AN EXPERIMENTAL STUDY OF BONE AND JOINT TUBERCULOSIS.*

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Much experimental work has been done with a view to demonstrating the origin and source of bone and joint tubercle, the paths by which the bacilli reach the bones, and the situations in which they develop.

Schüller (1) in 1880 described how he injected tuberculous material, sputum, glands, etc., through a tracheotomy opening into the lungs of dogs and rabbits, coincidentally injuring one of the knee joints of the infected animal; he succeeded in producing generalized tuberculosis and a tuberculous synovitis of the injured joint.

Müller (2) in 1886 injected the nutrient bone vessels of goats; apparently there resulted a multiple tuberculous osteomyelitis of the bone supplied by the infected blood vessel with accompanying infection of the neighboring joints. Müller quoted his results in demonstration of the hematogenous infection of osseous tubercle.

In 1891 Krause (3) published the results of his experimental work. He injected pure cultures of tubercle bacilli subcutaneously into guinea pigs and intravenously into rabbits. Directly before or immediately after the inoculation, or after a variable space of time, a joint was injured or a bone broken. In no case was there evidence of tubercle at the site of fracture, but in the instance of the joints many of them became tuberculous,—fifteen joints out of forty-four in guinea pigs, and fourteen out of twenty-eight in rabbits. The uninjured joints with one exception remained healthy.

A number of experiments upon rabbits were carried out by Benda (4) in 1899. He intimated his belief that the original lesion was an actual focus of tuberculous disease in the tunica interna of the blood vessel, and from such a focus there was a continuous liberation of bacilli into the blood stream. Lannelongue and Achard (5) (1899) found that it was by no means an easy matter to trace experimentally the source and origin of osseous or joint tubercle. They inoculated guinea pigs in various ways and directly afterwards or some time later they produced local injuries of the bones or joints, but they failed to produce tuberculous lesions.

With a view to investigating the hemic routes of infection, Friedrich (6) in 1899 introduced tuberculous cultures of low virulence into the left ventricle of rabbits. He succeeded in producing tuberculous joint affections. Certain

* Received for publication, December 12, 1912.

of the joints had been previously injured, but he found that those subjected to traumatism were less likely to become infected than the apparently healthy ones.

What practically amounted to the antithesis of this view was expressed by Pietrzikowski (7) (1903). According to this view 20 per cent. of all tuberculous affections of bones and joints were connected with some forms of injury; this rarely amounted to a fracture or dislocation but usually an injury of lesser degree, such as a sprain or bruise.

In 1904 Salvia (8) injected virulent cultures of tubercle intravenously into rabbits; simultaneously various parts of the body were subjected to traumatism. He found that in the flat bones the violence practically always decided the localization of tubercle; no localization could be obtained in the long bones of the limb, but slight injuries to the parts sometimes resulted in tuberculous disease.

When one attempts to epitomize the experimental results, one is met by a mass of contradictions, but underlying these there are two facts which are clear: (1) that it is difficult to reproduce experimentally tuberculous lesions of the bones and joints; (2) that trauma as a localizing factor is slight in degree rather than severe.

In the course of an investigation into the etiology and pathology of bone and joint tubercle, a number of experiments were performed with a view to establishing (1) the routes by which infection spread to the locomotory system, and (2) the factors which governed the localization of the lesion. It is a brief outline of these experiments and their results which I wish to record.

GENERAL INFECTION EXPERIMENTS.

Local bone or joint tubercle is rarely the result of a generalized infection. To illustrate this fact a series of guinea pigs was inoculated with known quantities of human tubercle bacilli by two of the commonest routes of infection, subcutaneous inoculation and infection by feeding.

The first series, ten in number, was infected with amounts varying from one to two milligrams of dried bacilli. The inoculation was made subcutaneously, and six weeks later the animals were killed and examined. Special attention was paid to examination of the bones and joints, and in most instances the bones were submitted to microscopical examination. The post-mortem examination revealed in each case the presence of a disseminated tuberculosis. In no instance was any lesion discovered in the bones or joints.

Infection by ingestion was carried out by feeding four guinea pigs for a period of three weeks upon food adulterated with tubercle bacilli.

The animals were permitted to live for six weeks and they were then killed and examined. Post-mortem examination revealed the presence of general tuberculosis, apparently primarily beginning as a mesenteric infection. Examination of the bone and joints yielded entirely negative results.

These experiments illustrate the fact that infection of bones or joints is not a likely possibility in the general dissemination of tubercle.

LOCAL DIRECT INFECTION OF THE BONE.

A most important question to be enquired into is the result of a direct inoculation of the bone. The infection was carried on as follows:

Technique.—The tibia was usually chosen. The animal was anesthetised with ether and with aseptic precautions the bone was exposed from its inner or subcutaneous aspect. For about a third of the circumference the periosteum was separated from the underlying bone until an area of 1.9 cm. was exposed. In this area the medulla was opened by raising a tiny trap door of bone. This may be done with strong, sharply pointed scissors, but considerable care must be exercised to prevent fracture of the fragile shaft. The medulla must be inoculated without infection of the soft parts. The most satisfactory method of ensuring this was to introduce into the medullary cavity with a fine probe a tiny plug of cotton wool infected with tubercle bacilli. The wound was then bathed with antiseptic lotion, and the trap door of bone and the raised periosteum were replaced.

Guinea pigs and rabbits were used in the experiment and the human bacillus was employed. I shall first describe the results obtained by infection of guinea pigs. A series of seven animals was employed, and at the end of a varying period each animal was killed and examined. The infected bone was removed intact and submitted to microscopical examination. The more important details are expressed in table I.

Results.—The duration of time which each animal was allowed to live after inoculation varied from ten to sixty days. The post-mortem examination revealed in every case, as was to be expected a general tuberculosis. The examination of the infected

bone showed that in four of the seven cases a definite tuberculous osteomyelitis had developed; but the negative results of the remaining three cases demand the chief attention, as microscopic examination afforded no sign of active tubercle; in one instance there existed what apparently was a healed tuberculous focus. In the negative cases one was convinced that there was no source of error in the original infection of the bone, but that they were instances of true recovery from infection.

TABLE I.

Local Inoculation of the Bone of the Guinea Pig.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 11	Oct. 20	10 days	Gland tubercle	Early T. B. Osteomyelitis.
B	Sept. 23	Oct. 17	20 days	General tubercle	T. B. Osteomyelitis.
C	Oct. 19	Nov. 22	30 days	General tubercle	Healed T. B. Osteomyelitis.
D	Oct. 4	Nov. 14	40 days	General tubercle	T. B. Osteomyelitis.
E	Oct. 2	Nov. 14	43 days	General tubercle	No tuberculous lesion.
F	Oct. 21	Dec. 10	50 days	General tubercle	No tuberculous lesion.
G	Oct. 8	Dec. 6	60 days	General tubercle	T. B. Osteomyelitis.

A similar series of experiments was performed upon rabbits. The rabbit possesses a considerable power of resistance to infection by the human tubercle bacillus, while it is unusually susceptible to inoculation with the bovine bacillus. In this series two rabbits were infected with a bacillus of human strain, while two were infected with a bovine bacillus. The details are given in table II.

TABLE II.

Local Inoculation of the Bone of the Rabbit.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Nov. 10	Feb. 20	102 days	Human	Slight pulmonary T. B.	No active tubercle.
B	Nov. 10	Feb. 28	110 days	Human	Slight pulmonary T. B.	No T. B. Osteomyelitis.
C	Nov. 30	Jan. 10	40 days	Bovine	No general T. B.	Slight T. B. Osteomyelitis.
D	Nov. 30	Dec. 20	20 days	Bovine	No general T. B.	Slight T. B. Osteomyelitis.

Results.—When rabbit bones are directly infected with the human tubercle bacillus there results no local development of disease. When the bones are infected with the much more virulent bovine bacillus, the virulence of which had been previously demonstrated, a slight lesion appears, but it is circumscribed and retrogressive. It is interesting to note that the rabbits which were inoculated with bovine bacilli showed no evidence of general tuberculosis. The explanation apparently is that the cellular action is so intense as to prevent any general spread of the disease.

These experiments were carried out in fully developed animals and the center of the shaft was the site of local infection.

LOCAL INFECTION OF THE METAPHYSIS OF THE RABBIT.

In order to reproduce more exactly the type of infection which clinically occurs, a series of experiments was performed in which young rabbits with growing epiphyses were used, and the bones were infected at what is considered the site of election of the disease; namely, the metaphysis.

Technique.—The bone was opened at its most accessible part, usually the center of the diaphysis. Along the interior of the shaft, and with as slight damage to the marrow as possible, a fine capillary tube was passed until the progress of the tube was arrested at the growing epiphyseal extremity. To the tube so placed a piece of fine rubber tubing and a syringe filled with tubercle bacillary emulsion was attached, and along the tube a single drop of the emulsion was injected. In withdrawing the tube it is important to prevent as far as possible further infection of the medulla, and this can, to a certain extent, be done if while the tube is being removed a negative pressure is maintained by slightly withdrawing the piston of the syringe. The details of the experiment are given in table III.

TABLE III.

Local Infection of the Metaphysis.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Dec. 10	Feb. 28	90 days	Human	Negative	Negative
B	Dec. 18	Feb. 18	62 days	Human	Negative	Negative

Results.—Both the above experiments yielded negative results. In neither case was there any development of the disease, local or general.

Conclusion.—The main fact illustrated by the above experiments is the remarkable difficulty which there is in infecting with tubercle the medulla of a healthy bone.

The guinea pig, the most susceptible of all animals to such an infection, shows a certain proportion of recoveries when the bone marrow is infected.

In the case of the rabbit its natural immunity to the human bacillus prevents entirely the development of a marrow lesion, and even when the bovine bacillus is inoculated the resulting disease is slight and retrogressive.

Evidence such as this suggests that clinically a primary infection of the bone medulla, a primary tuberculous osteomyelitis, is rare and unlikely.

DIRECT INFECTION OF JOINTS IN THE RABBIT.

The next question to consider is the result of direct infection of the joint cavity. With a view to elucidating this point a series of experiments was performed. Rabbits were chosen for the experiments and both human and bovine bacilli were employed in individual instances.

Technique.—The left knee joint was chosen, as being one of the most accessible both for inoculation and later observation. The interior of the joint can easily be entered if the knee is firmly flexed and the needle inserted immediately by the side of the ligamentum patellæ (table IV).

After the inoculation the animal was allowed to use the part as before.

TABLE IV.

Local Infection of Joints.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Type of bacillus.	General examination.	Local examination.
A	Feb. 2	June 20	138 days	Human	Negative	Chronic T. B. Synovitis.
B	Feb. 7	June 15	128 days	Human	Negative	Chronic T. B. Synovitis.
C	Feb. 14	Mar. 30	44 days	Bovine	Slight pulmonary T. B.	Acute T. B. Synovitis.
D	Feb. 14	Mar. 30	44 days	Bovine	Pulmonary tubercle	Acute T. B. Synovitis.

Results.—In striking contrast to the bones, the joints are readily infected with the tubercle bacillus. As is to be expected, the

degree of reaction which occurs is much more intense in the case of the bovine bacillus than in that of the human.

The probability is that the liability of the joints to infection as compared to the bones depends upon the absence in the former of resistive cell elements. The drawback to experiments like the above lies in the fact that such massive direct infections can never actually occur *in vivo*. In order to reproduce a typical clinical infection it is necessary to secure a more general and gradual dissemination and with this in view a further series of experiments was performed.

GENERAL INFECTION VIA THE MESENTERIC VESSELS IN THE RABBIT.

Children, the main sufferers from bone and joint tubercle, are infected in the majority of instances by the ingestion of tuberculous milk (Fraser (9), 1912); the primary focus lies in the mesenteric lymph glands, and from such a deposit, dissemination occurs.

To reproduce this type of infection a series of experiments was performed in which a quantity of tubercle bacilli was introduced into the circulation by injection of a mesenteric vein.

Technique.—Rabbits were employed in the experiments. The animals were anesthetised with ether and the abdomen was shaved and sterilized. An incision was made in the middle line and a loop of small intestines extracted. With a fine hypodermic syringe and needle one of the larger mesenteric veins was punctured and a quantity of human bacillary emulsion slowly injected. After withdrawing the needle the bleeding was sometimes so severe as to necessitate ligation of the punctured vessel. The abdominal wall was finally closed (table V).

TABLE V.

General Infection via the Mesenteric Veins.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	Result of experiment.
A	Dec. 7	Feb. 4	59 days	Tubercle of liver and peritoneum; bones and joints healthy.
B	Dec. 7	Dec. 22	15 days	Tubercle of liver and lungs; bones and joints healthy.
C	Dec. 15	Dec. 30	15 days	Tubercle of liver and lungs; bones and joints healthy.
D	Dec. 16	Jan. 14	30 days	Tubercle of liver and lungs; bones and joints healthy.

Results.—Uniformly the results have been negative. Infection of the mesenteric veins necessarily produced tuberculous foci in the

liver and lungs and occasionally in the overlying peritoneum, but in no instance was there any sign of infection of the bones or joints.

GENERAL INFECTION BY DIRECT INOCULATION OF THE HEART BLOOD.

It has been suggested that the incidence of bone tubercle owes its origin to a hematogenic infection, and a series of experiments was performed to demonstrate the possibility of its occurrence.

Technique.—I have previously alluded to the experiments of Friedrich in this relation. Friedrich infected the blood stream by passing a tube into the left ventricle along the carotid. The method which I adopted was somewhat different and simpler in execution. It is possible to enter the left ventricle by inserting a needle through the chest wall. Under an anesthetic this was done and the emulsion was slowly injected. It is rapidly disseminated by the arterial blood throughout the body. Of course the most obvious way to infect the circulation is by intravenous injection, but this leads to such a massive infection of the lungs that death results very early. It is by no means difficult to enter the ventricle through the chest wall and I have demonstrated by post-mortem dissection the certainty of the method (table VI).

TABLE VI.

General Infection via the Left Ventricle.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 29	Mar. 3	125 days	Pulmonary tubercle	Bones and joints healthy.
B	Nov. 7	Mar. 3	116 days	Pulmonary tubercle	Bones and joints healthy.
C	Nov. 12	Mar. 20	128 days	Pulmonary tubercle	Healed tubercle center right tibia.
D	Dec. 9	Mar. 3	84 days	Pulmonary tubercle	Bones and joints healthy.
E	Dec. 16	Jan. 7	22 days	Pulmonary tubercle	Bones and joints healthy.
F	Dec. 16	Jan. 8	23 days	Pulmonary tubercle	Bones and joints healthy.

Results.—In a total of six experiments a local infection developed in one instance, and this single positive result was indicated not by an active tubercle, but by a healing and retrogressive process. The value of these results lies in their demonstrating that a circulation of tubercle, a tuberculous septicemia, in fact, unaided by any predisposing factors, is unlikely in itself to produce a local tuberculous lesion of the bone or joint.

DIRECT INFECTION OF THE MAIN VESSEL SUPPLYING A LIMB OF THE RABBIT.

While a general arterial infection may yield negative results, it seemed possible that a more direct infection of the vessel supplying a limb might give more promising results.

Technique.—Rabbits were employed in the experiments. The femoral vessels were exposed upon the left side immediately beneath the groin, and the sheath of the vessel was opened and the artery separated for a distance of about 1.27 cm. For the purpose of injecting the vessel a specially prepared needle was employed, bent to a right angle about 1.9 cm. from its tip. The vessel lumen was entered with the needle and gradually about 0.5 c.c. of tubercle emulsion was slowly injected in the direction of the blood stream. The injected fluid was thoroughly distributed by a few pulsations of the heart. The bleeding subsequent to withdrawal of the needle was so severe as to necessitate in almost every case ligature of the punctured vessel. The wound was closed with sutures, and the animal allowed to use the limbs as before (table VII).

TABLE VII.

Direct Infection of the Main Blood Vessel of the Limb.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	Oct. 19	Mar. 3	135 days	Pulmonary tubercle	Bones and joints healthy.
B	Oct. 26	Jan. 5	63 days	Pulmonary tubercle	Tuberculous disease of left ankle.
C	Oct. 29	Mar. 3	127 days	Pulmonary tubercle	Tuberculous metatarsophalangeal joint.
D	Nov. 7	Mar. 3	117 days	Pulmonary tubercle	Bones and joints healthy.

Results.—These experiments show that it is possible to infect the joints of a limb by infection of the main blood vessel with tubercle.

The primary infection was of the joint, and any change which may have appeared in the neighboring bone was secondary to the synovial tubercle. This was demonstrated by subsequent microscopic examination. The infection had originated in that portion of the synovial membrane which lines more especially the reflection of the joint.

Histologically the changes in the joint subsequent to infection were those of an acute synovial tubercle.

DIRECT INFECTION OF THE NUTRIENT ARTERY OF THE RABBIT.

While infection of the main blood vessel leads to tubercle of the joints of that limb, the question arose as to the result of direct infection of the nutrient artery, the injection being so directed as to enter entirely the medulla of the bone.

Technique.—The methods employed were similar to those used by Cheyne (10). The experiments were performed upon rabbits. The nutrient artery supplying the interior of the tibia springs from the femoral artery, a short distance below the knee, and enters the tibia obliquely from above downwards, at the junction of its upper and middle thirds.

The nutrient vessel is much too fine to permit of direct inoculation, and therefore one has, so to speak, to procure its infection indirectly. The femoral artery is tied a short distance below the level at which the nutrient vessel is given off, the artery is exposed higher up just below the groin, and there inoculated with fine needle and syringe. The blood stream must therefore follow the only path which remains open to it, namely the nutrient vessel, and it carries with it into the interior of the bone the infected material.

TABLE VIII.

Direct Inoculation of the Nutrient Artery.

Animal.	Date of inoculation.	Date of examination.	Duration of disease.	General examination.	Local examination.
A	April 2	April 20	18 days	Pulmonary tubercle	Tibia healthy.
B	April 5	Sept. 7	5 months	Pulmonary tubercle	Tibia healthy.

Results.—In both cases the result of the local infection was negative. There was, of course, some evidence of general infection, but the infected tibia, when examined microscopically, showed a fatty change in the marrow, but no evidence of the development of tuberculous tissue.

GENERAL CONCLUSIONS.

While one can not assert that the conclusions which experiments appear to offer must of necessity correspond to the conditions which arise clinically in man, nevertheless these results, if definite, must tend to show the direction in which the truth lies.

The conclusions which may be drawn from this research are as follows:

1. Direct infection of the medulla of a long bone is unlikely to lead to the development of a tuberculous osteomyelitis.

2. Inoculation of the interior of a joint with tubercle bacilli readily produces tubercle of the synovial membrane.

3. From such an infected joint the epiphysis or metaphysis of the bone becomes diseased.

4. Infection of the arterial heart blood does not result in the local development of tubercle of the bones or joints.

5. Infection of the main artery supplying a limb leads to the development of tuberculous disease of certain of the joints of that limb.

6. Direct infection of the nutrient artery does not result in tuberculous osteomyelitis of the bone.

I wish to acknowledge my indebtedness to Dr. James Ritchie, Superintendent of the Laboratory of the College of Physicians, Edinburgh, for assistance in carrying on this work. Financial aid for the research was obtained from the Trustees of the McCunn Scholarship and Carnegie Funds.

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