pH 8.5 to 8.9 in the other) calcium carbonate producing a greater change than calcium sulphate in each case. It should be noted that the soils used produced filtrates in the alkaline range, and that acid soils might respond to these precipitants in a different way.

Heated Versus Unheated Soil Extract

A simple experiment was carried out to determine the effect of heat in preparing extract and sterilizing it on the number of bacteria developing on soil-extract agar. Two extracts were prepared from aliquots of one soil, each with double the concentration of extractives: the extract from 1 kg. soil was increased to 500 ml. (not to 1000 ml.). The first aliquot was heated to extract the solutes, filtered, and water was added to raise the volume to 500 ml. This extract was heat-sterilized. The second extract was prepared by soaking the other 1000 g. aliquot in 1000 ml. water at 45° C. for 3 days, adding water to compensate for evaporation, filtering, and adding water to raise the volume to 500 ml. This extract was Seitz-filter sterilized. The two extracts were used to prepare three soil-extract agars: one with heated extract, one with filtered extract, and one with half the quantity of each extract. The other portion of each medium, consisting of 500 ml. water, double concentration of dipotassium acid phosphate and of agar, and 0.2 ml. 1/10th normal hydrochloric acid,* was sterilized by heat, cooled to 50° C., and mixed with the extract, likewise at 50° C., immediately before the plating. The three media were used for counts of bacteria in five soils. The soils are defined and the results presented in Table III. Two points are apparent. The unheated extract provided the nutritional requirements for a significant portion of the population in each soil, but not for as large a portion as did the heated extract. The mixing of the two extracts failed to produce larger counts than did either of the extracts. Whether the populations on the three media were the same was beyond the scope of this study. The problem of determining the effect

TABLE III

EFFECT OF HEAT IN PREPARATION OF SOIL-EXTRACT AGAR ON NUMBERS OF BACTERIA. THREE MEAN COUNTS, EACH FROM 10 REPLICATE PLATES FROM ONE AND THE SAME DILUTION OF SOIL

Soil*	Heated	Filtered	½ H + ½ F
1	90	55	90
6	137	70	119
8	66	45	64
9	54	38	52
10	100	54	91

*1, 6, 8: See footnotes Table II.
9: Clay, virgin forest, Red River Association.
10: Garden.

*The amount per liter previously found necessary with extracts from this soil to give pH 7.0 in the complete medium.

of heat in releasing specific growth factors from soil, or in inactivating others, would involve detailed nutritional studies on large numbers of isolates from each medium. Lochhead and Chase (4) reported that only one of 63 cultures, known to require soil extract, produced maximum growth in a basal salts, glucose, yeast extract medium in which soil-extract ash replaced soil extract. This may be accepted as indicating that extreme heat inactivates most of the growth factors present in soil extract.

Dried Soil Extract

Soil extract, in liter quantities with dipotassium acid phosphate added, was dried at 60° C. for 4 days and stored at laboratory temperature and humidity for periods ranging from 13 to 25 weeks. Agar was not added before drying because of the problem of drying and pulverizing, and it was considered that for this study the addition of agar at the time of testing was simpler. For each test a dried extract was suspended in distilled water by boiling for a brief period. After filtering to remove sediment, agar was added and the medium sterilized and used along with freshly prepared soil-extract agar made from an aliquot of the soil from which the dried extract had been prepared. Comparative counts on 13 samples representing eight soils are presented in Table IV. The freshly prepared extract gave higher counts in 11 of the 13 trials.

TABLE IV

EFFECT OF DRYING SOIL EXTRACT ON NUMBERS OF BACTERIA DEVELOPING ON SOIL-EXTRACT AGAR. TWO MEAN COUNTS, EACH FROM 10 REPLICATE PLATES FROM ONE AND THE SAME DILUTION OF SOIL

Soil*	Weeks dried	Dried extract	Fresh extract
1*a*	13	58	78
b	19	35	47
c	21	61	125
d	25	54	83
2*a*	13	100	124
b	19	47	73
3*a*	13	42	37
b	19	21	21
4	25	63	143
5	25	86	172
6	21	70	90
7	21	50	56
8	21	39	64

*See footnotes Table II.

It should be noted that this finding would not necessarily apply to soil-extract agar prepared by methods used for other dehydrated media. The extract was dry, whereas previously opened commercial dehydrated nutrient broth contained 5.0% moisture, nutrient agar 5.0%, yeast extract 9.8%, and malt extract 19.0%.

Dipotassium Acid Phosphate in Soil-Extract Agar

Soil-extract agar prepared as outlined above with 0.02% dipotassium acid phosphate was used along with soil-extract agar without it to obtain counts of bacteria in five soils. Higher concentrations were not investigated because even though accurate information on the requirements of potassium and phosphorus is not available the hundred or fewer bacteria in 1 ml. of the dilution plated could not assimilate the amount of these elements in 12 ml. medium in the plate. The results are presented in Table V. The medium with dipotassium acid phosphate gave higher counts in all trials.

TABLE V

EFFECT OF 0.02% K_2HPO_4 IN SOIL-EXTRACT AGAR ON NUMBERS OF BACTERIA DEVELOPING ON PLATES. TWO MEAN COUNTS, EACH FROM 10 REPLICATE PLATES FROM ONE AND THE SAME DILUTION OF SOIL

Soil*	With K_2HPO_4	Without K_2HPO_4
1	88	61
6	111	84
8	58	42
9	50	34
10	35	20

*1, 6, 8: See footnotes Table II.
9, 10: See footnotes Table III.

The Preparation of Soil-Extract Agar

1. Select a field soil of high fertility.

2. Use 500 g. soil and the amount of water, predetermined for the soil used, necessary to yield 1000 ml. extract. That amount of water will depend upon the moisture-holding capacity and the moisture content of the soil. It will vary from 1200 to 1500 ml. This eliminates the need for adding water after filtration, which would lower the concentration of extractives. It yields the maximum proportion of extractives obtainable by one extraction from the amount of soil used. In 16 trials with three soils, when 1000 g. soil and 1000 ml. water were used, the volumes of extract recovered ranged from 270 to 480 ml., and contained only 27 to 48% of the extractives. In a single trial, when 500 g. soil and 1200 ml. water were used, the volume of extract was 580 ml., and 58% of the extractives were recovered. In six trials with different soils, when 500 g. soil and 1300 ml. water were used, the extractives represented from 53 to 62%. When in a subsequent trial 500 g. soil and 1500 ml. water were used 1000 ml. extract and 67% of the extractives were recovered. Undoubtedly, this reasoning established the proportion of soil and water used by Smith and Worden (7).

3. Heat in an autoclave at 121° C. for 30 minutes.

4. Filter through filter paper or cloth. Refilter the first cloudy portion of the filtrate by returning it to the same filter. If a precipitant has been found necessary for the soil used add 0.5 g. calcium sulphate or 0.5 g. calcium carbonate after heating and let stand for 5 minutes before filtering.

5. To 1000 ml. filtrate add 0.2 g. dipotassium acid phosphate.
6. Add 15 g. agar.
7. Heat to dissolve.
8. Adjust to pH 6.8.
9. Filter through cotton, and dispense in 100 ml. quantities.
10. Sterilize at 121° C. for 20 minutes.
11. Temper to 50° C. and use, or store, tightly sealed, at 5° to 10° C. for later use.

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