fresh soil gave the most brilliant test. Then a series was run as follows: check (synthetic solution), solution plus fresh soil, solution plus sterile soil and solution plus fresh soil. Three-tenths c.c. of nitrite culture was added to the first three. Fresh soil gave best results, sterilized soil not quite as good. Fresh soil wihout the culture gave a slight test after long incubation, showing that the beneficial result was not due to any nitrifying bacteria already in the soil.

It is evident that there is some substance present, in the bacteria that grow on nutrient agar, and in the soil itself, that stimulates these still impure cultures of the "nitrite" bacteria. It was thought that this substance might be in the nature of a vitamin. In order to test this out, to the synthetic solution, small amounts of the following were added: dried autolised yeast, dried yeast, yeast cake, alfalfa and soil. Two sets were run. One set was sterilized and the other not. It was found that all these substances, sterile or not, stimulated the "nitrite" bacteria. Larger amounts of nitrite were formed in all cases, in a given time, than in check. Since the above substances are rich in vitamin A and B, the writer feels there may be a definite relation between food assessory substances and the "nitrite" organisms.

## 149 (2109)

### Some temperature studies on B. acidophilus milk.

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Since the beneficial effects of *B. acidophilus* appear to depend upon a transformation of the intestinal flora, it follows that a mass inoculation is desirable. This means a maximum number of viable organisms per c.c. of pabulum. The usual recommendation on commercial preparations of fermented milk is that such milk be kept in a cool place preferably in the ice-box. Consequently, the influence of low temperature on the number of viable *B. acidophilus* in milk seemed worth investigating.

The number of viable *B. acidophilus* in milk held in the icebox at about 9° C. was determined daily. The viable organisms were killed as follows: after 1 day—about 50 per cent.; after 2 days—about 75 per cent.; after 3 days about 90 per cent., etc. The obvious importance of having a large number of viable organisms is manifested in the necessity for increasing dosage in severe cases. In fact, it is likely that in cases reported as failures, a sufficient increase in the number of viable organisms administered might have resulted in success. The practice of iceboxing *B. acidophilus* has little to recommend it beyond preserving the palatability of the culture and it is therefore more desirable to keep *B. acidophilus* at room temperature.

A study was made of the influence of time and pressure in autoclaving milk, prior to inoculation with *B. acidophilus,* with respect to the growth of the organism. Milk was sterilized at 15 and 20 pounds pressure in the autoclave for different periods of time and inoculated with *B. acidophilus.* Subcultures were made from each set of flasks for 3 days on milk identically sterilized, thus obtaining acclimatization. On the basis of these comparative tests it was found that milk to be used for inoculation with *B. acidophilus* should be sterilized in the autoclave at 20 pounds pressure for 20 minutes, or at 15 pounds pressure for 20 to 30 minutes. Under these conditions a maximum number of viable organisms is obtained after incubation.

It was necessary for certain experimental purposes to pasteurize *B. acidophilus* milk. By exposing *B. acidophilus* milk to different temperatures for different periods of time it was found that it could be completely sterilized in one litre portions in the Arnold steam sterilizer for 10 minutes (with a final temperature of 81° C.) In attempting to kill *B. acidophilus* in milk by freezing, it was found that after 6 days at about 3° C. the original number of viable organisms was reduced 99.95 per cent. This again emphasizes the drastic action of low temperature on this organism.