

EVALUATION OF ANIMAL INOCULATION TEST IN GENITAL TUBERCULOSIS

by

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It is very essential to establish the diagnosis of tuberculous endometritis before it ends up in permanent amenorrhoea and to start proper anti-tubercular treatment. The diagnosis of female genital tuberculosis is notoriously difficult and a great deal of attention has been paid to this question in recent years. Because of markedly varying manifestations and difficulties in diagnosis this problem has created considerable interest in the medical field. It is now generally agreed that most helpful measures in the diagnosis of female genital tuberculosis are bacteriological investigations and histology of the endometrium.

Material and Method

The cases studied in the present series belong to three groups — (1) those who had genital tuberculosis histologically proved, (2) those suspected to have genital tuberculosis with proved extra-genital tuberculous lesions, and (3) those in whom tuberculosis was strongly suspected on clinical examination.

It is generally agreed that the genital lesion is secondary to another focus, so special attention was given

to the family history of tuberculosis or contacts. Routine general examination as also systemic and pelvic examinations were done in each case. The special investigations done were total leucocyte count, differential counts, E.S.R., Hb.% and screening of chest. Wherever it was possible multiple strip endometrial biopsy was taken or the patient was subjected to curettage, specially in amenorrhoea group, as endometrial tissue in the latter group is usually difficult to obtain with an endometrial biopsy curettage.

The endometrium obtained was examined histologically and bacteriologically by guinea-pig inoculation and culture. The endometrium for bacteriological investigation was collected in autoclaved saline in two bottles — one for culture and another for guinea-pig inoculation. In the first 20 cases the method used was — crushing the endometrium in normal saline, centrifuging it with 2,000 revolutions per minute for 20 minutes and injecting the deposit intramuscularly in the medial aspect of the thigh near the inguinal glands of the tuberculin tested animal. A slide of the deposit was also prepared and stained with Zeihl-Neilsen's stain. Tuberculin test was done in guinea-pigs after 6 weeks of intramuscular injection and if negative the animal

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was killed after 6 weeks i.e. total 3 months. Any enlargement of regional or lumbar lymph nodes was noted, and the spleen, liver and lungs were carefully inspected for any enlargement or tubercle. If there were any lesions the organs were sent for histological examination to prove the tuberculous nature of the lesion. Culture was done on Lowenstein-Jensen media the results of which were read after 6 weeks.

As none of the guinea-pigs showed any positive lesions, though out of 20 endometria 7 were histologically proved tuberculous endometritis, another method was resorted to. In 5 cases endometrial tissue was directly implanted into the peritoneal cavity. As all the guinea-pigs died due to herniation of the intestines from the abdominal wound within 24 to 48 hours the method was abandoned. The next method that was used in 12 cases was directly placing the endometrium in the medial aspect of the thigh subcutaneously near the inguinal region, through a small incision, which was sutured with 2 or 3 stitches.

Table I shows the diagnosis of the cases selected for the trial. Those cases which were proved histologically as tuberculous endometritis, but had not received treatment for more than 3 months were included. Only one case of tuberculous endometritis (case of primary sterility), who had a full course of anti-tubercular treatment and had come for a check up, was included in this group. The endometrial report was secretory phase in this case.

In 5 cases no tissue was seen histologically. They were all from an amenorrhoea group. Only in 9 cases was the diagnosis of tuberculous endometritis confirmed on histological examination, and there was one case of tuberculous ulcer of the vulva. In the rest of the cases the histological reports were proliferative phase in 13, secretory phase in 3, metropathia in 2 and inflammatory reaction in 2.

It is interesting to note from Table II that 3 cases of abdominal tuberculosis had tuberculous endometritis. One case was diagnosed as tuberculous abdomen with subacute

TABLE I
Diagnosis of the cases, histological report and culture

Diagnosis	No. of cases	T.B. endometritis (Histological report)	AFB cultures (Positive)
T.O. mass	2	—	—
T.B. ulcer of vulva	1	—	—
T.B. cervicitis	2	2	—
T.B. abdomen	1	1	—
Primary sterility (old case of T.B. endometritis)	1	—	—
Secondary amenorrhoea	22	5	4
T.B. abdomen with Pott's spine	1	1	—
Primary amenorrhoea	1	—	—
Puberty menorrhagia	6	—	1

TABLE II
Type of extragenital tuberculosis

Extragenital T.B.	No. of cases	T.B. endometritis
Abdominal	4	3
Pulmonary (old healed lesion)	1	—
T.B. lymphadenitis	2	—

intestinal obstruction, another was bilateral pulmonary tuberculosis with encysted tuberculous peritonitis, tuberculous lymphadenitis and Pott's spine. These two cases had generalised manifestations of the disease with tuberculous endometritis. Two other cases of tuberculous abdomen were proved after laparotomy. One was a case of primary amenorrhoea where no endometrial tissue was obtained for histological examination and the other was secondary amenorrhoea with tuberculous endometritis; both had enlarged mesenteric glands, proved tuberculous after biopsy.

Table III shows that out of 5 positive cultures 3 endometria were histologically positive for tuberculosis. Out of 9 histologically proved positive cases only 3 showed positive culture and 6 were negative. Four of the cases, where culture was positive, were cases of secondary amenor-

TABLE III
Culture of endometrium and histological report on positive culture

Culture for mycobacterium tuberculosis	No. of cases	Histological report of endometrium
Positive	5	T.B. endometritis 3 No endometrium 1 Proliferative phase 1
Negative	32	

rhoea and one of puberty menorrhagia (Table I). It is interesting to note that out of 5 positive cultures, two endometria did not have any evidence of tuberculosis histologically.

Out of 37 guinea-pigs, 5 died within 24 to 48 hours after intraperitoneal implantation of the endometrium. On sacrificing the other guinea-pigs after 3 months, 2 showed tubercles on the spleen and one showed localised abscess over the thigh with regional lymph gland enlargement. On histological examination of these one of the spleens showed non-tuberculous abscess due to secondary infection and another spleen and regional lymph glands showed tuberculous lesions.

TABLE IV
Method of guinea-pig inoculation and tuberculous endometritis (histologically proved)

Method of guinea-pig inoculation	No. of cases	T.B. endometritis	Positive guinea-pig inoculation
Intramuscular injection in the thigh	20	6	nil
Implantation into the thigh subcutaneously	12	3	2

Discussion

The present study was carried out to evaluate the animal inoculation test in tuberculous endometritis. Different methods of inoculation were used, but none proved to be very helpful. In 9 histologically proved tuberculous endometritis none were positive for mycobacterium tuberculosis after Ziehl-Neilson's stain. Malkani (1959) also found this method disappointing. The presence of mycobacterium tuberculosis was demonstrated in 3 out of 140 cases of histologically proved endometrial tuberculosis.

Out of 9 histologically proved tuberculous endometritis only two of the guinea-pigs showed tuberculous lesions after inoculation. Five endometrial cultures were positive, three of which were histologically proved tuberculous endometritis; in one case of secondary amenorrhoea no endometrium could be seen histologically and another was a case of functional uterine haemorrhage where the report on endometrium was proliferative phase. In Sutherland's series (1952) out of 72 cases of histologically proved genital tuberculosis where guinea-pig inoculations were done, 43 showed positive results. Malkani (1959), out of 140 cases of histologically proved endometrial tuberculosis, found 13 cases positive for mycobacterium tuberculosis on culture and in 7 guinea-pig inoculations all were negative. Haines (1951) found 18 positive guinea-pig inoculations in 24 histological positive endometrial tuberculosis, but failed to find any evidence of tuberculous endometritis in 30 cases where it was suspected on clinical grounds. Millar

(1954) carried out guinea-pig inoculations in 33 cases of endometrial tuberculosis. He got positive evidence in 27 cases and negative in 6 cases where histology was positive twice. He found primary culture of the material most unreliable.

The aim of bacteriological investigation is to isolate mycobacterium tuberculosis by culture and animal inoculation and submit for typing of the strain and to test the sensitivity of the organism to the antibiotics. "Animal inoculation has been invaluable in checking the progress of patients. The method has obvious disadvantages: it requires biopsy or curettage and facilities for animal work. I have no personal data, but question the reliability of direct inoculation of culture media with endometrium" (Haines, 1952). "Bacteriologic studies are not very encouraging, but have a certain amount of value if supplemented with histologic studies" (Malkani, 1959).

Conclusions

It appears that the cheapest, easiest and quickest method of diagnosis of endometrial tuberculosis is by histological examination of the endometrium. Guinea-pig inoculation and culture for mycobacterium tuberculosis does not establish its superiority over histological examination. The only advantage is that drug resistance test can be done if the bacteriology is positive and appropriate anti-tubercular drugs can be given. The failure of bacteriological method in diagnosis of endometrial tuberculosis can be due to:

- (1) The endometrial strip that is

subjected to bacteriological investigation may not contain any mycobacterium tuberculosis.

(2) After implanting the endometrium in the thigh the tissue undergoes necrosis and mycobacterium tuberculosis cannot survive in such media and dies. Same is true in intramuscular injection where the endometrium after crushing and centrifuging becomes jelly-like material which is injected through a wide bore needle.

(3) In long standing cases of amenorrhoea it is difficult to get the endometrium even though it were primed by hormone therapy before the curettage and even when obtained was very scanty.

"It will be possible in a number of cases to make a diagnosis from a histological examination of the curettings, a saving of time which will favourably influence the treatment. We conclude that histological examination is as necessary as culture of the bacillus, for we have cases in which

culture was negative whereas the histological evidence was positive" (Berge, 1952).

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References

1. Berge, B. S.: J. Obst. & Gynec. Brit. Emp. 59: 736, 1952.
2. Haines, M.: Lancet. 1: 436, 1951.
3. Haines, M.: J. Obst. & Gynec. Brit. Emp. 59: 721, 1952.
4. Malkani, P. K.: Obst. & Gynec. 14: 600, 1959.
5. Millar, W. G.: J. Obst. & Gynec. Brit. Emp. 61: 372, 1954.
6. Sutherland, A. M.: J. Obst. & Gynec. Brit. Emp. 59: 738, 1952.