TABLE III.
Survival of Tubercle Bacilli in Solutions of Glycerol and Some of Its Derivatives.

Suspension of tubercle bacilli in	No. of colonies in subculture made after 2 wks	4 wks	6 wks	8 wks
6.0% glycerol	2	0	0	0
.1% glyceraldehyde	0	0	0	0
.1% dioxyacetone	0	0	0	0
.1% glycerophosphate	3	3	1	0
.1% phosphoglycerate	2	1	0	0
.1% glycerate	3	3	0	0
.1% lactate	2	1	0	0
.1% pyruvate	4	4	4	4
Distilled water	4	4	4	4

4—more than 100 colonies.
3—20-100 colonies.
2—5-20 colonies.
1—less than 5 colonies.

is maintained below 0.1%. At higher concentrations it inhibits growth. Tubercle bacilli multiply in pyruvate suspensions when Na-phosphate is present. Potassium and magnesium are not required as they are for the glycerol metabolism.

The formation of pyruvic acid in Long's culture medium during the growth of tubercle bacilli was demonstrated through the di-nitro phenylhydrazone. It is apparently an intermediary metabolite in the oxidation of glycerol by the tubercle bacillus.

Glyceraldehyde may be formed from glycerol by removal of 2 hydrogen atoms and pyruvic acid by the removal of 4 hydrogen atoms. Since pyruvate allows the tubercle bacilli to survive, it may well be that this attempted dehydrogenation is the direct cause of death for tubercle bacilli in glycerol water. A similar derangement of the oxidation mechanism may be responsible for the lack of survival of the tubercle bacillus under anaerobic conditions.

Summary. Tubercle bacilli die more or less rapidly in solutions in water of glycerol, glycerophosphate, phosphoglycerate, glycerate, lactate, glyceraldehyde, or dioxyacetone. Similar suspension in distilled water in solutions of inorganic salts and in pyruvate survive for a prolonged period.

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Growth Promotion of Bovine Tubercle Bacilli by Pyruvate.

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It has been shown in the preceding paper[1] that human tubercle bacilli die when suspended in a 6% glycerol solution while they remain viable for at least 8 weeks in distilled water or in a one-tenth of one per cent pyruvate solution. If phosphate is added to the pyruvate solution the tubercle bacilli multiply while a similar addition to the glycerol solution has no effect.

Glycerol is believed to be harmful for the growth of the bovine type of tubercle bacilli, and many strains of bovine tubercle bacilli grow very slowly or not at all on Long's medium. Addition of 0.1% pyruvate makes it suitable for the growth of the majority of the dysgonic type of bovine tubercle bacilli.

1 Boissevain, C. H., PROC. SOC. EXP. BIOL. AND MED., 1943, 54, 342.

Three strains of bovine tubercle bacilli were isolated on Feldman-Sweany medium from the liver of rabbits that had been injected with an emulsion of tuberculous calf lymph glands.* Transplants from Feldman-Sweany medium to Long's medium gave only very scanty growth and it was impossible to maintain these strains by serial transplants on Long's medium. Addition of 0.1% pyruvate enabled all 3 strains to grow well and even luxuriously on this medium. The pyruvic acid solution was brought to pH = 7.0 with N/1 NaOH, sterilized separately and then added to Long's medium. Addition of crystalline horse serum albumen[2] or of lipoid extract[3] previously described as growth factors for the human tubercle bacillus also enabled these bovine strains to grow on Long's medium. Pyruvate is however without effect on the growth of any of the 12 different human strains tested.

Three laboratory strains of "dysgonic" bovine tubercle bacilli were kindly supplied by Dr. H. J. Corper of the National Jewish Hospital, Denver. One of these strains grew fairly well on Long's medium but better after addition of pyruvate, the second did not grow on Long's medium unless pyruvate was added and the third strain did not grow on Long's medium, with or without pyruvate. Addition of horse serum albumen or lipoid extract had no effect on the growth of this last strain.

Summary. Three newly isolated strains of bovine tubercle bacilli that were unable to grow on Long's medium grew luxuriously on this medium after the addition of 0.1% pyruvate. Of 3 laboratory strains of "dysgonic" bovine tubercle bacilli, 2 showed a similar behavior. Addition of pyruvate had no effect on the growth of 12 human strains of tubercle bacilli.

* Obtained through the courtesy of Dr. C. L. Davis, U. S. Dept. of Agriculture, Bureau of Animal Industry.

2 Boissevain, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **44**, 110.

3 Boissevain, C. H., and Schultz, H. W., *Am. Rev. Tuberc.*, 1938, **38**, 624.