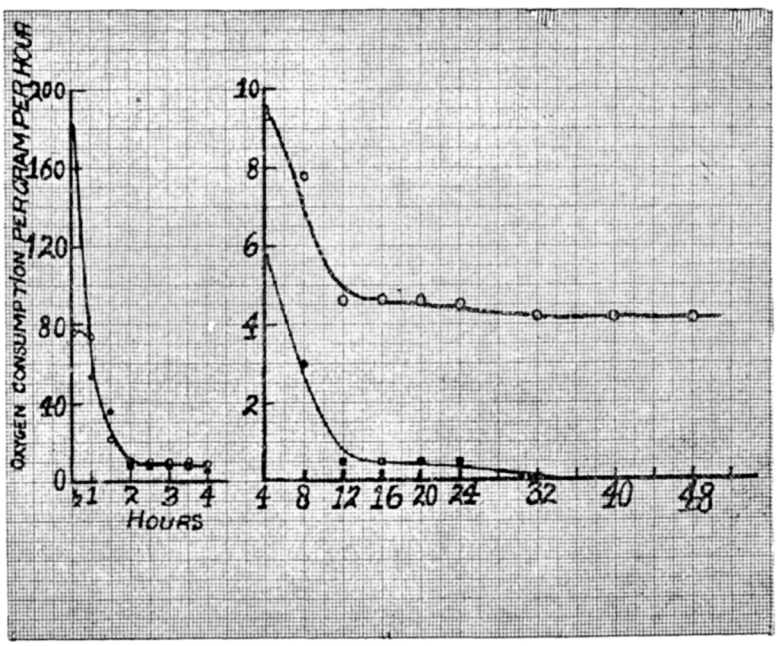

FIG. 1.

The effect of acidification upon gaseous exchanges of frog gastrocnemius muscle.
Ordinate—cu. mm. oxygen consumption per gm. of fresh weight per hour.
Abscissa—time in hours after acidification.
o oxygen consumption.
• carbon dioxide production.

the time at which carbon dioxide production ceases, but in all the terminal rate of oxygen consumption is surprisingly constant at 4 to 6 cu. mm. per gm. of fresh weight per hour for at least 76 hours. Boiling for one hour does not alter the character of the effect.

5711

Effect of Freezing on Microorganisms in Various Menstra.

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We showed[1] that prolonged action of freezing temperatures destroyed many yeasts and bacteria although with some species absolute sterility of the suspensions was not attained even after freezing at —15°C for 160 weeks. These investigations have been continued

[1] Tanner, F. W., and Williamson, B. W., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 377.

to determine the behavior of microorganisms in a large number of commercially frozen fruits and vegetables. Although the numbers of viable microorganisms have shown considerable reduction, there was no sterility even after storage at —16°C for about 2 years. Some 18 pure cultures of various microorganisms have been maintained in the frozen condition in 16 different menstra of varying hydrogen ion, salt and sugar concentrations as well as several fruit juices. To date the suspensions have been frozen for 19 months. In each case there has been a distinct decrease in the numbers of viable cells.

Escherichia coli, a short non-spore-forming rod from cherries, a similar organism from strawberries and the molds showed the greatest decrease in numbers. *Bacillus subtilis* and the spore former from strawberries showed the greatest resistance to prolonged freezing. The presence of acid seemed to have considerable effect on the destruction of bacteria by freezing. Microorganisms suspended in cherry juice and strawberry juice with a pH of about 3.8 and plain broth with a pH of 4.0 and 5.5 showed a greater decrease than in other menstra. While this same effect was noticeable with the yeasts and molds, it was not as pronounced as with the bacteria. Suspensions in strawberry and cherry juice, either sweetened or unsweetened, behaved very much like those in plain broth. Reactions on the alkaline side of neutrality were not as harmful as those on the acid side. High concentrations of sodium chloride seemed to cause a more rapid decrease in numbers of living cells especially in the higher concentrations used (6%). Suspensions frozen in distilled water and various concentrations of sugar showed very slow decrease. The molds died rather quickly but, after 16 months, there were still a very few living cells showing that storage at below freezing does not destroy all of the cells. Non-spore-forming bacteria, especially when frozen in an acid medium, were completely destroyed in from 5 to 10 months.

Alternate freezing and thawing was no more destructive to microorganisms than continuous freezing.

An attempt was also made to determine whether different degrees of cold might influence the death rate of pure cultures of microorganisms in distilled water and cherry juice. The suspensions were held at —16°C, —40°C, and —79°C. When the microorganisms were suspended in distilled water, there were no noticeable differences in death rates at the different temperatures. *Escherichia coli* in cherry juice showed slightly greater longevity at —79°C than at —40°C, and —16°C.

Experiments with *Clostridium botulinum* in several vegetables and a few fruits showed that spores of the organism survived freezing at —16°C for 14 months. The toxin also showed no decrease in toxicity when stored at —79°C for 2 months or at —16°C for 14 months. Vegetables to which detoxified spores were added before freezing at —14°C for 14 months, became toxic in from 3 to 6 days when allowed to thaw and stand at room temperature. This indicates that frozen fruits and vegetables must be considered as perishable products. They differ in this respect from sterilized canned foods. With frozen fruits, despite a pH which ordinarily prevents toxin formation by *Cl. botulinum,* toxin was formed in a few instances. Its formation may have been made possible by development of molds which altered the pH sufficiently to permit development of *Cl. botulinum.* The appearance of the foods in most of these cases would have indicated that they were abnormal due to incipient decomposition. Meyer and Gunnison[2] reported a similar situation with canned bartlett pears.

Experiments are in progress on the longevity of members of the colon-typhoid group in frozen cherries. When suspended in the clear juice and held at —14°C, the organisms died out in 2 weeks. However, when held at —16°C in the presence of both cherries and juice they remained viable for 5 months as proven by bacteriological and serological identification. However, after this time it has been possible to isolate organisms which culturally are like the organisms with which the experiment was started but they seem to have lost their ability to respond to agglutinin.

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Bone Marrow Volume in Rabbits.*

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Only a few papers pertaining to bone marrow volume can be found in medical literature. Töppich[1] determined the bone marrow volume

[2] Meyer, K. F., and Gunnison, J. B., *J. Inf. Dis.*, 1929, **45**, 147.

* Aided in part by a grant from the William W. Wellington Fund, Harvard Medical School.

[1] Töppich, G., *Arch. f. Anat.*, 1914, 9.