# THE ACTION OF « PENBRITIN » ON Brucella MICRO-ORGANISMS

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A report (Rolinson and Stevens, 1961) on 6 D (—)  $\approx$  aminophenylacetamido penicillanic acid  $\ll$  Penbritin  $\gg$  and its action on Gramnegative bacteria made us think it would be useful to test its activity on Brucella micro-organisms. This was done on 8 strains of *Brucella melitensis* all recently isolated in Malta, 5 of human and 3 of caprine origin. Parallel tests were also carried out with tetracycline hydrochloride and with chloramphenicol. Penicillin G was known to have little or no action on *Brucella* but was also tested for the sake of comparison.

# Technique and Results

The technique adopted was to test the drugs at concentrations ranging from 0.009 ug/ml in sterile distilled water up to one of 10 ug/ml. Cultivation was carried out on Difco tryptose broth. Since Brucella can be a slow grower, to test the duration of the effect of the antibiotics, and, in the light of past experience with similar tests, it was thought a truer picture of the value of the antibiotic being tested compared with the value of others would be obtained if cultures were obsreved for a period of 10 days. The strains were first passed from tryptose agar on to tryptose broth. After 48 hours, these were used to seed in the proportion of 0.12 per 100 ml, bulk volumes of tryptose broth of a 10/9 strength, which were then distribued in 4.5 ml volumes in tubes. To each of these tubes 0.5 ml of the antibiotic concerned in the required concentration were added. Penbritin, supplied by Messrs. Beecham Research Laboratories, was only available in a preparation of 80 % purity (Robinson 1961). Since these melitensis strains did not, in controls, give perceptible growth after 24 hours incubation, inhibition was only considered to have occurred if no growth was apparent after 48 hours. Penbritin was in no case inhibitory at 1.25 ug/ml. At 2.5 ug/ml, it inhibited 3 strains for 2 days and 1 strain for 4; at 5 ug/ml it inhibited 8 strains

TABLE 1

Number of strains of Brucella melitensis inhibited by varying concentrations of Penbritin for varying times

Concentration in ug/ml	10	5	2.5
2 days	8	8	3
3 days	8	4	1
4 days	5	1	1
5 days	. 3	0	0
6 days	•1	0	0
7 days	1	0	0

for 2 days, 4 for 3 and 1 for 4 days; at 10 ug/ml it inhibited 8 strains for 3 days, 5 for 4, 3 for 5 and 1 for 7 days. These results are given in Table 1. The strain which was most inhibited was the one most recently isolated being on its second transplant. Pencillin G inhibited 3 strains only at 10 ug/ml for 2 days, but no longer. The inhibitory action of tetracycline hydrochloride and chloramphenicol is given in Tables 2 and 3.

A report (Stewart, Harrison and Holt, 1960) that « Celbenin » had proved active on some strains of *Haemophilus influenzae* induced us to include it in tests on 3 melitensis strains. On these it was completely inactive.

A strain of *Brucella abortus* and one of *Brucella suis*, both of which had long been in laboratory culture, were also tested. These showed growth after 24 hours and therefore a 24-hour culture was used to provide the inoculum and inhibition of growth for 24 hours was also considered. Both species appeared more sensitive to the antibiotics tested than the melitensis strains, although, of course, this could only refer to the strains examined. The effect of antibiotics on these are shown in Tables 4 and 5.

TABLE 2

Number of strains of Brucella melitensis inhibited
by varying concentrations of chloramphenicol for varying times

Concen- tration in ug/ml	10	. 5	2.5	1.25	.625	.312	.156	.078	.039	.019
2 days	_8_	8	_8_	5	4	0	0	0	0	0
3 days	_8_	. 8	7	4	4	0	0	0	0	0
4 days	8	8	6	0	0	0	0	0	0	0_
5 days	8	_7_	1	0	0	0	0	0	0	0_
6 days	8	7	1_	0	0	_0	0	0	0	0
7 days	_8_	_5_	0	0	0	0	0	0	0	0_
8 days	8	_5	0	0	0	0	0	0	0	0
9 days	8	2	0	0	0	0	0	0	0	0_
10 days	8	0	0	0	0	0	0	0	0	0

Table 3

Number of strains of Brucella melitensis inhibited by varying concentrations of tetracycline hydrochloride for varying times

Concentration in ug/ml	10	5	2.5	1.25	.625	.312	.156	.078	.039	.019	.009
2 days	88	88	8	_8_	_8	88	8	4	1	1	1
3 days	8	_8	8	_8_	8 -	7	_2_	0	0	0_	0
4 days	_8	88	_8_	_8_	7_	3_	0	0_	0_	0	0
5 days	_8_	88	7_	_6_	3_	0	0_	0	0	0_	0_
6 days	_8_	88_	_6_	_5_	1_	0_	0_	0_	0_	0_	0
7 days	_8_	8	3	1	0_	0_	0	0_	0	0_	0
8 days	8	8	0	0	0	0	0	0	0	0	_0_
9 days	8	6_	0	0	_0	0	0	0	0	0	_0_
10 days	8	6 -	0	0	0.	0	0	.0	0	0	-o

TABLE 4

Number of days for which Brucella abortus was inhibited by various antibiotics at varying concentrations

	Penbritin	Pencillin G	Tetracycline H Cl	Chloram- phenicol
10 ug/ml	6	2	10	10
5 ug/ml	2	1	10	10
2.5 ug/ml	2	1	7	6
1.25 ug/ml	1	1	4	2
.625 ug/ml	11	1	4	1
.312 ug/m1	1	11	4	1
.156 ug/ml	1	1	2	1
.078 ug/m1	1	1	1	1

TABLE 5

Number of days for which Brucella suis was inhibited by various antibiotics at varying concentrations

	Penbritin	Pencillin G	Tetracycline H Cl	Chloram- phenicol
10 ug/ml	10	10	10	10
5 ug/ml	10	10	10	10
2.5 ug/ml	6	4	10	10
1.25 ug/ml	4	2	10	4
.625 ug/ml	2	1	7	2
.312 ug/ml	1	1	4	1
.156 ug/ml	0	0	4	0
.078 ug/ml	0	0	2	0
.039 ug/ml	0	0	2	O
.019 ug/m1	0	0	1	0
.009 ug/ml	0	0	1	0

## Conclusions

Penbritin at varying concentrations appears definitely more active on Brucella strains than Penicillin G but less active than tetracycline hydrochloride and chloramphenicol.

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#### Résumé

La Penbritine se montre plus active à diverses concentrations sur des souches de Brucella que la pénicilline G, mais moins active que la tétracycline ou le chloramphénicol.

## Zusammenfassung

Penbritin in verschiedenen Konznetrazionen erscheint entschieden aktiver gegen Brucellastämme als Penicillin G, aber weniger wirkungsvoll als Tetracyclinhydrochlorid und Chloramphencol.

### REFERENCES

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