

A RAPID METHOD FOR THE DIAGNOSIS OF RENAL
TUBERCULOSIS BY THE USE OF THE
X-RAYED GUINEA PIG.

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The value of animal inoculation in the diagnosis of tuberculosis has been recognized since Marcet's¹ experiments in 1867. Klebs² confirmed this work in the following year, and the constant employment of the guinea pig test since that time has proved it to be the safest diagnostic method that we have.

It is natural that this method should be given special importance in cases of renal tuberculosis where there is always the complicating smegma bacillus to rule out on microscopic examination, and where the demonstration of the tubercle bacillus is a matter of considerable difficulty. In the past year Crabtree³ published his technique for high centrifugalization of the urine, an important step forward, but in the same article he admits that guinea pig inoculation must be the final test in all doubtful cases.

Damsche⁴ was the first investigator to use the guinea pig for the diagnosis of renal tuberculosis. From his time to the present there has been little change in the technique employed. In the Massachusetts General Hospital a subcutaneous injection of 15 to 20 minims of suspected urine is made into the abdominal wall of the animal and after 5 weeks an autopsy is done. The diagnosis is made both by gross lesions and by microscopic examination of film preparations. Barney and Young's⁵ summary of 197 cases from this hospital shows

¹ Marcet, W., *Tr. Med. Clin.*, 1867, 437.

² Klebs, E., *Virchows Arch. path. Anat.*, 1868, xlv, 242, 296.

³ Crabtree, E. G., *Surg., Gyn. and Obst.*, 1916, xxii, 221.

⁴ Damsche, *Deutsch. Arch. klin. Med.*, 1882, xxxi, 78 ff.

⁵ Barney, J. D., and Young, E. L., Jr., *Boston Med. and Surg. J.*, 1911, clxiv, 917.

that the reports previous to operation have been correct in 99.5 per cent of the cases.

The method is, however, open to criticism on account of the time that is lost while waiting for the lesions to develop in animals.⁶ Any modification, therefore, that will allow us to shorten the time appreciably should be of practical value. With this in mind, the following experiments were undertaken.

Murphy and Ellis⁷ have shown that white mice which have been exposed to x-rays are made markedly more susceptible to bovine tuberculosis than normal animals. They explain this fact by the destruction of the lymphoid tissue which they claim constitutes an important agent in the defensive mechanism against tuberculosis. It seemed reasonable to assume that the guinea pig could be rendered less resistant than it is normally by a similar procedure. Accordingly, guinea pigs were given various doses of Roentgen rays to determine how much they could stand and whether the resistance to known tuberculous urine could be reduced.

It was found that guinea pigs could tolerate a large amount of x-rays without apparent injury to their health. The average normal white blood count of the animals was from 12,000 to 15,000 cells per c. mm., of which 40 to 45 per cent were of the lymphocytic variety, and, with the exception of $\frac{1}{2}$ to 1 per cent of transitionals and large mononuclears combined, the remaining cells were polymorphonuclears. One massive treatment with x-ray sufficed to reduce the total count by over one-half (4,000 to 6,000), mainly effected by reduction in the lymphoid cells. The count remained depressed for a period of over 1 week, which was as far as it was followed. Animals treated in this way when inoculated with a known tuberculous urine developed marked lesions of the disease in a much shorter time than did normal animals. Further experiments showed that an x-ray exposure given shortly after inoculation was as effective in reducing the resistance as when given before the inoculation. This fact seems to be of practical importance as it obviates the necessity of keeping a stock of treated animals on hand. After having established a working dosage and the best time interval before killing the animals, a series of cases was studied in connection with the Genito-Urinary

⁶ Churchman, J. W., *Med. Rec.*, 1916, lxxxix, 511.

⁷ Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

Department of the Massachusetts General Hospital, in order to test the usefulness of the method.

It was considered necessary for practical reasons to confine the x-ray preparation to one exposure only, sufficiently massive to carry the animals through 10 days with depressed resistance. It is probable, however, as is shown by the experiments of Murphy and Morton,⁸ that repeated small exposures would be more satisfactory for experimental study when it is desirable to produce a maximum effect on the lymphoid tissue with a minimum damage to other structures. The following dosage was adopted as the most useful. The animal was placed in a pasteboard box of such a size that it could not move around, and then radiated for a period of 10 minutes with the Coolidge tube, the target being 12 inches distant from the base of the box. A 5 milliamperere current was passed through the tube, backing up $8\frac{1}{2}$ inches of spark between points. The Snook interrupterless was the type of machine employed. No aluminum or leather filters were used. From 1 to 2 cc. of urine were injected intraperitoneally according to the amount at hand. After 10 days the guinea pigs were killed and examined for the lesions of tuberculosis. As in the normal animals, the usual sites of election were the spleen, mesenteric lymph glands, and liver. The lesions varied with the severity of the infection,—sometimes the spleen was riddled with a mass of caseous nodules 2 to 4 mm. in diameter, while at other times the spots were more nearly the size of a pinhead but distinctly pathognomonic. The lymph glands were usually from 1 to 1.5 cm. in diameter and caseous, and, as a rule, the liver had scattered over its surface nodes similar in size to those of the spleen. Films taken from these situations and stained for tubercle bacilli were used to confirm the gross diagnosis. Microscopically the picture was one of coagulation necrosis and caseation with a zone of endothelioid cells but no formation of giant cells, and a striking absence of the small round cell infiltration. There was also no evidence of an enclosing fibrous layer such as is commonly seen in the slow growing tubercle.

The following cases were taken at different times from the Genito-Urinary Department, and the above method of making the diagnosis was used. The guinea pig findings were confirmed in every case as far as possible by all other data which would tend to throw light on possible error. The results are given in Table I.

⁸ Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

TABLE I.

Case No.	Urine.	Cystoscopy.	Normal guinea pig test.	X-rayed guinea pig test.	Operation.	Pathological report.	Clinical diagnosis.	Remarks.
1	Large numbers of tubercle bacilli in clumps. Culture not done.	Tuberculous bladder.	1. Killed in 5 weeks. Spleen full of nodules of tubercle bacilli. 2. Died during night.	Both animals died on following day.	None.	None.	Tuberculosis of both kidneys and of lungs.	One normal and two x-rayed animals died in 24 hrs. from a mixed streptococcus infection. One normal animal showed tuberculous lesions of spleen after 5 weeks.
2	Sediment, from left kidney showed tubercle bacilli; from right kidney few tubercle bacilli. Culture, no growth.	Tuberculous pus in left ureter. No pus in right ureter.	No test.	Killed on 10th day. Liver covered with patches. Spleen contained small nodules. Glands caseous.	"	"	Tuberculosis of left kidney. Tuberculosis of right kidney? Tuberculosis of lungs.	Question of right kidney excreting bacilli without disease. No pus in urine on right side. Tubercle bacilli in sputum.
3	Sediment, from left kidney showed few tubercle bacilli; from right showed tubercle bacilli. Culture, few cocci from left; no growth from right.	"Not diagnostic."	Not yet reported.	Killed on 7th day. Extensive lesions in liver, spleen, and glands.	"	"	Tuberculosis of both kidneys.	
4	Sediment, many tubercle bacilli. Culture not made.	Not done.	No test.	Extensive lesions in liver, spleen, and lymph glands.	Nephrectomy.	Tuberculosis of kidney.	Tuberculosis of kidney.	

5	Sediment, few tubercle bacilli after high centrifuge. Culture, no growth.	Could not catheterize.	"	Killed on 10th day. Spleen riddled. Glands caseous; size of 10 cent piece.	"	"	Tuberculosis of kidney or bladder.	
6	Sediment, negative for tubercle bacilli. Culture, no growth.	Nothing suggestive.	Positive for tubercle bacilli in 5 weeks.	Killed on 9th day. Spleen and lymphoid glands caseous.	"	"	Tuberculosis of kidney.	Two x-rayed guinea pigs used. First killed on 9th day with diagnostic lesions; second killed in 5th week with severe lesions.
7	Sediment, negative for tubercle bacilli. Culture, no growth.	Left ureter retracted. Tuberculosis?	Negative four times.	Killed on 8th day. Liver peppered with pin-point lesions. Spleen also involved.	"	"	Tuberculosis of kidney.	Four normal guinea pigs gave negative results.
8	Sediment, colon bacilli. Culture not made.	Ulcer near right ureter, tuberculous.	No test.	Killed on 14th day. Liver and spleen contained pin-point caseous nodules.	"	"	Tuberculosis of right kidney.	
9	Sediment, no bacilli. Culture not made.	Suggests bilateral tuberculosis.	"	Died on 8th day. Peritonitis. Film showed tubercle bacilli present.	"	"	Tuberculosis of left kidney. Pott's spine. General peritonitis.	Mixed infection with doubtful lesions.
10	Sediment, negative for tubercle bacilli. Culture, streptococci.	Not done.	"	Killed on 14th day. Marked lesions in liver. Spleen riddled. Glands caseous.	None.	None.	Tuberculous epididymitis. Tuberculosis of bladder or kidney?	

TABLE II.

Case No.	Urine.	Cystoscopy.	Normal guinea pig test.	X-rayed guinea pig test.	Operation.	Pathological report.	Clinical diagnosis.	Remarks.
1	Sediment, no tubercle bacilli. Culture, staphylococci.	Normal.	Negative for tubercle bacilli.	Killed on 14th day. Marked lesions in liver and spleen.	None.	None.	Pyonephrosis or pyelitis.	Film cleared diagnosis. Cocci. Negative for tubercle bacilli.
2	Sediment, no tubercle bacilli; colon bacilli. Culture, colon bacilli.	"Bladder looks like subsiding colon infection."	No test.	Killed on 12th day. Plastic peritonitis. No tuberculosis.	"	"	Cystitis.	
3	Sediment, no tubercle bacilli. Culture, staphylococci.	"Practically normal."	Negative for tubercle bacilli.	Killed on 10th day. No lesions.	"	"	"	
4	Sediment, no tubercle bacilli. Culture, staphylococci.	Impossible.	"	Killed on 10th day. No lesions.	"	"	Cystitis. Renal stone.	
5	Sediment, no tubercle bacilli. Culture, staphylococci.	Not done.	"	Killed on 10th day. General peritonitis.	"	"	Cystitis.	
6	Sediment, no tubercle bacilli. Culture, colon bacilli.	"Suggestive of tuberculosis."	"	Killed on 10th day. Foul general peritonitis. Culture, staphylococci.	"	"	Pyonephrosis. Cystitis.	

7	Sediment, no tubercle bacilli. Culture, colon bacilli.	Not done.	"	Killed on 10th day. Urine from left and right ureters negative.	"	"	"	Cystitis.	
8	Sediment, no tubercle bacilli. Culture, staphylococci.	"	No test.	Died on 7th day. General peritonitis. No tuberculosis.	"	"	"	"	
9	Sediment, no tubercle bacilli. Culture, streptococci.	Irritable bladder, small capacity. Could not catheterize ureter.	"	Killed on 10th day. Bladder urine gave no lesions.	"	"	"	"	
10	Sediment, left, few cocci; right, no tubercle bacilli. Culture, left, staphylococci; right, no growth.	Normal.	Negative for tubercle bacilli.	Killed on 10th day. Urine from left ureter gave small lesions. Film, cocci. Urine from right ureter negative.	"	"	"	Pyelitis.	Film rules out atypical lesions. No tubercle bacilli.

Cases 1, 2, and 3 were extensive renal tuberculosis, bilateral, and of course inoperable. They were used early in the series and are included to make the gradations of severity complete. Case 1 is instructive in that the x-rayed guinea pigs and one of the normal animals were killed by a mixed streptococcus infection. The other normal guinea pig survived, and when it was killed after 5 weeks, the spleen was full of tuberculous nodules. Case 2 presents an interesting possibility; namely, that the left kidney alone was involved, and the right was excreting tubercle bacilli without being affected. That this does occur at times is recognized in the literature (Brown⁹). Cases 4 and 5 were moderately severe and the bacilli were found in the urine by use of the high centrifuge method. Cases 6 to 10 were not advanced cases and no tubercle bacilli were demonstrated by microscopic examination of the sedimented specimens. Case 7 was tried four times in normal guinea pigs with negative results, probably because the ureter was occluded. On the first trial with the x-rayed guinea pig the test was successful in 8 days, an example of good fortune in getting a specimen which contained the bacilli. Case 9 had a mixed infection and the lesions were atypical. Acid-fast bacilli were demonstrated in films made from the animal, but the report would not have been made as positive without repetition except for the evidence of Pott's disease of the spine in this case. Most of these cases were reported within the 10 day limit, but three of the earlier ones were allowed to go 2 weeks before a diagnosis was made. These three cases would have undoubtedly fallen into the same period as the others, as the lesions were extensive in each instance.

A table of the non-tuberculous conditions in which it was necessary to rule out tuberculosis is also presented (Table II).

In these ten cases the diagnosis "negative for tuberculosis" has been confirmed by the clinical course, by tests with normal guinea pigs, and by all other methods available. Cases 1 and 10 (same individual) were the only ones which gave lesions in any way comparable to tuberculosis. In these cases there were multiple abscesses which in films showed no tubercle bacilli, but many small micrococci. Other guinea pigs in the series died early of plastic peritonitis from which pure cultures of the organism responsible were recovered.

⁹ Brown, L., *J. Am. Med. Assn.*, 1915, lxiv, 886.

DISCUSSION.

While the number of cases here presented is comparatively small, the uniformity of the results justifies a report at this time. The work is of value from the experimental side in that it confirms and extends the observation of Murphy and Ellis, in which they showed that white mice were made markedly more susceptible to bovine tuberculosis by suitable x-ray exposures. These experiments demonstrate that the same conditions hold for the human strain of the organism in the guinea pig. As to whether this lowered resistance is due to the destruction of the lymphoid elements as is suggested by Murphy and Ellis, the present work is perhaps not conclusive. For a demonstration of this point it would be better to use the fractional dose method as recommended by Murphy where a maximum effect may be obtained on the lymphoid elements with a minimum on the other structures. For obvious reasons, however, this method would not be practical for clinical use.

SUMMARY.

It is possible to reduce the resistance of guinea pigs to tuberculosis by x-ray exposures, so that when animal inoculation is required for a diagnosis a much quicker result may be had than by the use of normal animals. In renal tuberculosis when it is necessary to resort to the use of animals, it ordinarily requires from 5 to 7 weeks, while by the use of x-rayed guinea pigs the diagnosis can be made in from 8 to 10 days. The resistance can be sufficiently lowered by one massive dose of x-ray administered either shortly before or after the inoculation of the material to be tested. The lesions are so marked in these animals that the diagnosis is certain after the interval indicated above.

I wish to thank the members of the Staff of the Genito-Urinary Department for the interest they have taken and the assistance they have rendered in making this work possible.