetracycline. J.A.V.M.A. Vol. 130, No. 10, May 15, 1957: pp 445-446.
5. Brock, W. E., Pearson, C. C., Kliewer, I. O.: Anaplasmosis Control by Test and Subsequent Treatment with Chlortetracycline. 62nd Annual Proceedings. U.S.L.S.A. (1958).

Control of Anaplasmosis by Feeding an Antibiotic (Aureomycin)

M. J. Twiehaus

Splitter and Miller reported the eradication of anaplasmosis carrier infections in cattle by use of injections of aureomycin and terramycin (1). The successful eradication of the carrier infection of anaplasmosis in the bovine has been observed at the Kansas Experiment Station by feeding aureomycin at 5 mg. per pound of body weight for a period of 30 days (2). In this experiment, splenectomized animals with known carrier infections were treated by this procedure. The treated animals remained free of *Anaplasma marginale* infection for the duration of the experiment. These animals were examined routinely by the complement-fixation test, and animals inoculated into splenectomized animals for periods ranging from 300 to 800 days post-treatment. These experiments were terminated because of economical reasons and the test animals were challenged and again found to be susceptible to *A. marginale* infection. Inoculum consisted of blood from known carrier animals. The amount employed was a 5 cc. quantity.

During these trials it was observed that the complement-fixation titers on serums from these animals persisted for periods of 20 to 45 days post-treatment with aureomycin. The titers became negative after this date and remained so during the duration of the experiment. With these findings it was decided that we try this same procedure for controlling anaplasmosis infections in the field.

A herd was selected in which evidence of infection had existed for a period of three years. Death losses had occurred during this interval from anaplasmosis and also sick animals had been treated during this period by a local veterinarian. The herd consisted of 156 animals. Twenty-six reactors were identified by the complement-fixation test. The 26 reactor animals were placed on a feeding program consisting of silage, alfalfa hay, and crumbles. The crumbles contained 2 gm. of aureomycin per pound. The aureomycin crumbles were scattered on top of the silage by a

mechanical ensilage wagon. The crumbles were mixed in the silage and unloaded by a mechanical unloader into feed bunks. The 26 animals were allowed free choice of the ensilage containing the crumbles and hay.

The calculated dosage was estimated by weighing some of the animals in this group. The dosage calculated was 5 mg. per pound of body weight. This procedure of feeding was continued for a period of 30 days. The 26 animals were bled 35 days post-treatment and 5 of these animals gave a questionable titer at the particular time. One of the animals had a 2+ titer at this particular bleeding. This animal was purchased by the college to continue the study as to whether the demonstrable titer was actually due to carrier infection or a non-specific infection. This animal was maintained for a period of 10 months and a persistant titer remained at routine bleeding. Blood collected from this animal and injected into splenectomized calves failed to elicit a reaction in susceptible splenectomized calves. It was concluded that this titer was not due to *Anaplasma* infection.

The entire herd was tested one year later and found to be negative to anaplasmosis by the complement-fixation test. The herd was again tested the following year and found to be negative by the complement-fixation test. During the past two years, no clinical cases of anaplasmosis have been observed or diagnosed in this particular herd.

Summary

It appears, from these findings, that anaplasmosis carrier animals may be treated successfully with aureomycin by feeding levels of 5 mg. per pound of body weight for a period of 30 days.

References

1. Splitter, E. J., and Miller, J. G.: The Apparent Eradication of the Anaplasmosis Carrier State with Antibiotics. Vet. Med. Vol. 68 (1953) p. 486-8.
2. Twiehaus, M. J., and Anthony, H. D.: The Successful Control of Anaplasmosis in an Infected Herd by Feeding Aureomycin Crumbles. (Unpublished data) Twiehaus, M. J. and Anthony, H. D.

Protective Measures Against Anaplasmosis in Jamaica for Imported Animals

J. G. Miller

Anaplasmosis has been a major problem associated with the importation of adult cattle into Jamaica. The disease is enzootic in the island, although it is not frequently seen in local cattle, probably due to the rapid spread amongst young stock by the ubiquitous insect vectors. Susceptible adult cattle readily become infected with a resulting high mortality rate and a difficult acclimatization for the survivors. In an effort to avoid these problems, there has been a definite trend towards the importation of young animals below 18 months of age. This incurred other problems, because acclimatization took place in the growing period and full growth was frequently not obtained, also it made ineligible many desirable older animals with proven "show" and breeding performance.

The following procedures were adopted in an effort to utilize Anaplasmosis susceptible adult seed stock in a breeding program.

The objective of this protective protocol is to control anaplasmosis at a sub-clinical stage, on the assumption that an immunity and carrier status would result that would perpetuate itself in the absence of drugs. The conditions under which these animals were kept precluded the use of laboratory data to determine when therapeutic intervention was necessary to control the anaplasmosis infection at the sub-clinical level. Also, local conditions dictated grazing rather than grain feeding, so that it was impractical to utilize medicated feed.

The protocol utilized, consisted of the intramuscular administration of 5 mg/lb. of chlortetracycline or 2 mg/lb. of oxytetracycline every 3 weeks for one year. No attempt has been made to determine the minimal dose or minimal length of time necessary to maintain this protocol. The dosages selected have been widely used as a therapeutic level of these drugs, and assuming an effective therapeutic level of 3 days, this would provide a span of 18 days for new infections of *Anaplasma marginale* to take place and reach what may be an antigenically significant level before the next therapeutic intervention. The infections that are therapeutically inhibited would tend to have their incubation period prolonged significantly beyond the period of the next drug administration.

It is conceivable that a single or small number of therapeutic doses of the tetracycline drugs timed with the aid of blood studies for the late preclinical phases of anaplasmosis would be sufficient to accomplish this objective. It is also possible that the intermittent feeding of a tetracycline medicated feed would be effective. Continual feeding however, may thwart the immunological objective by never allowing the anaplasmosis infection to reach an antigenically significant level. This protocol also assumes that repeated infections will take place by means of natural insect

vectors during the period of drug intervention. It may be advisable to obtain evidence of an exposure by means of a periodic C. F. test.

During the last 10 years the use of this procedure has successfully averted both morbidity and mortality from anaplasmosis in animals up to 5 years of age and 185 lbs. weight. It is presented as a minimal program that accomplishes an adult immunization against anaplasmosis under the conditions existing in Jamaica. It is entirely possible that modifications and refinements would allow a similar protocol to be effective under other conditions with the objective of protecting and immunizing adult susceptible cattle introduced into an anaplasmosis enzootic area.

A Review of The Treatment of Anaplasmosis

T. E. Franklin, F. C. Heck and J. W. Huff

The authors will consider this subject under four headings—introduction, experimental therapy, field therapy and non-specific therapy.

Introduction

Parkin (25) of South Africa states "in anaplasmosis one is dealing with a widely distributed parasite of considerable variations in virulency." This observation has been verified by others, Mott (23) and Mullins (24).

Experimental evidence pointing out a variation in virulency of two *Anaplasma marginale* strains was reported by Schmidt and Franklin (31). They also pointed out the importance of a tested strain of high virulence for evaluating therapeutic agents. Ristic (29) mentions that the virulence of the etiologic agent is probably the most important factor to be considered in drug treatment.

Lotze (18), in a study of experimental anaplasmosis, mentions that anaplasmosis varies greatly in severity, symptoms and mortality. He states that these variations are generally attributed to the five following conditions:

1. Age and condition of the affected animals.
2. Individual resistance of animals to the disease.
3. In experimental animals, the amount of inoculum and whether it was from an acutely infected animal or carrier.
4. The ability of the affected animal to regenerate red blood cells.
5. The virulence of the specific agent.

To this list we would add a sixth condition—environmental temperature, an effect which has been observed in anaplasmosis antigen production, Franklin *et al.*, (16). An environmental temperature of 75 - 80°F or over noticeably increases the rate of *Anaplasma* body multiplication and thereby shortens the incubation period under experimental conditions. A temperature of 60°F or lower has the opposite effect. Schmidt (30) states that "in cool weather, the severity of the disease is much less."

Miller (22) reported that at least 80 drugs had been proposed for the treatment of anaplasmosis. He classified them as arsenicals, antimalarials, antimony derivatives and dyes. He mentioned that the suggested value of these compounds had often been based on a small number of clinical cases with no data presented as to their specific activity.

Christensen (8) mentioned such compounds as sodium cacodylate[1], neoarspenamine[1], paludrine[1], quinoline diphosphate[1], acaprin[1], aralen[1], aricyl[1], and other drugs[1] as having favorably reported for the treatment of anaplasmosis. However, he said, "in all instances the results of treatment have been judged by the percentage of treated cattle that recover, a criterion that may lead to error when the great variation in the susceptibility of cattle to the disease is considered." Previous to these two reports, Dykstra, *et al.* (10) in a report on approximately 20 compounds including many of the sulfa drugs and most of the compounds previously mentioned, showed them to be of no value or at best of questionable value. Stiles (33) stated that sulfa drugs and penicillin are without value in anaplasmosis. Foote (14) mentioned that arsenicals, antimalarials, penicillin, streptomycin and chloromycetin have not proved effective in treatment.

Experimental Therapy

Lotze (18) in a detailed study of over 50 experimental cases of anaplasmosis stated that the period of rise of infection "patent rise" is fairly constant. Usually after one percent of the RBC's are visibly infected with marginal bodies, the percentage of infected erythrocytes approximately doubles every 24 hours until a peak is reached. He suggested that the patent rise be used as a standard to test the effectiveness of various chemotherapeutics in the treatment of this disease.

[1]Considered as non-specific compounds in the treatment of anaplasmosis.

Miller *et al.,* (20) used this criterion in reporting on a method of screening drugs in splenectomized calves. His report concerned the use of chlortetracycline (aureomycin) and oxytetracycline (terramycin) and demonstrated that both drugs halted the increase in *Anaplasma* bodies when administered in the early phase of anaplasmosis. There was 100 percent recovery in 32 treated animals while 80 percent of the 24 controls died. Aureomycin was administered daily and in single dose treatments intraveneously iv at the level of 5 mgs/pound body weight (mgs/lb). Whereas, terramycin was given similarly at 2 mgs/lb levels. However, this treatment did not always prevent the development of a serious anemia.

The treatment of 4 acutely infected adult cattle with a single dose of 2½ gms aureomycin iv confirmed their results obtained with splenectomized calves. Eradication of the carrier state from two adult cattle was indicated after daily intravenous treatment with 2½ gms of aureomycin for 20 days or the use of 1 gm of terramycin similarly for 16 days.

Previously, Foote *et al.* (12) had shown aureomycin to have an inhibiting effect on anaplasmosis. Foote and Wulf (13) used relatively large dosages (27.5 to 47.5 grams) of aureomycin and apparently destroyed the infection in 3 cows. However, Pearson and Brock (26) later reported on the animal treated with 47.5 grams and showed that the transmissive ability was temporarily abated up to 67 days but not destroyed (negative phase). Brock *et al.,* (3) reported on the treatment of 6 cattle (acute, convalescent and carriers) with large doses of aureomycin. He obtained variable results. After treatment with 100 gms, one carrier did not transmit at 296 days post treatment whereas, a similar animal given the same dosage remained a consistent carrier. Cows receiving more than 30 gms. passed through a negative phase (non-infectious) but were later shown to be infectious. Cows receiving less than 30 gms, were constant carriers. Splitter and Miller (32) reported on the treatment of 10 carrier cattle varying in age. Eight animals receiving either aureomycin at 15 mgs/lb/day for 16 days or terramycin at 5 mgs/lb/day for 12 to 14 days remained free of anaplasmosis for periods of 60 to 365 days or longer. Two of the ten animals which received low daily dosages (1 to 2.5 mgs/lb for 16 to 20 days) developed only a temporary negative phase lasting less than 60 days. Brock *et al.* (4) in an experiment with 13 splenectomized calves showed that tetracycline hydrochloride (polyotic) was effective in treating acute anaplasmosis. A single dose of 3 mgs/lb was injected. No difference was shown between calves injected intramuscularly im or iv. Christensen and Harrold (9) demonstrated inhibition of *Anaplasma* bodies in a group of eleven, 2-year-old heifers with terramycin using 3 mgs/lb/day, im.

Pearson *et al.* (27) in a study of carrier cattle found that daily intramuscular injections im of 5 mgs/lb tetracycline (polyotic) for ten days eliminated the carrier stage of infectious anaplasmosis. He pointed out that the maintenance of the antibiotic level in the animal is all important in the destruction of the carrier state. Brock *et al.* (5) and Brock (6) reported that 5 mgs/lb daily dosage of tetracycline compounds given orally for 60 days destroyed the carrier infection.

Ranali, *et al.* (28) administered tetracycline to 47 cows undergoing premunition for piroplasmosis and anaplasmosis. He reported 100 percent cure in 12 cows injected iv with 2.5 mg/kg/body weight of tetracycline hydrochloride whereas the same amount given im resulted in the cure of only 5 of 8 animals (62.5%).

In addition to the previous references there have been a number of reports in the foreign literature Farber (11), Balbo (2), Vallejo and Rostom (34), indicating the value of the tetracycline compounds in the treatment of both experimental and field cases of anaplasmosis.

Field Therapy

Miller *et al.* (21) reported on a field study in Louisiana involving 132 acutely infected cattle which were treated by practicing veterinarians cooperating with the research laboratory. Aureomycin was injected iv in 60 head, terramycin iv in 52, while 20 were treated with non-specific drugs. There was an overall recovery rate of 81.8 percent for aureomycin treated; 80.7 percent for terramycin; while 50 percent of the non-specific treated group recovered. Of the animals which had a low hemoglobin value (below 4.0 gms/100 cc Hb) 76.5 percent recovered with aureomycin treatment; 74.4 percent with terramycin while 52.9 percent recovered with non-specific treatment. In contrast 100 percent of 47 animals with high hemoglobin values (over 4 gms/100 cc Hb) recovered with aureomycin treatment, 85.8 percent of 31 animals recovered with terramycin whereas in three non-specific treated animals, only one survived. It is quite apparent from this report that treatment, to be most effective, should be administered at the first sign of the disease.

Carricaburu (7), a California practitioner, reported that the use of terramycin in the treatment of 64 acutely infected cattle resulted in the recovery of all but 4 cases (93% recovery) whereas, in 36 untreated cases, there were 24 deaths (33% recovery). He recommended a dosage of 3 mgs/lb/bw at a single injection as being the most practical treatment under range conditions.

Arline (Arline and Mamelli, 1), a Mississippi practitioner, reported on a field and laboratory study on 47 cattle treated with oxytetracycline (terramycin). The amount used was single doses of one to three mgs/lb/bw. Treatment resulted in an increased erythrocyte volume (hematocrit) in 62 percent of the treated animals and increased hemoglobin values in 42 percent. The most notable responses were in cattle with very low hemoglobin and hematocrit values. In 15 animals with hematocrits 20 or under at treatment, only one animal showed a drop following treat-

ment. Two animals died in 24 hours following treatment while 21 untreated animals died within a week after testing. In addition an abortion rate of 25 percent ceased completely one week following treatment.

Non-specific Therapy

Besides the drugs previously mentioned as showing no specific activity against the *Anaplasma* organism, the use of whole blood transfusions has been favorably reported by Lotze (19). Dykstra *et al.,* (10) recommends 5 cc per pound of body weight of whole blood as being beneficial. Experimental data on the use of whole blood treatment is limited. Franklin and Schmidt (15) failed to demonstrate any value of whole blood transfusions in a study involving 7 cows when whole blood was injected iv in doses of 1500 to 2600 cc per day for a 3 day total of 3000 to 6200 cc per head. Koger (17) recommends one to two gallons of whole citrated blood at a transfusion with possible repetition. Carricaburu (7) mentions the impracticability of transfusions in wild or range cattle and states that its use should be confined to gentle cattle. Mullins (24) states that he administers 1 quart to 1 gallon of citrated blood to a weak animal. Brock (6) in speaking of blood transfusions says that small doses up to 2000 ml appeared to be ineffectual while larger quantities may overload the heart.

Additional supportive and symptomatic therapy may be recommended when such treatment does not cause undue stress or excitement in the affected animals. Intravenous injections of 50 percent glucose and electrolyte solutions are used to combat weakness and dehydration. Various drugs to stimulate rumen activity have been recommended. Hematinics and the use of mineral supplements to combat the anemia and pica which occurs in the recovering animal are also advocated. The availability of good pasture and good nursing is very important in the treatment and recovery of affected animals.

Foote (14) and Brock *et al.* (5) and Ristic (29) mentioned the expense involved in treatment and for the eradication of the carrier state. Also it was pointed out that the acutely infected animal when routinely treated with tetracycline usually remains a carrier.

In conclusion then it is apparent that the tetracycline compounds aureomycin and terramycin are capable of exerting a specific inhibiting effect on the *Anaplasma* organism with the effectiveness of treatment being greatest at the first clinical sign of the disease. Non-specific drugs have been advocated and are used in treatment by many practitioners. Although the experimental data on blood transfusions are very sparse, its use has been reported to be beneficial and is used by many practitioners. It is suggested that its greatest value would be in gentle animals. To the author's knowledge, no drugs or chemical compounds other than the tetracyclines have been shown experimentally to possess specific activity against the anaplasma organism.

References

1. Arline, R. E., and Mamelli, J. A. (1958) Laboratory studies of anaplasmosis in cattle treated with oxytetracycline (terramycin). J. Am. Vet. Med. Assoc. 133: 517-519.
2. Balbo, M. (1955). L'Aureomicina Nella Terapia Dell' anaplasmosi Dei Bovine. Atti. Soc. Ital. Scienze Vet. IX: 657-658.
3. Brock, W. E., Pearson, C. C. and Kliewer, I. O. (1953). High-level aureomycin dosage in anaplasmosis. Am. J. Vet. Res. 14: 510-513.
4. Brock, W. E., Pearson, C. C. and Kliewer, I. O. (1955). An experiment in the treatment of acute anaplasmosis with tetracycline hydrochloride. North Am. Vet. 36: 547-550.
5. Brock, W. E., Pearson, C. C., and Kliewer, I. O. (1958). Anaplasmosis control by test and subsequent treatment with chlortetracycline. Proc. 62nd Ann. Meeting U. S. Livestock San. Assoc.: 66-70.
6. Brock, W. E. (1959). Anaplasmosis control and treatment. The Oklahoma Vet. 6: 3, 8-11.
7. Carricaburu, J. B. (1956). Observations on the use of terramycin in clinical anaplasmosis. California Vet. 9: 24, 25, 43.
8. Christensen, J. F. (1956). Anaplasmosis. Diseases of Cattle. 657-666. American Veterinary Publications. Evanston, Illinois.
9. Christensen, J. F., and Harrold, J. B. (1956). Inhibition of *Anaplasma marginale* infection in cattle with oxytetracycline hydrochloride. Proc. 60th Ann. Meeting, U. S. Livestock San. Assoc. 69-76.
10. Dykstra, R. R., Roderick, L. M., Farley, H., McMahan, V. K., and Splitter, E. J. (1948). Studies in anaplasmosis. II. (1938-1948) Kansas Agr. Expt. Sta. Tech. Bull. 66: 1-24.
11. Farber, J. (1955). Anaplasmosis—an experiment to combat the disease by use of terramycin, glucose, vitamin C and acetylmethionine. Refuah Vet. 12: 465.
12. Foote, L. E., Farley, H., and Gallagher, B. (1951). The use of aureomycin in anaplasmosis. N. Amer. Vet. 32: 547-549.
13. Foote, L. E., and Wulf, M. (1952). Anaplasmosis carrier infection destroyed with aureomycin. N. Amer. Vet. 33: 406-408.
14. Foote, L. E., (1957): Treatment of anaplasmosis carriers. Proc. 3rd Natl. Research Conf. Anaplasmosis, Manhattan, Kansas, pp 1-9.
15. Franklin, T. E., and Schmidt, H., (1954) Whole blood in the treatment of anaplasmosis. Southwestern Vet. 8: 1, 42-43.
16. Franklin, T. E., Huff, J. W., and Heck, F. C. (1962): Large scale production of anaplasmosis antigen. Southwestern Vet. 15: 2, 131-139.
17. Koger, L. M. (1954). Blood Transfusion in Cattle Under Range Conditions. 91st Ann. Meeting, Am. Vet. Med. Assn.
18. Lotze, J. C. (1947a). Variable and constants in experimental bovine anaplasmosis and their relationship to chemotherapy. Am. J. Vet. Research 8: 267-274.
19. Lotze, J. C. (1947b). Blood transfusions in bovine anaplasmosis. Am. J. Vet. Research. 8: 284-288.

20. Miller, J. G., Levy, H. E., Torbert, B. J., and Oglesby, W. T. (1952). A method of screening drugs to be used in the treatment of anaplasmosis—results of testing with aureomycin and terramycin. Proc. Book Am. Vet. Med. Assoc. 89th Ann. meeting, 160-167.

21. Miller, J. G., Levy, H. E., Torbert, B. J., and Oglesby, W. T. (1953). The treatment of anaplasmosis in Louisiana with aureomycin and terramycin. J. Amer. Vet. Med. Assn. 122: 390-392.

22. Miller, J. G. (1956). The prevention and treatment of anaplasmosis. Ann. N. Y. Acad. Sci. 64: 49-55.

23. Mott, L. O. (1957). The nature of anaplasmosis. Proc. 3rd Natl. Research Conf. Anaplasmosis, Manhattan, Kansas, 1-9.

24. Mullins, J. G., (1959). Anaplasmosis in the Mississippi Delta. Mod. Vet. Practice. 40: 12, 36-37.

25. Parkin, B. S., (1935). A short study of bovine anaplasmosis with special reference to the chemotherapy. Onderstepoort. J. Vet. Sci. Animal Ind. 4: 269-280.

26. Pearson, C. C., and Brock, W. E. (1953). Further studies on the use of aureomycin in anaplasmosis carrier infection. N. Amer. Vet. 34: 408-412.

27. Pearson, C. C., Brock, W. E., and Kliewer, I. O. (1957). A study of tetracycline dosage in cattle which are anaplasmosis carriers. J. Am. Vet. Med. Assoc. 130: 290-292.

28. Ranali, E., Gonzalez, G. S., and Koerber, W. L. (1957) Chemotherapeutic control of experimental babesial and anaplasmal infections in Brazil. Proc. 3rd Natl. Research Conf. Anaplasmosis, Manhattan, Kansas, 108-112.

29. Ristic, M., (1960). Anaplasmosis. Advances in Veterinary Science, 6: 111-192. Academic Press, New York and London.

30. Schmidt, H., (1936). Anaplasmosis in cattle. Vet. Med., 31: 10, 427-429.

31. Schmidt, H., and Franklin, T. E. (1951). Experimental bovine anaplasmosis and its treatment with trifloryl. Proc. 55th Ann. Meet. U. S. Livestock San. Assoc. 260-270.

32. Splitter, E. J., and Miller, J. G. (1953). The apparent eradication of the anaplasmosis carrier state with antibiotics. Vet. Med. 48: 486-488.

33. Stiles, G. W. (1946). Anaplasmosis in cattle. U.S.D.A. Circular 154, Washington, D. C.

34. Vallejo, J. and Rostom, V. H. A. (1959). Acute anaplasmosis in fields free from ticks. Tetracycline therapy. Rev. Med. Vet., B. Aires 40: 159-163.

Questions and Answers

QUESTION—Dr. Howarth, Davis, California

I would like to ask Dr. Franklin a question. Now this may not be a fair question, concerning one of the references you quoted, the one where there is a high abortion rate. In reading that article, was there any proof that that was anaplasmosis, and was there any proof that it was not leptospirosis?

ANSWER—Dr. Franklin, College Station, Texas

It was not given in the article. However, a number of years ago on our premises at College Station where we expect very little transmission, we had an angus cow come up one morning that had aborted, and if we hadn't been in the habit of making blood smears on every animal that came along in those days, we would have probably overlooked anaplasmosis. We attributed the abortion to anaplasmosis. She recovered and had several calves, and we've never recognized leptospirosis in our herd. Now that's the only experience we've had in our own cattle with this problem. On talking to practitioners, they have associated abortion in certain instances with anaplasmosis. I think it's quite likely that in many cases it's leptospiremia. I grant you that it's not a constant symptom.

QUESTION—Dr. Howarth,

I unfortunately cannot remember all the details of the article that you quote. But I think it would be of importance to know whether they actually found anaplasma bodies in those animals in that particular series.

ANSWER—Dr. Franklin, College Station, Texas

Yes, they did confirm anaplasmosis by both C-F and blood smears.

COMMENT—Dr. Howarth, Davis, California

I can tell of one happenstance in California where several range cows aborted and of these, several died of anaplasmosis two weeks later. In those fetuses which were collected before the cows died, virus abortion was isolated. So these cows actually had two diseases. They died of anaplasmosis as a consequence, possibly, of the abortion due to a virus.

QUESTION—Anonymous,

I wonder if Dr. Miller would again tell us about the intramuscular tetracyline treatment. How many doses and the duration?

ANSWER—Dr. Miller, Springfield, Virginia

Well, the dosages again are arbitrary. We have been using aureomycin at a regular therapeutic dosage for anaplasmosis, mainly for a lack of any other information. That is 5 milligrams per pound for aureomycin and down to around two for terramycin. However, when you get into a large animal, some of these brahmas run close to 2,000 pounds, you run into reactions at the site of intramuscular injection. We cut the dosage in half for larger animals and still had very good results. If you recall from the early work on the tetracyclines against anaplasma, nobody really ever went into a titration to determine how low a dosage you could get. And I feel quite sure that the arbitrary dosages that we used at that time based on the other infections are probably far in excess of what may be necessary or effective against anaplasma.

QUESTION—Dr. Silva, Reno, Nevada.

Do any of you gentlemen favor intra-peritoneal injections of your supportive medications?

The reason I bring that angle up is because handling is such a problem with so many of these border line cases; cases that are just coming out in the early stages. Their disposition seems, very often, to change from a rather easily handled animal to the animal that is difficult to handle and medication becomes quite a problem. Your route of medication becomes something that seems to be quite a decisive factor. I've routinely used 10 or 20 percent saline and glucose intraperitoneally in animals that I could just pen up somewhere and use it in the flank area and had fairly good results provided we didn't get too much excitement with it. Along with that, we've used the Liquamycin in one gram doses and 10 cc of Aralen along with it, intramuscularly, and handled the animals as easily as possible and had fair results with it. I don't know mortality-wise how good it was but I'd say that probably 75 percent of the cases we treated that way have responded favorably.

Control Measures

Regulatory Aspects of Anaplasmosis

E. E. Saulmon

The livestock industry of the United States has gained a reputation over the years of not living with an infectious, communicable disease that affected their industry when it was possible to apply the knowledge and available tools so that they could live without it. This philosophy has been greatly responsible for the development of a livestock industry that is the envy of most nations throughout the world.

Also, this philosophy has been responsible to a large degree for the development of the efficient regulatory phase of veterinary medicine that this country now enjoys. The presence and threat of contagious pleuropneumonia and the economic effect it was having on our cattle industry was directly responsible for the establishment of our organized Federal Regulatory Veterinary Service in 1883. The former Bureau of Animal Industry was established the following year by an Act of Congress and charged with the responsibility of not allowing the interstate movement of livestock known "to be affected with any contagious, infectious, or communicable disease." This Act of Congress has been the basic authority under which the Federal Government has cooperated with the different States in livestock disease control and eradication programs since that time. State livestock disease control officials had or have been given similar authority to control and eradicate diseases within their States.

Through the years, as the success in elimination and control of diseases has strengthened organized regulatory veterinary medicine, the responsibilities for safeguarding the animal health of this Nation have increased.

As has been recognized here at this meeting, anaplasmosis is a disease that is of great economic concern to a large percentage of the cattle producers of the country. However, there are some States and portions of other States where the disease has not become established. Cattle owners and regulatory officials of these areas recognize that with our modern transportation facilities and with the great movement of cattle, anaplasmosis carrier animals may be introduced into herds in these areas and bring about establishment of the disease.

Those in research on anaplasmosis are to be commended for the work that has been accomplished in connection with this disease. It would appear that as additional knowledge is gained, we are approaching the time when this disease can be added to the list of those diseases that our livestock industry has decided they can do without. We are all looking forward to the day when it is determined that we have the knowledge and the tools to attack anaplasmosis on a broad scale so that its annual costs can be eliminated. However, we are not yet ready for an all-out eradication program. We must recognize, though, that because of the nature of the disease and its distribution, attitudes vary according to areas and incidence.

The complement-fixation test has proven itself as a reliable tool in eliminating anaplasmosis in the individual herd, if prescribed management practices are adhered to. As more laboratories become proficient in the techniques of this test, we can expect it to be applied in more and more herds working toward anaplasmosis-free status.

Most regulatory officials in those States where anaplasmosis is infrequently diagnosed have taken steps to eliminate it when it is found. Some States have talked of having anaplasmosis-free status as a requirement for entry of cattle for other than immediate slaughter purposes. Hawaii has had this requirement since 1954, soon after the inauguration of their cooperative anaplasmosis eradication program. They now require that all cattle must be anaplasmosis CF test negative prior to entry into the State. These cattle must pass another test before release from quarantine, after arrival in the Islands, with a follow-up test after entry into the herd.

Some of the North Central and Northeastern States have considered similar requirements. New York does not have such a requirement but has contacted all importers of cattle and informed them of the dangers of bringing anaplasmosis carrier cattle into the State. The following is a quote from a letter outlining their policy: "Anaplasmosis testing, prior to shipment, is not at present an official requirement for cattle entering this State. However, this Department strongly recommends that importers require a negative complement-fixation test for all cattle imported from areas where the disease is well established." They define the area in this policy statement as "most States south of the Mason-Dixon Line and the Ohio River and in most of the Plains States west of the Mississippi River and in the Rocky Mountain and West Coast States". They also point out that those animals imported from this area not tested prior to shipment can be tested at destination at State expense and that reactors and suspects will be quarantined. This policy has been in effect since March 1, 1961.

Dr. H. H. Hoyt, College of Veterinary Medicine, University of Minnesota, compiled a report summarizing the cases of anaplasmosis brought to the

attention of the Livestock Sanitary Board of Minnesota since 1949. This report shows that 14 herds have been confirmed as having the disease since that time. The increased incidence over the years stimulated a survey of beef herds in one county which showed 217 herds negative, 18 with suspects only, and 5 with reactors. He concludes his report, "The survey results indicate endemic infection in one county with fairly low cattle population of predominantly beef herds. This confirms past suspicion that although the incidence is low in Minnesota, there are undetected cases providing a potential for more serious losses in the future. An effort now to detect these carriers and to avoid further introductions deserves serious consideration."

One of the complications, from the regulatory standpoint, of applying the test to herds or individual animals where the owner is notified of the test results is the restrictions that need be placed on the movement of those animals found to be infected. One of the recognized primary methods of restricting the spread of any disease is to control the movement of the known-infected animal. This should be followed in the case of anaplasmosis. It is essential that all reacting animals be identified as reactors. Methods of reactor identification have varied from the ear tag-to-tag and tattoo-to-brand. To me, the important thing is to be sure that those animals known to be carrying the infection are identified in such a manner that they will be recognized as such. All segments of the industry and the veterinary profession are charged with the responsibility of protecting the unsuspecting recipient from animals that have, for some reason or other, been determined to be infected with anaplasmosis. On the other side of the coin, every precaution must be taken to prevent those unscrupulous owners who have known infected cattle from selling them, or even giving them away, without their true status being made known.

One corollary that will illustrate the point I wish to make—: The brucellosis agglutination test was developed in 1909. Many herds were tested from that time until proper controls were placed on the animals that reacted to that test. In attempting to help the individual herd owner in his particular problem, regulatory officials as well as practicing veterinarians were parties to allowing known diseased animals to move in normal channels of trade without restriction and thereby spreading the disease to many other herds. Those of you who can remember will recall that in those early days, when brucellosis reactors were disclosed, they were very often promptly offered for sale to unsuspecting buyers. I have no doubt in my mind but that this compounded the brucellosis problem in this country. We cannot allow this to happen with anaplasmosis.

I mentioned earlier that the basic authority under which the Federal Government operated in the field of livestock disease control and eradication prohibits the interstate movement of livestock that are affected with any contagious, infectious, or communicable disease. That Act has been amended on two occasions —one, to allow the interstate movement of tuberculosis and paratuberculosis, and the second time to allow the movement of brucellosis-infected animals for immediate slaughter. At the present time we do not have this authority in connection with anaplasmosis. This has created problems in areas where testing has been performed and local slaughter markets are limited. Owners have been unable to move their reactor animals across State lines where competitive bidding could be received. It must be understood that we in Federal regulatory service have no choice in this matter. It is prohibited by law, not by regulation.

With the development of the C-F test for anaplasmosis, it has been appreciated that provision needed to be made for the orderly movement across State lines of reactors for immediate slaughter. These things do not happen overnight, but I am pleased to report that a bill has been introduced into this session of Congress which, if adopted, will allow such movement. This bill would amend the Act of May 29, 1884, and allow regulations to be promulgated that would permit the interstate movement of anaplasmosis-infected cattle in a manner that would not endanger other cattle. This authority will be beneficial to those owners who desire to do something about the disease in their herds. It will overcome one of the problems that has been encountered in areas where any large amount of herd testing has been accomplished.

The regulatory official has a real responsibility in regard to anaplasmosis. He must help the owner who has the problem in his herd with all the knowledge and tools that are available to him. But he must also provide the owner of the susceptible herd with all the assistance and protection that he can muster to safeguard him against acquiring that problem.

Study on The Complement-Fixation Test For Anaplasma Antibodies

F. C. Heck, T. E. Franklin and J. W. Huff

The complement-fixation (CF) test has been established as a useful tool for the diagnosis of anaplasmosis. With due consideration for the inherent technicalities of the CF test, the researcher has an instrument available to assist in probing the vast mystery encompassing this disease.

The specificity and attainable degree of duplication of this test has been well established by Price, Brock, and Miller, 1954; Gates, Roby, and Mott, 1955; and Roby *et al.* 1956. Test accuracy is relative to many factors of which technical error is probably the most significant. Denaturation or destruction of homologous antibody during preparation of the test sera is an obscure consideration. However, disruption of the protein moiety during pre-testing treatment remains a possibility. This supposition is supported by observation made in our laboratory during the past three years, and is indicated from results of testing specific sera on which reproduceable results were difficult to obtain. Variations in the CF results of replicate serum samples which had been phenolized, as opposed to non-treated samples, affirmed our suspicion. This work was completed to determine the effect of phenol on the serum antibody and to find whether or not an accurate CF result is obtained from testing phenolized sera.

Blood samples were obtained from 541 head of range cattle in an endemic area of South Central Texas. After centrifugation, two, 2 ml aliquots of cell-free serum were obtained from each sample. One fraction was treated with phenol to a final concentration of 0.5 percent, the other fraction was not treated. After storage of from 24 to 48 hours at 4°C, the sera were tested by the CF method adapted by the USDA, ADE, (1958).

A statistical analysis on the results of 541 phenolized samples and the corresponding non-phenolized replicates indicates that the computed (t) value is significant (Figure 1). Since the variation is not due to sampling, this result reflects the effects between treatments.

Treatment	Samples	Total Reaction	Mean	(t)
Phenolized	541	1429	2.64	6.42
Non-treated	541	1276	2.35	

Figure 1. Calculated value (t) indicating the difference between treatments.

To demonstrate the dissimilarity between phenolized and non-treated samples, all phenolized samples were grouped according to their CF result, that is, 4+, 3+, 2+, 1+, (±), and negative. The non-treated replicates of all corresponding phenolized samples of a particular category were tested and results between treatments were compared. The comparative data from testing the non-phenolized replicates corresponding to phenolized samples in the 4+ category are given in Figure 2. Eighty-two percent of these non-treated samples had 4+ CF reactions. Nine percent had 3+ reactions. Of particular significance is the 1.3 percent of non-treated samples which were negative. This occurrence is meaningful when these data are interpreted in respect to an observation by Willers (1957), that transmission of the causative agent could not be accomplished from all animals' blood of which the serum CF antibody level was 4+.

More variation was observed from results of the comparative study on 3+ replicates (Figure 3) than

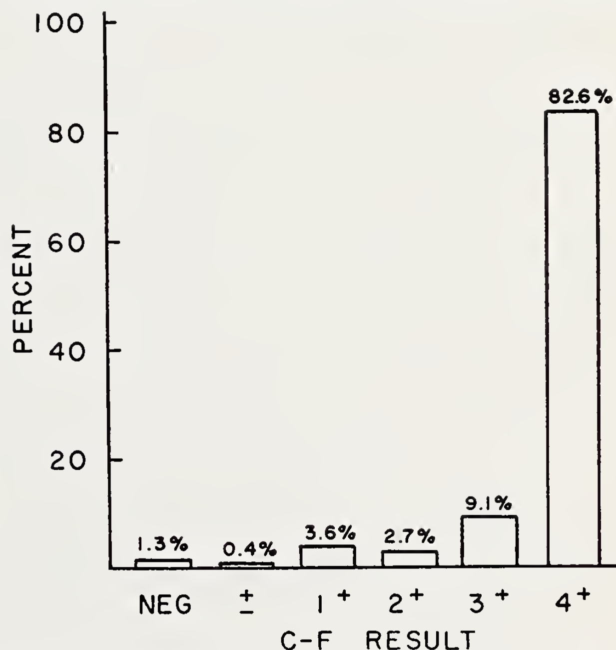

Figure 2. Difference between treatments of phenolized samples with 4+ CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) phenolized 4+ sera. The CF results of the corresponding non-treated fractions are shown (abscissa).

was observed in the 4+ replicate group. Nine percent of the 3+ replicates were negative, while 15.6 percent had the same reaction as the corresponding phenolized samples.

The CF results of the remaining 3+ replicates, approximately 75 percent of the corresponding non-phenolized samples, were arrayed from (±) to 4+.

The comparative results of the 2+ phenolized group with the corresponding non-treated samples are shown in Figure 4. Twenty-seven percent of the samples in this group had 1+ reactions while 22 percent duplicated the reaction of the phenolized sample. A weaker reaction in the non-treated samples is apparent from these data.

Only 17 percent of these replicate samples which corresponded to the phenolized 1+ group had the same results (Figure 5). A weaker reaction in the non-phenolized samples was evident in 67 percent of the samples tested.

This graphic representation shows comparative results from the phenolized (±) group and the corresponding non-treated samples (Figure 6). Sixty-four percent of the samples had a reaction which was lower than the result of the corresponding phenolized sample. Only 27 percent of the replicates had the same results. There has been interest concerning the problem of which animals in the (±) category should be considered suspicious and which should be considered anaplasmosis negative or clean animals. The indication from these data is that more than half of the (±) animals, whose serum was tested as a phenolized or preserved sample, are anaplasmosis negative.

The data from a comparative study of the phenolized-negative group with the corresponding non-treated sample are shown in Figure 7. Good agreement is indicated, as 83 percent duplication was attained.

It has been determined (Heck, Huff, and Franklin, 1962) that transmission of *Anaplasma marginale* was not accomplished from animal's blood, of which the phenolized serum CF reaction was more than (±) but less than 3+. An interpretation of this observation can be made from these data which indicate that 1.3 percent of the phenolized 4+ group, 9.3 percent of the phenolized 3+ group, 12 percent of the phenolized 2+ group, 35 percent of the phenolized 1+ group and 64 percent of the phenolized (±) group

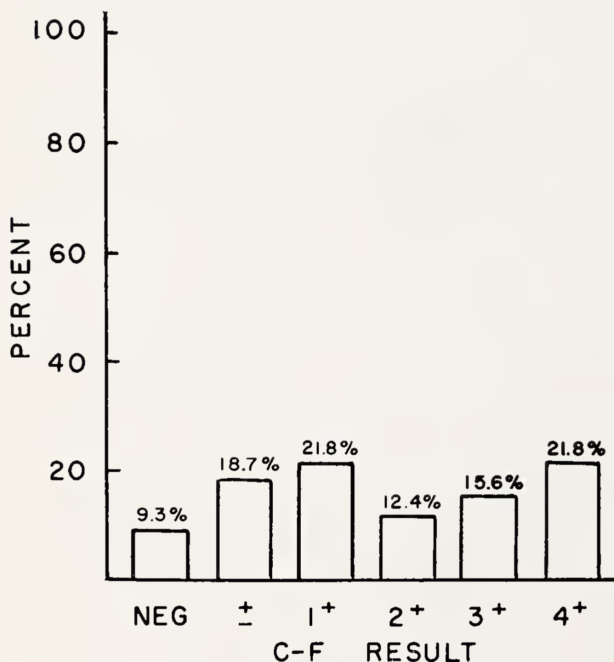

Figure 3. Difference between treatments of phenolized samples with 3+ CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) phenolized 3+ sera. The CF results of the corresponding non-treated fractions are shown (abscissa).

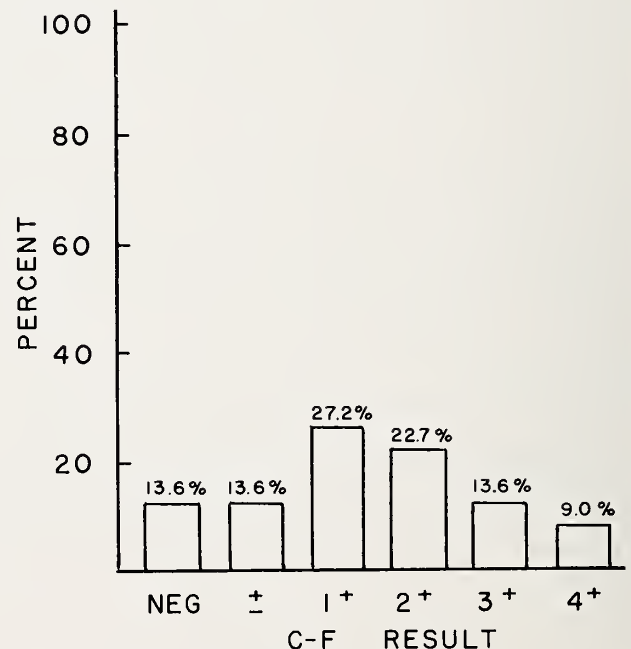

Figure 4. Difference between treatments of phenolized samples with 2+ CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) phenolized 2+ sera. The CF results of the corresponding non-treated fractions are shown (abscissa).

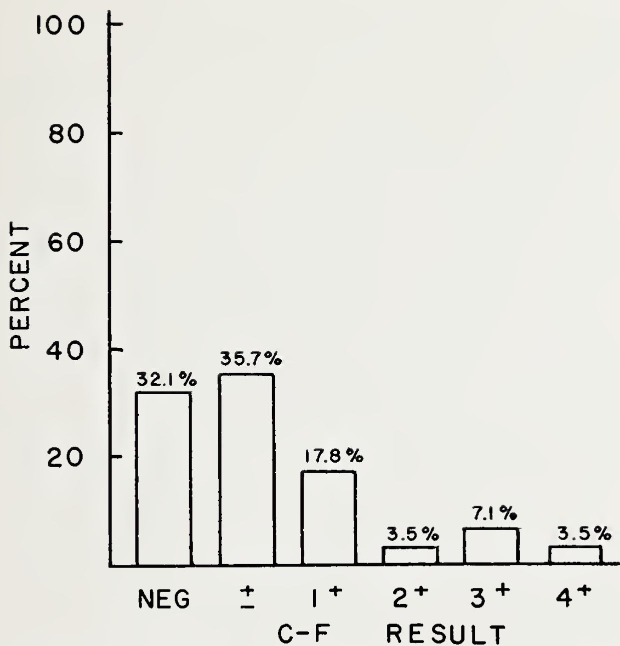

Figure 5. Difference between treatments of phenolized samples with 1+ CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) phenolized 1+ sera. The CF results of the corresponding non-treated fractions are shown (abscissa).

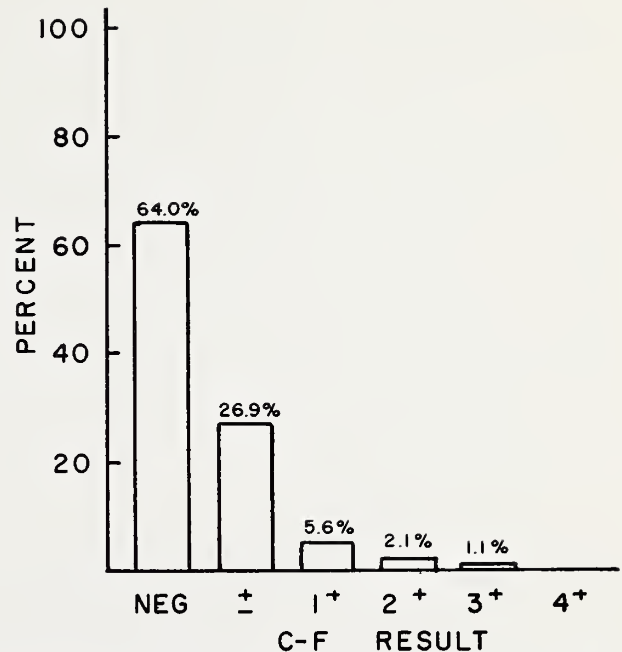

Figure 6. Difference between treatments of phenolized samples with ± CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) phenolized ± sera. The CF results of the corresponding non-treated fraction are shown (abscissa).

had a negative reaction in the corresponding non-treated sample. It can be concluded, subject to inoculation studies, that the indicated CF result from phenolized serum may not be a true indication of the presence of homologous antibodies.

Other studies were conducted concerning the storage problems arising from testing sera which has not been treated with a bactericide. Duplicate samples were obtained from ten animals of known CF status. One set of samples, clot included, was stored at 4°C for twelve days, the other set remained at room temperature for the same period. No special precaution was taken to protect the sera with the exception that a rubber stopper was placed in each tube. Each day, 0.2 ml of serum was extracted from each tube and tested. There was no variation in the test results between days or between treatments over the twelve-day period. Obvious bacterial growth was evident after five days in the blood samples which remained on the laboratory bench. The presence of these bacteria did not affect the test results on these samples.

The significance of incorporating Mg^{++} in complement-fixation tests was shown by Levine (1953). However, in the CF test for *Anaplasma* antibodies there was no variation in the CF result of sera tested in a system containing 0.1 percent magnesium chloride when compared with the result of the same sera tested in a system without this divalent ion. There was increased complement activity (20 percent) in the presence of magnesium, requiring restandardization of the entire component system.

In accordance with these data it was apparent that a bactericide need not be added to serum prior to testing. However, because of the deviation in results between treatments and to insure uniformity in test results between laboratories, it should be determined whether to test the sera as a phenolized sample or to test without phenolization as suggested.

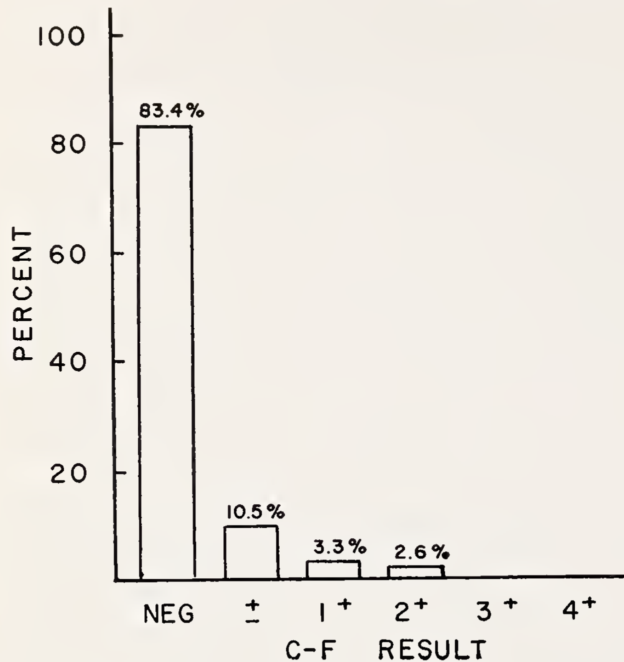

Figure 7. Difference between treatments of phenolized samples with Neg. CF reaction and the corresponding non-phenolized samples. The ordinate is equivalent to the total number (percent) Neg. sera. The CF results of the corresponding non-treated fractions are shown (abscissa).

References

1. Gates, D. W., T. O. Roby, and L. O. Mott. 1955. Studies on the specificity of the complement-fixation test for anaplasmosis. Proc. 59th Ann. Meet. U. S. Livestock San. A. 89-97.

2. Heck, F. C., J. W. Huff, and T. E. Franklin. 1962. A Study on the transmissibility of *Anaplasma marginale*. In press.

3. Levine, L., K. M. Cowan, A. G. Osler, and M. M. Mayer. 1953. Studies on the role of Ca++ and Mg++ in C-F and immune hemolysis. J. Immuno. *71*:359-366.

4. Price, K. E., W. E. Brock, and J. G. Miller. 1954. An evaluation of the complement fixation test for anaplasmosis. Am. J. Vet. Res. *15*:511-516.

5. Roby, T. O., W. H. Martin, D. W. Gates, and P. A. Madden. 1956. The evaluation of the complement-fixation test for anaplasmosis in field control and eradication studies. Proc. 60th Ann. Meet. U. S. Livestock San. A. 60-68.

6. Willers, E. H. 1957. Eradication of anaplasmosis in Hawaii. Proc. 3rd Natl. Research Conf. Anaplasmosis in Cattle. Manhattan, Kansas. 40-56.

Recent Findings in Anaplasmosis CF Antigen Production

T. E. Franklin, F. C. Heck, and J. W. Huff

Production of Highly Infected Anaplasma Blood in Four Days

It became apparent after producing over 4,000,000 test doses of anaplasmosis antigen that three factors are most important in successful antigen production, Franklin *et al.* (1). They are as follows:

A large volume inoculum of infected erythrocytes was our standard inoculum dose—1cc of packed RBC's per pound of body weight (lb/bw);

A highly infected inoculum—70 percent infected RBC's or higher;

Maintenance of an environmental temperature of 72° to 75°F for the inoculated animal.

After it became apparent that these factors—volume, infectivity, and temperature were most important in the production of antigen, these facts were checked on seven normal cows (table 1). This study was made in the early summer of 1961 after completion of our antigen production contract.

However, we increased the inoculation dosage to 1½ cc of packed RBC's lb/bw and beginning with the fifth animal inoculated. The environmental temperature was increased and maintained at a constant 80°F or higher. The inocula were varied from 82 to 100 percent infected RBC's. The hematocrits of the recipients remained average (21) to high (27) at exsanguination, a factor which determines the volume of packed cells obtained at harvesting (table 1).

It has been observed that the percentage of infected red blood cells at exsanguination is approximately the same as the percentage of infected cells injected—provided the environmental temperature, inoculum dosage, and incubation period are standardized, Franklin *et al.*, (1). A high titer Sharples and CO_2 antigen was produced from the highly infected blood obtained at exsanguination (table 1).

CO_2 Antigen Studies

In connection with this undertaking, beginning with a few previously inoculated animals, we compared the relationship of low anaplasma infected blood (low count) to the titer of its respective CO_2 antigen. Also, the same comparison was made with highly infected blood (high count) and both relationships were studied in the same animal using a total of six animals (table 2).

It was found when the infected RBC's were under 50 percent, the CO_2 antigen was weak or sub-standard. On the other hand, when the infected red cell count was 80 to 90 percent or higher the antigen had a high antigenic titer (table 2).

Table 1—Volume, Infectivity and Temperature Data for Antigen Production[1]

Animal	Breed	Weight (lbs.)	A.E. Temp.	Vol. of Inoc. cc	% Inf. RBC's Inoc.	% Inf. RBC's at Bld.	Days to Bleeding	Pre-Inoc. Hemat.	Hemat. at Bld.	Vol. Blood Drawn (L.)	Titer Sharples A.	Titer CO_2 A.
9	Jer.	750	74.8[4]	1125	90	94	5	32	26	13	-----	-----
339	Hol.	1250	77.7[4]	1880	94	84	5	29	21	26	4+.05 1+10	-----
80	H. C.[2]	815	77.8[4]	1300	84	96	5	42	27	14+	4+.1 1+15	4+.15 1+5
24	Jer.	800	80.1[4]	1200	30	93	7	32	21	15+	4+.15 1+7	4+.15 1+3
Temperature Maintained at Constant 80°F												
28	Her.	650	80.0	1000	93	95	4	33	21	12	4+.1[3] 1—+10	4+.15 1+5
29	Jer.	800	80.0	1200	82	90	4	35	26	11+	4+.1 1+10	4+.1 1+5
540	Jer.	940	80.0	1800	100	93	4	34	25	15	-----	-----

[1]All inoculations were approximately 1½ cc. lb./bw of highly infected packed RBC's.
[2]Hereford Cross
[3]CO_2-Sharples antigen only
[4]Average environmental temperature
----- No antigen made

Table 2—Data on High and Low Count CO_2 Antigens[1,2]

Animal	Breed	Wgt. (lbs.)	A.E. Temp. F.	RBC's Inoc. lb/bw	% Inf. RBC's Inoc.	% Inf. RBC's L.C.	Days to[3] L. C. Bleeding	% Inf. RBC's H.C.	Days to[4] H.C. Bleeding	Preinoc. Hemat.	L.C. Hemat.	H.C. Hemat.	Vol. Blood Drawn (H.C.)	Titer L.C. A.	Titer H.C.A.
80	Her.C.	815	77.8	1½	84	67	2	96	5	42	36	27	14+L	2+.15 1+2[5]	4+.15 1+5
24	Jer.	800	80.1	1½	30	53	4	93	7	32	28	21	15+	3+.15 1+3[5]	4+.15 1+3
28	Her.	650	80.0	1½	93	64	3	95	4	33	24	21	12	3+.15 1+2[5]	4+.15 1+5
27	Jer.	730	80.0	1cc	95	52	3	82	4	33	30	23	16+	4+.15 1+3	4+.15 1+7
143	Hol.	1000	70.+	1cc	93	40	4	90	6	31	26	17	20	±.15 1+2[5]	4+.15 1+4
329	Hol.	1140	70.+	¼cc	91	24	5	50	8	34	22	17	18+	±.15 1+2[5]	4+.1 1+3

[1]High Count (H.C.) Highly Infected RBC's for CO_2 Antigens
Low Count (L. C.) Low Infected Blood for CO_2 Antigens.
[2]4+.15, 1+2 dilution is minimum strength for acceptable CO_2 antigens (ADPRD ADED)
[3]One liter venous blood drawn for Low Count Antigens
[4]Days to exsanguination.
[5]Antigen too weak for official poolings

CO_2-Sharples (CO_2-S) Antigen

The authors produced a new type of antigen, CO_2-S, which compares with the Sharples antigen in antigenic strength. This new procedure was made possible by the production of a CO_2 lysate reported by Gates *et al.,* (2) which was passed through the Sharples centrifuge. This method of production has one great advantage over the regular Sharples method, that is, in the reduction of man hours necessary for producing CO_2-S antigen. A more rapid flow of CO_2 lysate through the Sharples centrifuge without antigenic loss is possible than with the water lysed material. Also, we have been able to process CO_2 lysate of subminimal titer through the Sharples centrifuge and produce a satisfactory antigen.

One disadvantage of the CO_2-S antigen is that it often precipitates after final dilution but we believe this can be remedied by further research. Also, by this method one is able to rapidly concentrate large numbers of anaplasma bodies. Other than for antigen production the potential of such a highly infected concentrate remains to be determined.

Discussion and Summary

It is apparent that from the data presented here and previously that the consistent production of a highly infected anaplasma blood is possible. This makes it possible to produce a higher antigenic Sharples, CO_2 or CO_2-Sharples antigen.

Three important factors to be considered in production are inoculum volume, infectivity of inoculum and environmental temperature of the recipient. With these factors in operation, one can produce a highly infected blood in the shortest possible time. This methods results in greater yield and economy in antigen production.

A method (CO_2-Sharples) of producing a highly infected anaplasma concentrate is discussed and with further research, this could likely be the preferred method of antigen production. Consistent production of a satisfactory CO_2 antigen is possible and the economy of producing this type of antigen should be considered by anyone interested in future antigen production.

References

1. Franklin, T. E., Huff, J. W., and Heck, F. C. (1962). Large scale production of anaplasmosis antigen. Southwestern Veterinarian, 15:2, 131-139.

2. Gates, D. W., Mohler, W. M., Mott, L. O., Poelma, L. J., Price, K. E., and Mitchell, James. (1954). A comparison of antigen production methods and complement-fixation. Procedures for Diagnosing Bovine Anaplasmosis. Proc. U. S. Livestock Sanitary Assoc., 58th Ann. Meeting, 105-114.

Comparison of C.F. Test Results in Official Laboratories

Walter H. Martin

The last decade has seen rapid advances in the accurate laboratory diagnosis of bovine anaplasmosis, primarily through the development of a satisfactory complement-fixing antigen. This antigen, though relatively crude, is a remarkably accurate diagnostic material when compared to other purified antigens of a similar nature. It has been found to be approximately 97 percent accurate when testing phenolized serum from known positive and negative animals.

Many of the errors inherent in standard complement-fixation test procedures are magnified when using the anaplasmosis antigen due in part to the extraneous material present in the product. For example, turbidity, which is not a problem in normal procedures, is a major factor in interpretation in the anaplasmosis C.F. test. Because of this and other technical factors, Division policy has been to furnish anaplasmosis antigen only to those state, federal and research laboratories which have sent personnel to Beltsville for a training period in anaplasmosis C.F. procedures, usually lasting about three weeks. Upon completion of the training period, the technician must then purchase necessary equipment and supplies, and a period of from three to six months frequently elapses before preliminary testing actually begins. Once the laboratory begins routine testing, all samples coming into that laboratory are checked and then forwarded to Beltsville for confirmation. After a proficiency of approximately 95 percent agreement has been attained during this check-testing period, that laboratory is released for routine testing. Thereafter the Division requires that each laboratory forward to Beltsville a given number of positive and/or suspicious samples found during routine testing plus an equal number of negative samples from the same herd. In this way a monthly evaluation of the testing efficacy of that laboratory can be made.

In 1958, the Department of Agriculture began distributing samples of known positive and negative serum samples to all laboratories engaged in anaplasmosis testing. These were carefully chosen to present a diversity of reactions and titers, each sample being tested many times to establish its correct reaction or titer before including it with the test sera. Each laboratory was requested to report the reaction observed on every sample and to determine serum antibody levels (titers) on all positive sera; thus, two numerical evaluations could be made, one based on correct identity of the samples and another on the correct titer.

We feel that probably one of the best criteria of testing accuracy is in the ability of the technician to correctly determine the antibody levels (titers) of positive serums. As in any immunological test system, reproducibility is usually limited to within plus or minus 5 percent; thus, it is our opinion that a laboratory should be able to reproduce titers of positive samples to one dilution higher or lower than the standard.

In order to compare the efficacy of testing techniques in the various state, federal and university laboratories engaged in anaplasmosis testing or research, a number of samples were selected from a large collection of serums from known positive and negative cattle held at the Animal Disease Station, Beltsville. These sera had been collected, preserved with 0.5 prcent phenol, and stored for several years during which time they had been tested many times with little or no change in the C.F. reactions or titers.

Before evaluating the accuracy of a laboratory, each sample selected was first tested until no question could be raised as to its correct reaction. Every sample distributed was checked at least twenty times and titers of positive sera determined each time. Those positive sera whose reactions or titers appeared to vary were discarded and only samples which had reproducible C.F. titers were used. In order to then evaluate the results of other laboratories which received the test sera, a system was devised wherein numerical values were assigned to various complement-fixation reactions similar to the method described by Gates, *et al.* (1954) [1]. In evaluating the titers of positive serum samples a similar method was used. For example, a titer reported one dilution higher or lower than the standard, was said to be 95 percent accurate. The greater the variation in the reported titer from the standard, the lower the percent agreement.

The results of the 1961 comparative test on the samples distributed to twenty-four laboratories are shown in Table 1. The identity of the cooperating laboratories has been coded and each is informed of his own results without divulging the identity of the other laboratories. Included in this group of thirty-one sera were positive, negative and anticomplementary samples. No suspicious samples were included; however, several positive sera had low titers (1:5) approaching the suspicious category. Thirteen of the twenty laboratories reporting showed a 95 percent agreement or better with the standard serums. Generally, those laboratories which test daily, or do large numbers of tests, had the highest degree of accuracy

[1]Gates, D. W.; Mohler, William M.; Mott, L. O.; Poelma, L. J.; Price, K. E.; Mitchell, James: A Comparison of Antigen Production Methods and Complement-Fixation Procedures For Diagnosing Bovine Anaplasmosis. Proceedings U. S. Livestock Sanitary Association—58th Annual Meeting. November 1954. pp. 105-114.

Table 1—Comparative Test Results on Reference Serum Samples in Field Laboratories

	Percent Agreement	
Laboratory	Identity of Samples	Serum Titers of Positive Samples
A	100.0	98.3
B	100.0	97.4
C	100.0	97.0
D	100.0	96.2
E	100.0	94.5
F	99.2	98.3
G	99.2	93.3
H	98.4	90.2
I	98.4	77.9
J	97.6	96.4
K	97.5	97.8
L	96.8	91.9
M	96.0	91.9
N	94.4	96.4
O	93.3	82.9
P	93.3	63.3
Q	89.5	72.0
R	87.1	86.0
S	83.9	67.1
V	82.3	80.2

and, in addition, also showed a higher percent agreement on titrations of the positive serum samples.

The comparative monthly check test results received from an individual laboratory are shown in Table 2. This laboratory is one which consistently runs many thousands of samples monthly and has a high degree of test proficiency. The agreement on both the identity of samples and serum titers was almost perfect. This table is included to show that laboratories can show remarkable agreement even on unknown field samples using the complement-fixation test.

Table 3 shows the results of the comparative testing on standard serums during the last three years. These results are based on the correct identity of serums only and not on titrations of reacting sera. Again those laboratories which have the highest percent agreement are the ones which test the largest number of samples.

During the past five years, thirty-six individuals from twenty-seven states and territories and two South American countries have been trained in the conduct of the complement-fixation test; the majority of these individuals were college trained and many had advanced degrees in bacteriology and veterinary medicine.

The Animal Disease Eradication Laboratory at Beltsville conducts approximately 100,000 tests for anaplasmosis yearly on samples submitted from almost every state in the Union. At the present time we are assisting eight states in conducting anaplasmosis surveys using serum from brucellosis samples prepared in the regional laboratories, to determine the extent of the disease in those areas and to arrive at practical means for control and eradication of this disease.

During the past year, 346,300 test doses of anaplasmosis antigen were distributed to twenty-five state, federal and research laboratories by this Division.

Table 2—Comparative Tests With One Laboratory (D) on Samples Submitted Monthly

	Percent Agreement	
Number Of Samples	Identity of Samples	Serum Titers of Positive Samples
100	99.0	98.0
75	99.9	100.0
95	99.5	99.5
50	100.0	99.3
100	99.5	97.1
100	99.4	98.0
100	99.9	98.0
99	99.5	100.0
100	100.0	98.5
100	99.0	99.5
100	100.0	98.5

Table 3—Comparative Test Results of Field Laboratories for the Last Three Years on Identity of Samples

	Percent Agreement		
Laboratory	1959	1960	1961
A	---	---	100.0
B	95.1	---	100.0
C	78.1	95.7	100.0
D	89.7	100.0	100.0
E	96.0	100.0	100.0
F	94.0	98.8	99.2
G	88.8	98.8	99.2
H	98.6	100.0	98.4
I	---	91.1	98.4
J	---	---	97.6
K	91.1	98.2	97.5
L	92.9	93.9	96.8
M	---	93.3	91.9
N	90.2	96.3	94.4
O	---	95.1	93.3
P	95.5	99.4	93.3
Q	96.0	86.0	89.5
R	---	---	87.1
S	---	---	83.9
T	75.4	98.8	---
U	91.1	82.1	---
V	---	84.6	82.3

A Capillary Tube Agglutination Test for Anaplasmosis

Miodrag Ristic

This investigation was supported in part by research grants from the National Institute of Allergy and Infectious Diseases, United States Public Health Service (E-3315) and from Diamond Laboratories, Inc., Des Moines, Iowa.

A distinguishing characteristic of *Anaplasma marginale* infection is that following disappearance of microscopically visible marginal bodies from the erythrocytes, recovered animals enter the carrier phase of the disease. During this phase the blood of carrier animals remains infectious for long periods. In one instance, a carrier lost the ability to transmit the infection 12 years after recovery from the clinical disease. More frequently however, carriers apparently retain infectious status during their entire life span. Anaplasmosis carriers are an important reservoir of infection for arthropod vectors and other transmitting agents which are capable of carrying the infection to *Anaplasma*-free cattle. Carrier cattle cannot be differentiated from noninfected animals on the basis of clinical symptoms or blood examination. Thus development of a serologic test for identification of these animals is an essential prerequisite for any effective prevention and control of anaplasmosis.

During the last decade workers in the United States Department of Agriculture produced an antigen from infected erythrocytes which proved useful for the detection of a complement-fixing antibody present in the blood of carrier animals (1). The complement-fixation (CF) test constituted a valuable serologic aid for research in anaplasmosis. However, inherent variables and difficulties in the CF test have greatly hampered development of a popular and effective program for identification of *Anaplasma* carriers on farms and ranches. The most serious objections to the CF test are found in the recent reports (2, 3) which indicate that false positive reactions have been observed with this test.

Under these circumstances it was apparent that there is a real need for additional research that may lead to the development of a more accurate and simpler test for the diagnosis of anaplasmosis. This need is well emphasized in the 1960 report of the Committee on Anaplasmosis of the U. S. Livestock Sanitary Association (4).

In the course of our studies of biophysical properties of the *Anaplasma* organism it became apparent that a technique could be devised to obtain a purified *Anaplasma* antigen from infected erythrocytes and that such an antigen could be then employed in a simpler test, e.g., agglutination test for detection of serum antibodies found in carrier animals.

The present report describes such a serologic test for anaplasmosis which is both a simple and a specific one.

Materials and Methods

More comprehensive and detailed information concerning the methods of antigen production and performance of the CA test is due to appear in the Journal of Am. Vet. Med. Assoc. The present report will describe only the main sequences of these methods. The *Anaplasma* antigen used in the CA test was liberated from acutely infected bovine erythrocytes following treatment of these cells by sonic oscillation for 10 minutes. The sonicated mixture was then centrifuged at 6950 X G for 30 minutes. The sediment was then washed in at least 20 volumes of veronal buffered saline, pH 7.2 and centrifuged in the same manner 2 more times. The final sediment was admixed with 3 volumes of veronal buffered saline and frozen at —65°C. After 24 or more hours of freezing, the suspension was thawed at room temperature, homogenized in approximately 8 volumes of veronal buffered saline and centrifuged at 1750 X G for 45 minutes. The resulting sediment was suspended in sufficient veronal buffered saline solution to make the volume equal to that of the original frozen material and centrifuged at 1750 X G for exactly 30 seconds. The supernatant contains the CA antigen. Formalin was added to a concentration of 0.2 percent and the antigen was stored at 4°C.

The serologic potency of each new antigen preparation was also determined. This was accomplished by setting in a "box titration system," serial dilutions of the new antigen with the known "reference" immune serum. An antigen with an OD of 0.070 reacted in the CA test with the "reference"* serum when the latter was diluted to 1:64 serial dilution.

Preparation of Serums

Serums were inactivated in a water bath at 56° C. for 30 minutes. Phenol (U.S.P.) was added to a final concentration of 0.25 percent and the serums were stored at 4° C. until tested. If the serums were to be tested immediately, addition of phenol was not required. The reference serum used for potency determinations of each new batch of antigen was inactivated by heat and kept in 1 ml. quantities at —65° C. Before use this serum was thawed and then phenolized.

Bacterially contaminated and turbid serums were clarified by centrifugation before testing.

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*The reference serum was obtained from an anaplasmosis carrier cow 8 months after experimental infection in April, 1960.

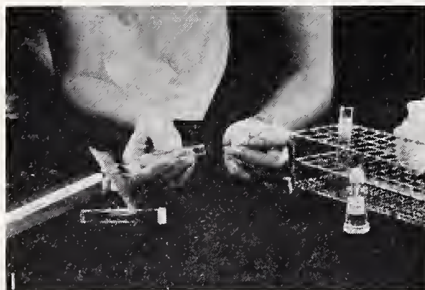
Performing the Test

Glass capillary tubes, approximately 10 cm. long and 0.5 mm. in diameter, were used. By capillary action, approximately one-third of the tube was filled with the antigen. The remaining portion of the tube was filled with the serum and the tube was set in a vertical position in clay or wax with the antigen at the bottom. The top of the tubes was sealed with nail polish in order to slow evaporation (Fig. 1). Tests were held at room temperature, and the final reading was recorded after 24 hours. High titer serums caused agglutination within 5 to 10 minutes following the setting of the test. Aggregates of agglutinated antigen were clearly visible when the tubes were viewed against a window. Reading of the test was greatly facilitated by use of a black background and a source of light situated above the tubes. A test reading box has been designed for use in diagnostic tests for anaplasmosis in the field (Fig. 1). Aggregates of antigen agglutinated by specific antibody remained visible for two to three weeks after the test had been completed.

Results

The accuracy of the capillary tube agglutination (CA) method was determined by examination of paired serum samples obtained before and after infection from 198 experimentally infected cattle. These serums were accumulated and kept at —65° C. in the course of 5 years of experimental studies of anaplasmosis. The results of examination of these serums by the CA method are shown in Table 1. In the majority of cases, the earliest CA reactions were observed before the peak of an acute infection. At the time the first agglutinating antibodies appeared, the blood examination by the Giemsa staining method usually revealed that 3 to 6 percent of the erythrocytes were harboring marginal *Anaplasma* bodies. At the peak of infection, which was the time when an animal showed the greatest number of parasitized erythrocytes, only 14 of 198 animals were still negative by the CA method. Nine of these 14 animals had been used for CA antigen production after infection by injection of massive doses of acutely infected blood. This procedure resulted in a peak of infection being reached within 4 to 6 days after inoculation. All 14 animals reacted to the test within 10 days following the peak of infection.

A comparison of the CA and the CF tests on approximately 3,000 animals of known anaplasmosis history and animals from disease free herds showed a relatively close agreement between the results ob-

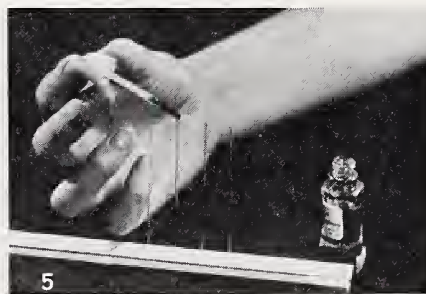


By means of capillary action approximately one-third of the tube is filled with antigen.

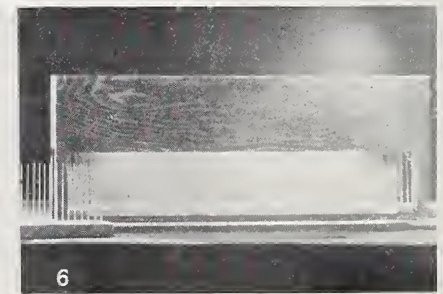
The antigen is wiped from the outside of the tube with cheese cloth.

The same end of the capillary tube is then set in the serum and the remainder of the tube is filled with serum. The antigen has now reached the opposite end of the tube.

The tube with the antigen end down is set in a vertical position in clay or wax.



The top of the tube is sealed with nail polish to stop evaporation.



A test reading box for use in anaplasmosis field diagnostic serology.

Figure 1. Performance of the capillary tube agglutination (CA) test (Illustrations 1-5) and the test reading box (Illustration 6).

Table 1 — Capillary tube agglutination test (CA) with serums of cattle experimentally infected with (A. marginale)

Cattle Age in Months	No. Serums Tested	CA Results			
			After Infection		
		Before Infection	Before the Peak[1] of Infection	At the Peak of Infection	After the Peak of Infection
2-5	82	Neg.[2] 82 (100%)	Pos.[3] 70 (85%)	Pos. 78 (95%)	Pos. 82 (100%)
6-12	94	Neg. 94 (100%)	Pos. 78 (83%)	Pos. 86 (91%)	Pos. 94 (100%)
12 and older	22	Neg. 22 (100%)	Pos. 18 (82%)	Pos. 20 (91%)	Pos. 22 (100%)
Total	198	Neg. 198 (100%)	Pos. 166 (84%)	Pos. 184 (93%)	Pos. 198 (100%)

[1]Peak of infection was the day an animal showed the greatest number of parasitized erythrocytes.
[2]Neg. = No agglutination observed.
[3]Pos. = Agglutination observed.

tained with individual serums tested in these two tests. The numbers of positive reactors were as follows: In the CF test 342 (excluding suspects in the CA) and 348 (including suspects in the CA); in the CA test 327 (excluding anticomplementary and suspects in the CF) and 371 (including anticomplementary and suspects in the CF test).

Discussion

The accuracy of the CA test is clearly demonstrated by its ability to classify pre- and post-infection serums from 198 cattle as negative and positive, respectively.

Reproducibility and specificity of reaction are also important attributes of a good serologic test. In connection with disease control in several cattle herds in the southern portion of the state of Illinois, an annual collection of blood serum from animals of the herds has been practiced during the past 3 years. Serums from individual anaplasmosis carrier animals, some also collected semiannually, were consistently positive when tested with a single or different batches of the CA antigen. In order to determine the specificity of the CA method, antiserums produced in rabbits against the following potential pathogens of cattle have been examined by this test: *Brucella abortus, Leptospira pomona, L. canicola, L. icterohemorrhagiae, Pasteurella multocida,* parainfluenza virus, *Vibrio fetus* and *Coxiella burnetii.* In no instance was there a positive or suspect result.

There are several comments one can make concerning the comparison of the CA and the CF test. Undiluted serums were used in the CA test and the serums diluted to 1:5 were used in the CF test. The latter dilution is the lowest that can be accurately examined with the CF technique, since nonspecific reactions are frequently encountered with undiluted serums. Observations of CA test results indicate that undiluted serum does not produce nonspecific reactions. Tests made with undiluted serum could be clearly read as positive or negative. The CA method has the distinct advantage that undiluted serum can be used, thus permitting detection of lower titers of antibody than with the CF test. This advantage was demonstrated with 14 serums which exhibited doubtful reactions in the CF test but gave clear-cut reactions in the CA test. Frequent occurrence of doubtful reactions, with the CF test, i.e., in 421 serums of 2380 tested is another inherent defect of this test which, at its best, is incapable of classifying uninfected cattle as such with complete accuracy.

The choice of method for serum inactivation and preservation is of great importance for adequate comparative studies and evaluation of the CA and the CF methods. The manual of the U.S.D.A. for the preparation of a serum to be tested by the CF method calls for addition of phenol to a 0.5 percent final concentration prior to serum inactivation at 58° C. for 35 minutes. The CA antibody is almost completely destroyed with a 0.5 percent or greater phenol concentration. This result is explained by the fact that denaturation and inactivation of antibodies in phenol or formalin treated serum is accelerated by heating. It is therefore important that the serums are heat inactivated before phenolization and the instructions concerning further serum preservation and testing are followed exactly as described here.

The CA test serves primarily to determine whether an animal is or is not actively infected. This information is regarded as paramount from the practical and economic standpoint. Figure 2 illustrates 3 different degrees of reaction obtained with serums from 3 different animals. Preliminary studies have shown that positive serums exhibiting 2+ and 3+ reactions can be titrated, thus making the CA technique valuable also for research purposes requiring titer evaluation (Fig. 3). The titer of this serum was 1:16. The next serial serum dilution tube (1:32) contained very minute aggregates which adhered to the wall of the capillary tube and were barely visible to the naked eye. Undiluted serums which react in such a manner are designated as suspects.

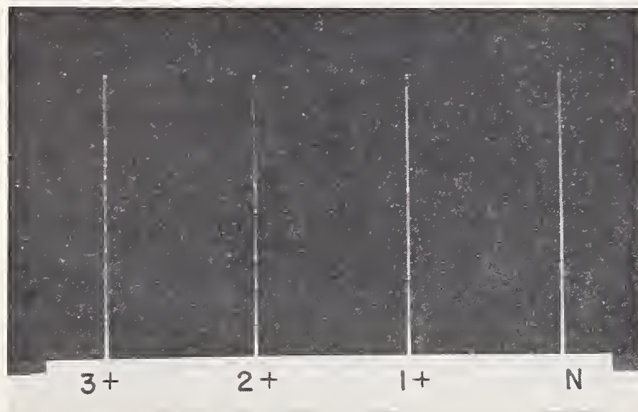


Figure 2. Anaplasmosis capillary tube agglutination reactions. From right to left: N=negative; 1+ = positive; 2+ = strong positive; 3+ = strongest positive.

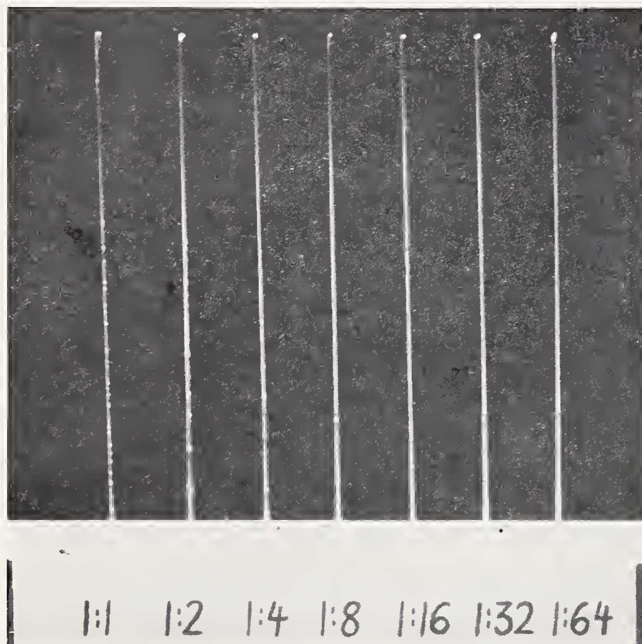


Figure 3. Titer evaluation of a serum from an anaplasmosis carrier animal. The titer of this serum is 1:16. The next serial serum dilution tube (1:32) contains very minute aggregates adhered to the wall of a capillary tube and barely visible by the naked eye. Undiluted serums reacting in such a manner have been preliminarily designated as suspects.

The greatest advantages of the CA test in comparison with the CF test are its simplicity and the rapidity with which it can be performed. The problem of anticomplementary activity of serums does not arise in the CA test and hemolyzed serums, frequently observed during the summer months, can be easily tested by the CA method. In addition, the CA test requires only limited equipment and minute quantities of antigen and serum. The test does not require specially trained personnel for its performance and interpretation. Thus, the CA test can be used for survey purposes to determine the prevalence of anaplasmosis; it should be especially convenient for use by the practicing veterinarian.

Summary

A capillary tube agglutination (CA) test for the diagnosis of anaplasmosis has been developed. By this test 198 artificially infected cattle have been accurately identified. On the basis of tests of approximately 3,000 serums, the sensitivity and specificity of the CA test for detection of anaplasmosis carrier animals were shown to be comparable to those of the complement-fixation (CF) test.

The advantages of the CA test are simplicity, economy, and speed of performance. The CA test appears to be useful in the prevention and control of anaplasmosis.

References

1. Gates, D. W., Mohler, W. M., Mott, L. O., Poelma, L. S., Price, K. E. and Mitchell, J.: A comparison of antigen production methods and complement-fixation procedures for diagnosing bovine anaplasmosis. Proc. 58th Ann. Meeting, U. S. Livestock Sanitary Association (1954):105-114.

2. Heck, F. C.: Studies on the complement-fixation test. Presented 4th Nat. Anaplasmosis Conference, April 26-27, Reno, Nevada.

3. Osebold, J. W.: Experience with Anaplasmosis in Species other than Cattle. Presented 4th Nat. Anaplasmosis Conference, April 26-27, Reno, Nevada.

4. Report of the Committee on Anaplasmosis, Proc. 64th Annual Meeting, U. S. Livestock Sanitary Association, October 18-21, (1960):102-104, Charleston, West Virginia.

Demonstration of Anaplasma marginale with Acridine Orange

J. H. Gainer

An article concerning a method of demonstrating *Anaplasma marginale* with acridine orange (AO) was published in the September, 1961, issue of the American Journal of Veterinary Research (AJVR). In brief, the article stated that erythrocytes, as smears or whole blood, are fixed 24 hours in 10 percent formalin-saline and are stained ten seconds in 1:1000 acridine orange in 0.1 N. H. Cl. These smears are then observed with a fluorescence microscope, and, with appropriate filters *A. marginale* appears as a brilliant orange object in the faint green erythrocyte. Leucocytes, bacteria, or other blood parasites stain varying intensities of orange. Immature erythrocytes are a dull orange. Thrombocytes are yellowish green. Residual plasma protein is light green. Fibrin strands are long yellowish green structures. The procedure of fixation and staining is very simple, the formalin-fixed blood keeps indefinitely, the stained smears retain their stain for long periods when kept in the dark, as e.g. in a black slide box; and, at least in the active disease state, the organisms are readily recognized.

Comparison between this staining method and the complement-fixation (CF) test was made to determine the relative accuracy of the procedure to detect carrier animals. Nine herds consisting of 1310 animals were studied; herds varied in size from 14 to 472. Prof. Kliewer, Dr. Ristic, and Dr. Martin kindly performed the CF tests. The study revealed that only 46 percent of the samples were both AO+ — CF+ or AO— CF—; 36.3 percent were AO+, CF—; and 10.8 percent were AO— CF+; suspects in both tests accounted for the remaining percentages. One dairy herd of 14 animals, which had not had clinical anaplasmosis for 3 years, correlated 85.7 percent—all 14 were CF negative, 2 were AO postive. Another dairy herd of 60 animals revealed only 30 percent correlation with 41/60 animals possessing AO+CF— reaction. When the largest herd in the study, consisting of 472 dairy animals, was divided into varying age groups, the calves and yearling groups correlated by 25.6 percent, the adults correlated 45.1 percent, and a group of cows considered to have had anaplasmosis the preceding year revealed 62.5 percent correlation; overall this herd had had a 41.8 percent correlation. Of the 80 cases of suspected disease the preceding fall (about 6 months prior to these tests), CF the following spring showed 71.3 percent +, AO revealed 68.8 percent +. AO and duplicate CF's were done on 52 samples from this herd by Prof. Kliewer and Dr. Ristic. Seven of these 52 had been reported to have had anaplasmosis the preceding year; 5 (71.4%) reacted + to AO, 2 (28.6%) CF+ by Prof. Kliewer, and 1 CF+ (14.3%) by Dr. Ristic.

Another dairy consisting of 319 samples revealed a total correlation of 53 percent. When these were broken down into known cases of anaplasmosis based upon clinical diagnosis by a competent practicing veterinarian, correlation was 55.1 percent, AO still read 65.5 percent +, CF only 41.4 percent +. In the same herd when 22 suspected cases were compared, AO-CF correlation was 49.9 percent, AO called 72.5 percent +, CF called 50 percent +.

This acridine orange staining method is a specific histochemical reaction based on the fact that under a strong hydrogen ion concentration nuclear materials (nucleic acids in particular) have a strong affinity for the dye. Based upon this assumption, it was predicted early in the development of this staining procedure that any blood cell parasite would stain readily with acridine orange. Additionally, it was already observed that leucocytes readily fluoresce because they contain nuclei, whereas adult mammalian erythrocytes contain no such nuclei. Immature erythrocytes and thrombocytes are also readily discernable. Thus far, our predictions have held true in so far as they have been examined: (1) *Eperythrozoon spp.* and *Hemobartonella spp.* stain readily, (2) *Piroplasma caballi* as seen in Florida is readily demonstrable, (3) *Babesia argentina* readily stain as described in a personal communication by Dr. C. G. Ludford, Senior Serologist, Animal Research Institute, Yeerongphilly, Queensland, Australia, (4) *Babesia canis* has been readily identified.

Discussion and Summary

From the comparison study, it is apparent that either the AO stain is demonstrating many false positive reactions or the CF test is giving many false negative reactions. Checking for the discrepancies between the tests by animal inoculation has not been done. Based upon studies of the CF test by earlier workers, it would appear that the CF test is possibly at fault in only a low percent of cases, whereas the AO stain is falsely positive in a high percent of the cases. Ready differentiation between *A. marginale* and the Howell-Jolly body by AO must not, therefore, be possible; this may be especially true in younger animals where one might anticipate a higher percent of Howell-Jolly bodies than in the older mature animal. On the other hand, when one examines the cases where clinical disease was reported to have occurred, even though validity of the diagnosis may be questioned, the AO stain seems much better and, in fact, reveals a higher percentage of carriers than does the CF test. On theoretical grounds one might expect that looking for the parasite would be a better means of diagnosis than is looking for an antibody to the parasite; such is especially true in this infection where chronicity of infection exists in spite of the presence of CF antibody. The percent of residually

circulating infected cells in a carrier precludes as to the efficacy of this AO method; by our method one cell among 10,000 must be infected.

The acridine orange stain far surpasses the Wright's or Giemsa stain in demonstrating *A. marginale.* Worrisome stain deposits as exist with these stains are practically non-existent with AO. Under the conditions described, AO readily stains *Babesia* or *Piroplasma* which, in our hands, stains very faintly with Giemsa or Wright's stain. Most blood particulate materials may be readily differentiated by this staining procedure.

Immunity in Anaplasmosis

William A. Summers

Sufficient evidence has been accumulated through the studies of numerous investigators to confirm that anaplasmosis is an infectious disease of cattle and other ruminants. Theiler (1) established that this disease was caused by a microorganism which parasitized the erythrocytes, that it was distinct and separate from *Piroplasma bigemina* and in 1910 gave it the name *Anaplasma marginale.* Subsequently, he and others demonstrated that the primary infection in cattle resulted in considerable resistance to reinfection and that survivors continued to be carriers of the microorganism, inasmuch as their blood remained infectious for non-immune cattle for long periods.

On the basis of this demonstration of acquired immunity, several attempts were made by Theiler (2) and other (3), (4), (5), (6), (7) to induce artificial active immunity in cattle by means of vaccines. This proved to be unsuccessful although some investigators have reported a lengthening of the incubation period and slight alterations in the course and severity of the disease following the use of vaccines.

Based upon the impressions gained from the studies of many investigators in the past, as well as from his own studies, Ristic (8) recently described in a general way the functioning of the immune mechanism in anaplasmosis as follows: "In those animals which are destined to recover, the gradual increase of circulating antibody and the intense proliferation of the fixed and migrating macrophages would render the infection of normal erythrocytes increasingly difficult until, with the onset of convalescence, infection would be confined to a smaller percentage of erythrocytes. From time to time some of these infected erythrocytes, destroyed by the anaplasma growing within them, would disintegrate, liberating the organisms, most of which would meet and after uniting with antibody, would be prevented either from entering susceptible erythrocytes or, having entered would be unable to multiply. Certain numbers of the organisms, however, would escape the antibody and enter and continue perpetuation of the infection within other erythrocytes. At the same time, these intermittent invasions of the *anaplasma* would provide the antigenic stimuli necessary to maintain humoral and cellular anti-anaplasma forces at an effective level." Ristic visualizes the eventual disappearance of *anaplasma* with the waning of the acquired immunity and a return of the animal to susceptibility.

It should be obvious that this description of the course of events in anaplasmosis in the intact animal has been offered as a working hypothesis. It is a reasonable explanation of the functioning of the immune mechanism, but like any hypothesis makes certain assumptions and draws certain inferences for which there is either insufficient evidence or no evidence. In essence the hypothesis states that, following infection, antibodies directed against the organism, and host defense cells having the inherent ability to phagocytize, increase in amount and number respectively, until they reach a peak after which their activity subsides. Following a variable period of asymptomatic latency immunity disappears and susceptibility to infection returns. In some respects this immunological pattern is similar to that known to occur in many infectious diseases, each differing from the others in terms of antigenicity and virulence of the parasite, in the ability of the host to react favorably or unfavorably, in the duration of immunity and in the duration of latency of the infection.

Acceptance of this hypothesis will require critical analysis and evaluation of the immunological data offered in its support. In addition, precise experimental data must be obtained to support or deny the assumptions embodied in it. Thus in analyzing the hypothesis we find a number of biological events described which require experimental proof and on the other hand, some for which there seems to be adequate experimental proof. In the latter category are the following:

1. The infectious nature of *A. marginale* and its presence in the erythrocytes of infected cattle.

2. The development of a latent infection or carrier state characterized by the persistence of the organism in the blood.

3. The antigenicity of *A. marginale* with the development of serologically detectable antibody.

4. The intense proliferation of reticuloendothelial cells, particularly fixed and circulating macrophages, Kupffer cells and reticular cells of the bone marrow, during the acute stage.

Particular aspects of this hypothetical consideration of the functioning of the immune mechanism for which there is either no precise experimental evidence or inadequately confirmed experimental data are as follows:

The conception of the life cycle which portrays *A. marginale* confining its activities to the erythrocytes as it was proposed by Lotze and Yiengst (9) and later amended by Ristic (8). This does not take into account the possible role of other cells and tissues as sites of infection and propagation in spite of the fact that many such cells and tissues are known to be affected during the course of the disease. In various studies specific reference has been made to the histopathology seen in lymph nodes, spleen, bone marrow, vascular endothelium, liver, kidney, mucous membranes of the gall bladder etc., and yet few suggestions have been offered as to an underlying mechanism for the drastic changes. In view of this it seems imperative that studies be undertaken to determine whether or not infection with *A. marginale* is concerned solely with erythrocytes. The immunological and pathological characteristics of anaplasmosis may very well be the result of the influence of the organism upon cells and tissues other than erythrocytes. Furthermore there has been very little experimental data offered to indicate that *A. marginale* actually causes the disintegration of infected erythrocytes as assumed in the hypothesis.

The assumption that phagocytic cells, which increase markedly during the acute phase of the disease, phagocytize and thus clear the blood and tissues of the organism per se, lacks convincing proof. What has been observed has been the presence in certain of these cells of hemosiderin and even intact infected erythrocytes, but it has not been made clear as to whether such erythrocytes have been phagocytized because they were physiologically abnormal or because of some specific antigen-antibody reaction involving *A. marginale* per se, such as might be expected in opsonization. Ristic and Sippel (10) suggested that the complement-fixing antibody might act as an opsonizing antibody to render the parasite more susceptible to phagocytosis but there has been no critical experimental data presented to support this contention. The gathering up and destruction of aged or altered erythrocytes is supposedly a normal function of many of these cells under ordinary conditions.

Jiminez de Asua (11) *et al,* attempted to evaluate the role of the reticuloendothelial system in acquired immunity in anaplasmosis by blocking its activity with India ink. In the use of this method the assumption is made that mechanical blockage of the RES cells prevents further phagocytic activity, thus permitting any injected infectious agent to grow in an uninhibited fashion. They observed that all types of RES cells except the splenic monocytes became filled with the ink granules. In such animals the severity of the disease was not greater than in unblocked animals. The authors concluded that blockage of the RES has little or no influence upon the severity of the disease. There has been no reported confirmation of these observations.

More recently Ristic (12) *et al.* investigated the effect of cortisone upon intact and splenectomized anaplasma-infected cattle. Their experimental premise was based on the known depressive action of cortisone for inflammatory cells. They anticipated that relapse would occur in cortisone-treated cattle deprived of the protective function of these cells. Instead, following splenectomy, they observed continued elevation of the complement-fixing antibody, no increase in numbers of anaplasma and a high percent of survival among animals that had received cortisone for several weeks before splenectomy. Animals that received cortisone beginning on the day of splenectomy, or not at all, relapsed and died of anaplasmosis. In them the parasite increased and the complement-fixation titer dropped.

The authors interpret from these observations that cortisone had stimulated extra-splenic tissues sufficiently to take over anti-anaplasma activity after removal of the spleen. Thus in the study of Jimines *et al.* drastic interference with the phagocytic system by the method employed did not appear to predispose anaplasma-infected cattle to death, a fact which may indicate the possible presence of other anti-anaplasma forces in the animal body. In the study of Ristic *et al.* cortisone appeared not to interfere with the protective function of the spleen but rather contributed to the resistance of the animals by causing a marked increase in extra-splenic anti-anaplasma activity.

Interesting as these experiments may be, they raise more questions than are answered. Both are based upon experimental methods in which the presumed active mechanism is incompletely understood and known to produce variable results in different species of animals. It is questionable whether the kind of precise data that is needed for a more complete understanding of the immune processes in anaplasmosis can be obtained by such methods. Furthermore, before the results can be finally evaluated other investigators should be encouraged to repeat these studies. Evidence of the protective

role of the host's phagocytic cells must be obtained by demonstrating the presence of the organism within them microscopically. This might be done by the use of available, relatively simple methods such as the immunofluorescent antibody technique or by the *in vitro* demonstration of the opsonizing action of bovine convalescent serum in conjunction with bovine reticuloendothelial cells.

The assumption made in the hypothesis that Anaplasma organisms are affected in some way by circulating antibody which prevents their entry into or multiplication within new erythrocytes lacks substantiating proof. In fact Rosenbusch and Gonzalez (13) showed that anaplasma convalescent serum had no effect on the infectivity of *A. marginale* following *in vitro* exposure. It is not difficult to detect a possible flaw in their experiment, however, inasmuch as they exposed intact infected erythrocytes, instead of liberated organisms to the convalescent serum. This technique may have prevented the union of antibody with the organism during the 24-hour period of incubation. Thus no one has actually demonstrated that the immune serum possesses any appreciable influence upon the infectious agent.

Many serological activities have been determined for an antibody developed during the course of infectious diseases. One or more activities such as agglutination, precipitation, inhibition of reproduction, neutralization of infectivity, toxin neutralization, fixation of complement, lysis or opsonization may characterize such an antibody. Some, or all of these *in vitro* activities of antibody can be correlated with protective action against the homologous infectious agent. However, the mere serologic demonstration of the presence of such antibody activity in anaplasmosis, or in any other infectious disease, does not constitute proof of its protective function in the host. There is little doubt that *A. marginale* is antigenic, inducing the formation of antibody detectable by complement-fixation, immunofluorescent and possibly other techniques. The development of antibody indicates a response to an antigenic substance associated with *A. marginale* but the evidence for a protective role for this antibody is not very convincing.

In addition to the serological detection of antibody in anaplasmosis, demonstration of its ability to passively transfer resistance to normal animals, and to have its protective property absorbed from the serum by *A. marginale* resulting in loss of ability to transfer resistance, is needed before its protective function can be evaluated.

A further criterion required to demonstrate a possible protective role of antibody in infectious diseases is that concerned with its production by artificial immunization. Failure to be able to do this has characterized the results of anaplasma-investigators in this area since the time of Theiler. The best that has been attained in this respect has been a slight increase in incubation period, and some alleviation of the severity of the disease. Animals immunized in this fashion are subject to challenge infection, undergo a somewhat milder course, develop latency, and according to Kuttler (7) develop complement-fixing antibody. In general, several reasons may be offered in possible explanation of the failure to produce protective immunity by artificial immunization. These are:

a. The antigen normally may not be able to induce the formation of protective antibody.

b. The route, dosage and duration of vaccine administration may seriously affect the nature and amount of antibody produced.

c. The immuno-chemical nature of the antigen may be altered by procedures and techniques that have been used in its preparation.

d. Potentially protective antibody, though it may be present in the circulation, may not be able to combine *in vivo* with the disease producing agent because the latter may be largely inaccessible to it, residing more or less continuously in various host cell types.

Theiler (14) observed that immunity following initial infection with *A. marginale* was not complete. He mentions the fact that an animal may be successfully inoculated more than once, although the second inoculation produced only a mild reaction. Furthermore, according to Theiler, the latent infection of carrier animals, may reappear in a milder form under the influence of other diseases of cattle.

There seems not to have been many recent attempts to study the degree of immunity to anaplasmosis in naturally or experimentally infected cattle. In this connection, however, Kuttler (7) found that low antibody titers could be produced by vaccination of cattle with an anaplasma antigen. Although such animals were always subject to challenge infection some beneficial effects of prior vaccination were noted. It is significant that following challenge, vaccinated animals developed higher complement-fixation titers than did unvaccinated animals, a fact which suggests the occurrence of an anamnestic response.

Thus, the period of latent inapparent infection associated with the presence of antibody as observed in anaplasmosis is not a unique phenomenon. This has been described as "non-sterile" immunity or infection-immunity and is seen in such chronic infectious diseases of man as syphilis, tuberculosis, typhoid fever and some mycotic diseases. To a lesser extent infection-immunity occurs in some viral and rickettsial diseases of man and animals.

Summary

An hypothesis, relative to the functions of the immune mechanisms in bovine anaplasmosis, presented by Ristic (4), is supported in part by well established data. Future studies aimed at testing the validity of the hypothesis will be required, especially with respect to (1) the life cycle of *A. marginale* in the bovine host and (2) the role of the cellular and humoral defense mechanisms in immunity.

References

1. Theiler, A. (1910) *Anaplasma marginale* (Genus nov. et species nov.) Un nouveau protozaire du betail. Bull. Soc. pathol. exotique 3: 135-137.
2. Theiler, A. (1912) Weitere Untersuchungen uber die Anaplasmosis der Rinder und deren Schutzimpfung. Z. Infections krankh. parasit. Krankh. u. Hyg. Haustiere. 11:193-207.
3. Lignieres, J. (1919) La vaccination des bovides contre l'anaplasmose. L'Anaplasma inocule au mouton et a la chevre s'attenue dans l'organisme de ces especes animales et leur sang est alors un excellent vaccin pour les bovides contre l'anaplasmose la plus grave. Bull. Soc. pathol. exotique. 12: 765-774.
4. Lignieres, J. (1928) Sur la vaccination des bovides contre le piroplasmose, la babesiellose et l'anaplasmose. Comparison des procedes employes en Argentine et en Algerie. Bull. so. pathol. exotique. 21: 371-378.
5. Pearson, C. C., Brock, W. E., and Kliewer, I. O. (1953) Studies on the u-e of biologics in control of anaplasmosis. Vet. Med. 48: 435-437.
6. Mott, L. O. (1957) The natuie of anaplasmosis. Proc. 3rd Natl. Res. Confeience. Anaplasmosis, Manhattan, Kansas. pp. 1-9.
7. Kuttler, K. L. (1961) Anaplasmosis immunization studies. J. Am. Vet. Med. Assn. 139: 1306.
8. Ristic, M. (1960) Anaplasmosis. Advances in Veterinary Science 6: 111-192. Academic Press.
9. Lotze, John C., and Yiengst, Marvin J. (1942) Studies on the nature of Anaplasma. Am. J. Vet. Research 3: 312-320.
10. Ristic, M. and Sippel, W. L. (1958) Effect of Cortisone on the Mechanism of Anaplasma Immunity in Experimentally Infected Calves. II. Studies of Pathological Changes. Am. J. Vet. Res. 19: 44-50.
11. Jiminez de Asua, F., Dios, R. L., Zuccarini, J. A. and Kuhn, M. J. (1927) Intervencion del sistema reticuloenthelial en "tristeza" (piroplasmosis y anaplasmosis bovine). Rev. Asoc. Arg. biol. 3: 541-546.
12. Ristic, M., White, F. H., and Sanders, D. A. (1958) Effect of Cortisone on the Mechanism of Anaplasma Immunity in Experimentally Infected Calves. I. Hematological and Immunoserological Studies. Am. J. Vet. Res. 19: 37-43.
13. Rosenbusch, F., and Gonzalez, R. (1925) Beitragzum Studium der Tristeza. Archiv fur Protistenkunde. 50: 443-485.
14. Theiler, A. (1910) Anaplasma marginale (Genus et spec. nov.) The marginal points in the blood of cattle suffering from a specific disease. Rept. Govt. Vet. Bacteriol. Transvall, S. Africa 1908-09., pp. 7-64.

Observations of Possible Immune Responses in Cattle to Anaplasmosis Not Associated With Active Infection

K. L. Kuttler

Studies have been conducted using killed anaplasmosis organisms with adjuvants to produce a vaccine in an attempt to induce an immune response to anaplasmosis in susceptible cattle. Work to date indicates that vaccination with such products will produce an immune response which will reduce the severity of clinical anaplasmosis but will not prevent the development of the carrier state.

Figures 1 and 2 show a typical response in splenectomized calves, following challenge with 5 ml. of carrier blood, in a previously vaccinated animal (Figure 2) and a non-vaccinated control (Figure 1). Table 1 summarizes the effects of challenge with 5 ml. carrier blood subcutaneously in 17 vaccinated and 13 unvaccinated controls. Significantly longer incubation along with less severe anemias occurred in vaccinated animals. The number of anaplasma bodies observed

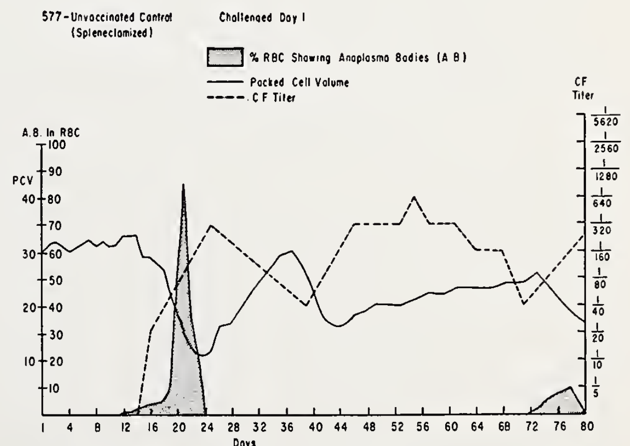


Figure 1.

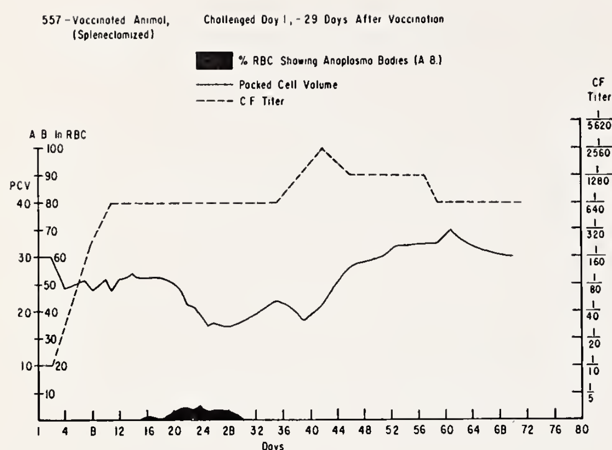


Figure 2.

in red blood cells during the infection were fewer in vaccinated animals than in non-vaccinated controls.

Field vaccination, in cooperation with Dr. L. M. Koger, Ontario, Oregon, followed by CF surveys indicated that all animals responded to the extent of developing CF titers. Complement-fixation results 15 and 101 day post vaccination are presented in Table 2. All animals were positive to the complement-fixation tests 15 days after vaccination with titers ranging from 1/5 to 1/160. Observations of the same animals 101 days after vaccination showed most to be negative or to have less than 1/5 titers.

Survey complement-fixation testing (CF) of a cow-calf herd in an enzootic area has shown 6- to 8-month-old calves have a much lower rate of infection than adult cows even after exposure to infection. Table 3 charts the rate of transmission in calves. In August, after range exposure during the first summer season of their lives, an incidence of 8% was recorded, which compares with 74 percent incidence in August a year later. This observation suggests the possibility of some degree of immunity or transient low level infection among calves during their first exposure. Weaned calves of similar age, when given an artificial exposure in the course of anaplasmosis experiments, have been shown to contract anaplasmosis and become carriers with subsequent CF positive reactions. Clinical manifestations among these young animals are generally mild.

A study of calves born to cows which were carriers of the infection ,shows that within a few days after birth a high percent were positive to the complement-fixation test (Tables 3 and 4). At an average age of 9 days, 78 percent were positive. This decreased to 54 percent at 27 days of age and to only 4% reactors at 57 days of age. Maximum serum titers to the compliment-fixation test were reached at 6 to 10 days of age followed by a gradual decline. An analysis of cow vs. calf (less than 20 days of age) CF titers revealed significant correlation with high calf titers being associated with high cow titers, along with the reverse. Calf CF titers were significantly lower than cow titers, however.

These observations suggest a passive transfer of CF antibodies, probably by way of colostrum, from the carrier cow to the calf. Attempts to demonstrate infection in calves up to 30 days of age, reacting to the CF test have to date been negative. Blood from 13 calves, positive to the CF test, have been subinoculated into susceptible animals without producing any infection. Evidence of the probable transfer of colostral CF antibodies and of increased resistance to exposure in calves during the first year suggest the possibility that immune antibodies are also transferred to the calf via the colostrum, producing a degree of non-infective immunity.

Table 1—Effects of Vaccination* on Splenectomized Calves and Adult Cows When Challenged With Virulent Blood

	No. of Animals	Average Low PCV**	Time interval in Days from challenge to the development of low PCF
Vaccinated Animals	17	20.1%	35.9
Unvaccinated Controls	13	14.3	30.7
Diff.		5.8	5.2
Possibility of Error		<.01	<.05

*Table includes cattle vaccinated with 5 different vaccines. Some were better than others, but all produced a CF response.
**Packed Red Cell Volume

Table 2—Summary of Field Trial in Which 50 Yearlings Were Vaccinated for Anaplasmosis

		Reaction to the Complement-Fixation Test							
Controls	Total No. of Animals	Neg. or 1/5 Reaction	1/5	1/10	1/20	1/40	1/80	1/160 and over	PCV*
Day 1**	50	50	0	0	0	0	0	0	35.0
Day 15	48	0	20	4	4	13	6	1	32.8
Day 101	45***	28	11	2	0	0	0	4	37.4
Unvaccinated controls									
Day 1	50	50	0	0	0	0	0	0	34.7
Day 15	50	50	0	0	0	0	0	0	32.8
Day 101	45***	42	0	0	0	0	0	3	36.9

*PCV—Packed Red Blood Cell Volume
**Animals vaccinated on Day 1
***Between Day 15 and 101 the cattle were on range. The drop in numbers is due to the mechanics of handling. No known deaths occurred in the experimental groups.

Table 3—Seasonal Incidence of Complement-Fixation Reactions Among Native Spring Calves* in an Area of High Incidence**

	No. of Yrs. tests were conducted	No. of Calves tested	Percent CF Positive	Average Age (Days)
April	1	36	78%	9
May	2	71	54%	27
June	1	23	4%	57
July	1	34	6%	88
August	1	26	8%	119
No tests conducted in September, October and November				
December	3	90	16	241
January	3	96	23	272
March	3	98	22	331
May	3	103	25	392
June	3	96	53	423
August	3	95	74	484

*Calving began in March and continued into May.
**Adult cows showed an anaplasmosis incidence of 88%, based on the CF test.

Table 4—CF Titers in Calves Born to Anaplasmosis Carrier Cows at Various Ages

				Serum Control	Dilutions in Which complement-fixation occurred				
Calf Age	No. of observations	Negative	Suspicious	1:5	1:10	1:20	1:40	1:80	Avg. Titer
Less than 24 hrs.	12	1	3	2	2	1	3	0	1/15
1-5 days	13	1	3	4	1	0	4	0	1/15
6-10 days	13	0	1	1	1	3	4	3	1/37*
11-15 days	9	0	0	6	0	2	1	0	1/12
16-20 days	8	2	2	1	3	0	0	0	1/5
21-25 days	16	4	1	9	0	1	1	0	1/7
26-60 days	32	13	5	11	0	2	1	0	1/5

*Significantly different, $P < .01$ from all other values

Additional Observations on Immunity and Immunization The Relation of Antibody Response and Clinical Anaplasmosis To Injection With C-F Antigen

O. Kliewer, C. C. Pearson, W. E. Brock

In 1961 Kuttler (1) reported that injections of anaplasma antigen prior to infection with anaplasmosis reduced the clinical symptoms and death losses from the disease. Since this method appears to offer some hope of reducing losses from anaplasmosis we investigated the relation of the antibody response following stimulation by complement-fixing antigen to the degree of anemia and clinical illness developed in anaplasma infected cattle.

Procedure

Twenty, 2-year-old Hereford cattle were randomly divided into 4 groups of 5 cattle per group. One group was given antigen 89 days before infection with anaplasmosis. A second group was given antigen 6 days after infection with anaplasmosis. A third group was given antigen 138 days prior to a second dose of antigen. The second dose of antigen was given at the same time the other groups were exposed to anaplasmosis. The fourth group was infected with anaplasmosis without receiving any injections of the antigen.

Each dose of antigen consisted of 250 mg. of the Price-Poelma C-F antigen suspended in 5 ml. of mineral oil. All of the cattle in the three anaplasmosis infected groups received 10 ml. aliquotes of the same sample of carrier blood on the same day. Sera for titration were obtained daily from each animal following injection of either antigen or carrier blood. After the injection of carrier blood, Wright's stained blood smears were examined every other day for the presence of anaplasma infected red blood cells. When these blood smears showed 1 percent anaplasma infection, hemoglobin, packed cell volume and erythrocyte counts were run every other day until convalescence of the animal was fully established.

Results

In the analysis of the results of the titrations, means of dilutions giving a 4+ reaction for the 5 animals in a group were taken for each day. These results are shown in figure 1. Both groups in which the antigen was given prior to the time of infection show an initial antibody response to the antigen on the eighth day. The highest titer reached in this initial reaction in either group is 1:40. After a few days the titer decreases to 1:10 where it is maintained for 45 to 50 days. There is, after this time, essentially no measurable antibody until after the second injection with anaplasma organisms or C-F antigen.

Both of these groups show an antibody response to the second injection of antigen on the 6th day. The antibody response to the carrier blood increases gradually over a period of 33 days to reach a maximum titer of 1:2560. After two days the titer returns to approximately 1:1280 where it remains during the next 35 days.

Antibody response to the C-F antigen is more rapid, attaining its maximum titer of 1:1280 in 5 days which, with some variation, is maintained for 15 days. The titer then varies between 1:160 and 1:80 during the next 50 days.

Antibody response in the group receiving the C-F antigen six days after injection with carrier blood is indicated by an initial titer of 1:20 on the 12th day after injection of the carrier blood. The magnitude of this response increases over the next ten days to reach a titer of 1:1280. The titer of 1:1280 is essentially maintained for the next 24 days after which it gradually declines during the next 35 days to a titer of 1:160.

The group receiving only carrier blood shows an initial titer of 1:20 on the 13th day after inoculation. The antibody response increases rapidly over the next 9 days to reach a maximum titer of 1:1280 which is maintained during the next 15 days. The titer then decreases during the next 40 days to a minimum of 1:160.

These results indicate that a killed anaplasma antigen given 3 or 4 months prior to infection with anaplasmosis decreases by approximately one-half the time required by the body to produce an antibody against the infectious organism. Comparison of the 3 days required to show antibody response after the initial stimulation with killed antigen in the pre-infection groups with the 12 and 13 days required by the other groups may indicate a more rapid initial response from this dosage of C-F antigen than from the living organism. The combination of C-F antigen and subsequent anaplasmosis infection produces the greatest and most prolonged antibody response.

Comparison of the antibody data between the post-infection group and the control group shows little difference which may be significant. The post-infection antigen group shows a 1-day shorter response period and a more rapid rise to a titer of 1:320 than the control group. Otherwise, in magnitude and duration the antibody titers are very similar.

Figure 2 shows the mean values of the packed cell volumes to indicate the degree of anemia, and the percentages of anaplasma infected red blood cells to

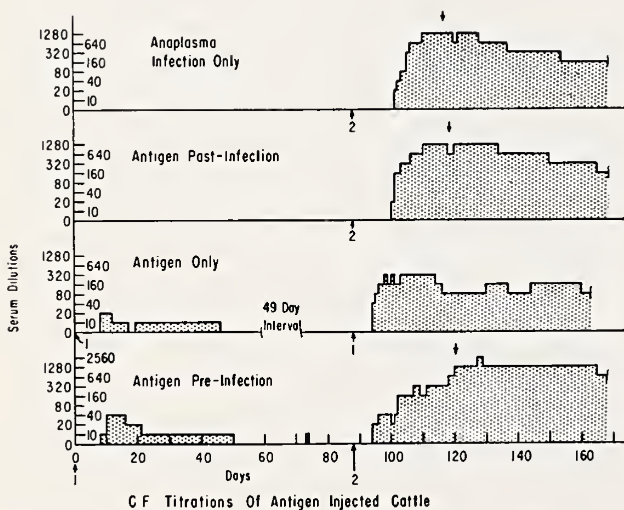


Figure 1. Average complement-fixation titers of the 4 groups of cattle. The number 1 on the time axis indicates the time of injection with antigen and the number 2 indicates the time of injection with carrier blood. The arrow above the titrations indicates the time of maximum erythrocyte infection with anaplasma.

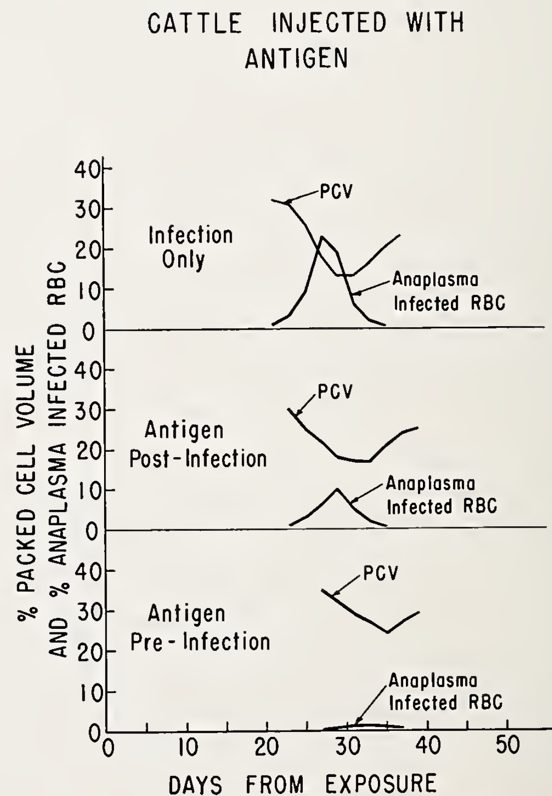


Figure 2. The average percentage of anaplasma infected erythrocytes and packed cell volumes found in the 4 groups of cattle.

indicate the degree of infection in the three groups of animals infected with anaplasmosis. Day zero is the day on which the animals received carrier blood.

The incubation period for the group receiving antigen prior to infection is 27 days; that of the group receiving antigen 6 days after infection is 23 days; that of the group which did not receive antigen is 21 days. The packed cell volume of the group receiving antigen before infection shows a low value on the 35th day of 24 percent. The percentage of anaplasma infected red blood cells in this group shows a high of slightly more than 1 percent between the 31st and 33rd days. The cattle in this group showed no clinical illness.

The group which received antigen after infection shows low packed cell volume of 17 percent on the 31st to 33rd days. This group has a peak percentage of anaplasma infected red blood cells of 10 percent on the 29th day. Some cattle in this group showed clinical signs of anaplasmosis.

The control group which did not receive antigen shows a low packed cell volume of 13 percent between the 29th and 31st days. The peak percentage of anaplasma infected red blood cells in this group is 23 percent on the 27th day. These cattle became definitely ill with marked anemia and characteristic signs of anaplasmosis.

Summary

In summary, the injection of a C-F anaplasma antigen 3 months prior to infection appears to markedly reduce the anemia and the percentage of anaplasma infected red blood cells. The C-F antigen injected 6 days after infection may reduce the anemia produced by anaplasmosis in some cases.

References

1. Kuttler, K. L.: Anaplasmosis Immunization Studies. Proc. U. S. Livestk. San. Assoc. 65: 79-87. 1961.

Observations on Immunization Trials for Bovine Anaplasmosis

T. O. Roby and D. W. Gates

Immunity to anaplasmosis constitutes a challenging and formidable research problem. Infected carrier animals are recognized as having considerable resistance to reinfection. However, such animals can serve as the source for further dissemination of the disease. When carrier animals are sterilized by treatment, or if autosterilization should occur, they are again susceptible. Thus the immunological problem in anaplasmosis is the absence of immunity after recovery from the carrier state. A practical and effective procedure for inducing immunity would greatly aid in control of the disease.

Experiments designed to study the possibility of inducing immunity to anaplasmosis have been conducted at the Beltsville Parasitological Laboratory. The immunizing substances (vaccines) that were used in these trials consisted of nonviable concentrated preparations of *Anaplasma marginale* made from the blood of cattle in the acute stages of the disease. The methods of preparing these vaccines were similar to those used in making anaplasmosis complement-fixing antigens (1). The experimental vaccines so prepared, in doses of 50 ml. and 100 ml., were administered intramuscularly to splenectomized calves and to adult nonsplenectomized cows (2). Single and repeated doses, the latter at intervals of seven days, were used in different experiments; as many as four injections were made in one trial. Most of the trials were done without adjuvants in the vaccine.

At intervals of 21 to 80 days after the last dose of vaccine, the immunity of the test animals was challenged by inoculating blood containing approximately 1,000 infective doses of *A. marginale*. Unvaccinated animals of comparable ages, sex, and breeds were used as controls, and received challenge inoculations in the manner just described.

The following is a brief summary of the findings.

As measured by complement-fixation (CF) reactions, the serological responses of most vaccinated animals fell to undetectable levels 35 to 40 days after vaccination. Consequently, in subsequent trials, vaccine adsorbed on 13 percent aluminum hydroxide was used (3). These adsorbed vaccines prolonged the serological responses of vaccinated animals as long as 70 to 80 days. The positive CF serum titers ranged from 1:10 to 1:40 dilution during this period.

These studies failed to demonstrate an appreciable immunity resulting from the vaccines used. Anaplasmosis resulted in all vaccinated and control animals following challenge inoculation. In several cases it was observed that, following challenge, incubation periods

were somewhat longer and clinical symptoms were less severe in the vaccinated animals. However, the apparent protection was so slight that it could not definitely be attributed to the immunization procedure. It was concluded that a positive serological response in these vaccination trials, as revealed by the complement-fixation test, was not in itself indicative of immunity to anaplasmosis.

References

1. Gates, D. W., Mohler, W. M., Mott, L. O., Poelma, L. J., Price, K. E., and James Mitchell. "A Comparison of Antigen Production Methods and Complement Fixation Procedures for Diagnosing Bovine Anaplasmosis." Proc. 58th Vet. U.S.L.S.A. Nov. 1954, pp 105-114.
2. Roby, T. O., Gates, D. W., and Mott, L. O. "The Comparative Susceptibility of Calves and Adult Cattle to Bovine Anaplasmosis." Am. J. Vet. Res. 22, Nov. 1961, pp 982-985.
3. Gill, E., Sullivan, J. F., Stone, H. D., and Hundemann, A. S. "Role of Adjuvants in Immunogenicity of Killed Newcastle Disease Vaccines." Am. J. Vet. Res. 20, March 1959, pp 357-365.

Anaplasmosis Transmission Studies With Dermacentor Variabilis (Say) and Dermacentor Andersoni Stiles (= D. Venustus Marx) as Experimental Vectors

D. W. Anthony and T. O. Roby

In 1957 the Animal Disease and Parasite Research Division and the Entomology Research Division of the Agricultural Research Service initiated a cooperative research project to study the transmission of anaplasmosis. This research has been conducted in facilities of the former Animal Disease Station, now the Beltsville Parasitological Laboratory, at Beltsville, Maryland. So far, studies have been limited to ticks as vectors of anaplasmosis. The main objectives have been to (1) confirm previously reported experimental transmission results, (2) develop methods for detecting *Anaplasma marginale* Theiler, and (3) elucidate the life cycle in ticks infected with and transmitting the disease. This report is concerned with the progress on the first objective.

Colonies of *Dermacentor variabilis* (Say) and *D. andersoni* Stiles (=*D. venustus* Marx) were established in 1957 and 1959, respectively, from stock specimens received from the Entomology Research Division laboratory at Orlando, Florida and the Department of Health, Education, and Welfare laboratory at Hamilton, Montana. The specimens forwarded to Beltsville from these laboratories were colony stocks maintained for research purposes and believed to be free of cattle pathogens, especially *Anaplasma marginale.* That the ticks were free of this pathogen was confirmed with both species in tests with splenectomized calves.

Anaplasmosis-transmission investigations have been carried out in a small isolation unit designed to provide maximum security against the escape of infected ticks. This building consists of an animal room with three stall spaces and a laboratory room with small anterooms connecting both main rooms to the outside. The laboratory room is equipped with heat and air conditioning for constant-temperature control, and with daylight fluorescent lamps which operate from a 2-way interval timer so that the photoperiod can be controlled. The building is surrounded by an oil-filled moat, and the entire unit is enclosed by a chain-link fence.

The ticks are confined in small plastic boxes with muslin tops kept in dessicator jars. A saturated solution of ammonium sulfate in the bottom maintains a relative humidity within the jar of approximately 80 percent at a room temperature of 23-25° C.

Noninfected ticks are usually fed on guinea pigs. However, at times when large numbers of adults must be fed, they are confined in capsules on anaplasmosis-free calves. In infection or transmission studies, adults are confined on cattle in screwtop plastic capsules cemented firmly to a cleansed and clipped area on the hip. Larvae and nymphs are confined on cattle in a double muslin sleeve attached to a cleansed and clipped portion of the tail. In the transovarian transmission experiments, intervening stages in the life cycle that are not to be tested on cattle are fed on guinea pigs.

Calves 3 to 6 months of age which had been splenectomized 45 to 60 days previously were used in the transmission experiments. Each animal was bled 3 times weekly from the date of splenectomy, and hematological and serological observations were made on blood from each bleeding. All animals failing to

show evidence of transmission after 90 days were challenged with *Anaplasma marginale* infected blood to prove their susceptibility.

Transmission Experiments With Dermacentor variabilis.

The results of 13 transmission experiments with *D. variabilis* are presented in tables 1, 2, and 3. The data in tables 1 and 2 agree closely with the earlier work of Rees (5, 6), Sanders (10), and Rees and Avery (7), which indicates that *D. variabilis* larvae and nymphs can become infected with *A. marginale* and transmit the disease in the nymphal and adult stages. However, transovarian transmission was not demonstrated.

Results of tests in which ticks were macerated and injected into susceptible calves are given in table 3. Transmission of anaplasmosis by inoculation was obtained only with males ground in normal bovine serum. Failure to transmit the disease with an inoculum of macerated females is difficult to explain. These females were one-third to one-half engorged when removed from the infected animal and should have contained more anaplasmas than the males which take very little blood.

As can be seen from table 3, transmission of anaplasmosis was obtained with 2 males 42 days after their removal from an infected animal. This result suggests that male *D. variabilis* could be vectors of the disease. It is quite possible that males could feed briefly on animals, detach to search for females, and while detached, transfer to another animal or drop to the ground to await another host. If the first host animal was in either the clinical or early convalescent stage of the disease, these displaced males should be capable of transmission. Unfortunately, there are no data available to prove this supposition. Dikmans (3) pointed out that the question of *D. variabilis* as a natural vector of anaplasmosis cannot be settled until adult-to-adult transovarian transmission has been proved. Transmission by males at different periods after infection is, of course, another possibility. It would seem that *D. variabilis* should be considered a potential vector of the disease, but its importance as a natural vector is not known.

Transmission Experiments with Dermacentor Andersoni

Many workers consider *D. andersoni* to be an important vector of anaplasmosis in the western United States. In several respects this species meets the requirements of an efficient vector. It is relatively abundant throughout its habitat. The adults have a wide host range and occur on deer, moose, elk, and most large wild animals as well as on livestock. This species has a long life span—some adults are capable of living 2 years or more. Transovarian passage of the anaplasmosis agent has been demonstrated by Howell *et al.* (4). In certain localities the seasonal incidence of anaplasmosis coincides with the seasonal occurrence of *D. andersoni.*

Table 1—Stage-to-stage transmissions experiments with (D. variabilis.)

Stages of ticks used in experiments					
Larva:	Nymph:	Adult:	Method of test	Results	Challenge Reaction
A	B		Feeding	+	not req.
A	B		Injection of saline suspension	−	+
A	B		Feeding	+	not req.
	B	B	do.	−	+
	A	B	do.	+	not req.
	A	B (males only)	do.	−	+
	A	B (females only)	do.	−	+

A=Stage engorging on infected host. B=stage tested on susceptible host.

Table 2—Transovarian transmission feeding experiments with (D. variabilis.)

Stages of ticks used in experiments							
Nymph:	Adult:	Egg:	Larva:	Nymph:	Adult:	Results	Challenge Reaction
	A		B			−	+
	A				B	−	+
A	B[1]			B		−	+

[1] Trans-stadial transmission positive.
A=Stage engorging on infected host. B=stage tested on susceptible host.

Table 3—Transmission tests with (D. variabilis) adults by delayed inoculation and feeding.

Delay in test (days)	Test Method	Number of Specimens and Sex	Results	Challenge Reaction
2	inoculation[1]	15 females	−	+
2	inoculation[1]	25 males	+	----
42	feeding	2 males	+	----

[1]Ticks ground in normal bovine serum in Ten Broeck tissue grinders and the resulting suspension inoculated subcutaneously.

Cooley (2) indicated that the larvae and nymphs of *D. andersoni* infest small rodents, such as field mice and ground squirrels, but are not found on cattle or large animals. Dikmans (3) pointed out that the importance of *D. andersoni* as an anaplasmosis vector cannot be fully evaluated until transovarian passage of *A. marginale* from adult to adult has been demonstrated.

Stage-to-stage transmission of anaplasmosis by *D. andersoni* has been demonstrated by Rees (5 and 6) and Boynton *et al.* (1). Tests at Beltsville have confirmed nymph-to-adult transmission.

Transovarian transmission of the anaplasmosis agent was reported by Howell *et al.* (4) with the larval progeny of an engorged female collected in Wyoming. Transovarian transmission trials by Rees and Avery (7) and Rozeboom (8) were not successful. Because of these conflicting results, much of the work at Beltsville has been concerned with transovarian transmission studies and with studies of transmission by males at different intervals after infection.

Data on our transovarian transmission trials are given in Table 4. All the trials were negative, but three of five test animals on which first-generation larvae fed, were resistant to challenge. In a personal communication, Dr. D. E. Howell of Oklahoma State University suggested that perhaps in nature those females that transmit the disease to their progeny are injured. He pointed out that a slight rupture of the gut would allow a greater amount of infective material from the gut to come in contact with the developing ova. To test this hypothesis, 12 females that fully engorged by feeding on an animal in the acute stage of anaplasmosis were separated into 4 groups, each containing 3 specimens. The groups were handled as follows:

1. Each female was punctured three times with *minuten* pins.
2. Each female was dropped five times to a cement tile floor from a height of 4 feet.
3. Each female was vigorously shaken 10 minutes in a small plastic box.
4. No treatment was given; each female was placed in a separate plastic box and allowed to oviposit.

Only the punctured specimens showed any adverse effects from the treatments. One of these died before oviposition and the other two deposited egg masses that were smaller than usual but hatched normally. All of the others laid egg masses of approximately normal size and hatch. The larval progeny from the females in each group were pooled and each of the pooled groups allowed to feed on a splenectomized calf.

Results of the tests, summarized in table 5, showed that there was no transovarian transmission. The number of engorged larvae recovered from the test animals ranged from 23, from vigorously shaken parent females, to more than 1000 from parent females dropped.

Transmission of *A. marginale* by allowing previously infected ticks to feed again was first reported by Sanborn *et al.* (9). In one experiment these workers effected a transmission with both sexes of *D. andersoni* by allowing an interval of 6 days to elapse between their exposure to the infected animal and susceptible host. In another successful experiment, they used one male, transferred directly from a clinical case of anaplasmosis to a susceptible host. Rozeboom *et al.* (8) reported a positive transmission with one male after 41 days had elapsed between removal of the

Table 4—Transovarian transmission feeding experiments with (Dermacentor andersoni.)

Stages of ticks used in experiments								
Nymph:	Adult:	Egg:	Larva:	Nymph:	Adult:	Number of tests	Results	Reaction to Challenge
	A ——		B			1	—	+
			B ——	B		1	—	+
				B ——	B	1	—	+
	A ——		B			5	—	3 of 5 resistant
	A ——		——	B		5	—	+
	A ——		——	——	B	3	—	+
A ——	B[1] ——		B			1	—	+

A=Stage engorging on infected animal.
B=Stage tested on susceptible animal.
[1]Trans-stadial transmission (nymph-to-adult) positive.

Table 5—Transovarian transmission feeding experiments with progeny of injured (Dermacentor andersoni) females.

Stages of ticks used in experiments					
Adult:	Egg:	Larva:	Treatment of parent female	Results	Reaction to challenge
A ——	——	B	punctured	—	+
A ——	——	B	dropped	—	±[1]
A ——	——	B	shaken 10 min.	—	resistant
A ——	——	B	no treatment	—	+

A=Stage engorging on infected host. B=stage tested on susceptible host.
[1]Susceptibility of test animal to challenge questionable. Animal developed C.F. reaction but never showed hematological or clinical signs of the disease.

tick from the clinical case and its placement on the susceptible host.

The early work at Beltsville was hampered by failure of the males to survive longer than 60 days after feeding and mating. However, active males which attached and fed for 7 to 10 days without females lived considerably longer than those that had mated.

The results of the experiments are given in table 6. Test group A originally consisted of 80 mated males of which only 5 were alive 60 days later, and these ticks failed to transmit the disease. Test group B originally consisted of 25 unmated males fed on an animal in the clinical stages of anaplasmosis. Anaplasmosis was transmitted by this group after 60 and 108 days, but not after 150 days; however, only 1 of the 2 specimens tested attached to the animal in the 150-day test. Test group C originally consisted of 150 unmated males infected for 10 days on an animal with acute anaplasmosis. Seventy-five of the specimens were forced into hibernation by progressive reduction of photoperiod and temperature. The remaining 75 were maintained at usual laboratory conditions. After 9 weeks the ticks were removed from hibernating conditions and held at laboratory environment. At the time the experiment was conducted, 197 days after the infective feeding, 18 of the 75 specimens forced into 9 weeks of hibernation were alive and active. Fourteen of these attached and transmitted anaplasmosis to the test animal. None of the 75 specimens not subjected to hibernation lived 197 days.

Table 6—Delayed feeding transmission experiments with presumably infected males.

Test group	Interval (days)	Number males tested	Results	Reaction to challenge
A	60	5	−	+
B	60	10	+	------
B	108	6	+	------
B	150	2	−	+
C	197[1]	14	+	------

[1]Ticks placed in hibernation 63 days.

The need for further studies with *D. andersoni* as a vector of anaplasmosis is strongly indicated. The failure of these two experiments to show transovarian transmission can be interpreted in two ways: (1) the frequency of transovarian transmission may be so low that it would be very difficult to demonstrate in the laboratory. (2) Laboratory conditions under which ticks are reared differ so greatly from conditions occurring in nature that a true indication of the infection rates and transmission potential as they occur naturally cannot be obtained.

The demonstration of transmission by unmated males after hibernation suggests another possible reservoir of infection. However, it must be pointed out that this theory cannot be evaluated at this time owing to the lack of knowledge regarding transmission under natural conditions.

References

1. Boynton, W. H., W. B. Herms, D. E. Howell and G. M. Woods. 1936. Anaplasmosis transmission by three species of ticks in California. J.A.V.M.A. 88:500-502.
2. Cooley, R. A. 1932. The Rocky Mountain wood tick. Montana State College and Agric. Experiment Sta. Bulletin 268.
3. Dikmans, G. 1950. The transmission of anaplasmosis. American Journal Vet. Res. 11(38):5-16.
4. Howell, D. E., G. W. Stiles and L. H. Moe. 1941. The hereditary transmission of anaplasmosis by *Dermacentor andersoni* Stiles. Amer. J. Vet. Res. 2(3):165-166.
5. Rees, C. W. 1932. The experimental transmission of anaplasmosis by *Dermacentor variabilis.* Science 75:318-320.
6. Rees, C. W. 1934. Transmission of anaplasmosis by various species of ticks. U.S.D.A. Tech. Bull. 418.
7. Rees, C. W. and J. L. Avery. 1939. Experiments on the hereditary transmission of anaplasmosis by ticks. N. Amer. Vet. 20:35-36.
8. Rozeboom, L. E., G. W. Stiles, and L. H. Moe. 1940. Anaplasmosis transmission by *Dermacentor andersoni* Stiles. Jour. Parasit. 26:95-100.
9. Sanborn, C. E., G. W. Stiles, and L. H. Moe. 1938. Anaplasmosis transmission by naturally infected *Dermacentor andersoni* male and female ticks. N. Am. Vet. 19:31-32.
10. Sanders, D. A. 1933. Notes on the experimental transmission of bovine anaplasmosis in Florida. J.A.V.M.A. 88:799-805.

Factors Complicating The Control of Bovine Anaplasmosis In California

John F. Christensen

Anaplasmosis in California is chiefly a problem in beef cattle that spend a considerable part of each year on the hill and mountain rangelands. Studies have been concentrated during the last few years on determining the factors responsible for the high incidence of infection in this environment. This paper is a summary of work and observations conducted in the Coast Range area of the state, where the disease is best known, and a discussion of the factors which appear to complicate the problem of controlling anaplasmosis in California, and probably other western states.

Geography and Cattle Husbandry Practices

The Coast Range of California lies between the Pacific ocean and the Central Valley, and is approximately 400 miles long and averages about 50 miles in width. This area represents roughly one-third of the available rangelands of the state and provides pasture for about 600,000 beef cattle annually.

Geography and cattle husbandry practices appear to be important factors in the maintenance of anaplasmosis as an important infection in cattle in this area. The terrain varies from rolling hills to low, steep mountains and is covered by grasses in various combinations with brush and woods. The winter and early spring seasons are characterized by rainfall and temperatures mild enough to permit growth of grasses, hence they are the chief pasture seasons for cattle. Summers are hot and dry, the grasses die, and cattle are usually moved to valley locations for pasture or supplementary feeding. Topography, climate and vegetation favor an abundant wildlife, including a good share of the state's estimated 1,500,000 deer, which have been shown to be carriers of latent *Anaplasma marginale* infection. The numerous large and small mammalian hosts favor the propagation of ticks, which are believed to be the principle vectors of *Anaplasma* infection.

The economy of beef cattle production in the Coast Range area depends on the use of rangelands during the winter-spring season when the grass is growing. When the grasses are grazed down or die from lack of moisture in the late spring and summer, cattle are usually moved to valley pastures or feedlots. During the period of several months on rangeland pastures the cattle are widely dispersed and unavailable for the frequent handling necessary for effective chemical control of ectoparasites.

Acknowledgment is made of the observations of many veterinarians, in practice and government work, who responded to a questionnaire on anaplasmosis in California.

There is little chance that there will be significant modification of usage of California's rangelands or cattle management practices that would reduce or minimize the anaplasmosis problem in cattle. Despite growing human populations, large areas of the state appear destined to be used permanently for grazing cattle.

The Problem of Carrier Deer

Work at the University of California over the past several years has demonstrated that a large percentage of the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) occupying the rangelands with cattle are carriers of latent *A. marginale* infection (1, 2). It was shown by inoculation of blood that the organism obtained from deer could be readily transferred from deer to deer, deer to calf, calf to calf, and calf to deer (1, 2). Subclinical and mild infections were also produced in deer by inoculation of blood from bovine carriers of naturally-acquired infection (3), and these deer were shown to be carriers 11 months later (4). There can be no doubt that similar transfers of infection between deer and cattle occur in the rangeland environment through the bites of suitable natural vectors.

The presence of large numbers of carrier deer on rangelands grazed by cattle constitutes the most difficult obstacle in the way of controlling anaplasmosis in cattle. The esthetic and sporting importance of deer to the people of California make it very unlikely that measures will be taken designed to reduce deer population to aid in the control of anaplasmosis of cattle. Segregation of cattle from deer by means of fencing is an economic and practical impossibility. Destruction of carrier infection by means of drugs, which has been accomplished in cattle through the feeding of tetracycline antibiotics (6), is unlikely to be attempted in deer because of cost and inherent practical difficulties. The existence of this large source of infection in deer constitutes an imposing obstacle in the way of controlling the disease in cattle by removal of reactors to the complement-fixation (CF) test. Replacement of reactor cattle by infection-free animals would result in an explosive disease problem.

The Problem of Natural Vectors

Transmission of anaplasmosis under rangeland conditions is accomplished chiefly by natural vectors. Man-made transmission is not considered to be a significant factor in transmission of the disease in this environment. Cattle are not subjected to procedures

that might effect mechanical transmission of the infection for a period of several months while on range, yet cases of anaplasmosis occur long after the usual incubation period of about 5 weeks reported for outbreaks in feedlot cattle traceable to such procedures as vaccination, implantation of stilbestrol and castration (6).

Blood-feeding insects such as horseflies, deerflies, mosquitoes and others are undoubtedly responsible for some transmission of anaplasmosis under rangeland conditions, but are not considered to be vectors of major importance. The following factors support this contention: 1) The time interval during which an insect can transmit infection after feeding on an infected animal lasts only a few minutes, since the insect mouth parts must carry moist infected blood when it resumes feeding on a susceptible animal (7). 2) It is difficult to effect transmission from a carrier to a susceptible bovine animal with insects even under the most ideal experimental conditions (8). 3) There is closer correlation of clinical anaplasmosis with the seasonal abundance of ticks than with blood-feeding insects. 4) Cattle from many valley locations have been shown by CF testing, required before shipment to Hawaii, to have a very low incidence of carrier infection[1], despite the abundance of blood-feeding insects and greater crowding of cattle.

Maintenance of *Anaplasma* infection at the high levels known to exist in native cattle in the Coast Range area indicates a high rate of exposure. The sporadic nature of clinical anaplasmosis in older cattle suggests that most animals are exposed during calfhood, when cattle are relatively resistant to clinical infection. Cases of the disease in older cattle may be accounted for on the grounds that some animals escape exposure during calfhood and are susceptible to clinical infection when exposed later. The high incidence of the disease in susceptible adult animals such as purebred bulls brought from tick-free ranches for addition to native herds also points to a high exposure rate.

The exposure rate for beef cattle is not known on a broad basis, but CF testing on one ranch typical of the area studied indicates the high exposure potential[2]. Of 41 calves tested at the end of one season on rangeland pasture, nearly two-thirds were positive, while testing of 203 animals that had spent 2 to 14 seasons on pasture revealed 80 percent positive and 15 percent suspicious reactions. It is probable that most of the suspicious reactions associated with this high percentage of reactors represented exposure to infection.

Ticks are believed to be the only natural vectors in the area capable of maintaining exposure to *Anaplasma* infection at the high levels demonstrated for cattle and deer. Of the 9 species of ticks known to attack both cattle and deer in the Coast Range area, the Pacific Coast tick, *Dermacentor occidentalis,* appears to have the best qualifications to be an important natural vector. This tick has been shown to be capable of transfer of infection through the eggs to the next generation (9), resulting in multiplication of the infective agent by the production of many hundreds of larvae from one female tick. Ticks of this species obtained from deer have been allowed to resume feeding on cattle, producing anaplasmosis in 2 of 5 trials, thus proving the presence of *A. marginale* in ticks in the rangeland environment and the ability of these ticks to infect cattle (10). *D. occidentalis* attacks both cattle and deer in the adult stage and is abundant throughout the Coast Range area and other parts of California where the disease occurs.

The possibility that other ticks may be vectors cannot be excluded until further studies are made, but most of them can be eliminated or considered to be of little importance in transmission through factors of host preference, biological capabilities and habits. On the basis of available evidence, *D. occidentalis* appears to be an important natural vector in California, and probably the most important.

The possibility of aiming control measures for bovine anaplasmosis at ticks is complicated by the wide dispersal and unavailability of cattle while on rangeland pastures, the presence of numerous large and small wild mammals to serve as alternate hosts, and prohibitive economic considerations. Application of suitable chemicals to cattle, assuming the feasibility of such a program, might be expected to reduce exposure to the bites of tick and insect vectors if performed with sufficient thoroughness and frequency, which is impossible under the conditions described. An answer to tick control might be found in the development of chemicals that will protect cattle from exposure to the bites of these vectors throughout the long rangeland pasture season following a single application, but there appears to be little hope for this in the foreseeable future.

Conclusions

Experimental work and observations in the Coast Range area of California have revealed formidable obstacles in the way of controlling anaplasmosis of cattle on the rangeland pastures through eradication. The geography of the areas involved, the cattle management practices, the presence of large numbers of deer carrying latent *A. marginale,* and the presence of efficient natural vectors combine to maintain infection at a high level in native cattle. Available evidence points to the probability that the Pacific Coast tick, *Dermacentor occidentalis,* is the most important vector.

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[1]Data supplied by Dr. E. E. Saulmon, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture.

[2]Blood samples were collected by Dr. J. B. Carricaburu, Santa Ynez, California. CF testing was done by the Agricultural Research Service, United States Department of Agriculture.

Additional studies in California and other western states should be conducted to determine the presence and incidence of latent *A. marginale* infection in deer, the incidence of carrier infection in cattle, and the role of natural vectors, particularly ticks, in transmission. If these studies indicate situations on a widespread basis similar to those reported for the Coast Range area of California, it may be necessary to abandon the traditional philosophy of eradication as the approach to control of anaplasmosis in cattle in areas where the disease is enzootic. An alternative would be control by immunization, even though this might prove to be possible only through the creation of solid carrier herds through calfhood exposure to infection.

References

1. Osebold, J. W., Christensen, J. F., Longhurst, W. M., and Rosen, M. N.: Latent *Anaplasma marginale* Infection in Wild Deer Demonstrated by Calf Inoculation. The Cornell Vet., 49, (1959): 97-115.
2. Christensen, J. F., Osebold, J. W., and Rosen, M. N.: The Incidence of Latent *Anaplasma marginale* Infection in Wild Deer in an Area Where Anaplasmosis is Enzootic in Cattle. Proc. 62nd Ann. Meet. U. S. Livestock San. Assoc., Nov. 4-7, 1958, (1959): 59-65.
3. Christensen, J. F., Osebold, J. W. and Rosen, M. N.: Infection and Antibody Response in Deer Experimentally Infected with *Anaplasma marginale* from Bovine Carriers. J. Am. Vet. Med. Assoc., 132, (1958): 289-292.
4. Christensen, J. F., Osebold, J. W., Harrold, J. B., and Rosen, M. N.: Persistence of Latent *Anaplasma marginale* Infection in Deer. J. Am. Vet. Med. Assoc., 136, (1960): 426-427.
5. Brock, W. E., Pearson, C. C., and Kliewer, I. O.: Anaplasmosis Control by Test and Subsequent Treatment with Chlortetracycline. Proc. 62nd Ann. Meet. U. S. Livestock San. Assoc., Nov. 4-7, 1958, (1959): 66-70.
6. Crane, C. S.: Anaplasmosis in a Feedlot. Modern Vet. Pract., 40, (1959): 41-43.
7. Howell, D. E.: Transmission of Anaplasmosis by Arthropods. Proc. 3rd Nat. Res. Conf.: Anaplasmosis in Cattle, (June 12-13, 1957): 14-16. (Mimeographed)
8. Dikmans, G.: The Transmission of Anaplasmosis. Am. J. Vet. Res., 11, (1950): 5-16.
9. Carricaburu, J. B.: Control of Anaplasmosis by Acquired Immunity. No. Am. Vet., Sect. 1, (Feb. 1, 1958): 46-47. (An Interview)
10. Boynton, W. H., Herms, W. B., Howell, D. E., and Woods, G. M.: Anaplasmosis Transmission by Three Species of Ticks in California. J. Am. Vet. Med. Assoc., 41, (1936): 500-502.
11. Osebold, J. W., Douglas, J. R., and Christensen, J. F.: Transmission of Anaplasmosis to Cattle by Ticks Obtained from Deer. Am. J. Vet. Res., 23, (1962): 21-23.

Control of Vectors–Equipment and Insecticides

R. A. Hoffman

If it is assumed that the current concept of mechanical transmission of anaplasmosis by insects is correct, we can theorize that any of the insects or ticks that feed on the blood of cattle could be vectors of the disease. It is probable, however, that in any given region the primary vector or vectors are rather specific depending, perhaps, on their numbers or feeding habits. Unfortunately, few critical studies have been completed that provide this information. In the Southern States, for example, the Tabanidae (horse and deer flies) are thought to be the principal vector group and there is, at least, circumstantial evidence to support this belief. But which tabanids? Dozens of species are present, many of which have overlapping adult-activity cycles. In addition, there has been no conclusive proof that mosquitoes, horn flies, stable flies, and biting gnats are not involved; in fact, the work of Dr. D. E. Howell and associates (1) in Oklahoma has demonstrated that some of these species could possibly be carriers.

Therefore, in speaking of control of anaplasmosis vectors, we take a broad outlook and conclude that in a region where anaplasmosis is endemic, any measure taken to control a blood-feeding external parasite is a step toward control of anaplasmosis. Within this concept many insecticides and application methods can be listed with which we are all more or less familiar—such diverse measures as mosquito-larvae control by air application of parathion, simulid-larvae control by application of DDT to streams, tick control by dipping cattle in vats containing toxaphene, and horn fly control by pen spraying of cattle with methoxychlor by means of an orchard-type power sprayer, or by allowing the cattle access to insecticide-treated rubbing devices.

Other measures include control of *Stomoxys* on dairy cattle by flit-gun application of pyrethrins, and tabanid control by air application of dieldrin granules to predetermined areas where larvae are concentrated.

This latter measure was demonstrated against *Tabanus nigrovittatus* Macq. in the coastal salt marshes both by Hansens (2) in New Jersey and Jamnback (3) in New York. DDT and other chlorinated hydrocarbon insecticides have been applied as area spray treatments for adult tabanid control by Dr. Howell's group (4) in Oklahoma and Dr. A. W. A. Brown (5) in Canada, with variable but generally short-term suppression of numbers. However, with the use of recently developed toxicants this latter technique does appear to have some possibilities in situations where insects congregate because of a limited resting habitat.

One of the more interesting innovations is that of a systemic insecticide fed or otherwise given to the host animal to make the animal's blood or body excretions toxic to insect pests. Control of the horn fly on cattle fed ronnel is an example of this technique, from the standpoint of toxicity both to feeding adults and to larvae in the manure.

Vector-control experiments specifically designed to demonstrate the effect, if any, of insect control on anaplasmosis dissemination have been rare, but such tests have been conducted in Mississippi, Louisiana, and Arkansas in recent years. Data from the Mississippi tests conducted in 1959 and 1960 have been published as a Mississippi State University Information Bulletin (6). The design for the Mississippi trials consisted of establishing herds containing both anaplasmosis complement-fixation positive and negative animals and, by means of photocell-actuated sprayers, applying pyrethrin insecticides to each animal whenever it entered or left a fenced area containing the only source of water. The anaplasmosis infection status of herds that received monthly residual insecticide treatment by the pen-spray method was observed in direct comparison. The results of the 1959 and 1960 studies were sufficiently encouraging that the test was repeated in 1961. Dr. R. H. Roberts of the USDA-ARS-ERD laboratory at Stoneville, Mississippi, will present data from the latter study as an illustration of this method of vector control.

References

1. Howell, D. E., Stiles, G. W., and Mol, L. H. The Transmission of Anaplasmosis by Mosquitoes (Culicidae). J.A.V.M.A. 99, (1941): 107-110.
2. Hansens, E. J. Granulated Insecticides Against Greenhead (Tabanus) Larvae in the Salt Marsh. Jour. Econ. Ent. 49: 401-403 (1956).
3. Jamnback, H., and Wall, W. Control of Salt Marsh *Tabanus* Larvae with Granulated Insecticides. Jour. Econ. Ent. 50: 379-382 (1957).
4. Howell, D. E., Eddy, G. W., and Cuff, R. L. Effect on Horse Fly Population of Aerial Spray Application to Wooded Areas. Jour. Econ. Ent. 42: 644-646 (1949).
5. Brown, A. W. A., and Morrison, P. E. Control of Adult Tabanids by Aerial Spraying. Jour. Econ. Ent. 48: 125-129 (1955).
6. Hoffman, R. A., Smith, K. O., Collins, J. C., Mott, L. O., and Scales, J. W. Summary of 1959 and 1960 Mississippi Experiments Relative to the Influence of Insect Control on Transmission of Bovine Anaplasmosis. Miss. State Univ., Agric. Exp. Station, Information Sheet No. 699 (April 1961).

Control of Anaplasmosis Through Insect Control, Washington County, Mississippi, 1961

R. H. Roberts

In 1961, a third experiment in the control of anaplasmosis through insect control was conducted in the Delta area of Mississippi. Cattle belonging to the "G" plantation were divided into two herds, each containing a similar ratio of anaplasmosis positive and negative animals. In addition, 10 susceptible adult cattle from a known negative-source herd were added to each group as a possible indicant of disease tolerance in the native cattle.

In order to obtain similar insect densities, the two herds were placed in adjoining pastures separated only by a single fence. The fence was so placed that no tree areas or other natural resting sites for the cattle were common to both pastures. The nearest group of other cattle was approximately 1 mile away.

The test herd was sprayed with 0.05 percent pyrethrins plus 0.5 percent piperonyl butoxide formulated in kerosene, by means of a photocell-actuated sprayer set in a fence surrounding the only available water source in the pasture. A counter attachment showed that the number of sprayings averaged about two a day per animal. The sprayer reservoir was checked daily and filled as necessary. At intervals of approximately one month, the control herd was treated for horn fly control with 0.5 percent methoxychlor by the pen-spray method, the standard farm management practice in the area.

Weekly observations of insect activity on the two herds were made by counting the number of horn flies and horse flies on 15 animals in each herd. In conjunction with these weekly observations, the relative density of the tabanid species of the area was determined by weekly 30-minute collections from a horse. Mosquito densities were determined by biweekly light-trap collections from five locations—one in the test herd's pasture, three at the Delta Branch Experiment Station, and one at another plantation in Washington County.

The infectivity of the animals was determined by the anaplasmosis serological complement-fixation test. Two determinations were made before the beginning of the experiment; and on April 3, 1961, when the cattle were divided into two herds and the automatic sprayer was started, a third series of blood samples was taken. During the period from April to November, blood samples were taken from each animal at monthly intervals. The sprayer was in continuous operation from April until November 8.

A summary of the seasonal status of anaplasmosis in the two herds is presented in table 1. According to the complement-fixation test, a total of four new positive anaplasmosis cases appeared in the test herd and 18 in the control herd. Two of the 18 control animals died and nine required treatment with terramycin, whereas in the test herd, no deaths from anaplasmosis occurred and only one animal required treatment. No real difference in susceptibility was apparent between the cattle added to each herd and the susceptible native cattle, but of course the numbers of animals involved were too small to be conclusive. In the test herd, only one, or 10 percent of the introduced cattle as compared with three, or 12.5 percent of the 24 susceptible native cattle, developed anaplasmosis. In the control herd five, or 50 percent, of the introduced cattle and 13, or 59 percent, of 22 native cattle developed the disease.

Table 1—Anaplasmosis status of the cattle in the treated and control herds, based on the complement-fixation test

	Treated Herd Number			Control Herd Number		
Bleeding Date	Negative	Positive	New Positive	Negative	Positive	New Positive
2-2-61	34	14	0	32	15	0
3-9-61	34	14	0	32	15	0
4-6-61	34	14	0	32	15	0
5-4-61	34	14	0	32	15	0
6-30-61	34	14	0	31	16	1
8-1-61	34	14	0	24*	21	5
8-30-61	32	16	2	17**	27	6
10-4-61	30	18	2	13	31	4
11-8-61	30	18	0	11	33	2

*One animal removed from herd; one died of acute anaplasmosis.
**One animal died of acute anaplasmosis.

Pre-spray insect counts on April 3 indicated an average of 25 horn flies per animal on the test herd and 18 on the control herd; an occasional horse fly was noted on cattle of both herds. After spraying was started on April 5, no horn flies were observed on the test herd for the remainder of the season. Horse fly populations were generally lower on the test herd, which averaged 2.8 per head for the entire season, as compared with 4.1 on the control herd. Nevertheless, the daily insecticide treatments did not appear to be as effective as in preceding years. During 1959, horse flies averaged 3.13 per head on the control herd and 0.99 on the test herd, and similar results were obtained in 1960 (1). However, it should be noted that the average number of horse flies given refers only to the flies present on the animals, and does not necessarily indicate that the flies were actively feeding. In fact, the transmission data strongly suggest that insect feeding on the treated animals was reduced.

During the 7-month period of the experiment, approximately 11 gallons of a 10% piperonyl butoxide-1% pyrethrins concentrate, diluted 1:19 for a total of 220 gallons of spray, was used in the insect-control program. At a market price of $16.00 per gallon, the total cost of insecticide was $176.00, or about 84¢ a day to treat 48 animals. However, the monetary loss in the control herd represented by the death of two animals and the cost of treating the sick animals would have been more than enough to cover the cost of insecticide, diluent, and labor involved in sprayer maintenance.

References

1. Hoffman, R. A., Smith, K. O., Collins, J. C., Mott, L. O., and Scales, J. W. Summary of 1959 and 1960 Mississippi Experiments Relative to the Influence of Insect Control on Transmission of Bovine Anaplasmosis. Miss. State Univ., Agric. Exp. Station, Information Sheet No. 699 (April 1961).

The Relationship of Horse Fly (Tabanidae) Populations to the Incidence of Anaplasmosis in Louisiana

Bobby H. Wilson[1], E. C. Burns[1], W. T. Oglesby[2], R. B. Myers[2], G. T. Dimopoullos[2], James Wimberly[3], A. G. Pass[4], and F. B. Wheeler[5]

Various workers have shown nine species of horse flies to be capable of mechanical transmission of anaplasmosis. A direct relationship between horse fly abundance and incidence of anaplasmosis appeared to exist in Louisiana. Preliminary work in 1960 using synergized prethrin plus repellents dispensed in an automatic self spraying device indicated that the incidence of anaplasmosis could be reduced by horse fly control. In view of these facts work was continued during the summer of 1961 to determine the effect of horse fly populations in relation to the incidence of anaplasmosis.

A treatment and control herd of cattle were selected in two environments. Treatment herds were sprayed daily with synerigized pyrethrins plus MGK repellent 1207 in an oil solution at the rate of approximately 2 oz. per animal per day. Treatment was made with a self spraying device through which the animals were forced to pass in order to water. The effect of treatment on incidence of anaplasmosis was determined by subjecting pre-season and post-season blood samples of all animals to the complement-fixation test. Horse fly counts were taken bi-weekly.

In one area horse fly populations were relatively low in both the treatment and control herds and no valid comparison in the increased incidence of anaplasmosis could be made. In the other area during the period of maximum horse fly activity there was an average of 5.9 flies per animal in the control compared with 1.3 in the treatment. 40.9 percent of the animals in the control herd were found to be reactors in the pre-season test with 73.9 percent reactors in the post-season test. This was a 33 percent increase in reactors. The number of reactors in the treatment herd increased from 32 percent to 50.9 percent for an 18.9 percent increase. Thus a 14.1% difference occurred in the rate of increase between treatment and control herds. Twelve deaths occurred, presumably due to anaplasmosis, in the control herd, whereas only a single death occurred in the treatment herd.

[1]Entomology Research Department, Louisiana State University.
[2]Veterinary Science Department, Louisiana State University.
[3]Agriculture Engineering Department, Louisiana State University.
[4]Animal Disease Eradication Division. ARS USDA.
[5]Louisiana State Veterinarian.

Fly Control and Prevention of Anaplasmosis Transmission

J. L. Lancaster, Jr.

Two seasons of work have been completed in Southeast Arkansas in an on-the-farm situation, utilizing a simple self-sprayer for the control of biting flies in an effort to prevent transmission of anaplasmosis. This paper presents the results of that work.

A detailed review of the literature to show the relationship between the biting flies and anaplasmosis seems unnecessary before this group. It will suffice to say that the Tabanids, particularly, although not exclusively, have been incriminated as *THE* important vector group in some geographic regions. Dikman's[1] review summarizes the known information up to the time it was published and no new vectors have been recognized.

1960 Test

In this test a cow herd was CF tested and separated into two lots with equal numbers of reactors and negatives in each lot. The water source was fenced off and a self-sprayer placed in the gateway. This assured at least once a day treatment as the animals came to water. An experimental repellent (949) was used in one lot and synergized pyrethrins in the other lot. All the sprays were oil base sprays and the amount used per animal treatment was approximately two fluid ounces.

A group of heifers was given ¼ mg./kg. aureomycin in cottonseed meal free choice calculated on the basis of average weight and expected intake. These heifers were also sprayed by the owner with DDT or toxaphene for horn fly control as was his normal practice. The animals were blood tested before and during the test period. The heifers served as fly control checks.

[1]Dikmans, G. 1950. The Transmission of Anaplasmosis. American Journ. Vet. Res. XI: pp. 5-16.

Results for 1960

Fly counts were made regularly and are summarized as a fly index for horn flies, horseflies and stable flies. This figure is the average number per animal count for the season. These data are presented in Table 1.

The complement test was run on blood samples drawn May 5; August 5 and October 7 with the following results (Table 2).

No clinical cases occurred in 1960 and it appeared that highly satisfactory results were obtained, particularly with the synergized pyrethrins since no increase in the percentage of carriers occurred.

1961 Test

Some of the same cattle were used in the following season but heifers had been added to the cow herd and additional young stock had been purchased.

Table 1—Index of Fly Control McGehee 1960

Treatment	Fly Index		
	Horn	Horse	Stable
Synergized* Pyrethrins	14.34	.52	3.20
Repellent 949**	20.72	.35	1.70
Owner Treatment*** for hornflies	135.8	1.32	1.65

*Piperonyl butoxide 1% Pyrethrins .1 percent.
**Synergized pyrethrins added at .015 percent Pyrethrins.
***Aureomycin ¼ mg./kg. (calculated) free choice in cotton seed meal.

Since the feeding of a calculated ¼ mg./kg. aureomycin had not prevented the development of additional carriers, this treatment was dropped and Co-Ral spray was used as necessary for horn fly control. Concentration of interest was directed toward the synergized pyrethrin spray. The treatments then consisted of the split cow herd, as before, utilizing the self sprayers with the 1 percent piperonyl butoxide, .1 percent pyrethrins in one lot and one-half this concentration in the other lot. A check herd for fly counts was used but blood sampling of this herd was not accomplished.

Results for 1961

Fly counts were again made regularly throughout the season. These data are summarized as a fly index and can be compared directly to the previous season's data (Table 3).

The complement test was run on samples drawn April 14, in mid-summer and September 6. The summer test gave no useful data since all samples gave an anti-complement or positive reaction.

The results for 1961 are shown in Table 4.

One cow died of acute anaplasmosis September 5 in the best treatment and four others were treated by the veterinarian the next morning. One heifer also died from an acute case. Many additional carriers developed in all treatments in contrast to the previous seasons results, even though the fly control was approximately the same. Inquiry revealed that no clinical cases and no losses occurred in the herd used for fly control check.

Table 2—Results of Complement-Fixation McGehee 1960

Treatment	Number Animals	May 5		August 5		October 7	
		Number	Percent	Number	Percent	Number	Percent
		6R	25.00	7R	29.17	6R	25.00
Synergized	24	5S	20.83	4S	16.67	7S	29.17
Pyrethrins		10N	41.67	10N	41.66	4N	16.67
		3AC	12.50	3 unacct. for	12.50	4AC	16.67
						1 no test	4.16
			100.00		100.00	2 unacct. for	8.33
							100.00
		6R	25 00	7R	29.17	9R	37.50
Repellent	24	6S	25.00	4S	16.67	7S	29.17
		11N	45.84	12N	50 00	5N	20.84
		1AC	4.16	1 unacct. for	4.16	1 no test	4.16
						2 unacct. for	8.33
			100.00		100.00		
							100.00
Owner	34 (5/3)	8R	23.53	17R	36.96	19R	41.30
Treatment		5S	14.71	10S	21.74	6S	13.05
	46 (8/5)	7AC	20.58	2AC	4.34	8AC	17.39
	(10/7)	14N	41.18	17N	36.96	1QNS	2.17
						5N	10.87
			100.00		100.00	7 unacct. for	15.22
							100.00

Table 3—Index of Fly Control McGehee 1961

Treatment	Fly Index		
	Horn	Horse	Stable
1.0% Piperonyl butoxide .1% Pyrethrins	4.53	.82	1.40
.5% Piperonyl butoxide .05% Pyrethrins	7.97	.97	1.30
.25% Co-Ral Spray	15.08	1.56	1.02
Check	355.80	3.49	2.06

Table 4—Results of Complement-Fixation McGehee 1961

Treatment	Number Animals	April 14		September 6	
		Number	Percent	Number	Percent
1 percent Piperonyl butoxide .1 percent Pyrethrins	40	15R	37.50	24R	60.00
		5S	12.50	10S	25 00
		9AC	22.50	4N	10.00
		1QNS	2.50	1 unacct. for	2.50
		10N	25.00	1 died	2.50
			100.00		100.00
.5 percent Piperonyl butoxide .05 percent Pyrethrins	45	15R	33.33	29R	64.44
		6S	13.34	3S	6.67
		2QNS	4.44	1AC	2.23
		12AC	26.66	12N	26.66
		10N	22.23		
			100.00		100.00
.25 percent Co-Ral Spray	72	17R	23.61	21R	29.16
		9S	12.50	29S	40.28
		44AC	61.11	5AC	6.94
		1QNS	1.39	15N	20.84
		1N	1.39	1 no test	1.39
				1 died	1.39
			100.00		100.00

Discussion

There were two essential differences between the circumstances involved in the two seasons. First, the owner purchased a new bull and placed him in a lot adjoining the best treatment. This bull died of an acute case of anaplasmosis on June 26. This case undoubtedly provided a high level of infectivity in very close proximity to the better fly control treatment. Second, there was an over abundance of water and heavy mosquito populations at about the time the bull died. No estimate of mosquito control or lack of control was made.

The work reported suggests that practical fly control in the absence of an acute case may retard the development of the carrier state in animals in the same pasture. It also emphasizes the necessity for sharper delineation of the primary vector species. Fly control in general may not be indicative of the species control required to prevent transmission especially when highly infective sources are available.

Appendix—Horsefly Species Collected McGehee 1961

	June	July	Aug.	Sept	Total
Tabanus atratus	6/1	1/2	3/		22
Tabanus americanus	27/	10/			37
Tabanus styguis	1/	1/			2
Tabanus molestus	1/	1/			2
Tabanus venustus	3/		1/		4
Tabanus abdominals	1/				1
Tabanus proximus	1/	48/	15/	5/	69
Tabanus vittiger schwardti	96/	99/	53/	20/	268
Tabanus mularis	4/	8/	6/	4/	22
Tabanus cymatophorus	1/	57/	6/		64
Tabanus sulcifrons		9/	7/	25/	41
Tabanus lineola			2/		2
Tabanus giganteus				4/	4
Leucotabanus annulatus		1/			1
Chrysops flavida	14/	1/	1/		16
552 Total Specimens					
15 species					

Control of Tabanidae

D. E. Howell

Earlier speakers have presented the details of experiments designed to evaluate the control of Anaplasmosis by the application of synergized pyrethrins to cattle several times a day by automatic sprayers. This discussion is concerned with experiments designed to evaluate other methods of control.

It should be stressed that the biology and ecology of tabanids may vary in different areas and the data reported here refer to Oklahoma conditions.

Alteration of the Environment

Any procedure that will make the environment less satisfactory for larval horsefly development may be expected to reduce the amount of breeding and help reduce tabanids. The most obvious approach is changing the larval breeding areas by drainage, even though it can be expected to work on a limited number of species only.

Experiments were set up using 10 farm ponds of approximately uniform construction ranging in size from 1-2 acres. Prior to treatment the larval populations in the seepage area below the dam of each pond were determined by carefully screening twenty square feet of the soil and returning the larvae found to the plot.

By careful ditching the seepage areas of five of the ponds were dried as much as possible. The other five ponds were left for a control. Post-treatment sampling 3 months later showed the larvae in the drained area were concentrated in the greatly reduced moist area but the total number of larvae found had not appreciably decreased. Counts of adults on cattle grazing close to the ponds were slightly lower on animals in the vicinity of the test ponds. The species breeding in moist areas such as *T. atratus* Fab. and the *T. lineola* complex were lowered more than "dry land" breeders such as *T. abactor* Phillip and *T. sulcifrons* Macquart, but the results were not significant.

The following year the moist areas which had been ditched below the ponds were thoroughly treated with a 1.0% lindane emulsion so that the soil was wet for at least 4 inches. Sampling one month later indicated that the larvae were almost completely eliminated from the test areas and remained absent for over two years but the experiments were discontinued because of the insecticides residue problem, the marked toxicity to most invertebrates in the area and the high cost of treatment.

Extensive sampling along the margins of ponds devoid of vegetation and subject to wave action indicated few flies bred in such areas. Pond margins with vegetation, relatively clear water, and little variation in water level were favored areas for breeding for several species.

Large Water Empoundments

The construction of Tenkiller dam in eastern Oklahoma provided an excellent opportunity to determine the effects of large water empoundments on horsefly populations. Three years prior to the filling of the lake, three study areas were established. The first was located on Barron Fork Creek, approximately seven miles above the furtherest extension of the lake when the water reached spillway level. The second area was adjacent to the stream between the spillway level and expected maintenance level. The last area was approximately 5 miles below the second area where the lake water was expected at all times.

The number of flies and the species complex in each area was determined by collecting the tabanids found on comparable herds of dairy cattle each day between 4:00 and 5:00 p.m. during the horsefly season for three years prior to the filling of the lake and three years after.

The total relative numbers of flies remained about the same for the entire period but the species complex changed materially.

Prior to empoundment *T. fairchildii* Stone, was found in moderate numbers in all three areas; following empoundment it was only present in the highest area. Species such as *T. atratus* and *T. lineola* whose larvae develop in free water became much more common in the two lowest stations. *T. abactor, T. sulcifrons* and *T. equalis* Hine were essentially unchanged.

Based on current information concerning the ability of the species of tabanids to transmit anaplasmosis, it is probable that large water empoundments increase the probability of anaplasmosis transmission.

Adult Fly Control

Previous speakers have reported on the successful use of the potent, safe synergized pyrethrins applied by automatic sprayers. This approach is excellent under proper conditions but unfortunately it is of little value in many areas. It may be impossible to fence all water so that cattle are forced to pass through a sprayer to get to water. Where wind velocities exceed ten m.p.h. much of the finely divided spray is blown away and does not protect the cattle. When the vegetation is high in pastures the spray is quickly removed by abrasion or washing off and species which feed by preference on the legs or underline are deterred for short periods only.

Several workers have attempted to increase the effectiveness and duration of pyrethrins by the addition of repellents. To date the data are not conclusive but materials such as MGK 1207 and Phillips 874 show some promise in our small scale field tests.

In pastures relatively free of brush and trees, rubbing devices have been quite effective in freeing cattle of hornflies but our data have not indicated much effect on horsefly populations.

Area Control

In many cattle grazing areas horsefly adults rest during most of the daylight hours in the trees and bushes associated with small creeks. They seldom move far from these areas unless following their hosts or attracted by the presence of host animals. Cattle in the open grasslands may be almost free from attack while animals along the creek are seriously bothered.

Several workers have tried to control fly populations by spraying these areas but only mediocre results have been reported. Earlier work in Oklahoma with several chlorinated hydrocarbons showed almost no reduction in population. Because of the high toxicity tabanids, the relative safety to mammals and low residue hazard of DIBROM, area treatment with this quick acting organic phosphate insecticide seemed to merit evaluation.

A mixed grass prairie area of approximately 1200 acres which contained three small creeks was selected in the Oklahoma State University range unit near Stillwater. The wooded areas along the creeks varied in width from 30 to 200 feet. The vegetation was mainly hackberry and elm with some pecan and oak. The selected area was divided into three approximately equal areas, each containing about 14 acres of wooded area and separated from one another by at least ½ mile.

Tabanid fly populations in the wooded creek bed areas were sampled by leading horses through the wooded creek bed areas. At approximately 100 yard distances all possible flies bothering the horses were collected for species determination. Collections were made from 7:00 a.m. to 5:00 p.m. during a five-day period. Six trips were made through each area each day. Fly populations were essentially equal.

One area was sprayed by plane flying at treetop level with 0.5 lb./acre of DIBROM in kerosene. The second area received 0.25 lb./acre and the third was left as a check. Sampling by the same procedure continued until the horsefly populations in all three areas were approximately equal. A second test was run later in the same area, using 0.4 lbs./acre and 0.2 lbs./acre.

Within ten minutes after spraying most of the winged insects were affected. Horseflies were killed rapidly and within 15 minutes no live horseflies could be seen in the treated area. No live flies were found in the treated areas during the first day, while 223 flies were collected in the untreated area. Almost equally good results were obtained the second day but the flies were more numerous the third day and little control was evident thereafter.

During 1961, under similar conditions, but somewhat lower populations, control was appreciably less. Only during the day of application were the flies in the area treated with 0.4 pounds per acre reduced. These data are summarized in table 1. Reasons for the variations in results are not clear.

Summary

It is clear that no method of horsefly control will provide adequate control in all situations. Many tools are available but extensive research is needed before they are ready for use.

Table 1—Horsefly populations present in treated and untreated areas following aircraft spraying with DIBROM

	1960			1961		
Days after spraying	0.5 lb/A.	0.25 lb/A.	Check	0.4 lb/A.	0.2 lb/A.	Check
1	0	0	223	60	99	97
2	0	2	203	107	92	105
3	21	26	191	88	84	91
4	105	121	198	93	81	87
5	133	141	185	68	65	74

Application of CF Testing to Control

R. S. Sharman

Just for the sake of having it said loud, clear, and simple, the basic purpose of this group is to find one or more methods of reducing losses from anaplasmosis to the lowest possible point, as cheaply as possible and as quickly as possible. The ideal solution, of course, would be complete eradication from the United States. Tomorrow, the day after, a year from now, a revolutionary discovery may point an easy way to this end.

It is unlikely, however, that even a new breakthrough in the laboratory would enable us to immediately jump in and subdue or eradicate this disease unless we acquire more precise knowledge concerning its incidence and abundance in the United States.

Planning for a program to cope with the disease on a county, State, or national level provokes many questions in the minds of regulatory officials, State and Federal legislators, livestock groups, and others who must support such a program in one way or the other. If indemnities were paid for reactor animals, someone would be sure to come up promptly with a question regarding the quantity of money needed for indemnity purposes. Livestock association groups would be anxious to know the extent to which their herds might be depleted through disposal of reactor animals. Legislators would ask, "Is there enough infection in enough herds to warrant the cost of a county, State, or national program?" They would undoubtedly like to know the estimated dollar losses occasioned by anaplasmosis in different States. Those

responsible for planning a program would be immeasurably aided by knowing how widespread the disease was in herds in different areas and the percentage of infection in infected herds. An individual farmer, eliminating the disease from his herd, would like to know the relative risk of reinfection stemming from heavy or even light infection that might exist in the few or several herds in his neighborhood.

There are many other good reasons for acquiring more precise knowledge about the percent of herd infection and the relative number of herds infected in different parts of the country.

A number of States have conducted CF testing which might be classified as random sampling for the purpose of learning more about the extent of anaplasmosis. Hawaii was the the first State to conduct such work. This was done through collection and testing of blood samples in all cattle slaughtered in Hawaii. Out of a little over 2,000 herds tested from 1954 through 1957, involving 85,000 animals, 134 were found to be positive to CF testing. The infection rate was approximately .0015%.

During 1956 and 1957, a total of 6,700 serum samples representing 388 Wyoming beef and dairy cattle herds were tested for anaplasmosis, disclosing a little over 10 percent of the animals to be reactors and about 2½ percent as suspicious. It was interesting to note that in one county about half the cattle tested in eight herds were found to be infected, although only one of the herds had been known to be an anaplasmosis problem herd. Nine thousand cattle tested in Montana disclosed approximately 10 percent to be reactors. During 1955 and 1956, 8,500 cattle were tested in California, Missouri, Oregon, Tennessee, Virginia, and Washington, and 1,788 were found to be reactors, although this figure probably does not represent random sampling.

Two years ago a supply of antigen was acquired by the Animal Disease Eradication Division through cooperation with the Texas A & M College, and, as reported by Dr. Walter Martin, a number of State and Federal employees have been, and are being, trained to utilize the antigen. Last year, arrangements were made with North Carolina to conduct a pilot survey for the purpose of working out survey techniques that could be applied nationwide, as it became desirable or necessary, to conduct similar surveys in other States. Blood samples used in the survey were those submitted by veterinarians doing brucellosis testing, and the samples were selected in a random fashion following the technique used by Thomas in Wyoming in 1956 and 1957.

Results of the survey in North Carolina are shown in figure 1.

The survey represents twelve months' testing, from March 1961 to February 1962, in which almost 45,000 animals were tested; 1,728 animals were positive to the test for a statewide percentage of infection of 3.9.

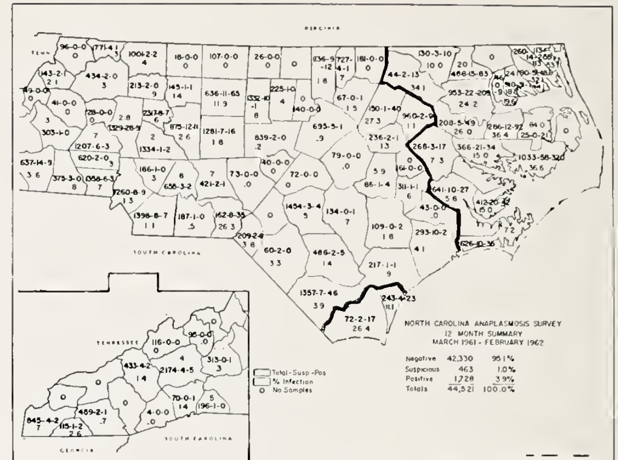

Figure 1. Results of an Anaplasmosis pilot survey conducted in North Carolina, March, 1961 to February, 1962.

Although some counties are not represented by substantial numbers of cattle tested, there does seem to be a trend that generally confirms the accuracy of earlier reports of the existence of anaplasmosis within the State.

Heaviest infection was found in the tidewater region, east of the heavy lines on the map. Infection in the extreme western portion of the State is relatively light or virtually non-existent.

The cost of such a survey, if not conducted on a crash basis, can be held to a low figure through utilization of brucellosis samples and the spare time of laboratory personnel for preparing the samples for shipment to a central laboratory for testing or doing such testing locally.

It would be interesting to conduct entomological field studies along with such a survey to determine the incidence and relative abundance of biting insects within the State during different periods of the year. An effort will be made to conduct some such work in one State as soon as possible, utilizing the services of insect-identification personnel at Beltsville, Maryland.

Surveys similar to that in North Carolina have been initiated in several other States. A special survey of this type was conducted in New York during 1961, in which 3,486 animals were tested from 179 herds. Two reactors and 25 suspicious animals were found for a total of .7 percent infection. In Ohio, 2,289 cattle have been tested in 229 herds with 100 positives and 29 suspects. In Georgia, 1,991 in 38 herds have been tested, with 78 reactors and 31 suspects, showing a percentage of 5.4. In Alabama, 1,629 animals in 23 counties have been tested in which 13 reactors and 28 suspects were disclosed for a percentage of 2.5 infection. Seventy-two herds have been included in this survey to date.

It is realized that the number of animals tested in these last few States is still too small to be relied upon to accurately depict the anaplasmosis picture in those States, but it has served to give a rough idea of what the trend might be. Florida and South Carolina have only recently undertaken a survey, and no results are yet available to establish any sort of trend in these two States.

I have some cards on individual herds in Tennessee, North Carolina, Indiana, and Virginia, in which CF testing has been carried out, primarily because the owner wished to do something about anaplasmosis in his herd. In most cases the owner has followed a test and segregation and/or slaughter program. There are uncontrolled and unknown factors associated with each herd since efforts to deal with the disease have been cooperative and voluntary ones. In many cases the status of nearby herds is unknown. Many times the owner was unable to follow the recommended bleeding schedule. There are undoubtedly instances where infected animals have gained access to clean, segregated herds by jumping fences, because of breeding purposes, etc., and these may have affected the results. The results do appear to bear out the contention that it is practical to eliminate anaplasmosis in many herds through the use of the CF test.

It would be interesting to see how effective test and segregation and/or slaughter would be over a period of time in a region known to have heavy infection in surrounding herds.

OWNER________ ADDRESS________ STATE N.C.

TYPE: Beef__ Dairy__ COUNTY Nash VETERINARIAN________

Date Bled	Date Tested	Number Tested	C.F. Reactions			Empty	Comments
			Neg.	Susp.	Pos.		
7-18-60	7-21-60	12	2	0	10		
	1-26-62	105	45	15	45		
3-15-62	3-20-62	85	80	3	2		—
4-3-62	4-10-62	75	75				

Figure 2. Sample of card used in CF testing.

Even with an accurate CF test for diagnosing anaplasmosis, we still do not have an eradication program that can be recommended for adoption by a state suffering substantial loss from anaplasmosis. Progress, however, is being made by research groups in applying the tetracycline group of antibiotics to anaplasmosis carriers. More research and more field studies are urgently needed, but perhaps this approach to the disease may yet be the most promising.

Questions, Answers and Comments

QUESTION—Dr. Gates, Washington, D. C.

I have a question for Dr. Franklin. How many pools were made and what percentage of individual antigens failed to meet the requirements?

ANSWER—Dr. Franklin, College Station, Texas.

Dr. Gates, I'm going to see if I can pass the buck to Mr. Heck. — Mr. Heck, College Station ,Texas.

I can't answer your question specifically, Dr. Gates. We observed, as you know, quite a few CO_2 precipitated antigens that did not meet the antigenic potency test as set up by the contract. I would estimate during the first 30 animals used in production that we did not obtain good CO_2 type antigens from more than 50 percent of these animals. However, with the Sharples water-lysed type antigens we were able to obtain approximately 98 percent good antigen. We were concerned chiefly with the anti-complementary effect of each individual antigen and we didn't have any problem with this. We have never determined why. It was non-existent and we didn't fool with it at all. In the Series-Two type antigen, as you well know because this was produced after your visit with us, we had extremely good luck with our CO_2 precipitated antigen. Again, statistically, I can't say. But we had very good luck with the Series-Two antigen as far as producing strong antigenic activity in this product.

COMMENT—Dr. Franklin, College Station, Texas.

Dr. Gates, in connection with your question, I don't want to put in a plug, I've already referred to our article that just came out in the Southwestern Veterinarian on large scale production of the antigen. I believe that will help clarify this. It won't directly answer your question but it will show that as our infectivity, both of inoculum and infectivity of exsanquinated blood, went up, we learn more about

our CO_2 antigenic patterns. In other words you have your most satisfactory CO_2 antigen production along with that increased infectivity.

QUESTION—Dudley Campbell, Denver, Colorado.

I have a question for Dr. Saulmon. During the last day and a half we've had some very astute discussions about the total picture of anaplasmosis and what you people are doing to find solutions to it. Undoubtedly, there are many areas that are quite gray. We don't know much about it. We have a test developed, but as far as practitioners' advice to the ranchers as to what to do when they have an infection these are simply opinions. It occurs to me that we might be a bit premature in developing rules and regulations for control of the disease when we know so little about it. Is it your intention, Dr. Saulmon, to draft regulations for interstate movement based only on the tests, when we could influence a test for example with antibiotics, I understand?

ANSWER—Dr. Saulmon, Washington, D. C.

My answer that would be, I don't think anyone claims the test as 100 percent infallible. It is a reliable test to disclose the infected animal. Under our basic authority as of now, this animal that was tested and disclosed as a reactor animal cannot legally move interstate. I am not advocating programs here. Testing up to now has been on a voluntary basis. The owner who was having the problem and wanted to do something about it requested and received the test. Let's face it, a lot of owners that have tested have been relieved of the economic impact of anaplasmosis in their herd. I do think there should be a regulation that could allow the movement of the animals that are disclosed as reactors and infected animals, so when they go to market they can go to the best market they can get. That would be the only regulation that I would assume, or even think in terms of, at the present time.

QUESTION—Dudley Campbell, Denver, Colorado.

One more question, Dr. Saulmon. Let's assume that we use something comparable to the brucellosis program, a small "a" on the jaw of infected animals. There is some indication that after high level treatment with certain antibiotics that this animal is no longer a carrier. Well, then, what do you do about the identification?

ANSWER—Dr. Saulmon, Washington, D. C.

Let's assume we use an ear tag instead of the hot iron brand you mentioned on the jaw. The ear tag identifies an infected animal then, when he is proved not to be an infected animal, the tag can be removed. I don't think that's an insurmountable thing.

COMMENT—Dr. Riemenschneider, Okla. City, Okla.

We have a little program going on with this very thing and when a man decides to go under the program he has many choices. It's strictly voluntary, and one of the choices is to treat these animals. There's a provision there made for the animals to be identified by an ear tag. After they pass the test, ear tags are removed. If they do not clear up then a hole is punched in the ear identifying them in that manner.

QUESTION—Dudley Campbell, Denver, Colorado.

One more question, Dr. Riemenschneider. What is the consensus of research people and practitioners in advising ranchers or dairy people what to do when they have high infections of anaplasmosis? Are we going to have a cull and slaughter, an isolation, identification-isolation program? What is the most accepted procedure to eliminate anaplasmosis from our herd, be it dairy cattle or beef cattle?

ANSWER—Dr. Saulmon, Washington, D. C.

In all probability, the degree of infection in an individual herd would make a difference. In some locations it will make a difference. In our north-central, north-east states where there's little anaplasmosis and in some states there's no known anaplasmosis, they'll look at it from one angle. While in your enzootic areas it's a different proposition entirely. It's very possible that in that case they will go on recommended antibiotic treatment that was mentioned here yesterday as successful. So I think it can vary, it may vary greatly from different areas and different situations.

COMMENT—Dr. Franklin, College Station, Texas.

I'm from a state, Texas, where we've had our share of anaplasmosis, although I don't think we've had all of it by any means. We have a lot of different types of ecological, and climatological areas. We've gone into some areas where herds have tested 60 percent and up, other areas we're surprised to find that the infectivity level is less than 5 percent and not over 30 percent in some areas which have a high vector level. We feel that the approach there would be different for a tick area than in the Gulf Coast area and in an infected flying insect area. We're doing some work with a herd there now and we don't have any recommendations at this time. We have approached it by segregating the reactors. The owner is continuing to run the reactor herd as a separate herd. We think that is very important for this to be carried out with proper management because we think the key here is the vectors plus the number of reactors and their possible infectivity level.

COMMENT—Dr. Pearson, Pawhuska, Oklahoma.

In Oklahoma we have a testing program available to our people. We have, for several years, done considerable work screening this testing procedure. We think there is good to come from our program. We wouldn't advocate adoption of our particular pro-

gram, in all parts of the country. We feel in our area, and in areas where ticks are not a vector, anything short of the testing program is simply appeasement. We've all heard in the past day or so, and in other meetings of this kind, that there are other methods of controlling this disease. We think that, when all the cards are on the table, testing will be the answer in those areas where the ticks are not a real serious vector. Now we have in our area a program in effect in which we offer our people this testing service in co-operation with the state veterinarian and federal department. Dr. Hanson and Dr. Riemenschneider and our department have conferred on this thing many times and we have quite a few thousand cattle in Oklahoma that are tested and we're getting real good results. This program has been going now for 9 years, first in our laboratory, and at present the tests are made in the state laboratory. We certainly wouldn't hesitate to recommend to any one, or any group of people, that anaplasmosis can be controlled in a given herd, or in a given area, by the testing program if ticks are not one of the important vectors.

QUESTION—Dr. Breen, Laramie, Wyoming

Dr. Saulmon, I want this primarily for the record, we do not want to put you on the spot. The question is, what groups or what actions were taken to give the ADE the impetus to promulgate their memorandum, I have forgotten the number but I think you're acquainted with it, in which you set up the provisions for controlling antigen distribution in regard to the regulatory phase?

I do want to explain this because Dr. Saulmon and I talked about this and I don't want to ask a loaded question. The background on this is our feeling in the west that, with the anaplasmosis being quite a problem there and actually looking at it from the cattlemen's viewpoint, we are wondering whether there were enough groups, such as the American National Cattlement's or other groups, directly involved, if they had enough of a background or enough of an educational exposure in order to help pass on recommendations.

I will go a little bit further on this without trying to grab the floor. We respect Dr. Saulmon and all of his group's responsibility to the basic law, and yet we have these things such as tuberculosis and brucellosis where specific memorandums or regulations come out. We have many other things all of us are concerned with, whether it be coccidiosis or other things that can be transmited or are communicable. But, when a division or a responsible group like ADE comes out with a memorandum specifically stating the troubles and the complications of anaplasmosis, we feel it gives it a much more regulatory wedge. We think it puts the cattlemen in a very embarrassing situation and we wondered how much of the say so he had in promulgating this memorandum.

ANSWER—Dr. Saulmon, Washington, D. C.

Let me get a little background for the record. You recall, at our meeting five years ago, a committee was appointed in which the conference recommended that a standard complement-fixation antigen and a standard test technique be developed.

That committee then was made as a sub-committee of the United States Livestock Sanitary Association. I believe I have my facts right. This committee came up with the standard technique for the test, and maybe antigen production. A part of the recommendation was that one central source of antigen be made available, and the Disease Eradication Division furnish this antigen. We went on and contracted with Texas A&M and the antigen was produced.

As I've explained, we operate under a basic Act of Congress. It's not an arbitrary thing, it's spelled out. We did not feel that, as regulatory officials, we could be a party to designating by reliable tests that specific animals were infected and then ignore their movement if they should be carried across state lines. We thought it was our responsibility to state our policy. As I explained to Dr. Breen yesterday, we had some considerable soul searching on this, but let's face it, it would have been easier to have done nothing. Test cattle and let them go everywhere! Certainly we're here to help the livestock industry in every way possible. That's our primary purpose. We don't feel we can be a party to discovering infected animals and then allowing them to move indiscriminantly in channels of trade. Of course, our authority is only in interstate channels.

QUESTION—Dr. Breen, Laramie, Wyoming.

Can I just add one more part there? I appreciate Dr. Saulmon's answer and I'm very thankful. I wanted a little more information, looking at it from the cattlemen's viewpoint. How much effort has gone out to actually contact them and look at it from the cattlemen's viewpoint, realizing they know your responsibilities? But when action is taken are they specifically consulted? I realize there are state regulatory officials which are United States Department of Agriculture Livestock and Sanitary Boards, and I'm sure you're acquainted with all the other regulatory phases and there have been controversies. But are these groups brought in? We, as veterinarians, sometimes are doing a little too much governing in the light of the stockmen's viewpoint without him being properly oriented in what we're trying to do for him. And I'm just wondering how much effort has been made to try to get responsibile stockmen's organizations backgrounded so they can advise these groups?

ANSWER—Dr. Saulmon, Washington, D. C.

I'm sure you have laws in Wyoming that say you can't rob a bank and I don't know who you consult with to rob a bank. I'm not trying to be facetious, but I don't think you appreciate what I said. It's a

basic Act of Congress. We have no choice in the matter. Even regardless of that, there are many livestock owners and regulatory officials that are insistant that an anaplasmosis reactor be identified. As I indicated, there are a number of states that have been thinking seriously of requiring an anaplasmosis free test to enter their state. It's because it's a disease that is not established there, but can we stand up and say they don't have vectors of anaplasmosis there. This day and age, with international travel being what it is, maybe New York state doesn't have a vector now. About a year ago, maybe a little more, we found the African red tick that, I believe, is a vector of anaplasmosis. It was found in the state of New York, fortunately in a wild animal game compound, and was eradicated before it became established. But don't let anaplasmosis get established up there just because we don't think we have vectors in the state like that. Those states are thinking seriously of it. They are thinking seriously, and they would say that we're not carrying our responsibility if we took the other stand. So, since the basic law says that known infected animals cannot be moved interstate, we have no choice but to take that stand until it should be changed.

COMMENT—Dr. Riemenschneider, Oklahoma City, Oklahoma.

I think every state has to ask for the privilege of having the antigen, the privilege of running the test. In our state at least, every man that wants to participate, does it on a voluntary basis. So he enters the thing voluntarily, for his own benefit, with those restrictions.

COMMENT—Dr. Pearson, Pawhuska, Oklahoma.

Dr. Riemenschneider, I think maybe we're talking about something here and assuming that some things haven't happened that have already happened. We, in Oklahoma, had a meeting with our representatives of the Oklahoma Cattlemen's Asosciation, with our state veterinarian and with representatives of our federal department. It was my good fortune to serve on that committee as a representative of our state cattlemen's association and also at the request of the state veterinarian.

This thing that we've been talking about here was hashed over in that meeting. Oklahoma at least has been one of the states where our state cattlemen's association has asked that the bureau do something about provisions for interstate shipment of these reactor animals. We on the committee from the cattlemen's association decided that no constructive, good-sized, progressive group could take a stand against some form of control of this disease and that came, of course, from the cattlemen in our area. We did ask, because it would be a handicap to them and because slaughter plants are not plentiful in Oklahoma, that we be permitted to ship these cattle interstate for slaughter only. Now, I think, that we're assuming here that the cattleman hasn't been considered to any degree at all, but he has. They sat in with us in this same group in which we drew up our agreements that are signed by all of our cooperators and agreed 100 percent in all the methods of control that we have in Oklahoma. We were fortunate in having the federal department represented there and our agreement was approved before the meeting was over. This was very satisfactory from the point of view of cattlemen's association as well as the regulatory officials. This thing can easily be worked out, I think. All we need is a little cooperation from the parties involved.

QUESTION—Dr. Koger, Ontario, Oregon.

First, may I humbly suggest that we may already have a product that would permit the introduction of susceptible cattle into enzootic areas, with marked reduction of death loss pending the development of a better product. I would like again to pose the question as to what place in the thinking of the regulatory officials does the future possible use of some agent to raise the resistance of our cattle against anaplasmosis occupy? I direct the question to Dr. Saulmon.

ANSWER—Dr. Saulmon, Washington, D. C.

If a good reliable immunizing agent were developed, I see no reason why it would not be used with anaplasmosis. Now if you're talking in terms of premmunization, something of that nature, you might run into a little difficulty from regulatory officials. But, if it is a true immunizing agent that does give resistance and protection against the disease, I think we are all looking for that. I would hope that we would get it someday.

COMMENT—Dr. Koger, Ontario, Oregon.

I wanted to get it in the record that a vaccine was being considered. I think it will be encouragement to people at home. They see the problem quite differently than the people in Oklahoma perhaps.

COMMENT—Dr. Saulmon, Washington, D. C.

I might say further that we would hope that a good immunizing agent, something to break the chain of infection, could be developed. Relating it again maybe to brucellosis, it's doubtful if we would have had the success we're having with the brucellosis program if the Strain 19 vaccine had not been developed. So I would hope something as good or better could come someday.

QUESTION—Dr. Ristic, Urbana, Illinois.

Mr. Chairman, I would like to ask Dr. Welter, since I know that he has done some cooperative work with several states using the capillary tube agglutination test, if he would like to make some comments on his results.

ANSWER—Dr. Welter, Des Moines, Iowa.

First of all, I would like to thank Dr. Riemenschneider for giving me an opportunity to just add a few comments here. I might just state a little bit about the biophysical stability of the antigen. We have shown it to be very remarkably stable to heat. It is not affected by heating for 60° for an hour. We have incubated it for 16 weeks at 37°C and further heat studies have been carried out to show that it is protein in nature and that we can go up over 60° and get some interference with it's ability to react in the CA test. It is also quite stable to pH changes. We have shown this in buffered solutions ranging from 4.6 to 8.6. Incubated for approximately 10 weeks there has been no reduction in antigen potency. And this has been characteristic all the way through. We have other stability studies and characterizations on suspending the antigen in various environmental conditions, and shown it to be remarkably stable.

Now, we have some studies carried out in conjunction with Wyoming and they have run the tests on 235 serums and found an 87 percent correlation with the CF test. Now, of these serums, roughly 50 percent of them were CF reactors. We have very consistently found that we get a very high correlation between CA and CF negative or CF positive and CA positive correlation has been round 65 percent. In addition to this, I might just go a little bit further, and state some of the other studies we've done. We have infected 80 cattle at our own research facilities, taken paired serums, pre-inoculation and post-inoculation. These serums were tested both in the CF test and the CA test. There was very close correlation on the pre-inoculation serum. Of these 80 calves and cattle, they represented both, all of them became CA positive a week to 10 days after the peak observed infected red cells. Now we routinely check our post-inoculation serums, about a week after the peak of infected cells because of the time of appearance of the CA antibody.

QUESTION—Dr. Franklin College Station, Texas.

I would like to ask either Dr. Welter or Dr. Ristic if they have compared the relative specificity of the CA antigens in relation to the infectivity, say, for an antigen made from an animal with infectivity of 30 percent as opposed to one with a 90-95 percent infectivity? Maybe this was discussed previously but if so I failed to get it.

COMMENT—Dr. Welter, Des Moines, Iowa.

Why don't you say a word first, Dr. Ristic, and I might just add a little bit about specificity.

ANSWER—Dr. Ristic, Urbana, Illinois.

I would just like to say that cell infections of 40 percent to 90 percent, of course they are found only during the acute stage. I pointed in my table that CA, that is a glutamic antibody, is a less firm developing antibody so I would expect that at time of infection that a 40 percent animal still did not reach its peak as far as glutamic antibody is concerned. We only want the highest peak of these glutamic antibodies usually at the end of acute infections rather than at the middle.

COMMENT—Dr. Welter, Des Moines, Iowa.

To carry this a little bit further, after the antibody has appeared we have some, oh about a half dozen, calves going now where we check the CA titer every week and it remains very even once it has been established. We have never seen it drop to a very low level. Now, along the specificity lines, we have checked a number of serums, roughly 200 I believe by now, on serums containing antibodies specific for a whole host of other bovine pathogens. This includes the vibrio, leptospira, several virus diseases, and some of the other protozoan or rickettsial type infection. I'm referring here to *Anaplasma ovis*, eperythrozoan, and so forth.

QUESTION—Dr. Hoyt, St. Paul, Minnesota.

I'd like to direct a question to Dr. Summers. All due respect to Dr. Saulmon, we do get increasing resistance from the Brucella 19 vaccine. I'd like to raise the question, in regard to anaplasmosis, a disease which has been recognized as producing a very high degree of carrier state, whether or not we can anticipate that we will have a vaccine developed which will give us true immunity rather than perhaps some increase in resistance?

ANSWER—Dr. Summers, Indianapolis, Indiana.

If I understood, the question was whether we can anticipate the development of an antigen, immunizing antigen, in anaplasmosis. From the theoretical point of view, I would say yes. Because I believe that there is in the anaplasma antigen or organism, something, some portion of the antigen, which is stimulatory of protective antibodies. Organisms are made up of numerous antigenic forces.

I can conceive that in the case of the anaplasma organism that all of its components do not stimulate the formation of protective antibodies. It's possible, maybe possibly using techniques that have been described here, to remove the organism from the red cell stroma. Then following fractionation of the antigen, to isolate from the organism a highly antigenic by the presence of other antigens. As we know, or I'm sure you all know, that the development of immunity in animals or man to disease is not going to be a lifetime proposition. Of course, in the case of cattle, the life expectancy is much shorter and I firmly believe that if some method can be obtained to improve the antigenicity or the protective aspect of the antigenic organism that we can expect to protect animals, to immunize them artificially.

QUESTION—Dr. Hoyt, St. Paul, Minnesota.

I believe that answers the question except I would raise this question. Why does not an animal that is infected then, which surely should have all the antigenic components of this organism present, have a complete recovery instead of developing a latent infection or carrier state?

ANSWER—Dr. Summers, Indianapolis, Indiana.

Now this is a mystery that I'm ill equipped to answer. Why does it happen in tuberculosis in man or brucellosis or some other diseases? The organism stays in the body, is infective for other animals. Many times it's a reproduction from the infection and it may protect against the amount of antibodies that terminate or protect against subsequent inoculation. I don't believe I can answer that, maybe someone else would like to try.

COMMENT—Dr. Kuttler, Reno, Nevada.

I certainly don't know the answer either, but I do have some thoughts on it. Possibly, rather than describe what happens in adult cattle where they remain carriers, I might refer to our observations in calves, where it appears that they have a passive immunity. Now, I don't think we have really proven this. But they are born with, or shortly after birth, develop a complement-fixation reaction which subsides to negative after about 50 or 60 days, and then in spite of future exposure, they do not become infected with anaplasmosis. So possibly they do have a passive immunity here that might protect them. Now as the calves become adult we know they are no longer immune. They're susceptible to anaplasmosis. Possibly if we could introduce the antigenic substance in such a way that it would be present in the body and still be producing antibodies without infection, we might be able to reproduce the carrier state without infection.

COMMENT—Dr. Ristic, Urbana, Illinois.

Mr. Chairman, may I make a short comment on the same subject please. I would just like to say that listening to the discussions the whole immunity is based on antibody here. Unfortunately, I would like to say that I agree very much with Dr. Roby and his results because we have done the same work two years ago but was never published because we do not feel encouraged to show any practical protection whatsoever. What I would like to say is that Dr. Summers has quoted me very well regarding my hypothesis on immunity. I would like to discuss further what he has quoted. And that is, I have pointed very clearly that a cellular system, fixed and migrating macrophages, I think they play a high, a more important role in immunity against anaplasmosis than the particular antibody we are talking about. And I think that is a basic point in our failure to produce good immunity against anaplasmosis using a dead vaccine. Because this vaccine, while they produce the antibody against the organism itself, they do not engage or stimulate in specific manner those fixed and migrating macrophages needed to really produce immunity against anaplasmosis.

COMMENT—Dr. Seger, Baton Rouge, Louisiana.

I would like to make the suggestion that in these young calves the increased resistance might be due to the increased efficiency of the phagocytic system and hematopoietic system rather than the true immunity. There's just more resistance to infection, in the young calves Dr. Kuttler was talking about.

COMMENT—Dr. Kuttler, Reno, Nevada.

I certainly agree with Dr. Seger, and if you recall I didn't say I believe we have proved anything here. I just presented it as an observation. On the other hand, we do have the corollary evidence of the complement-fixation reaction which is not necessarily the measure of the immunity but I think it does indicate some connection there.

COMMENT—Dr. Seger, Baton Rouge, Louisiana.

Yes, I just wanted to point that young calves have probably larger reticuloendothelial systems in proportion to their body weight. And their hemotopoietic system is more efficient.

COMMENT—Dr. Roby, Beltsville, Maryland.

I don't think anybody has even theoretically answered Dr. Hoyt's question, satisfactory to him. And I think you are going in circles on this theoretical basis and I think that it's very encouraging that more effort is being given towards a better understanding of the disease and the organism, its chemical constitution and that the only thought that I would like to leave is that sometimes our concepts have already changed and I think those of us close to the problem from an experimental standpoint are optimistic enough to hope that the old concept of the impossibility of producing an immunity will in the future someday be changed.

QUESTION—Dr. Avery, Alberta, Canada

I'd first like to say how nice it has been to be here, and secondly, thank goodness, as yet we haven't got it up there. I would like to ask one question. Is there any difference in the lack of immunity following the natural waning of the carrier state and that when you eradicate the organism following the use of antibiotics? Dr. Ristic said that the reason why we could not produce an immunity with the use of killed vaccines, was that we couldn't stimulate the reticuloendothelial system. Now this reticuloendothelial system apparently is stimulated, and are we doing anything different actually killing the organism using antibiotics with a system which remains stimulated than by allowing

this system, let us say, to die due to natural waning of immunity?

ANSWER—Dr. Ristic, Urbana, Illinois.

I really can't answer your question. All I can say is what I said a minute ago and that is after you treat with aureomycin or any of the tetracycline compounds the animal can again become susceptible to infection. You can reinfect them. To me it looks like if you want to have any stable immunity you just can't have all the basic factors that you have to have in a living active agent. This is nothing somebody didn't mention before. We know of so many infections where we just can't produce the immunity without an active agent. I don't believe you can in anaplasmosis.

COMMENT—Dr. Summers, Indianapolis, Indiana.

I might say one thing from experience. We know that immunity produced by certain types of toxoid materials require reimmunization at various periods of time in the life of the individual. It's possible, it seems to me, that if we could produce a sufficiently high immunity in young animals to prevent infection that they might be given booster shots, to restimulate both the cellular immunity as well as the humoral immunity that we speak so glibly about. There's another aspect of immunity, of course, that no one dares touch upon because we don't know very much about it. And that is immunity of the local tissues themselves. How much resistance do they have to continued infection? Are they able to recover and then never be reinfected by the organism? This is a very tenuous subject and I don't have any business talking about it. But it is another aspect in immunity and infectious diseases.

COMMENT—Dr. Hoyt, St. Paul, Minnesota.

I just wanted to respond, Dr. Riemenschneider, here briefly because I think Dr. Ristic and Dr. Roby certainly contributed to the thought that brought up the question in my mind originally. I used the comparison of Strain 19 because I think that this gives us a good example to work from. In other words, tissue immunity is a dynamic response and I think that our experience with Brucella as compared with Strain 19 points out that if we are successful in isolating a modified virilant organism so that this process can go on continuously and maintain this tissue resistance we have a good chance of developing an immunizing agent.

COMMENT—Dr. Franklin, College Station, Texas.

In response to Dr. Avery's question here, I might briefly cite a personal experience. About 10 years ago Dr. Schmidt injected a known carrier one morning with aureomycin, right now I couldn't quote the dosage but it was a relatively large dose, intravenously and that evening or maybe the next morning he drew blood from this carrier and from that time on we could never transmit from this known carrier which had been a previously known carrier. This animal was followed on the station for a number of years in the negative herd. It was then put in another herd in with some reactors, and lo and behold that animal came down with clinical anaplasmosis, recovered without treatment. I don't know whether that helps explain his question but I would think personally once an animal loses the infection regardless of how it would lose it, it would again be susceptible under certain conditions.

QUESTION—Dr. Chastain, California Department of Agriculture

I would like to ask Dr. Sharman, from the cards he showed on some of these herds, what was the fate of the suspicious cattle? Were they removed from the herd, or were they retested? What happened to them?

ANSWER—Dr. Sharman, Washington, D. C.

Our suspicious animals are retested. They are removed from the negative group. Segregation is practiced in the herd. They are not allowed to run with the negative group.