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.

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

or mass of 2 newly born infants and found that it was equivalent to approximately 2.3% of the body weight. He employed a method previously described by Wetzel,[2] in which the marrow volume was calculated from the dried weight of the skeleton, the total gross volume of the skeleton and the specific gravity of bone. As infant bone marrow consists practically entirely of red marrow, this figure represented the functioning mass. Wetzel[3] later reported a red marrow volume of 1419 cc. in the skeleton of a 20-year-old man. Mechanik[4] determined the marrow volume in 13 adult cadavers, using a method based upon the weights of the bones before and after maceration. The marrow mass varied from 1600 to 3700 gm., with an average figure of 2600 gm. He believed the red and yellow portions of the marrow were about equal, giving an average value of about 1300 gm. to the active portion, equivalent to approximately 2.3% of the body weight.

Since some idea of the bone marrow volume in rabbits was required in certain experimental work and since no figures could be found other than those relating to human marrow, 2 normal rabbits were killed and marrow volume determinations made with the results indicated below. The method used was essentially that employed by Töppich.[1]

The rabbits were killed by the intravenous injection of ether. The soft parts were cut away as cleanly as possible and the skeletons were covered with water and placed in an incubator (37.5°C). The water was changed twice a day. As many tendons and some muscle tissue still remained after 6 days' maceration, the skeletons were covered with veal infusion broth and 5.0 cc. of an actively growing culture of *B. histolyticus* were added. After 6 days' incubation the bones were completely cleaned of all tendons and muscle. Daily tests of the reaction showed no acid production. The bones were washed in running water for about 8 hours each day and covered with water and placed in the incubator for the balance of the time for 5 days and were then washed continuously in running water for 3 days. They were dried at 47°C to constant weight. After immersion in sulphuric ether for 24 hours, the bones were removed and were cleaned of all loose cartilage and fatty and waxy substances. They were immersed in fresh sulphuric ether for 24 hours and this was repeated 6 times. The bones, after each treatment with ether, were placed in a vacuum to draw out the old ether

[2] Wetzel, G., *Arch. f. Entwicklungsmech.*, 1910, **30**, 507.

[3] Wetzel, G., *Anat. Anz.*, 1920-21, **53**, 82.

[4] Mechanik, N., *Zeitschr. f. Anat. u. Entwicklungsgisch.*, 1926, **79**, 58.

and were again placed in a vacuum, after the fresh ether had been added, to ensure the filling of the marrow cavities. They were dried at 108°C to constant weight and the total weight of each skeleton noted. A boiling hot 4% watery solution of agar agar was poured over the bones and boiling was continued under reduced pressure for about 10 minutes. Keeping the agar agar solution well above the temperature of solidification, each bone was picked from the solution, rapidly rinsed in hot water (±60°C) and immediately plunged in iced water. After the surface water had dried, each bone was carefully examined to be sure that no spaces, except the marrow cavities, were filled by the agar agar. This applied particularly to the bones of the skull. In addition, obvious empty marrow spaces (those of the bodies of the vertebrae which opened into the spinal canal) were filled with vaseline. The lip of a tall glass cylinder was melted and drawn out and downward to a small tip. The cylinder was filled to overflowing with freshly distilled water and, after it had emptied to the level of the lip, all the bones of one skeleton were added one by one. Care was taken to be sure that no air bubbles or pockets remained on or in the bones. The weight of the water displaced was measured and the total gross volume of the skeleton calculated by applying a correction for temperature. The bones were again dried on the surface and the displacement redetermined.

For ascertaining the specific gravity of rabbit bone, a femur and humerus were each cut with a saw into three pieces of approximately equal length. These were cleaned, defatted and dried exactly the same as the skeletons. The weights in air and in distilled water were measured and the specific gravities calculated, applying proper temperature corrections. Previous to weighing in water the pieces were immersed in distilled water and placed in a vacuum to ensure the absence of air bubbles and pockets.

To estimate the relative amounts of red and yellow marrow, all the long bones of the extremities of another rabbit were split lengthwise and examined grossly.

The values for the specific gravity of rabbit bone, as determined by comparing the weights in air and in water of the 6 portions of femur and humerus, are given in Table I. The densities of the shafts are somewhat higher than those of the ends. The average specific gravity (2.302) is somewhat higher than the value (2.145) for human bone given by Wetzel.[2]

The measurements used in calculating the marrow volumes of the two rabbits are given in Table II. Knowing the weight of the dried bones, the volume of the bony substance is obtained by divid-

TABLE I.
Determination of Specific Gravity of Rabbit Bone.

Determination	Femur			Humerus		
	Shaft	Proximal End	Distal End	Shaft	Proximal End	Distal End
No. 1	2.430	2.288	2.187	2.360	2.183	2.268
No. 2	2.436	2.305	2.034*	2.425	2.257	2.291
Average	2.433	2.297	2.187	2.393	2.220	2.280

Average of all determinations = 2.302. * Omitted (probably an air bubble).

TABLE II.
Calculation of Rabbit Bone Marrow Volume.

	Rabbit No. 557 ♂	Rabbit No. 558 ♀
Body weight	2600 gm.	2115 gm.
Bone weight	89.3 gm.	74.3 gm.
Skeleton displacement	105.9 cc.	74.5 cc.
Bone volume	38.8 cc.	32.3 cc.
Marrow volume (weight)	67.1 cc. (gm.)	42.2 cc. (gm.)
$\frac{\text{Marrow weight}}{\text{Body weight}} \times 100$	2.59	2.00

ing the figure for the weight by the figure for the specific gravity of bone. The marrow volume is then obtained by subtracting the volume of the bony substance from the gross volume of the bones, as measured by displacement. This figure can be considered the marrow weight, for Mechanik[4] found that the specific gravities of both varieties of marrow were practically 1.000 (red slightly more, yellow slightly less). In terms of body weight the total marrow masses were 2.6 and 2.0%, respectively, an average of 2.3%. This average is identical with that of Töppich[1] for newly born infants and exactly one-half the value given by Mechanik[4] for adults.

Gross examination of longitudinal sections of the long bones of both extremities indicated that, although fatty marrow did occur, particularly in the radii and ulnae, there was relatively much more red marrow than in the long bones of normal humans. Accepting Mechanik's[4] statement that the red and yellow marrows are approximately equal in humans, it would seem reasonable to estimate that the marrow in the rabbit consisted of about 75% red marrow. This value would be equivalent to 1.7% in terms of body weight. It is appreciably lower than the figures given for infants[1] and adults,[4] which are identical (2.3%), but would be expected since the blood volume of rabbits in terms of body weight is considerably lower than that of humans.[5]

[5] Erlanger, J., *Physiol. Rev.*, 1921, **1**, 177.