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## A NEW AND RAPID METHOD FOR THE CONTROL OF URINARY TUBERCULOSIS: PRELIMINARY REPORT<sup>1</sup>

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This study was undertaken to determine whether there existed a more rapid and permanent control of inoperable or bilateral renal tuberculosis than the present therapeutic methods offered. It was not the purpose to supplant the existing treatment of unilateral upper tract tuberculosis with its secondary bladder symptoms nor lower tract involvement which is still a surgical procedure but rather to hasten the control of those cases which were treated by expectant hygienic measures in the preoperative stages of this disease (Fig. 1).

With the advent of streptomycin such a control seemed to be available. Hinshaw, Feldman and Pfeutze, and Poole and Cook, from whom I quote liberally, in their study of one hundred various types, arrived at the following conclusions: "That the Mycobacterium of tuberculosis is susceptible to streptomycin and that the course of the disease can be changed if an adequate dose is administered. However, the drug is bacteriostatic rather than bactericidal and has only a suppressive effect and that its lasting benefit would then depend upon the material resources of the body to promote recovery. The tubercle bacilli have a strong tendency to develop resistance against streptomycin and this usually occurs 1 month after the treatment is instituted and nearly always after the second month, and for these reasons the antibiotic should be used in rather early cases and in large daily doses to 3 gm." It must be also borne in mind that streptomycin is

<sup>&</sup>lt;sup>1</sup>Presented at meeting of the Western New York and Ontario Urological Society, Saranac Inn, Upper Saranac Lake, N. Y., September 5, 1947.

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a highly toxic substance and reactions to the preparation usually develop in individuals under really prolonged intensive treatment, as has been proven by the authors above quoted, and our own experience verifies this contention. The doses recommended by all investigators appear to be from 1 to 3 gm. a day; and with the present monetary stress attendant to the value of this preparation, the financial problems deserve consideration.

F. H. Redewill, and previously Schatz and S. A. Waksman, made complete and exhaustive surveys on the effect of streptomycin and other antibiotic substances in tuberculosis. Redewill reported the successful treatment of tuberculosis of the urinary bladder in 2 cases. His summary states: Streptomycin was used in 2 cases of bladder tuberculosis with dosage of 800,000 to one million units daily for 6 and 8 weeks respectively. One case, complicated by tuberculosis of the kidney and seminal vesicles, apparently cleared up under this treatment. He emphasized the use of the quartz light, with which this presentation has no interest, but in the text states that Feldman and Hinshaw (Proc. Staff Meet. Mayo Clinic, 19: 593, 1944) reported that extensive tests with streptomycin in the treatment of tuberculosis of guinea pigs and clinical tuberculosis indicates that such therapeusis is promising. However, again it is shown that resistance to the drug is an important factor. It is recommended that large doses be administered up to 800,000 units every 3 hours, though penicillin has no demonstrable effect on tuberculosis nor enhances the bacteriostatic effect. This method of treatment is promoted by Bigger who states that probably the penicillinase which is formed in the intestinal tract is destroyed by penicillin and checks bacterial resistance to streptomycin. Considerable animal experimentation has been done. Youman and McCarter, using mice with human organisms, report that results were striking in the mice treated with streptomycin but again it is noted in both in vitro and in animal experimentation and in vivo that large doses over prolonged periods of time are required.

The present studies were made since January 1, 1947 on 6 active cases of inoperable and bilateral renal tuberculosis with secondary bladder symptoms and were undertaken in vitro, through pig inoculations and in vivo. The results have been so striking that we believe we have arrived at a more rapid approach to the control of this disease by the method applied. In new cases the treatment was administered over a period of 30 days and in that period these proven positive cases have become negative bacteriologically, culturally, clinically and there has been spectacular improvement in each and every case.

Since the advent of the high powered microscope, which was confirmed by the present electron microscope, it is known by all bacteriologists that the Mycobacterium tuberculosis has a protecting waxy cell wall which protects the organism and had made resistant with the exception of material immunity of the individual, the extinction of this germ (Fig. 2). The same condition exists exactly in the Mycobacterium leprae and it is almost impossible to try to determine the difference between these two organisms except that in smears the leprae appear in more crowded clusters in the cells and are recognizable because of this feature. With this knowledge the author, before the advent of the present antibiotics, recognized these principles. To hasten the curative effects of encapsulating the organism through fibrosis, some 10 years ago I arrived at the hypothesis that if there were present a substance which would dissolve this protecting waxy wall of the M. tuberculosis, the material processes involved in inhibiting the growth of the lesions might be enhanced, and, knowing the existence of the same process in the M. leprae, the oil of chaulmoogra and its derivative esters was experimented with in a few chosen cases. Because of the instability of the preparation, results of the preliminary studies were distressing and unsatisfactory. The idea remained, however, until such time as a more stable, refined preparation was offered and which occurred shortly before the advent of streptomycin.

The present series was undertaken about January 1947 with the newer oil preparation. For 1 week the patient received daily injections, which were then supplemented by daily injections of streptomycin for 30 days only. Studies were continued in vitro and with pig inoculations while the clinical results were being evaluated. Tables 1, 2 and 3 disclose the results of these experiments.

#### IN VITRO STUDIES<sup>2</sup>

Effect of chaulmoogra oil upon streptomycin sensitivity in Mycobacterium phlei. This in vitro study was undertaken in order to determine the sensitivity of acid fast bacteria to streptomycin before and after exposure to varying concentrations of chaulmoogra oil.

The species Mycobacterium phlei was selected for initial

<sup>2</sup> The author wishes to acknowledge this study by Wilbert Spencer, Ph.D., Associate Professor of Biology, Department of Arts and Sciences, University of Buffalo. tests because it grows readily on ordinary laboratory media and gives a good growth in 24-48 hours. It is a harmless saprophyte found on vegetation and is therefore not dangerous to handle.

The organism was grown in broth for 24 hours, producing a rather coarse suspension, which on being well shaken up became cloudy as the clumps were broken up.

Dilutions of chaulmoogra oil. Dilutions of chaulmoogra oil in physiological saline were made as follows:

Solution A 2 parts oil in 5000 parts saline

Solution B 10 parts oil in 5000 parts saline

Solution C 20 parts oil in 5000 parts saline

These dilutions were well shaken and the oil became interspersed with the saline in fine droplets, giving a fairly even cloudy appearance.

*Exposure to chaulmoogra oil.* Twenty-four hour cultures of Mycobacterium phlei were inoculated into dilutions A, B, C of chaulmoogra oil in the proportions of 1 part culture to 40 parts diluted chaulmoogra oil. A saline control was also inoculated.

Samples from these dilutions were streaked upon blood agar base plates at the end of 24, 48, 72 hour intervals.

By comparing the result of the growth upon the four plates, it was clear that exposure to chaulmoogra oil caused considerable inhibition of growth. The least inhibition was noted after an exposure to oil for 24 hours.

These results, though not quantitative, were clearly evident.

Effect of streptomycin upon Mycobacterium phlei. The filter paper disk methods of determining the effect of streptomycin were used in which filter paper disks saturated with varying strengths of streptomycin are placed upon a culture plate previously seeded with the organism.

Seeding of plates. A 24 hour broth culture of M. phlei of 1:40 with solutions A, B, C and a saline solution for a control was plated on blood agar base. They were flooded with the dilute cultures, drained and set aside for a few hours in order to dry the surface.

The plates were divided off into 5 sectors with a wax pencil and the sectors were labelled 500, 125, 32, 8, 2 (micrograms of streptomycin respectively). See figure 3.-

Dilution of streptomycin. Streptomycin was diluted with saline to obtain a stock of 500 micrograms per cubic centimeter, after which it was diluted serially in tubes to give the above strengths.

The test. Filter paper disks with a diameter of 9 mm. were

dipped into the streptomycin dilutions and then placed on the proper sectors of the seeded plates which were then incubated for 48 hours.

The results are shown in tables 1, 2, and 3.

 TABLE 1.—24 hours exposure to chaulmoogra oil

 Zones of inhibition in Mm.

Units of Streptomycin	Control (Saline)	Chaulmoogra 2:5000	Chaulmoogra 10:5000	Chaulmoogra 20:5000
500	35	47	No colonies	No colonies
125	23	31	No colonies	No colonies
32	17	21	No colonies	No colonies
8	0	15	No colonies	No colonies
2	0	0	No colonies	No colonies

TABLE 2.-48 hours exposure to chaulmoogra oil

Zones	of	inhibition	in	Mm.
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Units of Streptomycin	Control (Saline)	Chaulmoogra 2:5000	Chaulmoogra 10:5000	Chaulmoogra 20:5000
500	39	No colonies	Contaminated	No colonies
125	31	No colonies	45	No colonies
32	17	No colonies	27	No colonies
8	0	No colonies	No colonies	No colonies
2	0	No colonies	21	No colonies

TABLE 3.—72 hours exposure to chaulmoogra oil Zones of inhibition in Mm.

Units of Streptomycin	Control (Saline)	Chaulmoogra 2:5000	Chaulmoogra 10:5000	Chaulmoogra 20:5000
500	33	No colonies	No colonies	No colonies
125	25	No colonies	No colonies	No colonies
32	21	No colonies	No colonies	No colonies
8	0	No colonies	No colonies	No colonies
2	0	No colonies	No colonies	No colonies

*Discussion.* It is evident from the tabulations that the exposure of Mycobacterium phlei to dilutions A, B, C for 48 hours or longer interferes with the growth of the organism to such an extent that too few colonies are produced to show a zone of inhibition around the disk saturated with streptomycin.

An exposure of 24 hours allows an abundant growth of colonies on the plate and the zones of inhibition in this case are readily compared with the zones on the control plate.

Here a tendency toward greater inhibition of growth of the treated organisms in comparison with the saline control is seen in the larger zones of inhibition and in the fact that the control strength of 8 micrograms per cubic centimeter of streptomycin fails to inhibit growth of M. phlei whereas in the treated organisms, a 15 mm zone of inhibition is produced.

Summary of in vitro studies. 1) Growth of Mycobacterium phlei is inhibited by exposure to chaulmoogra oil. 2) M. phlei is made more sensitive to streptomycin by exposure to 2:5000 dilutions of chaulmoogra oil for a period of 24 hours. 3) These experiments are to be continued using the M. tuberculosis by the same methods and will be reported on later.

**RESULTS OF GUINEA PIG INOCULATION RESULTS<sup>3</sup>** 

Pig No. 1. Control inoculated with tuberculosis; no treatment.

Pig No. 2. Treated with streptomycin alone 2 weeks after inoculation with tuberculosis.

Pigs Nos. 3 and 4. Treated with streptomycin and chaulmoogra oil 2 weeks after inoculation with tuberculosis.

*Pig No. 5.* Treated with chaulmoogra oil alone 2 weeks after inoculation with tuberculosis.

Pig No. 6. Inoculated with tuberculosis; no treatment; killed 2 weeks after inoculation and autopsied.

Pig No. 7. Inoculated with tuberculosis and started on treatment with streptomycin and chaulmoogra oil the same day.

Pigs Nos. 2, 3, 4, 5, were treated for 50 days, then killed and autopsied.

Pigs Nos. 2, 3, and 4 received streptomycin, 60 mg. daily.

Over the 50 day period each received 3 gm.

Pig No. 2 did not receive chaulmoogra oil.

Pigs Nos. 3, 4, and 5, received 0.2 cc chaulmoogra oil every other day for 24 doses. Each received a total of 4.8 cc.

Pig No. 5 received no streptomycin.

Pig No. 7 was treated with 60 mg. of streptomycin daily from the day it received its inoculation of tuberculosis for a total dosage of 3.78 gm. over a period of 63 days. The animal also received 0.2 cc chaulmoogra oil every other day for a total dosage of 6.2 cc for 31 doses over a period of 63 days. All injections were intraperitoneal.

Except for pig No. 6, all guinea pigs were killed the day following termination of treatment.

Pathological and microscopic results. Pig No. 1, grossly showed a spleen 2 times normal size incorporating several

<sup>3</sup>The author wishes to acknowledge these animal studies by Dr. P. A. Greco, Resident in Urology, Millard Fillmore Hospital, Buffalo, New York.

whitish nodules measuring up to 1 cm. in diameter. The celiac lymph nodes were grayish white, large, firm and matted. Microscopically, the spleen showed tubercles some of which were confluent and consist of epithelial cells, giant cells and central caseation necrosis. See figure 4.

Pig No. 2 grossly and microscopically showed no lesions. See figure 5.

Pig No. 3 showed no evidence of tuberculosis anywhere. See figure 6.

Pig No. 4, on microscopic examination, the spleen showed a suggestive early tubercle formation which was composed of large epitheloid cells and showed central caseation necrosis. However, no giant cells were present. See figure 7.

Pig No. 5, grossly and microscopically, the spleen, liver, omentum, lungs, were riddled with tubercles. However, heart muscle and kidney showed no tubercles. See figure 8.

Pig No. 6, on microscopic section, tuberculosis of a retroperitoneal lymph node and at the site of the intraperitoneal injection.

Pig No. 7 showed no gross or microscopic evidence of tuberculosis.

Summary of pig inoculations. Pig No. 1, inoculated with tuberculosis, received no treatment, and grossly and microscopically showed myriads of tubercles.

Pig No. 2, treated with streptomycin alone 2 weeks after inoculation with tuberculosis, showed grossly and microscopically no lesions.

Pigs Nos. 3 and 4, treated with streptomycin and chaulmoogra oil 2 weeks after inoculation, showed no evidence of tuberculosis.

Pig No. 5, treated with chaulmoogra oil alone 2 weeks after inoculation with tuberculosis, riddled with tubercles.

Pig No. 6, inoculated with tuberculosis, had no treatment. Autopsy 2 weeks later showed marked involvement.

Pig No. 7, inoculated with tuberculosis, was treated with chaulmoogra oil and streptomycin the same day, and showed no evidence of the disease, grossly or microscopically.

#### CLINICAL RESULTS

F, 24 years, married, was first seen on January 24, 1946 with a history of persistent loss of weight, marked frequency, urgency and nocturia 4-6 times; no history of hematuria. The patient was extremely nervous, excitable, weight 99 pounds. A catheterized specimen of urine was cloudy and loaded with white and red blood cells. Upon cystoscopy on January 25, 1946, there was positive evidence in the bladder of ulcerative tubercles and while the pyelogram disclosed no ulcerative lesions, direct smear from the bladder, right and left kidneys was positive for tuber-culosis, which was also confirmed by guinea pig inoculation. She was put on a rest cure in a private sanatorium until February 3, 1947.

Re-examination again disclosed positive urines from both kidneys and bladder although her weight had increased 60 pounds. She was put on combined treatment of chaulmoogra oil and streptomycin for 30 days. On April 3, 1947 she was symptom free with no nocturia over a period of 8 hours, urine negative, bacteriologically and by guinea pig inoculation. See figure 9, A.

M, 37 years, was first seen October 20, 1946 with frequency every half hour, nocturia, perineal pain and swollen left testicle, and loss of 20 pounds in weight. Examination disclosed a typical beaded left epididymitis and nodular prostate. The urines were positive on direct smear. On October 27, 1946, a left epididymectomy was performed. Cystoscopy was not done because of the presence of the active lesion in the prostate, and the epididymitis. Only intravenous urograms were made. On November 20, 1946, guinea pigs were reported positive for tuberculosis. The patient's health improved after the epididymectomy. He was not put on a rest cure but his urine never cleared up. On May 10, 1947, because of nocturia, the patient was hospitalized and put on Moogrol<sup>4</sup> and streptomycin for 1 month. At the end of 30 days the frequency had diminished to 4 hours, nocturia once, and on Petragnini culture of the urine July 15, 1947, reported negative. Direct smear, negative. See figure 9, B.

M, 20 years, since 1939 had frequency, dysuria, and nocturia 4-5 times. He had never been examined properly. In 1943 he was accepted in the Army for active service in spite of his symptoms and discharged in October 1946. Because of his complaints of nocturia on July 15, 1946, and pain in his right testicle, he was examined. A diagnosis was made of tuberculosis of the testicle which now disclosed a draining sinus. An orchidectomy was performed on November 19, 1946 and the left vas was tied. The urine was positive on direct smear. The prostate showed no activity. On November 26, 1946, cystoscopy was done. The pyelograms disclosed no caseous changes with plugged catheters, but tubercle bacilli were recovered from each side and confirmed

<sup>&</sup>lt;sup>4</sup> Ethyl ester of hydnocarpus oil.

by pigs a few weeks later. On December 1, 1946, he was again hospitalized, received 1 month's treatment of chaulmoogra oil and streptomycin. The symptoms disappeared promptly, nocturia diminished to twice nightly, and on January 8, 1947, the urine showed only occasional scattered tubercles. Treatment was repeated with Moogrol and streptomycin on April 4, 1947. On May 20, 1947, when re-examined, he was voiding every 4 hours during the day, once a night. A direct smear on urine was negative and guinea pigs injected were reported negative on May 20, 1947. See figure 9, C.

F, 26 years, was first seen November 27, 1946 with a history of progressive loss of weight, frequency, urgency and nocturia of 1 month's duration, and pain in the right lumbar area. A catheterized specimen of urine disclosed many red blood cells, white cells in clumps, and no tubercle bacilli on direct smear. Bilateral pyelograms disclosed no renal destruction and smears from both kidneys were positive. The bladder urine was again negative. Guinea pigs were reported on January 15, 1947 to be positive from both kidneys. She was then placed on chaulmoogra oil and streptomycin for 1 month, with prompt alleviation of her symptoms and a gain in weight. On April 14, 1947 she was re-examined. She had no nocturia or frequency. Guinea pig inoculations on June 5, 1947 were negative. See figure 9, D.

F, 29 years, a single Nisei nurse, 3 years ago found to have tuberculosis of the chest with effusion, and was treated by collapse of the right lung. In April 1946, hematuria developed and marked urinary symptoms. Cystoscopy disclosed a "frozen" left ureter. Bilateral renal tuberculosis was reported. The patient was in Chicago at the time, and has been on rest cure ever since. She was seen by me in Buffalo, May 21, 1947 complaining of frequency every 2 hours, and nocturia 3-4 times. Cystoscopy revealed active tuberculous ulceration of the bladder. Pyelograms disclosed considerable dilatation of both ureters without active degeneration of the kidneys, which, by direct smears, were positive for tuberculosis, confirmed later by positive pigs. She was immediately placed on Moogrol and streptomycin for 1 month with complete alleviation of all her symptoms, and no nocturia up to 9 hours. On June 27, 1947, the urine was absolutely negative on gross examination. Cystoscopy disclosed a normal bladder with smooth clean mucous membrane. A specimen was taken for pig inoculation. On August 7, the pigs were sacrificed and autopsy disclosed no evidence of tuberculosis. See figure 10.

F, age 29, first seen on July 7, 1947. For the past 2 years she had had marked urinary distress, frequency every half hour day and night, urgency and tenesmus. She was finally examined by a urologist, who made a diagnosis of Hunner ulcer. Treatment over a period of 4 months did not alleviate her symptoms. At this time she noticed blood in the urine and pain in her right lumbar area. She continued to lose weight but was not examined until seen in this office. There was no history of pulmonary disease or productive cough of any type. The patient's history was entirely negative of tuberculosis. Cystoscopy on July 8, 1947 showed that the capacity of the bladder was diminished to 4 ounces and the entire mucous membrane was a mass of ulcerated tubercles. The direct smear of rapidly spun sediment showed tubercle bacilli. The ureters were buried in this tuberculous ulcerated mass, and could not be catheterized. Intravenous urograms made with Diodrast were most unsatisfactory, showing poor renal concentration and output. Undoubtedly, there was present bilateral involvement. She was immediately hospitalized and put on Moogrol and streptomycin. Within 2 weeks her symptoms had improved to such an extent that she insisted upon being discharged from the hospital. Cystoscopy disclosed that the bladder was completely healed except for some ulceration around the ureteral areas which still could not be catheterized. The patient is to be watched at home and further treatment administered, but her improvement within 2 weeks has been so satisfactory that she refuses further treatment.

Therapy. The first 3 cases were treated with the regular oil of chaulmoogra, which is a fatty oil expressed from the seeds of Taraktogenos Kurzi. Later, however, it was noted by the Burroughs Wellcome Company, which manufactures the present product, that a more refined oil, which is painless, more readily absorbable and non-irritating, could be derived from the ethyl ester hydnocarpus oil which is the preparation which is now being used under the trade name of Moogrol.<sup>5</sup>

Each patient received 1 cc. of oil intramuscularly daily for 3 days, then increased to 2 cc. for 4 days. On the seventh day, the patient received 1 cc. of the oil and 1 gm. of streptomycin for 30 days. The antibiotic is added to 16 cc. distilled water or saline solution and administered in 8 doses of 2 cc. each, every three hours. All patients were hospitalized but ambulatory.

<sup>&</sup>lt;sup>5</sup>Moogrol was kindly supplied by the Burroughs Wellcome Co.

#### SUMMARY

The Mycobacterium tuberculosis has a protective cell wall which can be definitely dissolved, we believe, and proven clinically, by the use of esters of chaulmoogra oil. At the present time the more refined preparation of Moogrol is recommended because it is painless, more concentrated and stable. The clinical result of this "softening and conditioning process" over a 30 day period warrant further investigation, not only in the urological tract, but in all types of tuberculosis and especially where there is available an electron microscope to determine the effect of the dissolution on the waxy cell wall of the Mycobacterium tuberculosis. While the present series of cases is small, this study is preliminary and has only prevailed since early 1947, but the in vitro studies, the animal inoculation and the clinical results have been so striking as compared to prolonged hygienic, sanatoria or routine tuberculosis regime as to warrant publication and further investigation.

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