opment of starter cultures composed of penicillin-resistant strains of lactic streptococci is another possible means of overcoming this difficulty. Cysteine did not do this even when added at the rate of 1–10 mg per bottle. Pasteurization of milk containing varying amounts of penicillin failed to inactivate the antibiotic.

Where occasional mastitis-infected animals are treated with penicillin this problem of inhibition of starter activity in the milk may not arise owing to dilution of the penicillin in the pooled milk supply. However, where extensive use of penicillin is being made, its inhibitory effect can be a source of considerable concern to the cheesemaker. Whitehead (*1*) suggests that milk obtained from cows during the 3-day penicillin treatment, and for one day thereafter, be used for purposes other than cheesemaking. The loss of this milk could be obviated most simply by addition of penicillinase.

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A Convenient and Rapid Method of Calibrating Warburg Manometers

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Although several methods have been described for the calibration of Warburg constant volume respirometers (*1, 2, 6*), it is generally agreed that calibration with mercury is the most reliable. Present descriptions (*1, 5, 7*) advise filling the inverted manometers through the neck from above, which is often cumbersome in practice due to the trapping of air bubbles in the column of mercury. We have recently used a variation of this method in which mercury is allowed to flow up into the manometer from below, thus avoiding this difficulty.

Using the method illustrated in Fig. 1, the time required for calibrating a manometer is reduced to less than 5 min. The only experimental value needed for calibration is the total volume (V) of the cup-manometer system, which for convenience is measured in two separate weighings. A scratch is placed on the neck of the manometer about halfway between the ground glass joint and the glass prongs that hold the fastening springs. The volume below this scratch will be referred to as V_C or the volume of the cup; while that above will be referred to as V_M, the volume of the manometer. The sum of V_C and V_M yields (V). Since the change in density of mercury between 20° and 28° is not significant in the determination of Warburg constants to two decimal places, these volumes are obtained simply by dividing the weight of mercury by 13.54.

Once (V) has been obtained, k_{O_2} and k_{CO_2} at 25° and at 37° may be read directly off the nomogram published by Dixon (*3, 4*) for the determination of Warburg manometer constants. For this, a hair line is stretched from the extreme left hand (V) scale across to the extreme right hand (v_F) scale, where v_F stands for the arbitrarily agreed on volume of fluid in the manometer cup.

For the convenience of workers unfamiliar with manometer calibration, the present method will be described

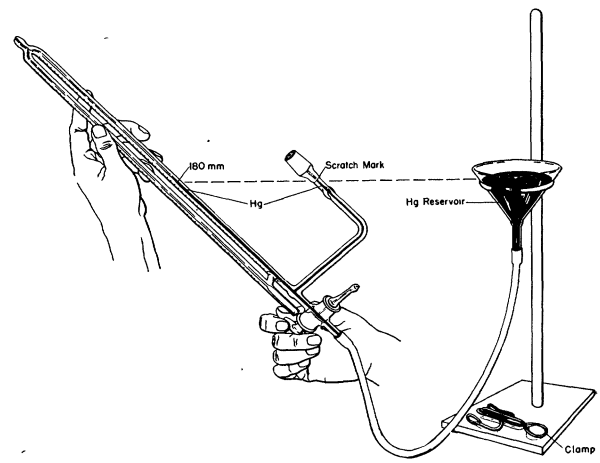

FIG. 1.

in detail. All weighings are made on a crude pan balance to the nearest tenth of a gram.

To obtain V_C:

1. Weigh the empty cup, stopper in place. Fill the cup completely with clean mercury, adjusting the level with a medicine dropper until the column of mercury just reaches the scratch mark when the cup is ground onto the manometer. Reweigh, and divide the net weight by 13.54 to obtain V_C.

To obtain V_M:

1. Attach a funnel to a ring stand as illustrated, and wire onto it a 3-ft piece of rubber tubing selected to fit snugly on the end of a manometer. Place a clamp on the end of the tubing and fill with about 100 ml of clean mercury.

2. Hold the inverted manometer above the level of the mercury reservoir and attach its lower end to the tubing. Remove the clamp and, slowly lowering the manometer, allow the mercury to flow evenly up into both the manometer limbs.

3. Leaving the mercury in free communication with the mercury in the reservoir, adjust the level of the mercury simultaneously in the two limbs until it just reaches the scratch mark on one side and the desired null point (180 mm for example) on the other. It is convenient to mark these two points with a thin red crayon for rapid identification.

The above adjustment is best done freehand with the elbows firmly planted on the table. The funnel should be adjusted to a convenient height depending on the operator. When the mercury columns are properly adjusted by combining the movements a) tipping the manometer, and b) raising and lowering it relative to the mercury in the funnel, the stopcock should be closed one-

eighth (not one-fourth) of a turn with the marking dot down.

4. Raise the whole manometer, keeping it slightly tilted, and turn the stopcock another eighth of a turn thus allowing air to enter the end of the stopcock and the excess mercury to flow back towards the reservoir. Detach the tubing from the manometer after replacing the clamp.

5. Allow the mercury to flow into a tared vessel and divide the net weight by 13.54 to obtain V_M. Disregard the small amount of mercury in the channel of the stopcock as this is below the level of significance. A permanent record of the V_M values should be made for future reference.

The above maneuver requires perhaps 15 min of practice before repeatable results are obtained, but once mastered permits rapid calibrations in duplicate.

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Effect of Radioactive Phosphorus (P^{32}) in the Culture Medium on Growth and Viability of *Phomopsis citri* and *Diplodia natalensis*

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Some interesting results were obtained while studying the effects of radioactive phosphorus on the physiology of *Phomopsis citri* and *Diplodia natalensis,* the fungi commonly causing stem-end decay in oranges.

Potato dextrose agar was prepared in two lots that were identical in every respect, except that one lot contained 20 ml per l of a solution of radioactive phosphorus. The radioactivity of the solution introduced into the agar ranged from 0.156 to 0.245 μc/ml, with a half-life of 14.3 days. The control lot of agar was treated with an equal amount of 0.1% solution of NaH_2PO_4. Mycelial tufts of the fungi were transferred from pure cultures to the center of the agar in Petri plates, and the daily rate of growth of the cultures was determined by measuring increase in diameter. During four replications of the experiment the P^{32} showed a tendency to retard the growth of *Diplodia natalensis,* whereas the results with *Phomopsis citri* were not very consistent.

When transfers of the organisms were made from the Petri plates to orange fruits, the fungi that had been grown on radioactive media were slower in producing decay than were the control lots. California oranges were selected for inoculation to avoid the possibility of natural infection in the fruits, since the two fungi under consideration are rarely found in oranges produced under irrigation. A total of 400 fruits were employed, 200 of each variety. There were four lots of 50 fruits in each

TABLE 1

STEM-END DECAY IN CALIFORNIA ORANGES INOCULATED WITH FUNGI GROWN ON RADIOACTIVE AND CONTROL MEDIA

Variety	Days following inoculation	Percent showing decay					
		Phomopsis citri			*Diplodia natalensis*		
		Grown on radioactive media	Grown on control media	Chi square	Grown on radioactive media	Grown on control media	Chi square
Valencia	4	48	80	11.0	70	88	5.1
Washington navel	6	18	52	12.7	26	52	7.1

experiment. Inoculations were made by removing the stem-buttons, cutting a slit in the exposed stem with a sterile scalpel, and then forcing some of the agar and mycelia into the slit. Navel oranges were inoculated with mycelia from cultures in which the P^{32} had shown definite inhibitory effects on the rate of growth of the two fungi. In the experiments in which Valencia oranges were used, the inoculum was taken from cultures in which no significant retardation of either fungus had been produced by the P^{32}. Tests were made with navel oranges in April and with Valencia oranges in November, so that each variety was inoculated near the end of its commercial season, when one would not expect much resistance on the part of the fruits to pathogens.

It will be noted in Table 1 that in all instances there was more decay in the oranges when inoculated with fungi from control media than when the inoculating organisms had been grown on radioactive media. The effect was not so pronounced with Valencia oranges as with the Washington navel variety. With the navels, the control fungi caused at least twice as much decay as did the fungi that had been cultured on radioactive media. However, the results in all instances were highly significant.

The ability of the fungi to cause decay was reduced by exposure to P^{32}, irrespective of whether or not growth of the organisms on culture media had been previously retarded. Furthermore, the radioactivity of the solutions employed was not great. The original solutions, with an activity of 0.156 to 0.245 μc/ml, were diluted 50 times in making up the culture media. At the time that the Petri plates were poured and inoculated, 1 ml of the agar emitted 10,000 to 11,000 counts per min. Inoculations were made into oranges a week or ten days later, and the amount of inoculum employed weighed approxi-