FURTHER STUDIES ON THE OPHTHALMO-TUBERCULIN REACTION IN CATTLE.¹

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PLATE XLVIII.

Report on Cattle Used in First Series of Experiments (7).—In our previous experiments the cattle used were divided into three groups. Class A contained five animals which appeared to be in the best of condition with the exception of No. 2A, which was beginning to show the clinical signs of tuberculosis. These animals were tested with tuberculin by the ordinary subcutaneous method eight months before the ophthalmo-tuberculin test was applied. It was stated that all these animals gave a most pronounced ophthalmo-reaction when first tested. Class B, containing five animals, was secured from a herd of thirty-four cattle, twenty-seven of which reacted to the usual subcutaneous tuberculin test. This test was applied only one month previous to the application of the ophthalmic test. In all these animals, as stated in our previous paper, an ophthalmo-reaction was observed, but in a very mild form. We made the tentative statement that the recent subcutaneous tuberculin test might possibly have inhibited the subsequent ocular reactions in Class B, but also stated that since the animals showed themselves clinically to be in the advanced stages of tuberculosis the generalization of the tubercular toxins in the body might possibly inhibit the ophthalmo-tuberculin reaction as it does occasionally the usual subcutaneous test and, consequently, produce only a slight reaction. From our later experiments we are inclined to believe that the ordinary subcutaneous tuberculin does inhibit the eye reaction in a cer-

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tain percentage of cases (50 per cent.) which are instilled the first time. We are inclined to believe also that the more generalized or advanced the tubercular process is, the longer the eye will be in a state of anaphalaxis to the tuberculin. It may be also stated here that it is important to differentiate between the natural and induced hypersusceptibility of the conjunctiva. While the ocular reaction may be readily induced several times, it will not be as pronounced unless the conjunctiva has become artificially hypersensitized by spaced instillations. Further experiments were made on Class A and will be referred to later. Necropsies were held on Class B soon after our first experiments were made. (See Necropsy Report, Class B.) It will be seen on referring to the accompanying report of the necropsies that all the animals were affected with advanced tuberculosis. In our previous report we called attention to the finding of *Bacillus tuberculosi*s in the faeces of No. 2B. We were able to demonstrate again the bacteria in the edges of the typical tubercular ulcers found in the large intestines of this animal.

Class C, containing twenty animals, was used as control. With the exception of No. 2C, all had been repeatedly tested with tuberculin by the usual method, the last time being six months before the ophthalmo-tuberculin test was applied. It was stated that No. 2C gave a very slight reaction to the ocular test. On being subsequently tested with tuberculin by the usual subcutaneous method No. 2C reacted, and on being necropsied showed tubercular lesions. (See Necropsy Report.) However, two other cattle among these controls, Nos. 8C and 10C, which did not react to the first ophthalmic test, on being subsequently tested with tuberculin by the usual subcutaneous method reacted to this test, as well as later to the ophthalmic test. On necropsy quite extensive tubercular lesions were observed. Undoubtedly these cattle were tubercular when the first ophthalmic test was applied. We can offer no explanation except that the conjunctiva of these cattle was not sufficiently anaphalactic or was antianaphalactic to tuberculin for some unknown reason, or what is more probable, that in these beginning tests our inaccuracy of observation was responsible for not seeing a slight reaction which possibly occurred. Both antianaphalaxis or immunity and very slight reactions of the conjunctiva have been observed during our recent experiments.
Cattle Used in Recent Experiments.—The cattle in Class A and Class C, and in addition a new group of tubercular cattle which we have designated as Class D, have been used. For the purpose of control we have used another group of twenty-six non-reacting cattle (temperature test), Class E. Classes D and E were last tested with tuberculin by the usual subcutaneous method in January, 1908.

Class D—Tubercular.
1. D Grade Shorthorn. 9. D Grade Shorthorn.
8. D Holstein (heifer).

Class E—Non-Tubercular (Control).
2. E Red Polled. 15. E Guernsey.
5. E Grade Shorthorn. 18. E Grade Shorthorn.
7. E Devon. 20. E Polled Angus.
10. E Grade Red Polled. 23. E Shorthorn.

Description of Experiments.—We have used in our recent investigations, as we did in our first work, the tuberculin as supplied by the Bureau of Animal Industry, United States Department of Agriculture. This tuberculin is quite stable and well suited for comparative tests. The glycerine present aids in the absorption of the tuberculous material. The tuberculin was used full strength in the majority of cases. We were able, however, to elicit a more pronounced reaction in some few cases with this tuberculin concentrated to one tenth when the first instillation only gave a slight reaction. The glycerine or preservative agents were not responsible for the reaction. These cattle showed on necropsy a few small tubercular lesions.
In our first work we instilled 0.25 cubic centimeter into the conjunctival sac. We have since found that a smaller amount of tuberculin serves equally well. It has been our practice in these later investigations to use about one tenth of a cubic centimeter. Using this amount there is less chance of expulsion, and by slightly manipulating the lids the tuberculin becomes well distributed before lacrimation can become excessive. It was found to be advantageous to draw the lower lid down and instill the tuberculin on the inferior portion of the bulbar conjunctiva. Before making the instillation of tuberculin into the eye its condition was carefully compared with that of the opposite eye.

Reaction. — We have found that the ophthalmo-reaction varies greatly in different cattle. The reaction described in our previous communication (7) is typical of the rather severe form (see figures). Milder reactions often occur. Calmette (4) describes the reaction in man as a slight hyperemia and swelling of the caruncle and conjunctiva, especially in region of the inner canthus. The reactions have been variously separated according to different writers. For example, Aubaret and Magne (2) and Aubaret and Laford (1) have separated the reactions into four divisions as follows: (a) mild form; (b) moderately severe form; (c) intense form; and (d) very intense form. In cattle we have divided the reactions observed into three divisions: (a) slight; (b) marked; (c) very marked. In recording the tests we have used a modification of the scheme suggested by Comby (5) and Baldwin (3):

(N) Negative = No difference observed in eyes.
(D) Doubtful = Slight redness of conjunctiva.
+ = Slight engorgement of palpebral conjunctiva with exudation.
++ = Marked engorgement of whole conjunctiva, photophobia, lacrimation and exudation.
+++ = Very marked reaction.

Reactions of varying intensity were observed in all tubercular animals used in this group of experiments. Since our first work on this subject we have been able to distinguish in some cases a very mild form of the reaction which is characterized only by an engorgement of ocular vessels and no exudation (+). We have found also that the reaction reaches a maximum in from ten to twelve hours after the tuberculin is instilled.
The tuberculin reaction, no matter how the tuberculin may be administered (ophthalmic, cutaneous, subdivided as cuti- and dermo-reaction, and subcutaneous) is due to the increased sensitiveness of the tissues of the tuberculous individual over the normal or non-tubercular. The hypersensitiveness has been termed anaphylaxis.

As pointed out previously, it has been known for some time that local reactions can be produced in infected individuals when the toxin from the organism causing the infection is introduced into...
the body. Von Pirquet (9) exhaustively studied the phenomenon in the case of revaccination for smallpox and called particular attention to its significance. In the case of tuberculin injections it has frequently been noted that in tubercular individuals there was a marked hyperaemia and swelling at the focus of inoculation even where the injection was made with the strictest aseptic precautions. (Epstein [1891], Escherich, Spengler, Hamburger.)

The condition of hypersensitivity which is present in tubercular individuals is an indication of an incomplete resistance on the part of the tissue cells of the body. This resistance does not produce complete immunity. When tuberculin is injected into an infected individual, at the focus of the infection a specific resistance is manifested and is characterized by hyperaemia and local leucocytosis.

Depending upon the extent of the tubercular process in the body and the amount of absorption of tubercular products, all the body cells become hypersensitive in varying degrees. The conjunctiva is a very delicate membrane, and is very easily affected by irritants. In other words the cells which compose it are normally extremely hypersensitive. In a tubercular individual the conjunctival cells are particularly sensitive to tuberculin or tuberculous material. We have here, as in all cells of the body, a condition of hypersensitivity and resistance combined. Von Pirquet has applied the term "allergie" to the condition of acquired immunity associated with anaphylaxis. As a result when tuberculin is instilled into the eye there is an attempt on the part of the conjunctiva to ward off the tubercular material. Consequently, there is a marked local hyperaemia and leucocytosis, with exudation in some cases. The intensity of the reaction will, as before stated, depend upon the extent of the tubercular processes in the body. In our investigations we determined that the local reaction is directly proportional to the extent of the tubercular lesions (compare Necropsy Report and Reaction Chart).

Undoubtedly the proteids derived from the tubercle bacillus are responsible for the reaction. Tuberculin is probably related to the nucleo-proteids, as is shown by the fact that it is destroyed by the tryptic enzyme, and by the slowness with which it is acted upon by pepsin. As would be expected, the washed proteid material from
Studies on Ophthalmo-Tuberculin Reaction in Cattle.

Ground-up bacteria of tuberculosis produces the local ocular reaction. It has been suggested that perhaps the beef proteids in the glycerine bouillon on which the bacteria were grown might influence the reaction. Our investigation demonstrates satisfactorily that this is not the case.

The instillation of full-strength tuberculin into the eye produced no serious results in the cattle used in our experiments.

Statement of Results of Last Series of Experiments; Ophthalmic Test.—It will be noted that the ophthalmic reaction occurred in varying degrees in all the tubercular cattle in Class D (see Reaction Chart). The reaction was most marked in those animals which showed on necropsy the most tuberculosis. This is, of course, different from the temperature findings in the usual subcutaneous tuberculin test which is usually most marked in those animals showing the least tuberculosis. Furthermore, it will be noted that in the majority of animals in Class D and also in Class A, which had been used in our first work, the reaction became more intense with subsequent instillations of tuberculin given at intervals of over two weeks, indicating a marked hypersensitiveness of the cells of the conjunctiva to tuberculin. In one case (No. 3D), when the four instillations were made at intervals of three days, the ophthalmic reaction did not appear after the last two instillations, indicating a local immunity (antianaphylaxis). We were unable to try the above experiment on any other cases. This animal showed on necropsy tuberculosis of both lungs and of the bronchial and mediastinal glands.

The Necropsy Report shows tuberculosis of the mediastinal lymph glands to be the most frequent locus of lesion.

We have found in a certain percentage of tuberculous animals, when a subcutaneous injection of two cubic centimeters of tuberculin is given at the same time that the eye is instilled, that the ophthalmic reaction is much more severe. In our first work we were inclined to believe that a recent tuberculin injection inhibited slightly the ocular reaction. We find this to be true in only about fifty per cent. of the cases tested.

We have never observed in cattle the delayed reaction which has been reported in man by Montagon (8) and others. We did not
find the opposite eye to the one instilled hypersensitive, as has been reported on human subjects.

In Class E (controls), which were last tested with tuberculin by the usual method eight weeks before, one animal, No. 4E, twice showed a typical ophthalmic reaction (+ +). This animal showed no clinical signs of tuberculosis; it had been repeatedly tested by the usual subcutaneous method, and gave no reaction. This animal had previously received two instillations of tuberculin in the eye, and had failed to react. We believe that this reaction was a case of artificially induced local anaphylaxis. We were able in one case to induce purposely this local anaphylaxis to tuberculin by spaced instillations of tuberculin.

Result of Ophthalmo-tuberculin Test on Calves.—The dams (Nos. 2A, 4A and 5A) of these calves had repeatedly reacted to the usual tuberculin test and several times to the ophthalmo-tuberculin test. Necropsy showed the animals to have generalized tuberculosis (see Necropsy Report). The ophthalmic test was tried on these calves to determine if any conjunctival hypersensitiveness had been acquired in utero. The instillation of tuberculin in the eye failed to elicit any reaction whatever. Two subsequent instillations likewise produced no effect. The calves evidently were not in a state of anaphylaxis to tuberculin. It has been noted in guinea-pigs that the sensitizing substances can be transmitted to the foetus in utero, and after birth the offspring give a reaction. It is possible that the calves were antianaphylactic or partially immune to tuberculin and tuberculosis. Subsequent experiments will be made with these animals.

It may be here stated in passing that No. 14D (shorthorn steer, one year old) was also the offspring of No. 2A. This steer on the first test made eight months after birth showed a slight reaction (+). Later the animal gave a pronounced reaction (+ ++). Necropsy on this animal showed tubercular lesions in both lungs, in the bronchial, mediastinal and inguinal glands, and in the intestines. It should also be noted that No. 2A had generalized tuberculosis with a tubercular abscess in the udder. No. 14D suckled No. 2A for four months and it was quite logical to expect intestinal tuberculosis in this animal.
Reaction to Ophthalmic, Cutaneous and Subcutaneous Tests Combined.—We were able to apply this combined test in only two cases. The eye was instilled and the subcutaneous and cutaneous tests were made as rapidly as possible in the foregoing order. About two minutes were consumed in applying these tests. In these animals the temperature curve was characteristic, but not high. The cutaneous reaction appeared in one of the animals, which was one year old, in about twenty-four hours and persisted for forty-eight hours. The reaction was characterized by a hyperemia of the skin of the flank and later by an edematous swelling. The ophthalmic reaction appeared in about eight hours and disappeared in twenty-four hours. There was not much fibrinous exudate in these cases. All three of these reactions were much lowered in their intensity.

Ophthalmo-tuberculin Test on Vaccinated Cattle.—Seven cattle were used in these experiments, six heifers and a young bull, of various breeds. These cattle had received intravenous injections of one, one and a half, and two cubic centimeters respectively of an emulsion of human tubercle bacilli in physiological sodium chloride solution. Guinea-pigs inoculated with the emulsion intraperitoneally died in four weeks. A second series of guinea-pigs died in five weeks. The cattle received three intravenous injections, six weeks apart. The method is that used by von Behring in Germany, and modified by Pearson in this country. When the ophthalmic test was applied to these vaccinated cattle it was noted that three gave on the first test a conclusive reaction which was not very pronounced (+) and four failed to react. On the second test after an interval of six weeks the three reacting cattle all showed an ophthalmic reaction of very low intensity (D). One heifer showed slightly more conjunctivitis than the others. Four animals failed to react. On the third test, six weeks later than the second, no reactions were observed on any of the cattle. When again tested in eight weeks, two of the three reacting cattle responded to the ophthalmic test. It would seem that at the time of the first test, as a result of vaccination, the three animals were in a state of anaphylaxis and as a result of the instillation of the tuberculin into the eye there was developed a condition of antianaphylaxis or local immunity.
are inclined to believe that this condition of partially acquired immunity as a result of the ophthalmic test is entirely local, for when these three cattle were subsequently tested with tuberculin, by the usual subcutaneous method they gave temperature reactions. When first tested by the usual subcutaneous test these three cattle gave typical tuberculin temperature reactions. When tested again in six weeks, two showed an atypical reaction and one failed to react. Four animals failed to react to the usual subcutaneous test. Pearson explains this fact by stating that the animals subsequent to vaccination are in a condition of hypersusceptibility to tuberculin (anaphylactic). This hypersusceptibility is present for varying lengths of time. It requires, therefore, several injections of tuberculin in those animals which are still hypersensitive, to use up or combine with this hypersensitive substance (anaphylactin).

We are not prepared to state whether all these vaccinated animals are tubercular or entirely immune to tuberculosis. We will investigate this matter by necropsy as soon as possible.

Cutaneous Reaction.—Our experiments with this reaction alone have been quite limited. Only five animals were used. The skin on the flank was shaved and the tuberculin rubbed in. In from twenty-four to thirty-six hours a slight hyperaemia followed by edematous swelling developed at the locus of inoculation in two cases. Subcutaneous injection of tuberculin by the usual method into the other three animals preceding another cutaneous test failed to elicit any cutaneous reaction. A typical temperature reaction was given in these cases. The cutaneous reaction is very difficult to observe on dark-skinned cattle.

We are, obviously, not prepared to state our views in regard to the efficiency of the cutaneous test on cattle at the present time. A distinction has been made by some writers between two methods of applying this test in man which are not applicable to animals. The term (cuti-reaction) is applied to the reaction following the rubbing of tuberculin on the shaved area of the skin and the term (demo-reaction) for the reaction which follows the application of tuberculin after the scarification of the skin. Whenever the thick skin of an animal like an ox is shaved there is, in a large majority of cases, some slight scarification, perhaps not easily visible. The
test, therefore, is made in the majority of cases after scarification. Further study will be given this test.

CONCLUSIONS AND SUMMARY.

Our conclusions differ in a few points from those of our first work on this subject. We are able to sum up our observations in this series of experiments as follows:

1. The ophthalmo-tuberculin test is of limited value in the diagnosis of tuberculosis in cattle. In some cases the reaction is very slight (hyperaemia). In others more pronounced congestion with profuse exudates are noted. Accuracy of observation is important. We are inclined to rely primarily on the results of the first instillation of tuberculin. Second instillations in a few instances elicit reaction in non-tubercular animals.

2. In the majority of animals tested the reaction increased in its intensity with each subsequent instillation of tuberculin. This fact indicates the development of a local hypersusceptibility or anaphylaxis associated with a partial immunity; von Pirquet calls this condition "allergie" (9).

3. It is possible in some cases to create a condition of "allergie" in healthy cattle, when spaced instillations of tuberculin are made. It is evident, therefore, that the result of the first instillation of tuberculin should be made the only basis of diagnosis. Rosenau and Anderson (11) have recently called attention to this point in regard to the human subject.

4. When repeated instillations of tuberculin are made on the conjunctiva at short intervals (twenty-four hours, etc.) a local immunity results (No. 3D et al.). If the instillations are separated two weeks or more anaphylaxis results.

5. We, therefore, hold that if tuberculin (0.1 cubic centimeter) is carefully instilled into the conjunctival sac and if careful comparison of the instilled eye with the opposite eye shows that a reaction of varying intensity results in from ten to twelve hours after the first instillation, a tubercular lesion is present.

6. In our first report (7) we were inclined to believe that subcutaneous tubercular injection given previous to the ocular test would slightly inhibit it. We have since become convinced that this
### Necropsy Report

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Studies on Ophthalmos-Tuberculin Reaction in Cattle.

**Necropsy Report.—Continued.**

*Class D.*

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*x = Lesions few and small.
xx = Lesions many and small.
xxx = Lesions large.
B = Tubercle bacillus demonstrated.
a = Both lungs.
o = No lesions demonstrated.
Intestine—S = small intestine; L = large intestine.

is true only to a limited extent, and that in some cases the ophthalmos-reaction is exaggerated by a subcutaneous injection of tuberculin.

7. The primary ophthalmos-tuberculin reaction is in direct proportion to the extent of the tubercular processes in the body. The more extensive the tubercular processes, the more anaphylactic the animal is. This is in direct variance with the condition in the usual subcutaneous tuberculin test. (See Necropsy Report.)
Eugene F. McCampbell and David S. White.

8. We are inclined to believe that the ophthalmo-tuberculin test will reveal tuberculosis at as early a stage as the usual subcutaneous test.

9. The ophthalmo-reaction is of no value in determining whether vaccinated cattle are actively tubercular or not, or in demonstrating any hypersusceptibility in the offspring of tubercular cattle.

10. The cutaneous test from our brief series of experiments does not seem to be as accurate as the ophthalmic test. This conclusion has been reached by several investigators.

BIBLIOGRAPHY.

2. Aubaret and Magne, Jour. de méd. de Bordeaux, 1907, xxxvii, 535.
5. Comby, Presse médicale, 1907, xv, 596.
8. Montagon, Province médicale, 1908, xix, 27.