Culture Possibilities of *Mycobacteria* at 5°C

72 strains of 'Bacilles paratuberculeux' (so-called atypical *Mycobacteria*) have been inoculated. These strains came from very different sources, both regarding material from which the initial isolation was made and countries of origin (Table I).

Table I

Number of strains	Origin	Temperature and mean periods of growth (as already known)
6	Japan (Prof. UYEDA)	20–37° (15 days) 45° (35 days)
8	USSR (Dr. OGLOBINA)	30–37° (15–20 days) 6 of them grow at 45° (35 days)
5	France (Miss NOUFFLARD)	20–37° (15–20 days) 3 of them grow at 45° (35 days)
20	Holland (Prof. MANTEN)	20–37° (15–20 days) 11 of them grow at 45° (35 days)
8	Canada (Prof. MANKIEWITZ)	20–37° (15 to 20 days) 5 of them grow at 45° (35 days)
5	Malmö (Sweden) (Dr. JUHLIN)	20–37° (15–20 days) 45° (35 days)
6	Australia (Dr. KOVACS)	20–37° (15–20 days) 45° (35 days)
9	Iran (Dr. TABATABAÏ)	20–37° (30 days)
4	Belgium (Dr. VERSTRAETE)	20–37° (30 days)
1	Austria (Prof. Diemhofer) (*M. vaccae*)	20–37° (15–20 days)

All strains have been isolated from various pathological and non-pathological products: expectorations, gastric washes, lymphatic ganglia, residual water. In no case was there a *M. tuberculosis* var. *hominis*.

All have been inoculated with Löwenstein-Jensen and Dubois media, and kept at 5°C (±1°C). For results see Table II. These results show first of all the necessity of inoculating bacteria on different media if studies of temperature influence on growth are made. Should this precaution not be taken, results tend to be inaccurate.

Development of cultures at 5°C appears much later than growth at previously known temperatures (Table I). For 71 of the strains, growth started at 5°C after 30 days, while an abundant growth was only apparent after 50 or 60 days. A single strain, *M. vaccae* (Bönicke), gave abundant growth within 20 days.

Coloration by Gram and Ziehl methods for strains grown at 5°C shows no difference from the coloration for the same strains grown at higher temperatures. Serial reinoculations are also possible at 5°C.

Discussions on possible variations in biological characteristics for strains grown at 5°C will appear in later publications.

Table II

	Cultures	
	Positive at 5°C	Negative at 5°C
Dubos medium	59 (82%)	13 (18%)
Löwenstein-Jensen medium	51 (71%)	21 (29%)

Résumé. Les auteurs ont constaté que sur 72 souches de Myobactéries atypiques 82% d'entre elles pouvaient développer à 5°C. Les cultures à cette température commencent à être visibles vers le trentième jour et sont abondantes vers le cinquantième ou le soixantième jour.

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The Application of Fluorescent-Antibody Test to Cysts of *Entamoeba invadens*

The fluorescent-antibody test has been used extensively by GOLDMAN[1] to study the antigens of *Entamoeba*. Although in an initial study he had successfully applied the test to both the cysts and trophozoites of *Entamoeba histolytica*[2], most of his work is based on trophozoites as antigens.

This is a preliminary report into the study of the antigenic relationship of *Entamoeba* using cysts as antigens. The cysts, although not as readily available in cultures, have certain advantages over trophozoites. They can be stored for long periods at low temperatures and can be washed almost entirely free of extraneous antigens and bacteria without losing their viability.

The strain of *E. invadens* (BC) used in this study was obtained through the courtesy of Dr. E. W. MCCONNACHIE, Cambridge (England). This strain was originally monoxemic, growing with *Escherichia coli*. At present it is contaminated with at least one more bacterium. The strain encysts in large numbers when grown in Difco's Endamoeba medium[3], 5–6 days after inoculation.

To prepare the antigens, the cysts were washed three times in sterile distilled water by centrifugation and stored at 5°C overnight. This process was repeated daily

[1] M. GOLDMAN, Exp. Parasit. *10*, 366 (1960).
[2] M. GOLDMAN, Am. J. Hyg. *58*, 319 (1953).
[3] L. R. CLEVELAND and J. COLLIER, Am. J. Hyg. *12*, 606 (1930).