attempted to show. If the plan has helped the unfortunates in some jurisdictions, and never been the subject of just attack, it is due the unfortunates of other jurisdictions that they be given the opportunities and privileges that it offers. Apart and aside from the professional viewpoint of the physician, not only do the insane command our sympathy, but as our fellowmen they present to us an obligation which we must and should assume. As members of a great race, it is incumbent on the strong to assist the weak, and this must be done not only because of the personal sympathy which applies toward some, but because the conservation of the body social makes necessary such assistance.

CONTRIBUTION TO THE DIAGNOSIS OF MALTA FEVER *

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On the island of Malta there has been endemic for an indefinite period a febrile disease of the inhabitants, termed "Malta fever," and also known as "Rock," "Mediterranean" or "undulant fever." It is a specific infectious disease caused by the *Micrococcus melitensis*, which was discovered by Bruce in 1887. This infection was so exceedingly prevalent among the British soldiers and sailors stationed on the island that, in 1904, a commission was appointed by the British government, under the supervision of an advisory committee of the British Royal Society, to investigate the possible sources of infection and advise methods for its control. The commission has since investigated the disease in all of its phases in a most exhaustive manner and, as early as in 1905, was led to consider that the milk from the native goats was an important, if not the main, factor in the dissemination of Malta fever among human beings. It was found not only that the goats were susceptible to artificial infection, but also that about 50 per cent. of them acquired the disease naturally and that the organisms were eliminated in their milk and urine. It was then decided to investigate the milk of such infected, though apparently healthy goats, with the result that in Malta 10 per cent. of the goats were found to be eliminating the specific coccus in the milk, and that this milk when fed to monkeys even for a day was able to produce typical attacks of Malta fever which ran a course parallel to that of the disease in man. The only logical conclusion which could be formulated from this work was that the Maltese goats were carriers of the virus of Malta fever and were one of the principal means of transmitting the disease to human beings through the ingestion of their milk. All the available evidence points to contaminated food as the vehicle by which these goats become infected with the virus of Malta fever. Furthermore, it has been shown that the urine of infected goats and of ambulatory cases in man at times contains the *Micrococcus melitensis*, so that goats feeding on material that has come in contact with such urine (which is not at all infrequent by the usual method of handling these animals) are readily infected. Thus the frequency and the method of infection in goats are quite readily explained.

By the recent investigations of E. R. Gentry and T. L. Ferenbaugh,[1] the existence of Malta fever in Texas has been definitely established. Its occurrence in human beings has been demonstrated bacteriologically among certain families in the goat-raising sections of Texas, and since goats have been incriminated as carriers of the infection to man, the serums of a number of these animals in the infected localities were subjected to the agglutination test with positive results. The isolation of the *Micrococcus melitensis* from these goats has thus far not been successful, and the agglutination test was therefore relied on for the diagnosis of Malta fever in these animals.

The existence of this disease in Texas is of great moment, inasmuch as the general opinion has prevailed that the United States is free of Malta fever, and that the only occasions when the disease has appeared in this country were isolated instances, occurring through importation. In 1905, the Department of Agriculture imported a number of Maltese goats from the island of Malta for the purpose of obtaining foundation stock for a milch goat industry in this country. Certain facts in the history of their voyage to this country, which came to our attention about this time, suggested that some of the imported goats were infected with Malta fever virus. The presence of this infection among the Maltese goats was subsequently demonstrated by Mohler and Hart,[2] both bacteriologically and by a series of agglutination tests conducted in this laboratory. As a result of these tests it was deemed advisable to destroy not only the imported goats, which during these investigations were kept in quarantine, but also their offspring, as it was found that even the kids gave positive reactions to the agglutination test in many instances.

The numerous investigations which have been carried out relative to the methods of diagnosis of Malta fever appear to be quite uniform as to the reliability of the agglutination test for the diagnosis of this malady. Nevertheless, the results of some of the investigators prove that the agglutination value is by no means constant in the suspected patients, and it has further been established that human beings, as well as goats, although apparently recovered from the disease, give an agglutination value of the serum indicative of Malta fever even years after the infection. It has also been found that the agglutination value in the presence of this affection is by no means constant, and occasionally it may even fail to indicate the presence of the infection.

Furthermore, there appears to be a diverse opinion among the investigators of Malta fever relative to the height of the agglutination value which should be considered as indicative of an infection. Thus, by some writers, an agglutination of 1 to 10 is considered as sufficient proof of the presence of the disease; others claim 1 to 20, while still others require 1 to 30 as the lowest value for a positive diagnosis. During the quarantine of the goats imported from Malta, referred to above, the Bureau of Animal Industry conducted a number of experiments in order to determine the agglutination value of normal goat-serum for the virus of Malta fever. It was found repeatedly that such serums from healthy goats, born and raised at the experiment station, gave an agglutination value of 1 to 40. Hence, in the tests of the quarantined Maltese goats, an agglu-

* From the Pathological Division, U. S. Bureau of Animal Industry.

1. Gentry, E. R., and Ferenbaugh, T. L.: THE JOURNAL A. M. A., Aug. 26, 1911, p. 730; Sept. 9, 1911, p. 889; Sept. 23, 1911, p. 1045; Sept. 30, 1911, p. 1127.
2. Mohler and Hart: Twenty-Fifth Ann. Rep., Bur. Anim. Ind., 1908, p. 270.

tination value of 1 to 70 within a time limit of one and one-half hours was required for a positive diagnosis. Kolle and Hetsch[3] state that the agglutination test is indicative of Malta fever only when its value represents a titer of at least 1 to 100.

In consideration of this difference of opinion regarding the agglutination value of serums from suspected and infected patients, it was deemed advisable to carry out preliminary experiments to determine whether the complement fixation test could be utilized for the diagnosis of Malta fever. Serums of a large number of goats from the experimental farm at Beltsville, Maryland, were obtained and tested both with the agglutination and complement fixation tests. In the meantime, two goats were subcutaneously injected with 0.5 c.c. of a washed agar culture of the *Micrococcus melitensis*.

The agglutination test was applied both by the microscopic and macroscopic methods, but the best results were obtained from the macroscopic method carried out by a procedure similar to that practiced for the diagnosis of glanders. For this purpose the test fluid is prepared from a four-day-old glycerin agar culture of the *Micrococcus melitensis* by heating the culture at 60 C. for one and one-half hours, which is then washed with phenolized salt solution, filtered and diluted to a desired density. This is established by comparative tests with serums of known agglutination values determined by microscopic agglutination. Once the titer of the agglutination fluid has been established by these comparative tests, the proper density of a newly prepared fluid is readily obtained by pouring a sample of each, respectively, into two beakers of equal size up to the height of about 2 cm. The density of the old and new fluids may then be compared by placing the beakers on print, preferably engraved print, and observing the legibility of the print through the fluid. The dilution of the new fluid is continued until the density becomes similar to that of the old fluid. The numerous comparative tests which have been undertaken with the microscopic and macroscopic agglutination tests show that a reliable uniformity has always been obtained. The technic of the macroscopic agglutination test is carried out as follows:

The suspected serum is diluted with phenolized salt solution in the proportion of 1 to 4 (0.5 c.c. serum to 1.5 c.c. phenolized salt solution). This constitutes the basic dilution, and from this all the dilutions of serums are made in test-tubes in such a way that with the added 2 c.c. of test-fluid (bacilli emulsion) the desired dilutions are obtained. Thus, 2 c.c. of test-fluid added to 0.2 c.c. of the basic dilution would give a serum value of 1 to 40. Any number of different dilutions can be prepared in this manner. The rack containing the test-tubes is then placed in the incubator for one-half hour, after which the tubes are removed and centrifugalized for ten minutes at 1,600 revolutions per minute. The test-tubes are then returned to the rack without further incubation and the results read after one to two hours. The sharply circumscribed lentil-shaped sediment in the center of the bottom of the test-tube with cloudiness of the upper portion of the fluid indicates the failure to agglutinate, while an irregular, veil-like clumping of the sediment over the bottom of the tube with a clearing of the upper part of the fluid is indicative of agglutination. The racks are so constructed that they have a conical opening on the lower shelf into which the bottom of the test-tubes fit, and through these openings the reaction is plainly visible, especially when placed on a dark background.[4]

This method of agglutination would come especially into consideration if large numbers of serums were to be tested, and particularly if it were desired to examine the blood of a great number of goats in certain localities for the control or possible eradication of the disease.

The serums of healthy goats from the experimental farm and that of twenty suspected goats from Texas, as well as the serums from the artificially infected animals, were carefully tested by the microscopic agglutination and also by the method just described. The results showed practically no variation in these two methods, and in consideration of the simplicity of the macroscopic method as described, it would appear that it should be given the preference over the microscopic method, especially in cases in which a large number of individuals are to be tested.

In the meantime, experiments have been conducted with the complement fixation test for the diagnosis of Malta fever with serums from normal as well as from infected animals. The hemolytic system consisted of sensitized rabbit-serum, serum from a guinea-pig, and a 5 per cent. suspension of washed sheep-corpuscles. An antigen was prepared from four-day-old glycerin agar cultures, and after being heated for two hours at 60 C., it was agitated for four days in a shaking-machine.

The extract was then placed in centrifuge tubes and centrifugalized for two hours at a speed of about 2,500 revolutions per minute. The clear fluid was drawn off and preserved with 10 per cent. of a 5 per cent. phenol (carbolic acid) solution. A titration of the antigen was then undertaken in order to establish the smallest quantity which would no longer prevent hemolysis. A dilution of 1 to 50 was found to be the proportion of antigen to be used in the tests.

The goat-serum to be tested was inactivated at 56 C. for thirty minutes. The complement was titered in each instance in order to establish the necessary smallest quantity required to produce complete hemolysis. Of the serums to be examined, 0.2 c.c. and 0.1 c.c., respectively, were used, and the customary control-tubes were always included in the test. Thus, in routine testing, four tubes were taken for the test proper, the first pair receiving 0.1 c.c. of serum, and the second pair 0.2 c.c. The second and fourth tubes served as controls for the serum, in order to establish that the serum without the antigen would not produce a fixation of complement.

The serums from the goats at the experimental farm failed to give a fixation in any instance, although, in several cases, it was observed that the hemolysis resulted slowly, and sometimes a very small quantity of blood corpuscles settled to the bottom of the tube, but in all these instances the reaction was the same in all four tubes; namely, in the tubes for the test proper, as well as in the control-tubes. Of the serums examined from the suspected cases sent from Texas, four gave positive complement fixation, and in all these instances the fixation was complete, even in the tube in which only 0.1 c.c. of serum had been used, while sixteen other examinations of serums from the same source gave negative results.

3. Kolle and Hetsch: Die experimentelle Bakteriologie und die Infektionskrankheiten mit besonderer Berücksichtigung der Immunitätslehre, 1911

4. A more detailed description of the technic of this method as applied to glanders, together with drawings showing the above-described appearances of the sediment in both positive and negative reactions, will be found in Circular No. 191 of the Bureau of Animal Industry, to be issued shortly.

The agglutination test of the serums from the cases in which a fixation of the complement was obtained showed a value in one instance of 1 to 50, in two, 1 to 15, and in the fourth it failed to agglutinate even at 1 to 10. The remaining sixteen cases failed to agglutinate in the proportion of 1 to 10.

The serums of the goats which had been artificially infected were drawn every day from the time of infection and examined both by the agglutination and the complement fixation tests. On the fifth day an agglutination of 1 to 40 was obtained, which on the subsequent days was marked at 1 to 500 and reached a height of 1 to 2,000, continuing to give an agglutination value of over 500 for the two months after infection, during which period the animals have been under constant observation. A partial complement fixation was first obtained on the seventh and ninth days, respectively, and from that time on a perfect fixation was obtained in all instances. In these tests even smaller quantities than 0.1 c.c. gave a fixation; thus, in establishing what would be the smallest quantity of serum which would give a complete fixation, it was found that on the twenty-second day after the infection in one goat, 0.04 c.c. of serum gave a complete fixation.

From the results of these investigations it appears that the complement fixation test can be utilized for the diagnosis of Malta fever, and in consideration of the fact that the agglutination test is not always reliable for such purposes, the complement fixation would be of great advantage as an adjunct in the diagnosis of this malady.

Additional samples of serums will be obtained from goats in those localities of Texas where the disease has been known to exist in human beings, and a further opportunity will therefore be presented to determine whether the very favorable results thus far obtained with the complement fixation test will be confirmed.

AN EPIDEMIC OF SEPTIC SORE THROAT IN BALTIMORE AND ITS RELATION TO A MILK-SUPPLY

A PRELIMINARY REPORT *

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During the month of February, 1912, it became evident to a number of practitioners of Baltimore that a throat affection presenting an unusual clinical picture was epidemic in the city. Cases apparently similar had been observed during the early winter months, but the sudden appearance of hundreds within a few weeks could not fail to attract attention.

Though children were chiefly affected, adults were not spared. The malady was characterized by a sudden onset, high and irregular fever, inflammation of the fauces of varying degree, marked enlargement of the cervical glands and a course much longer and much more severe than the usual type of tonsillitis. At first discussed indefinitely as "grip" it was not until the close of the month, when at least three fatalities were reported, that it became apparent that we were dealing with a "new and very terrible disease."[1]

At this point I was impressed by the uniform results of the cultures taken from patients' throats and of the examination of smears from the inflammatory exudates complicating the disorder. Thus, the discharge after paracentesis of the ear-drum in the case of a child 5 years old showed within leukocytes numerous pairs of Gram positive cocci, while a culture from the secretion of the opposite ear discovered the cocci in chains still retaining their diplococcic arrangement. Since then, this streptococcus has been obtained whenever it has been sought, often in pure culture. In this way, it has been recovered not only from the fauces and otitic exudate, but from the suppurating lymph-nodes, from the peritoneum during laparotomy and from the blood of the femoral vein at autopsy.

The organism is being studied in Dr. Charles Simon's laboratory by Dr. Simon and his associate, Dr. G. Howard White, and a full report of their findings will be published in due course of time. At present it may be said that in the direct smears it often appears as an end-to-end diplococcus, intracellular in some specimens. Each pair seems surrounded by a halo, but with the capsule stain of Welch, Burger and His, no envelope can be demonstrated. On agar it grows in dew-like droplets. It coagulates and acidifies milk and in this liquid it develops in long chains with end-to-end arrangement and sometimes in tetrad form, division having taken place in two planes. The organisms are not dissolved by bile salts. Blood-agar plates show a hemolytic zone around individual colonies. In broth the cocci cause uniform turbidity. They do not ferment inulin.

The organism was demonstrated to a number of colleagues Friday evening, March 15, 1912, and the following day the report of Davis and Rosenow appeared,[2] describing a "peculiar streptococcus" obtained in the course of an epidemic of sore throat prevailing this winter in Chicago. It is obviously the same organism and the same infectious disease.

A significant feature of the Baltimore epidemic is the large number of cases of severe type appearing suddenly within a few weeks in the month of February. Indeed, many of the cases appeared within a few days in widely separated portions of the city. Struck by this fact I ventured to assume that one of the more general carriers of epidemic disease, such as milk, might be at fault. Thereupon, inquiry was instituted in twenty-five households in which the type of sore throat under discussion prevailed with the noteworthy result that in every instance it transpired that the milk-supply was derived from the same dairy. These returns were submitted at the meeting of March 15, and, making allowance for statistical error, confirmatory evidence as to the coincidence of disease and dairy was added and appeared so convincing that it was deemed advisable to caution the public to boil all milk. Accordingly, through the medium of the newspapers the advice to boil milk was repeatedly promulgated during the week following March 16. Immediately, too, the dairy in question raised its pasteurization temperature to 160 F. and during the ensuing week changed its method from "flash" system to the "holding" device. In other words, instead of maintaining the milk for three minutes at a temperature of 145 it increased the duration of pasteurization to twenty minutes. It is of interest to note that at present, a week later, the new cases, which are apparently neither so severe nor so numerous, do not follow the milk-supply, but occur in prosodemic fashion; namely, they appear to be transmitted from individual to individual through various channels of communica-

* From the Medical Clinic of the Johns Hopkins Hospital.

1. Sedgwick, William T.: Boston Med. and Surg. Jour., Dec. 14, 1911, p. 910.

2. Davis and Rosenow: THE JOURNAL A. M. A., March 16, 1912, p. 773.