THE SENSITIVENESS OF TUBERCLE AND OTHER ACID-FAST BACILLI TO ACIDS.

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It was desired to determine the minimum amount of acid which would kill tubercle and other acid-fast bacilli, incidentally to note any distinction between human and bovine tubercle bacilli and between tubercle and other acid-fast bacilli in respect to sensitiveness to acid, and to find whether inorganic acid were more powerful in action than organic acid, and whether insoluble fatty acids were also effective.

Seven strains of human tubercle bacilli were used (H1, 20, 28, 67, 70, 79 and Arloing-Courmont strain), also four strains of bovine (B1, 4, 43 and 164) and five other acid-fast bacilli (Mist, Timothy Grass, Korn, Rabinowitsch and Smegma).

Method of Testing Sensitiveness to Acid.

In order to test the sensitiveness of these eleven tubercle strains, and the five other acid-fast bacilli to acid, standard solutions, $n/2$, $n/5$, $n/10$, were made up of sulphuric, acetic and citric acids. Corresponding solutions of stearic, palmitic and oleic acids in benzene were also made, of which, after being very slightly warmed to completely dissolve, 0·25 c.c. were measured out into small tubes, the benzene being evaporated off through the cotton wadding on a stove. Half-strength physiological saline solution 0·75 c.c. was then added (sometimes 0·25 c.c. of this saline was replaced by glycerin which, dissolving a trace of fat, can thereby act as carrier). The solid stearic acid and palmitic acid were then broken up into as small pieces as possible. This was done by drawing the mass over the glass surface of the tube above the saline, heating the glass and breaking the acid up with a strong needle. Similarly, 0·25 c.c. of the soluble acids, sulphuric, acetic and citric,

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was placed in tubes, 0·5 c.c. of saline solution added, and to all tubes 0·25 c.c. of bacillary emulsion. For bacilli other than tubercle 0·125 c.c. and 0·25 c.c. of $n/10$ acid only, was used. After 24 hours' contact at 37° C., and at room temperature, 4 drops of tubercle or 3 drops from the other bacillary-acid mixtures were taken and inoculated on egg-medium in the case of tubercle, or 3 % glycerin agar in the case of other bacilli. The results were strikingly uniform and showed a marked difference between tubercle and other acid-fast bacilli.

Table Illustrating Sensitiveness of Tubercle Bacilli.

											Acidity	Growth
1.	0·25 c.c.	$n/2$	H_2SO_4	+	0·5 c.c.	0·4 %	NaCl	+	0·25 c.c.	tubercle emulsion	$n/8$	0
2.	0·5 c.c.	$n/5$	,,	+	0·25 c.c.	,,	+	,,	,,		$n/10$	0
3.	0·75 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/13$	+ +
4.	0·25 c.c.	$n/5$	,,	+	0·5 c.c.	,,	+	,,	,,		$n/20$	+ +
5.	0·25 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/40$	+ +
6.	0·25 c.c.	$n/2$	acetic	+	,,	,,	+	,,	,,		$n/8$	0
7.	0·5 c.c.	$n/5$	,,	+	0·25 c.c.	,,	+	,,	,,		$n/10$	0
8.	0·75 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/13$	+ +
9.	0·25 c.c.	$n/5$	,,	+	0·5 c.c.	,,	+	,,	,,		$n/20$	+ +
10.	0·25 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/40$	+ +
11.	0·25 c.c.	$n/2$	citric	+	0·5 c.c.	,,	+	,,	,,		$n/8$	0
12.	0·5 c.c.	$n/5$	,,	+	0·25 c.c.	,,	+	,,	,,		$n/10$	0
13.	0·75 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/13$	+ +
14.	0·25 c.c.	$n/5$	,,	+	0·5 c.c.	,,	+	,,	,,		$n/20$	+ +
15.	0·25 c.c.	$n/10$	,,	+	,,	,,	+	,,	,,		$n/40$	+ +

The 11 strains of tubercle bacilli tested, were all killed in 24 hours in the presence of $n/10$ sulphuric, acetic and citric acids, but they were not killed in 24 hours in the presence of $n/13$.

These bacilli could also be killed by stearic, palmitic and oleic acids, if the solid acids were finely divided and sufficiently mixed with the bacillary emulsion. A curious phenomenon occurred when bacilli and these last three acids were mixed; a thick milky emulsion formed, in which the acid was prevented from separating out from the saline solution as it would otherwise have done. Bacteriolysis with these acids, though sometimes complete, was more often partial, depending as it did on the insoluble acid reaching the bacillus. No difference could be observed between bovine and human tubercle bacilli in their sensitiveness to acid.

Other Acid-fast Bacilli.

Method as before, 24 hours' contact.

		Acidity	Growth
1.	0·25 c.c. *n*/10 H_2SO_4 + 0·5 c.c. 0·4 % NaCl + 0·25 c.c. bacil. emulsion	*n*/40	0
2.	0·125 c.c. „ „ + 0·625 c.c. „ + „ „	*n*/80	0
3.	0·25 c.c. „ acetic + 0·5 c.c. „ + „ „	*n*/40	0
4.	0·125 c.c. „ „ + 0·625 c.c. „ + „ „	*n*/80	0 or trace
5.	0·25 c.c. „ citric + 0·5 c.c. „ + „ „	*n*/40	0
6.	0·125 c.c. „ „ + 0·625 c.c. „ + „ „	*n*/80	0 or trace

Compared to tubercle bacilli, other acid-fast bacilli are very much more sensitive to acid; they are killed in 24 hours in the presence of *n*/80 acid. On the other hand they seem less affected by insoluble fatty acids; carefully mixed with stearic, palmitic and oleic acids, the majority commonly survive.

Conclusions.

In sensitiveness to acid, both organic and inorganic acid, a very great difference between tubercle and other acid-fast bacilli was demonstrated. Tubercle bacilli were killed in 24 hours by *n*/10 acid, but could resist more dilute acid; other acid-fast bacilli were killed in *n*/80 acid. The Arloing-Courmont strain, although so different from a typically human bacillus, in cultural characteristics (rapid growth, not eating into media, soft, damp appearance), in emulsifying properties, and in its poor acid-fastness, large size (and non-pathogenicity), yet shewed its tubercular nature in this faculty of resistance to acid. Although apt to be partly decolourised by acid, presenting a beaded appearance, it was not killed by a weaker concentration of acid than *n*/10. No difference could be noticed between the action of inorganic and soluble organic acid upon these bacilli. Between soluble and insoluble fatty acid there was naturally some difference, the effect of the latter depending on its reaching the bacillus. If in a fine emulsion and shaken with the bacilli bacteriolysis did occur. Acid-fast bacilli, other than tubercle, although more sensitive to lower acids, were less affected by insoluble fatty acid. No difference in sensitiveness could be detected between human and bovine bacilli.

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