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Original Articles.

THE APPARENT IMMUNIZING VALUE OF ATTENUATED TUBERCLE BACILLI.*

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The recent attention which has been drawn to the difference in virulence of tubercle bacilli from various animals, and the publication by Behring of the production of immunity to tuberculosis in cattle by the use of tubercle bacilli of low virulence, have suggested that if perhaps a little more attention had been given to some work published in December, 1894, there would have been less tendency in some quarters, at any rate, to assume an absolute difference between tubercle bacilli obtained from men and those obtained from cattle. The published works of the writer and Dorset and Schroeder, Ravenel, Nocard and that of others, have shown so conclusively that it is possible to obtain tubercle bacilli, sometimes from the sputum of adults and frequently from intestinal lesions in children, which will produce tuberculosis in cattle when the latter are inoculated subcutaneously, that it seems now almost a waste of time to discuss a question which has been so positively proved. Considering further the numerous cases of accidental infection with bovine tuberculous material, and also that monkeys are more readily infected with bovine tuberculosis than with human tuberculosis, and it is well known that monkeys are more closely allied to man than any other animal, the possibility of the intercommunicability of bovine and human tuberculosis has been well established. The fact, further, that tubercle bacilli as well as other bacilli become more or less attenuated, depending on their growth on artificial media outside of the animal body or on their cultivation within the animal body, or in other words, the fact that they are easily influenced like other plants, by changes in soil and climate, has also been well proven by ourselves and others.

Our work, published in 1894,¹ was the first to show that guinea-pigs that had received large doses of attenuated human tubercle bacilli, those bacilli that had largely lost their virulence or power of producing disease, were rendered resistant and immune to subsequent inoculation with tuberculous material of bovine origin.

* Read at the Fifty-fourth Annual Session of the American Medical Association, in the Section on Pathology and Physiology, and approved for publication by the Executive Committee: Drs. L. Hektoen, F. B. Wynn and W. S. Hall.

¹ Medical News.

Futher, in 1894-5-6-7-8, cattle and various other large and small animals were injected with frequent doses of attenuated tubercle bacilli and in some cases subsequently tested by inoculation with bovine tuberculous material. While the earlier works on the effect of large doses and the apparent immunizing value of attenuated human cultures on cattle were reported in 1896, Bulletin No. 13, Bureau of Animal Industry, and in the Proceedings of the Association of American Physicians, 1897, the results of the practical tests on some of these animals with tuberculous material have never been recorded, but may be of interest now. The practical treatment of these animals was made for me at the Experiment Station of the Bureau of Animal Industry by Dr. E. C. Schroeder.

In one of our earlier experiments begun in 1894, an old cow, 285, from a tuberculous herd, which on physical examination appeared to be clearly tuberculous, was given large injections of tuberculin, subcutaneously. The first dose used was 25 c.c. and the amounts were gradually increased so that between August, 1895, and October, 1897, when the animal was killed, it had received altogether 19,500 c.c. of tuberculin, as much as 1,500 c.c. being given at one dose. After four months' treatment with tuberculin the cow seemed to improve considerably. This apparent improvement, however, did not persist and the autopsy, when made in 1897, showed a most generalized tuberculosis, all stages of the disease being apparent. If the treatment with tuberculin had any beneficial effects the disease was so far advanced when the treatment was begun that no practical benefit could be secured.

IMMUNITY TESTS ON CATTLE.

On July 26, 1894, cow 217 received an injection in the jugular vein of 16 c.c. of a suspension of an attenuated human tubercle culture then in the 17th generation. This injection produced a large swelling at the seat of inoculation, but no other evidence of tuberculosis was noted.

On Nov. 28, 1894, this same animal received an abdominal injection of 6 c.c. of the 23d generation of attenuated culture. On Jan. 25, 1895, it received an intrathoracic injection of 6 c.c. of unfiltered culture of the 26th generation. On March 5, 1895, it received an injection into the right side of the chest of 16 c.c. of the 33d generation of a tubercle culture. Between April, 1895, and July, 1897, the animal received subcutaneous injections of attenuated tubercle culture, as much as 2,000 c.c. being given at a single injection. Altogether, therefore, it had received one intravenous, two intrathoracic, two abdominal and thirty-nine subcutaneous injections of attenuated tubercle culture. As the animal appeared to be in good health and well on Feb. 22,

1898, it received an injection into the thoracic cavity of 10 c.c. of a turbid triturate of a tuberculous gland obtained from a cow. This gland contained many virulent bacilli, as proved by inoculation tests. On Sept. 20, 1898, this cow was killed, and postmortem examination showed it to be free from disease, indicating, therefore, that neither the attenuated human culture nor the bovine material had produced tuberculosis.

Another cow, 297, the offspring of the tuberculin cow just referred to, 285, was tested with tuberculin on Nov. 19 and 20, 1894, and found free from disease. On Nov. 24, 1894, it received an injection in the abdominal cavity of 6 c.c. of the 23d generation of a tubercle culture. The temperature as taken for ten days following the injection seemed to indicate no disease. Between Jan. 25, 1895, and July 25, 1895, it received five subcutaneous injections of attenuated tubercle culture, about 250 c.c. being given altogether. In August, 1895, it received an injection of a bovine culture. Beginning again Dec. 2, 1895, it received large subcutaneous injections of attenuated tubercle culture, about 13,500 c.c. of the material being given altogether. On Feb. 22, 1898, at the same time that 217 was inoculated, it received an intrathoracic injection of some of the same tuberculous material as that given to 217. Killed on Sept. 20, 1898, when apparently in good condition, the autopsy showed a few tubercular nodules in the udder, while the superficial lymph glands, liver, kidneys and spleen were normal. There were, however, some large tubercular masses on the pleura. The tubercular lesions appeared to have come from the first inoculation with bovine material.

In April, 1896, two calves were inoculated with smaller doses of attenuated tubercle culture, subcutaneously, two animals with serum from cow 285, which in its turn, as reported, had received large doses of tuberculin, and two animals were reserved as checks. These calves subsequently tested with bovine tuberculous material did not show the same amount of resistance as did those first inoculated. One of the calves which had received preventive inoculations of the serum from cow 285 seemed to possess immunity. So far as the records of the check inoculated with bovine material show (these latter records are unfortunately incomplete) the check contracted tuberculosis from which it died in September, 1896, while the preventively inoculated animals had shown considerable resistance. The established immunity was not as good as that obtained in the first experiments. In the first tests several intravenous inoculations, as well as subcutaneous, were made. In the second set only a small number of subcutaneous inoculations were made.

These experiments on cattle considered in connection with those on guinea-pigs showed that as it was possible to vaccinate against bovine tuberculosis by means of tubercle bacilli of human origin, then we are simply dealing with tubercle bacilli of varying degrees of virulence, and not tubercle bacilli that are really different from each other. The readiness, as we have shown, with which not only human but, as may be seen from the records attached, also bovine tubercle bacilli may be attenuated, and the ease with which, according to Nocard, avian tubercle bacilli may be converted into those of a mammalian character, all go to prove the intimate relationship of bacilli obtained from various sources.

Steer 242 was drenched with bovine tuberculous material on Jan. 10, 11, 13 and 21, 1902. On February 17, 18 and 26 of the same year, tested with tuberculin, a

positive reaction was given. On October 6, about nine months after the first drenching, this animal was killed. A few small foci of a tuberculous character were found in the mediastinal and in the lymph glands at the entrance of the thorax; otherwise no evidence of tuberculosis.

On Nov. 2, 1901, bull 223 was inoculated subcutaneously with 10 c.c. of bovine culture No. 3 (Smith). On Jan. 21, 1902, and April 18, 1902, reactions after tuberculin injections were noted. On Oct. 2, 1902, when the animal was killed, its general condition was excellent. At the seat of inoculation there was a patch of induration about six inches in diameter in which were imbedded small abscesses containing a soft, cheesy material. The shoulder glands on the side of infection, both deep and superficial, were enlarged and sprinkled with necrotic foci. The mediastinal glands contained numerous necrotic foci. No other lesions of any kind were present.

This animal showed very much less tuberculosis than that found after six weeks in a calf which received, subcutaneously, injections of material from tuberculous cultures isolated from children. Compared with the record of another heifer, 284, which had been inoculated with a different virulent bovine culture (a culture that had been isolated from a spontaneous case of disease occurring at the Experiment Station), and which showed after eight weeks very generalized tuberculosis, it is very evident that the bovine germ by cultivation loses in violence and in ability to produce disease just as well as the human bacillus. It becomes attenuated. This is another strong point and emphasizes the influences of environment on the virulence of bovine as well as human bacilli.

Our earlier work and results in this connection have therefore simply been confirmed by the recent experiments of MacFadyean, Behring, Pearson and Gilliland and others.

DISCUSSION.

DR. L. HEKTOEN, Chicago—Did I understand the author to mean by attenuated bacilli those that have been cultivated artificially for some considerable time?

DR. M. DORSET, Washington, D. C.—This culture referred to is one which lost its virulence after having been cultivated for several years and one which will not kill guinea-pigs even in large doses.

DR. JOSEPH MCFARLAND, Philadelphia—Is that a peculiarity of this bacillus or is the same true of all bacilli? Do they all become attenuated?

DR. M. DORSET, Washington, D. C.—This particular human culture is the most attenuated form we have. We have some human tubercle bacilli that have been growing for ten years without passage through an animal, and they are still quite virulent for guinea-pigs. This particular culture does seem to be an exception.

DR. VICTOR C. VAUGHAN, Ann Arbor, Mich.—I would like to ask the gentlemen present if it has been their experience that tubercle bacilli become attenuated after having been grown for many generations?

DR. JOSEPH MCFARLAND, Philadelphia—I brought back with me from Berlin in 1895 a tubercle bacillus that had been isolated by Koch many years ago, and I kept it growing for more than five years. At the end of that time it seemed to have retained its virulence. I never went through any quantitative method of determining this, but relatively its virulence did not seem to be diminished. Unfortunately I lost the germ and could not continue the experiment.

DR. VICTOR C. VAUGHAN, Ann Arbor, Mich.—In 1888, when I was a student in Koch's laboratory, Professor Fraenkel gave me a culture of the tubercle bacillus which he said was the

112th generation of one of the first cultures made by Professor Koch in 1882. I used that germ and its descendants in experiments on guinea-pigs and rabbits and it retained its virulence until about 1896. During the interval between 1888 and 1896 no year passed in which I did not inoculate animals with this germ, and in every case it was found to be virulent. In 1896 it began to lose its virulency for guinea-pigs, and soon ceased to have any effect even in large doses. Two years ago I sent to Dr. Lartigau a culture of this germ and a few weeks ago I had a letter from Dr. Baldwin of Saranac, saying that he had obtained the culture from Dr. Prudden and had experimented with it and that his experiments had confirmed my statements. The germ has completely lost its virulence so far as guinea-pigs were concerned.

NEW PHYSIOLOGIC APPARATUS.

THE FROG-BOARD MYOGRAPH AND THE MANOMETER TAMBOUR.*

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THE FROG-BOARD MYOGRAPH.

The term myograph was originally applied to the whole apparatus used in recording the contraction of a muscle. The pendulum myograph of Helmholtz included the muscle-clamp, the muscle-lever and the moving surface (pendulum) on which the myogram or muscle-tracing was recorded. After the rotating cylinder came into use as a recording surface under the name of the kymograph, the term myograph has been applied to that portion of the apparatus which represented the muscle-clamp and the lever with the load and the tracing point. Such an apparatus traces on the kymograph the muscle-curve or myogram.

The myograph has taken several forms: In the simple myograph of Helmholtz, in the muscle-signal of Du Bois Reymond, and in several other forms the muscle is exposed to the atmosphere. The changes wrought by the atmosphere soon put the muscle-nerve preparation into an abnormal condition and the responses to stimuli are not typical. In fact, the evaporation of moisture soon condenses the tissue-plasma sufficient to induce a typical "salt-tetanus." The application of normal saline solution can be temporarily, but only temporarily, avert such a result.

To reduce to a minimum the effect of atmospheric changes the so-called "moist-chamber" was devised. This consists of a glass chamber which enclosed the muscle and nerve and the electrodes with the muscle clamp. A wire or thread passes through the bottom of the chamber to the tracing lever. The air in the chamber is kept saturated with moisture by a wet paper or sponge within. This device overcomes to a large extent the difficulties with which one must contend in attempting to work with the exposed muscle. There is one serious difficulty, however, which has not been mentioned and which is not corrected by the moist-chamber myograph, namely, the cutting off of the muscle from the blood and lymph circulation. In the classic method of making a muscle-nerve preparation from the gastrocnemius muscle and sciatic nerve of the frog, the muscle and nerve, with a portion of the femur, are completely removed from the frog's body, thus not only exposing them to atmospheric changes, but removing them completely from connection with the circulatory system.

For fundamental experiments on the elementary tissues, and for work with drugs in pharmacology, it is of very great importance to have a myograph that is simple and inexpensive and which does not involve disturbing the circulatory system. The importance of the last point can not be over-estimated. The circulatory system not only brings a continuous supply of nutriment and oxygen to the muscle, but carries from the muscle the accumulating products of fatigue and thus enables the muscle to do much more work than would otherwise be possible.

In pharmacologic experiments it enables the experimenter to watch the influence of drugs carried to the muscle in the normal way, rather than applied in some artificial and abnormal way.

The frog-board myograph is a new form of myograph so constructed as to permit all experiments usually performed on the gastrocnemius-sciatic preparation without exposing the active tissues to the atmosphere or disturbing the blood supply. The instrument is constructed as follows: An oaken base about one-fourth of an inch in thickness supports a cork plate of equal thickness; the cork plate presents a surface about 10 by 25 centimeters. (Fig. 1.) The lever holder at the end of the plate is constructed of thin sheet steel and slips from side to side in order to bring it opposite either leg of the frog.

The distance from the axis of the elbow-lever to the thread-eye is the same as that to the weight, therefore,

FIG. 1.—FROG-BOARD MYOGRAPH.

S, the shaft which is clamped to the upright stand; B, the oaken base; C, Pl, the cork plate to which the frog is fixed; A, the lever axis and slide lever-holder; W, the weight; L, the light lever about 20 cm. in length; H, the tendon hook which is joined through the thread T, which passed through the eye and under spring catch (c); R, the lever rest.

the weight lifted by the muscle is the actual weight hung upon the weight link. When the lever passes a little below the horizontal position it comes into contact with the rest. This rest can be used in "after-loading" a muscle. For further description of the instrument, see the figure and its description.

In the use of the frog-board myograph one proceeds as follows: The frog is pithed and pinned, dorsum up, on the cork-plate, with the feet at the lever end. The tendo-achillis is exposed and loosened from the tarsal ligaments, the tendon-hook (H) is passed through the tendon and the length of the thread adjusted at (c). The skin on the thigh is opened to the extent of two centimeters and the biceps femoris muscle removed, the sciatic nerve carefully separated from the sciatic artery and placed on the insulated electrodes. Stimulation may be made from time to time for a period of several hours before the preparation becomes exhausted.

SUMMARY.

1. The frog-board myograph is a convenient and inexpensive myograph especially adapted for work in general physiology and in pharmacology.
2. Its advantages over other similar instruments are as follows:
 - I. The muscle-nerve preparation can be made in at most one-fourth the time required for the classic preparation.

* Read at the Fifty-fourth Annual Session of the American Medical Association, in the Section on Pathology and Physiology, and approved for publication by the Executive Committee.