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## A Method for Determining the Hardness of Nutrient Agar.

W. J. NUNGESTER. (Introduced by A. A. Day.)

*From the Department of Bacteriology, Northwestern University Medical School.*

The importance attached to details of colony forms in the work of recent years dealing with microbic dissociation, makes it imperative to recognize and control extrinsic factors affecting the structure of colonies. One such factor is the hardness of nutrient agar. Media prepared with agar from various sources has been found to cause detectable differences in the structure of the RS colony type of *B. anthracis*. This fact suggested the need of a method for determining the hardness of agar. Whipple<sup>1</sup> in 1902 studied the hardness or "spissitude" of gelatin. He also made a few tests on the spissitude of varying concentrations of agar. His method consisted in determining the depth a plunger 10 mm. in diameter would sink under a weight of 150 gm. in 5 seconds. The reciprocal of this value he termed spissitude. The sinking of the plunger was read without amplification.

The author has measured the hardness of agar by noting the time required for a brass plunger 0.500 cm. in diameter to sink into the agar 0.1 mm. under a given weight. The apparatus for accomplishing this consists of a vertical plunger attached to a horizontal lever in such a manner that one arm of the lever serves as a pointer against a scale, each unit of which represents 0.1 mm. of vertical motion of the plunger. The other arm, to which the plunger is attached near the fulcrum, serves as a beam on which a weight can be variously placed, thus altering the weight applied on the agar by the plunger. Making use of the mechanical features of an old microscope the above unit was mounted in place of the barrel. This facilitates lowering the plunger to the agar surface. When contact of the plunger and agar has been carefully established, a release is tripped, the weight of the system is applied through the plunger to the agar and the time for the plunger to sink 0.1 mm. is measured. The hardness of the agar is then expressed in units of hardness. The unit tentatively suggested is equal to  $T \times W / 100$  where  $T$  is the time in seconds required for the plunger to sink 0.1 mm.,  $W$  is the weight in grams exerted on agar by the plunger, 0.500 cm. in diameter, and 100 is a divisor to eliminate non-significant digits in the product  $T \times W$ .

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<sup>1</sup> Whipple, George C., *Tech. Quar.*, 1902, xv, 127.

Certain precautions are essential in order to obtain comparable results. (1) The agar must be poured at a stated temperature. (2) A uniform thickness of agar is necessary. This is insured by pouring 20 cc. of melted agar into petri dishes of uniform diameter (9 cm.) with a minimum of crowning in the bottoms. (3) Readings should be made at a uniform temperature  $25 \pm 2^\circ$  C. (4) The weight applied should be adjusted in order that the plunger will sink 0.1 mm. in less than 100 seconds and in not less than 40 to 50 seconds. The latter limit is to be particularly emphasized for more rapid sinking of the plunger is often accompanied by a cracking of the agar with resulting inaccurate readings.

Seven samples of various commercial agars were dried at  $100^\circ$  C. for 1.5 hours under a 28 inch vacuum. Two per cent solutions in distilled water were then prepared, dissolved by autoclaving, poured at  $90^\circ$  C. and tested after standing 24 hours. The results (Table I) indicate a wide variation in the hardness of agar prepared from various sources of dried agar.

TABLE I.  
Hardness of Various Commercial Agars (2%).

Sample	1	2	3	4	5	6	7
Weight	4.6	4.6	51.9	21.0	12.2	9.2	29.6
Time	55.1	100.1	66.0	92.7	63.5	63.8	58.7
Hardness	2.5	4.6	34.2	19.5	7.7	5.9	17.4

In a similar manner the hardness of various concentrations of a sample of agar were tested. The results are given in Table II.

TABLE II.  
Hardness of Various Concentrations of Agar.

Concentration	3.0%	2.5%	2.0%	1.5%	1.0%
Weight	120.0	91.2	51.9	34.4	12.2
Time	94.5	69.6	76.0	56.8	70.0
Hardness	113.4	63.5	39.4	19.5	8.5

Differences of 10 units in the hardness of nutrient agar has been found to affect the colony form of the RS type of *B. anthracis*.