The Diagnosis Of Glanders By Complement Fixation

#John R. (John Robbins) b. 1875 Mohler
THE DIAGNOSIS OF GLANDERS BY COMPLEMENT FIXATION.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., March 20, 1911.

Sir: I have the honor to transmit herewith a paper entitled "The Diagnosis of Glanders by Complement Fixation," by Drs. John R. Mohler and Adoph Eichhorn, of the Pathological Division of this bureau.

Since the discovery of the glanders bacillus in 1883 many efforts have been made to find a reliable method of making an early diagnosis of the disease. The mallein test and later the agglutination test have been and are at present in general use, but neither of these is sufficiently reliable to be entirely satisfactory. Schütz and Schubert, German investigators, recently called attention to the value of complement fixation as affording a more reliable method of diagnosing glanders, and within the past year this method has been carefully studied and tested in the Pathological Division of this bureau.

It will be seen from the details given in the accompanying paper that the authors have found the complement fixation test to be highly reliable as a diagnostic agent for glanders, and they present a thorough exposition of the method resulting from their searching experiments, including practical tests in a recent outbreak of glanders at Washington, D. C.

In view of the great economic and scientific importance of the subject, and as no work upon this new method has so far been published in the United States, I recommend the immediate publication of the paper in the bulletin series of this bureau, in order that the value of the method and the technique necessary for its application may be made more fully available in this country.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.
CONTENTS.

Introduction........................................................................................................... 5
Hemolysis................................................................................................................ 7
  Method of obtaining hemolytic amboceptor (rabbit serum)................................. 9
  Titration of hemolytic rabbit serum................................................................. 12
  Method of obtaining complement (guinea-pig serum)...................................... 14
  Titration of complement................................................................................... 14
Specific complement fixation (deviation).................................................................. 16
Method of obtaining serum of animals to be tested.............................................. 18
  Inactivating the serum....................................................................................... 19
Preparation of the antigen (glanders bacilli extract)............................................ 20
  Titration of the extract..................................................................................... 21
The complement-fixation test................................................................................. 23
  Application of the test....................................................................................... 24
  Controls.............................................................................................................. 25
  Interpreting results of tests.............................................................................. 27
Controlling glanders in an infected stable............................................................. 29
Results of practical tests with complement fixation............................................. 29

ILLUSTRATIONS.

PLATE I. Diagrammatic representation of complement fixation......................... 8
  II. Titration of hemolytic amboceptor (rabbit serum).......................................... 12
  III. Titration of complement (guinea-pig serum)............................................... 16
  IV. Titration of antigen (glanders bacilli extract).............................................. 20
  V. Final test showing positive reaction to glanders......................................... 26
THE DIAGNOSIS OF GLANDERS BY COMPLEMENT FIXATION.

INTRODUCTION.

The early diagnosis of glanders constitutes one of the most important and difficult tasks which confronts the veterinarian engaged in sanitary work. This of course does not apply to the clinical cases of glanders, as in such cases the diagnosis is usually made without much difficulty from the characteristic symptoms and lesions present. In those instances, however, where there are no positive indications of the disease, it is impossible to establish a diagnosis by physical examination, and only through the aid of some special diagnostic method or test can there be any hope of determining the presence or absence of the disease. Horses affected with occult or latent glanders, and in which the disease is not suspected, are undoubtedly great factors in the propagation of the infection. Indeed, there are many glandered horses which do not show positive symptoms until the later stages of the disease.

Since the discovery of the glanders bacillus in 1883 by Loeffler and Schütz the diagnosis of glanders has been the subject of numerous investigations, and as a result great progress has been made in its determination. After the isolation of the infective agent of the disease the diagnosis was confined to the demonstration and cultivation of the organism, or to the reproduction of the disease by inoculations of exudates or parts of diseased organs from affected horses into susceptible animals.

The first important step toward determining obscure and latent cases of glanders was made by the discovery of mallein. With the aid of this biological product of the Bacillus mallei a large proportion of latent and occult cases of glanders can be diagnosed, particularly when such tests are made by efficient and experienced veterinarians. There are, however, a considerable number of glanderous animals in which the mallein fails to give a typical reaction, and, on the contrary, a reaction may follow the injection of mallein in the absence of glanders. Thus mallein is not an entirely reliable diagnostic agent for determining glanders, nor has it ever been considered
as efficacious in the detection of this disease as tuberculin for the diagnosis of tuberculosis.

With the application of the agglutination test for glanders it appeared that a more satisfactory method had been found for the diagnosis of all types of infection with this disease. It was first suggested by McPadyen in 1896, after this investigator had observed the value of Widal’s typhoid-fever agglutination test, but was not generally adopted until the method was perfected by Schütz and Meissner, whose interesting results were published in 1905. This test has since been extensively employed in practically every country where glanders exists, and therefore ample opportunity has been furnished for drawing conclusions relative to its diagnostic value.

While there is no doubt that the agglutination test is of great value in all cases of recent infection, the blood in such cases possessing a very high agglutination power (1 to 1,000 and higher), nevertheless extensive experience has proved that horses affected with chronic glanders give occasionally a very low agglutination value, which in some cases is even lower than that of normal blood serum (1 to 400 or even lower). From this condition it appears evident that in certain cases of chronic glanders the disease can be determined only by repeated tests, and a diagnosis in such cases is only possible from the fluctuation of the agglutination value—either an increase or a decrease—as it is a well-known fact that this value remains stationary in normal horses.

Besides this difficulty, there should also be taken into consideration the fact that the blood of normal horses sometimes shows a high agglutination value (1 to 800 and higher), and that changes in the agglutination power have been observed even in animals free of glanders. Furthermore, repeated agglutination tests require considerable time, as at least two weeks should elapse between two tests. Therefore the agglutination test alone does not constitute an entirely satisfactory diagnostic method for glanders. However, as its great value has been proved beyond doubt in the early cases of infection, it may well be utilized as an adjunct to any other test which may be applied in connection with the diagnosis of suspected cases of the disease.

Hutyra compared the agglutination test with the mallein test from the tables included in the works of Schütz and Meissner and of Nevermann, and came to the conclusion that the application of the agglutination test alone has not decreased the number of faulty diagnoses. He believes that the principal difference in the results lies in the fact that a large number of horses which were classified as only suspicious by the mallein test are considered as actually infected by the agglutination test.
In further efforts to find a method by which an early diagnosis of glanders could be made, various investigators directed their attention to the precipitation reaction. This is based upon the fact that when blood serum comes in contact with a concentrated extract of glanders bacilli the precipitins or receptors, which are formed in the blood of infected animals from the time the infection first occurs, are bound to the bodies in the bacillary extract, producing a precipitation which is manifested by cloudiness at the point of contact of the two fluids. This method of diagnosing glanders has recently been recommended by Pfeiler \(^1\) in Germany and by Konew \(^2\) in Russia, but it has not been applied extensively in practice. This is probably due to the fact that the reading of the reaction is in some cases difficult, due to the indistinct ring which occasionally is formed at the line of contact between the precipitant and the serum.

In 1909 Schütz and Schubert \(^3\) published the results of their important work on the application of the method of complement fixation for the diagnosis of glanders. And since their experiments were followed by splendid results, exceeding by far the results obtained from either the mallein or the agglutination test, they recommended that this method of diagnosis in combination with the agglutination test be taken as the official test in Germany. This method, overcoming as it does the disadvantages of the mallein and agglutination tests, constitutes without doubt the most reliable method for the diagnosis of glanders which we have at our command at the present time. The complement-fixation test is, in fact, the most definite method known for determining specific infections and is as nearly perfect as a biological test can be. It has only recently been introduced in veterinary science and the publications concerning it are at present limited exclusively to foreign periodicals. The principle of this test is presented in the phenomenon of hemolysis, which was first discovered and studied by Bordet and Gengou, and extended by Ehrlich, Morgenroth, and Sachs.

HEMOLYSIS.

It is a well-known fact that if red blood corpuscles of one animal are introduced into another of a different species the blood of the latter acquires the power to dissolve the blood corpuscles of the

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former when mixed with them in a reagent glass. This reaction is termed hemolysis, which means the dissolution of blood corpuscles, thereby setting the hemoglobin free in the medium in which the corpuscles are suspended.

To illustrate this phenomenon, if a rabbit is injected intraperitoneally, intravenously, or subcutaneously with washed red blood corpuscles of a sheep, the blood of the rabbit will develop antibodies which possess a dissolving action for the sheep blood corpuscles; that is, the rabbit blood will contain specific hemolysins.

The acquired hemolytic property of the blood depends on two substances. One of these is present in the blood of every animal, and is known as the complement. It is thermo-labile, which means that it is rendered inactive after the blood or serum has been heated to 56° C. for half an hour. The other body, which is formed as a result of the injection of blood corpuscles, is thermo-stabile; that is, it resists heating even higher than 56° C., and is known as immune body, fixative, sensitizer, or hemolytic amboceptor. The name amboceptor is derived from the fact that it has an affinity on the one hand for the blood corpuscles of the species of animal with which the animal has been injected, and on the other for the complement, this union taking place only after the first-mentioned affinity has been satisfied.

These two substances, together with the corpuscles to be dissolved, comprise the hemolytic system, and their combination leads to hemolysis. (See Pl. I, A.) This means that an opaque suspension of blood corpuscles is rendered semitransparent or "laked." The hemolysis, strictly speaking, does not represent a complete solution, but only an action of the hemolysin on the stroma of the erythrocytes, which permits the escape of the hemoglobin of the red blood corpuscles.

The injection of blood corpuscles of one animal into another of a different species gives rise to the development of antibodies which confer upon the blood serum the hemolytic action. This phenomenon is somewhat similar to the production of receptors in the formation of antitoxins which are thrown off, but these receptors alone are not able to dissolve the red blood corpuscles, requiring also the presence of a ferment. This ferment, however, is a constant constituent of the blood and is known as the complement.

That both of these substances are constantly present in the hemolytic serum can be demonstrated in the following manner: If the hemolytic serum is heated to 56° C. for half an hour, thereby destroying the complement, this serum will no longer possess a hemolytic action; that is, it will no longer dissolve red blood corpuscles. This heating of the serum is known as inactivation. On the other hand, if to such inactivated serum there be added fresh untreated
Diagrammatic Representation of Complement Fixation.

A. Hemolytic system.
B. Bacteriolytic system.
C. Negative reaction with normal horse serum.
D. Positive reaction with glandered horse serum.