EXPERIMENTAL STUDIES ON THE DISTRIBUTION OF STREPTOMYCIN IN THE TUBERCULOUS FOCI OF BONE AND JOINT

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I. Introduction

Before the introduction of streptomycin, bone and joint tuberculosis was one of the most dangerous diseases for human being. M. C. Wilkinson[26] has stated that there were three important causes of death from bone and joint tuberculosis in pre-antibiotic days: that is, miliary tuberculosis, secondary septic infection and other complicated tuberculous lesions. J. Dobson[5] has mentioned that judging from his observation of 320 cases of hip joint tuberculosis, prognosis of patients with bone and joint tuberculosis becomes very serious when any tuberculous lesions develop into other organs. He has stated that miliary tuberculosis is the most common cause of death from bone and joint tuberculosis.

Since S. A. Waksman[22] has discovered streptomycin, causal treatments for tuberculosis have become possible. In the field of the treatment for bone and joint tuberculosis, the mortality was much decreased and various epochal treatments have been devised. In the former report[15] of the present author, the literature on the streptomycin treatment for bone and joint tuberculosis has been reviewed.

E. Kondo[13] has reported that the mortality of this disease has much decreased and the surgical treatment has become much safer with the use of streptomycin. R. Roaf[20] has also published that the surgical operation upon the tuberculous foci of bone and joint has become safer but he has suggested the occurrence of resistant strains in the caseous foci into which the penetration of the streptomycin was not enough to inhibit the bacilli. A. R. Allen[1] has described that the

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tuberculous foci can not be sterilized with streptomycin alone. F. Jansey[12] has expressed a similar opinion that streptomycin should not be used as a substitute for surgical cleansing. M. C. Wilkinson[27] has mentioned that streptomycin can not penetrate into the chronic tuberculous foci which are enclosed by fibrous tissue wall because of its avascularity, and that some surgical procedures to open a pathway are necessary for the drug to enter the foci. H. Stevenson[23] has insisted that in orthopaedic tuberculosis, diseased bone should be removed before the chemotherapy, if it exists. D. M. Bosworth[3] has the same opinion as H. Stevenson that surgical procedures are necessary in the treatment of bone tuberculosis, because streptomycin upon the closed foci is not so effective when it is used without surgery. The Public Health Service Co-operative Investigation[19] of the United States has expressed the same opinion as R. Roaf[21] and M. C. Wilkinson[27] that the drug can not penetrate into such foci based on the results of radiographic examination.

R. Harris[11], B. Mukopodadhaya[17] and E. T. Evans[6] have reported that the best results were obtained when surgical treatment and streptomycin were combined. R. K. Ghomley[8] has published that streptomycin is more effective when it is used with surgery than when it is used without surgery. M. S. DeRoy[4] has a similar opinion as Ghomley that the development of the closed bone foci can not be arrested by streptomycin alone and the surgical drainage is necessary with the drug.

These preceeding reports may be summarized as follows:

1) The mortality of bone and joint tuberculosis has been much decreased since the introduction of streptomycin.

2) The tuberculous foci themselves can not be cured by conservative treatment with the administration of the drug alone.

3) The most excellent results in the treatment for bone and joint tuberculosis have been obtained by the direct surgical procedure on the foci combined with the drug.

4) The direct operation has become much safer with the use of the streptomycin treatment.

The rapid decrease of mortality since the introduction of streptomycin may be explained by the prevention of the metastasis of the bacilli. It is clear that the danger of the bacilli dissemination increases when the focus is treated surgically, compared with the conservative treatment. Besides E. Kondo[13] and R. Roaf[20], J. A. Weinberg[25] and S. Orell[18] have reported that the surgical treatment becomes safer when the streptomycin is administered and the present author believes that these reports on surgical operation were originated from the

prevention of the bacilli dissemination by the drug. He[14] has assayed the streptomycin concentration in the circulating blood of seventeen cases of bone and joint tuberculosis at various intervals successively after the intramuscular administration of the drug. He has found that the effective concentrations of the streptomycin are maintained in the circulating blood during twelve hours after the administration of 250 or 500 milligrams of the drug.

The author[15] has also assayed the distribution of streptomycin in various pathological tissues of the foci: cold pus, granulation, caseous mass, sequestrum, scar, etc., which were removed from the foci of sixty-one cases of bone and joint tuberculosis at various intervals after the intramuscular administration. The results showed that in most cases streptomycin did not penetrate into such pathological tissues, above all dead ones.

Judging from these results streptomycin administered intramuscularly can inhibit the bacilli which have emigrated into the circulating blood to prevent the metastasis or dissemination both in conservative and surgical treatment. However, the foci themselves are not curable with streptomycin alone as mentioned above, because the sufficient concentration of the drug can not penetrate into the foci. Accordingly, the pathological tissues of the foci should be removed with surgery.

In these clinical experiments[14,15], the author had reservations about the results of low streptomycin concentration in the dead tissues of the foci by the following reasons. How does the dead tissue prevent the streptomycin from penetrating into the foci? Whether the dead tissues of the foci such as caseous mass destroy the chemical construction of the drug or adsorb the drug itself or the penetration of the drug is very poor[2,8,9]? In the former two cases, the exact assay of streptomycin concentration in the foci may not be done. Although the results of the clinical experiments[15] showed that the concentration in dead tissue was very low, a higher concentration of the drug might have penetrated into the foci. Following experiment was done to decide whether the caseous mass had any influences (chemical or physical) upon the streptomycin to decrease its titer or not.

II. Experiment

1. Material and Method

In this experiment caseous mass was used as a typical representative of the dead tissues, because it can be obtained from a cold abcess in a large quantity and in the same nature.

Cold pus was aspirated from the lesions of bone and joint tuberculosis of eleven cases in which no antibiotics had been administered for one week before

the aspiration, therefore, these materials were considered not to contain any antibiotics. One portion of each sample was homogenized, and the other was separated by centrifugation into the supernatant layer of pus serum and the sediment of caseous mass.

The method of the experiment were: A certain dosage of streptomycin was added to both cold pus and pus serum in a definite proportion. And then the concentration of the drug was assayed at definite times after the addition in order to examine the changes of the titer of the drug to answer the question of whether the caseous mass had any influence upon the streptomycin titer. The details are follows:

1) Four series of sterile test tubes were prepared for each aspirated sample, each series consisting of twelve tubes.

Series A: Homogenized cold pus was poured in the amount of 4.5 ml. into each tube. Therefore each of the tubes contained pus serum and caseous mass, all the elements of tuberculous cold pus.

Series B: The pus serum separated from the same sample as Series A was poured in 4.5 ml. into each tube.

Series C: M/15 sterile phosphate buffer solution at pH 7.8 was poured in 4.5 ml. into each tube.

Series D: Eleven tubes of this Series were made with serially diluted solutions of streptomycin ranging from 1,000γ per ml. in the first tube through 500, 250, 125, 63, 32, 16, and so on to 1γ per ml. in the eleventh tube dissolved in M/15 sterile phosphate buffer solution at pH 7.8. The twelfth tube contained the buffer solution alone.

2) 0.5 ml. of streptomycin solution from each tube of Series D was added to each tube of Series A, B and C, respectively, and then mixed by shaking. When the contents were mixed up and became homogenous, streptomycin concentration in the first tubes of Series A, B and C should be 100γ/ml. according to the following formula.

$$\frac{1{,}000\gamma \times 0.5}{4.5\,\text{ml} + 0.5\,\text{ml.}} = 100\gamma/\text{ml.}$$

In this way, the concentration in the second tubes should be 50γ per ml., and in the eleventh tubes of the three series, it sould be 0.1γ per ml. The first tubes of each Series were marked as A_1, B_1, and C_1; the second tubes, as A_2, B_2, and C_2, respectively, and in this way, the eleventh tubes were marked as A_{11}, B_{11} and C_{11}. The twelfth tubes were used as controls to which streptomycin was not added.

3) As widely known, a cavity of a tuberculous abcess with cold pus has very poor metabolism. Therefore, the condition of the pus in the test tubes resembles that in a cavity of a cold abcess, if the test tubes are kept at human body temperature, 37°C. According to this idea, the test tubes of Series A and B were plugged and sealed with wax and were kept in an incubator for one and for twenty-four hours, so that the caseous mass acted upon the streptomycin added to it for these periods. Series C was also kept in the incubator for the same time and under the same conditions.

4) Thereafter, streptomycin concentration in each tube of Series A and B was assayed by the vertical diffusion method[7,16,24]. In Series A, the cold pus was centrifuged and then the assay was done on the pus serum and in Series B, the assay was done on the pus serum. Series C was used as a standard streptomycin series for the assay.

2. Results

If the streptomycin is destroyed or adsorbed by the dead tissue of caseous mass, the concentration in Series A should be lower than Series B, because the former Series contains caseous mass and the latter does not. If this is true, streptomycin concentration in Series A should be lower after twenty-four hours than after one hour, consequently the difference between the two series should become increasingly larger.

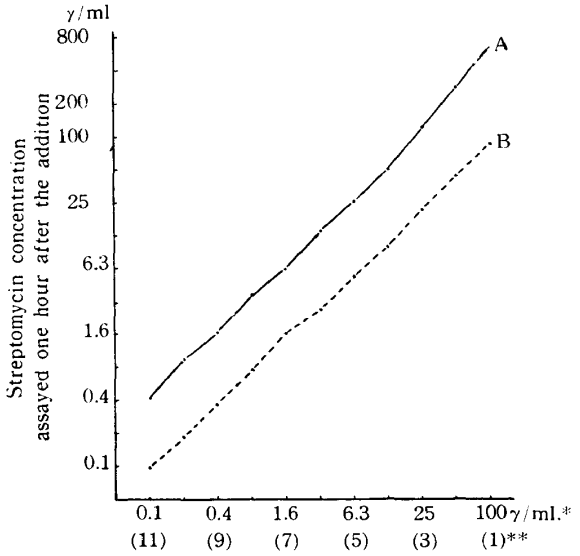
From the results of the assay, it became evident unexpectedly that the concentration in Series A was higher than in Series B, and the difference between the two series after twenty-four hours was less than that after one hour.

In the following figures, logarithmic scales were used to demonstrate the results; the assayed streptomycin concentrations were shown on the vertical axis and the number of tubes, namely the calculated concentrations were on the horizontal axis.

Streptomycin concentrations in Series A and B after one hour are shown in figures from 1 to 6. In all cases the concentrations in Series A were higher than in Series B.

The concentrations assayed after twenty-four hours are shown in figures from 7 to 15. In these cases, the differrence of the concentration between both series became less remarkable than those after one hour. Thus, the anticipated results of the experiment have been disproved. In the experiments shown in figures 4 and 12, 5 and 14, and 6 and 15, each pair represents cold pus obtained from a single patient at one time. Since a large quantity of cold pus was obtained in these three cases, the concentration after one hour and after twenty-four hours could be assayed and the comparison was made in the same material. The results are therefore believed to be more reliable.

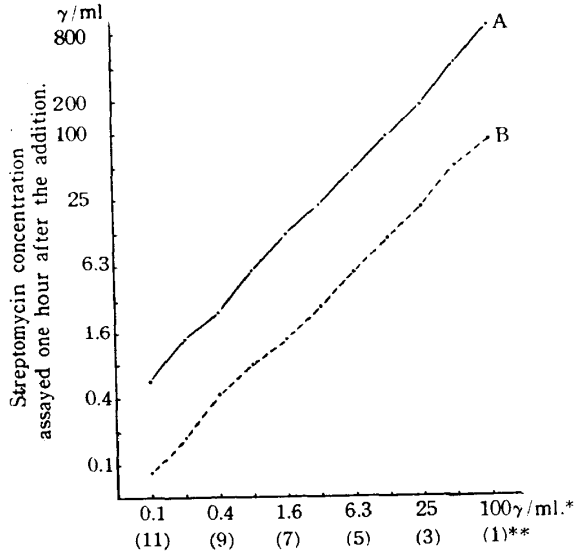
Fig. 1. Case 1., K.Z., 28 years-old man, with gravitation abscesses at the both iliac grooves from tuberculosis of the lumbar spine. The material of the experiment was obtained from the right iliac abcess.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.44	0.88	1.61	3.72	6.22	12.7	24.6	50.91	121.7	290.3	603.1
	B	0.1	0.19	0.36	0.7	1.59	2.71	5.2	10.12	20.9	43.7	91.2

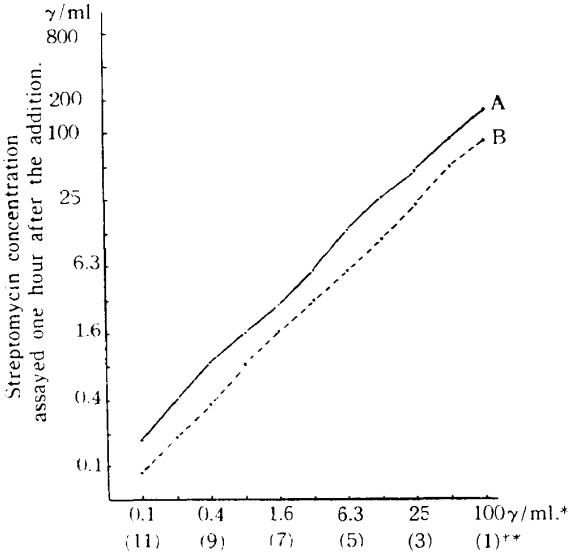
Fig. 2. Case 1., K.Z., 28 years-old man, with gravitation abscesses at the both iliac grooves from tuberculosis of the lumber spine. The material of the experiment was obtained from the right iliac abcess.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.57	1.4	2.79	5.96	12.5	22.2	48.1	92.2	197.0	420.2	912.1
	B	0.08	0.17	0.44	0.8	1.4	2.92	5.8	10.0	21.2	49.7	87.3

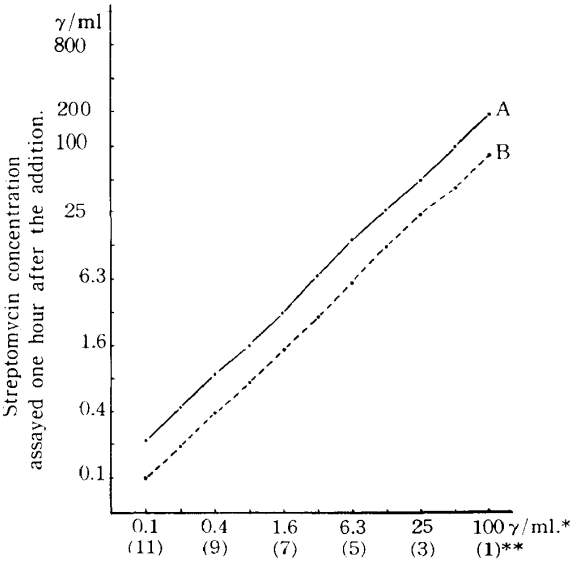
Fig. 3. Case 2., K.O., 52 years-old man, with a gravitation abcess at the right thigh from sacro-iliac joint tuberculosis.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.17	0.4	0.81	1.59	3.0	5.92	13.5	95.1	46.7	90.3	177.2
	B	0.09	0.19	0.37	0.89	1.7	3.2	5.91	11.12	23.1	50.7	97.2

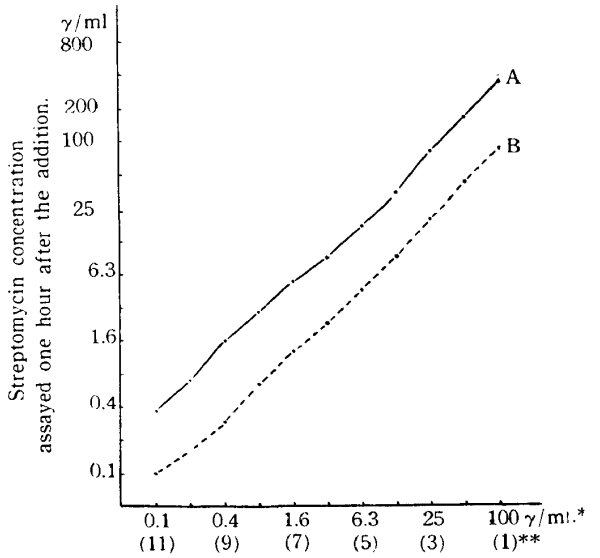
Fig. 4. Case 3., H.R., 30 years-old man, with a gravitation abcess at the left iliac groove from unknown origin.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.22	0.44	0.91	1.62	3.3	7.11	14.2	27.12	49.6	99.2	189.5
	B	0.11	0.19	0.39	0.78	1.5	2.95	6.0	12.3	24.2	44.2	87.7

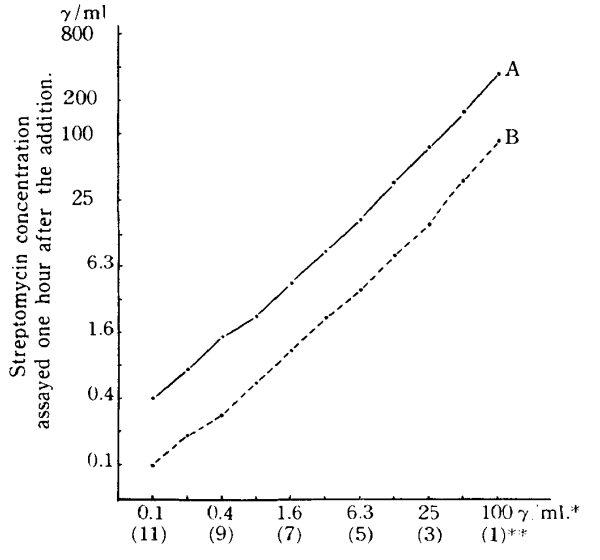
Fig. 5. Case 4., K.B., 32 years-old man, with a gravitation abcess at the left iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.36	0.76	1.57	2.9	5.2	9.01	18.4	37.3	78.2	165.3	379.0
	B	0.1	0.16	0.29	0.6	1.31	2.32	4.8	9.0	21.12	44.3	90.19

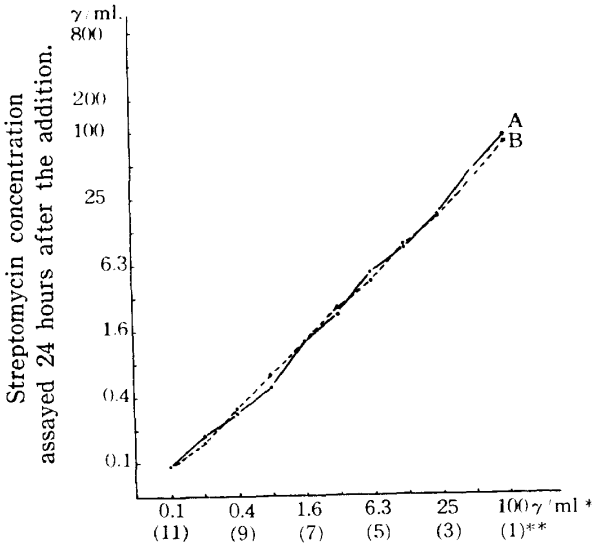
Fig. 6. Case 5., N.H., 37 years-old man, with a gravitation abcess at the right iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.38	0.7	1.37	2.3	4.61	9.0	18.21	32.12	78.3	165.7	367.7
	B	0.1	0.18	0.29	0.54	1.1	2.4	3.67	7.9	15.22	40.0	85.3

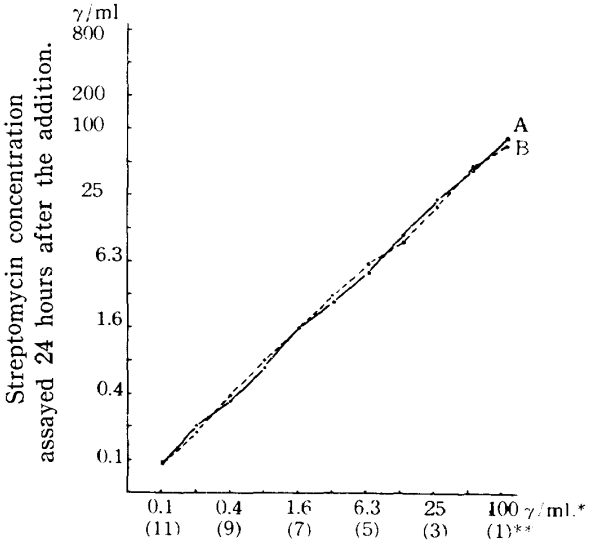
Fig. 7. Case 6., F.J., 22 years-old man, with a gravitation abcess at the right iliac groove from tuberculosis of the pelvis.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.11	0.18	0.29	0.5	1.35	2.51	5.2	9.2	17.9	42.2	92.7
	B	0.1	0.15	0.3	0.6	1.35	2.7	4.9	9.51	18.2	34.9	85.0

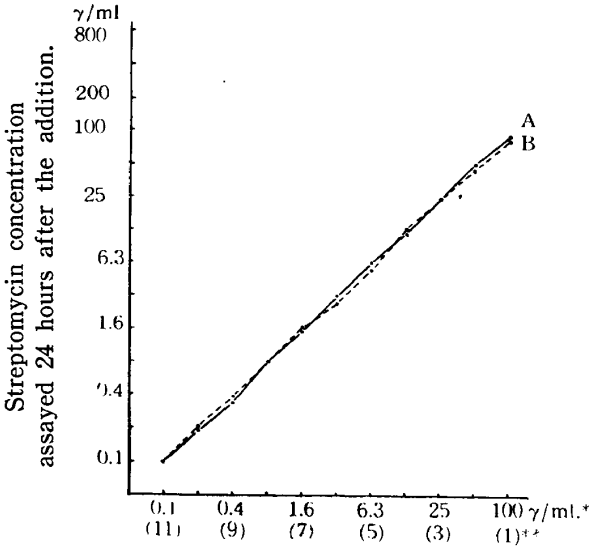
Fig. 8. Case 7., H.S., 27 years-old man, with a gravitation abcess at the right thigh from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.095	0.2	0.39	0.71	1.6	2.9	6.21	11.97	25.2	47.5	92.71
	B	0.097	0.18	0.4	0.8	1.57	3.2	5.4	10.5	22.9	50.0	89.2

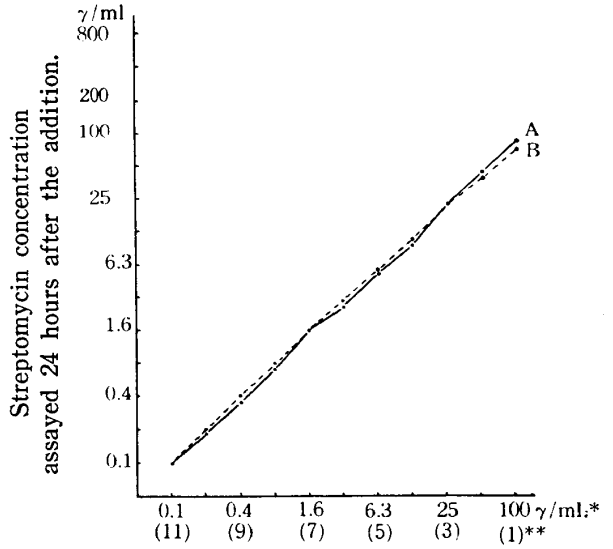
Fig. 9. Case 8., K.M., 24 years-old man, with a gravitation abcess at the right iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.18	0.34	0.8	1.49	3.2	6.27	11.99	24.2	50.0	92.5
	B	0.1	0.2	0.38	0.8	1.6	2.9	5.6	12.5	25.0	46.7	88.7

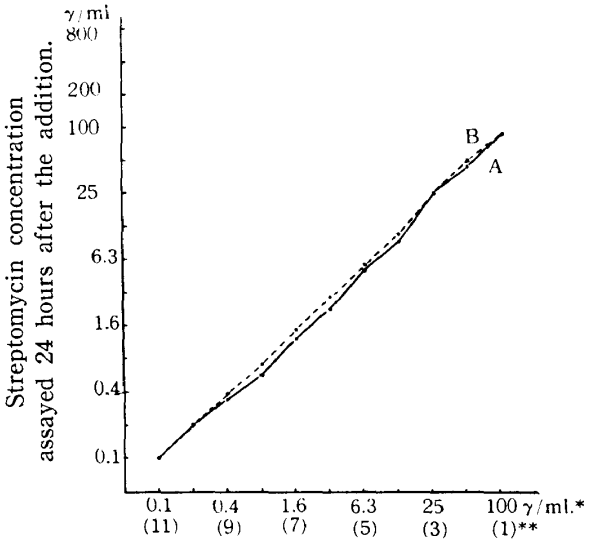
Fig. 10. Case 9., K.Y., 50 years-old woman, with a gravitation abcess at the right low back from tuberculosis of the thoracic spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.18	0.34	0.7	1.6	2.8	5.41	9.5	22.0	45.7	92.0
	B	0.097	0.2	0.4	0.77	1.52	3.0	5.67	10.06	21.7	44.0	81.73

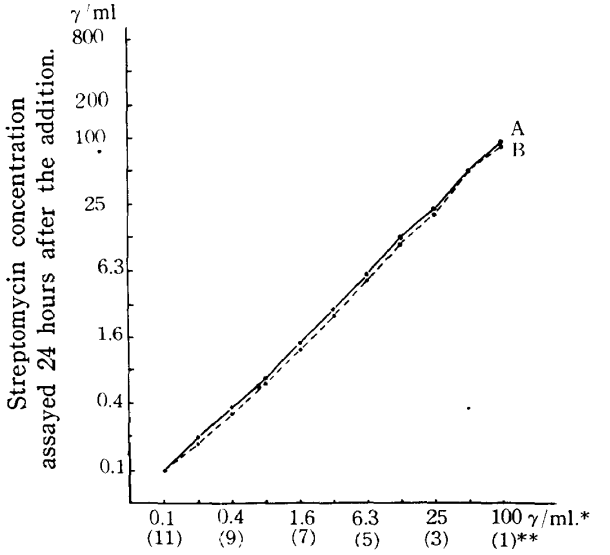
Fig. 11. Case 10., G.M., 42 years-old woman, with a gravitation abcess at the left thigh from tuberculosis of the left hip joint.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.19	0.34	0.56	1.22	2.4	5.4	9.7	24.71	46.8	93.7
	B	0.1	0.2	0.39	0.7	1.4	2.71	5.7	11.0	23.9	50.0	92.0

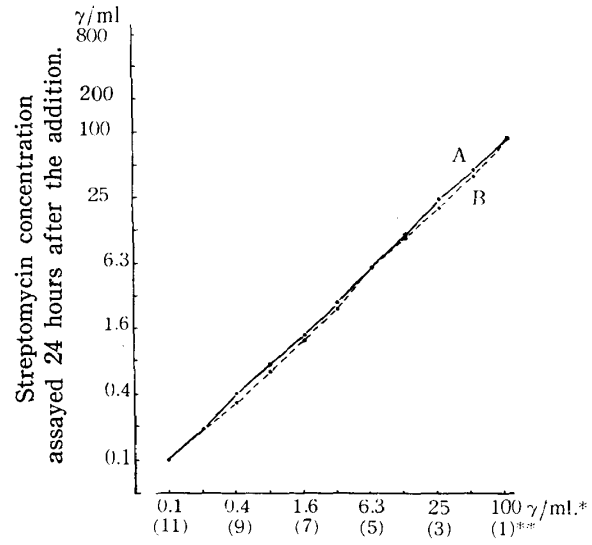
Fig. 12. Case 3., H.R., 30 years-old man, with a gravitation abcess at the left iliac groove from unknown origin.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.2	0.38	0.72	1.48	3.0	6.05	12.3	24.0	50.0	97.5
	B	0.1	0.18	0.36	0.71	1.4	2.85	5.8	11.7	22.73	50.0	89.7

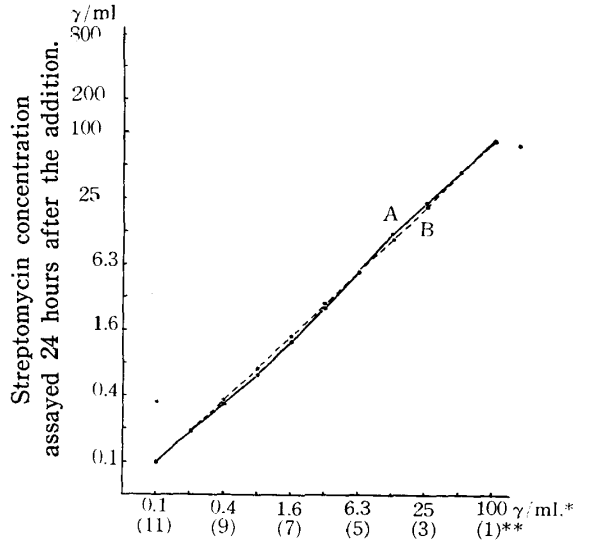
Fig. 13. Case 11., H.H., 39 years-old woman, with a gravitation abcess at the right iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.19	0.39	0.75	1.4	2.9	5.92	11.6	25.0	48.7	930
	B	0.1	0.18	0.35	0.64	1.29	2.81	5.87	11.6	22.9	42.0	942

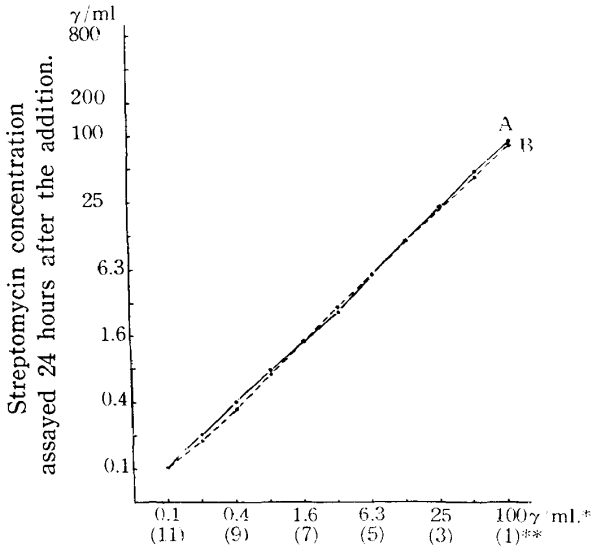
Fig. 14. Case 4., K.B., 32 years-old man, with a gravitation abcess at the left iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.19	0.37	0.67	1.2	2.52	5.6	12.5	24.06	47.5	92.37
	B	0.094	0.19	0.381	0.74	1.48	3.0	5.6	11.5	22.7	48.1	92.26

Fig. 15. Case 5., N.H., 37 years-old man, with a gravitation abcess at the right iliac groove from tuberculosis of the lumbar spine.



* The concentration calculated at the addition.
** (Number of test tubes)

No. of tubes		11	10	9	8	7	6	5	4	3	2	1
The concentration assayed (γ/ml)	A	0.1	0.2	0.39	0.78	1.45	2.85	5.91	11.56	23.0	50.0	96.2
	B	0.1	0.18	0.36	0.73	1.45	2.9	5.9	11.5	23.0	47.0	94.6

III. Discussion

The reason why the concentration of streptomycin in Series A is higher than that of Series B after one hour might be explained that the penetration velocity of streptomycin into caseous mass is very slow and the majority of the drug might still have remained in the pus serum in the test tubes of Series A, while the drug itself can neither be destroyed nor adsorbed by the tuberculous dead tissue of caseous mass. In the pus serum of Series B, the streptomycin had been dispersed throughout the larger volume of pus serum.

With lapse of time, the quantity of streptomycin penetrating into the caseous mass might have increased. After twenty-four hours, the drug became evenly distributed in each tube of Series A. Therefore the concentration in the separated pus serum in Series A approached that of Series B, in which the distribution had been homogenous from the beginning of the incubation.

In other words, from the results of this experiment, the hypothesis about the difficulty of streptomycin penetration into caseous dead tissue was verified *in vitro*. It was also proved that the difficulty of penetration was not caused by a chemical reaction of the caseous mass.

Moreover, in this experiment, the cold pus was homogenized in order to provide each test tubes of Series A with material of uniform quality. Accordingly, penetration of the drug into the caseous mass of the focus in human body might be more difficult.

The results of this experiment support those of the clinical experiment[15] in which the bacteriostatic concentration of the drug was not found in the dead tissues of the tuberculous foci. Thus, the difficulty of streptomycin penetration into the dead tissues has been demonstrated *in vivo* and *in vitro*. Namely, it may be said that besides bacteriostatic action, an essential requirement for a tuberculostatic is the ability for the drug to penetrate into the tissues of the focus.

Recently, various tuberculostatics other than streptomycin have been introduced. The penetrating ability of these drugs can be tested and compared with each other by the method mentioned above: the time which is needed for the concentration of Series A to approach and correspond to Series B can be compared if the assay is done with same cold pus for each drug.

The results of such experiments will be reported in the next paper.

IV. Conclusion

As published in the previous report[15], the considerable concentration of streptomycin was sometimes found in pus serum of the foci when the drug was administered intramuscularly. Of course, the higher concentration of streptomycin would be found in the pus serum if the drug is administered directly through injection into the cold abscess. But it seems difficult for streptomycin to penetrate into caseous mass or dead tissue of the foci, sufficiently to inhibit the bacilli, even if considerable concentration were maintained in the pus serum, either by intramuscular injection or by injection into the foci.

This opinion has been supported by the experiment *in vitro*. Accordingly, it seems difficult for the drug to act upon the bacilli surrounded by caseous mass, sequestra, and other dead tissues of the foci.

These results in the experiment *in vitro* also suggest the limitation of the conservative treatment for bone and joint tuberculosis, which has been discussed in the clinical experiment[15].

From the results in experiments both *in vivo* and *in vitro*, it has become evident that debridement of the foci, removal of dead tissue, is necessary so that the caseous foci of bone and joint can be completely healed in a shorter period of time. These surgical procedures should be performed as completely as possible so that no citadels of tubercle bacilli which can not be attacked with streptomycin alone will remain.

In former days, one of the most important problems and the most difficult obstacles to overcome was that of finding a drug which could penetrate into the wax capsule of tubercle bacilli. After the introduction of streptomycin, it has become possible for the bacilli to be attacked directly. The most difficult hindrance seems not to penetrate into the wax capsule, but to penetrate the fibrous wall, necrotic substance, and caseous dead tissues surrounding the foci.

These results also suggest that the penetration ability of antibiotics is one of the most important factors in chemotherapy, above all for tuberculosis. Under certain circumstances, the ability of a drug to penetrate is more important than chemotherapeutic index itself. However effective the bacteriortatic action of the drug may be, it can not act upon the bacilli, unless enough concentration of the antibiotic penetrates into foci to inhibit them. This condition seems more important in chronic tuberculosis than in other diseases because in chronic tuberculisis the foci contain caseous mass and dead tissues which are separated from general circulation, whether the foci are extra-pulmonary or not. Nevertheless, the principal object in studies on antibiotics so far, including tuberculostatics, has been confined to the chemotherapeutic index alone. Penetration of the drug into foci has been studied in an animal or a human body previously administered with the drug.

On the contrary, by the application of the present mothod, measurement of the penetration ability of several tuberculostatics into caseous foci has now become possible *in vitro*, whether the drug is used at present or may be discovered in the future. Accordingly, it is desirable that this study should be examined, estimated, and utilized by many researchers who are studying tuberculosis and antibiotics.

V. Summary

To explain the low concentration of streptomycin in the foci of bone and joint tuberculosis examined by the vertical diffusion method, above all in the dead tissues, the author did an experiment *in vitro*. The experiment was carried out with cold pus and pus serum each of which streptomycin was added to. The effect of the caseous mass contained in the cold pus upon streptomycin was examined using the pus serum as a control. The results of this experiment show that the streptomycin is neither destroyed nor adsorbed by the caseous mass, while the penetration ability of the drug into caseous mass is very poor.

Accordingly, the accuracy of the vertical diffusion method for the assay of streptomycin concentration in the dead tissue of the tuberculous foci of bone and joint is supported by this experiment.

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