termined by the McCrady most-probable-number method, using 3 tubes of beef tea for each dilution. There was a drop from one million to 10,000 bacteria per cc. at the end of one day. The succeeding days gave the same count of approximately 10,000 per cc.

3. The technic was the same as in 2. At monthly intervals a tube was removed and the number of survivors was determined by the most-probable-number method, and by the plate-count. This was continued for a period of 13 months and further counts were made after 16 and 19 months.

The plate-counts and the most-probable-number sometimes agreed and at other times disagreed widely. There was shown no consistent decrease in bacterial numbers over the period of 19 months. The counts varied from 2.5 per cc. to 9500 per cc. over the period. The difficulty in getting consistent counts was apparently due to this: In the process of freezing, the liquid in the tubes freezes from the bottom up; as ice crystals form, the bacteria are pushed toward the surface and finally there is a layer of frozen bacteria at the surface. On standing this film becomes tough and a uniform suspension of bacteria is not obtained when the contents of the tube are melted and shaken.

Saline suspensions of bacteria that survive the mechanical effects of freezing are still viable after 19 months at about 83° absolute.

8896 P

A Study of Milk Coagulation as a Differential Feature of *Monilia albicans* and *Monilia candida.*

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Though *Monilia albicans* and *Monilia candida* have so far been shown to give the same serological reactions,[1] nevertheless in disagreement with Stone and Garrod's conclusion,[2] we consider[3, 4, 5, 6]

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1 Almon and Stovall, *J. Infect. Dis.*, 1934, **55**, 12.

2 Stone and Garrod, *J. Path. and Bact.*, 1931, **34**, 429.

3 Stovall and Bubolz, *J. Infect. Dis.*, 1929, **45**, 463.

4 Stovall and Bubolz, *J. Infect. Dis.*, 1932, **50**, 73.

5 Stovall and Bubolz, *J. Lab. and Clin. Med.*, 1933, **18**, 890.

6 Stovall and Pessin, *J. Clin. Path.*, 1933, **3**, 347.

that there are sufficient cultural, biochemical, and pathogenic differences to justify placing the organisms in 2 different species. A prominent difference is the coagulation of milk by *M. albicans* in striking contrast to *M. candida's* inability. The purpose of this study was to determine the conditions under which coagulation occurred and to see if in all cases a constant, definite difference in the species was observable. In addition, another objective was to demonstrate that the rennet-like coagulation of the *albicans* type is probably distinct from the adherent mat or mycelial pad formation of *M. candida* which produces a pseudo-coagulative effect.

In the work with coagulation the procedure for the preparation of the calcium-lactate milk medium is that formerly described.[8]

While it was found that coagulation could readily be accomplished through the use of any of the *M. albicans* cultures themselves yet the utilization of an extract from that species would permit a better comparison of clotting results with commercial rennin. Now in order to perform experiments with extracting agents it was necessary to obtain a heavy cell yield, and for this purpose large Blake bottles containing Trommer's malt-extract agar were used. After several extracting agents had been employed without marked success, sterile distilled water in 20 to 25 cc. portions per Blake bottle was used to wash down the growth. The suspension, kept at room temperature, was shaken at intervals and centrifuged at the end of a week. When 1 cc. of the clear supernatant was added to 10 cc. of the calcium-lactate milk, a coagulative effect was observed in less than 4 to 5 hours. The usual time heretofore was 2 to 3 days. Acid coagulation was ruled out, for electrometric pH determinations showed no change upon addition of the extract. Since the filtered *M. albicans* product induced clotting, the presence in solution of a coagulating agent free from cells seemed certain.

Other preparations were subsequently shown to contain the coagulating enzyme in even greater amount. Desiccated macerated cells yielded a potent preparation clotting of milk occurring within a few minutes after addition of the centrifuged supernatant. In addition the filtered liquid from a 4 weeks' growth in 75 cc. of malt-broth displayed very pronounced coagulative action in the *albicans* but not in the *candida* cultures.

Having obtained in solution an agent from *M. albicans* which clotted milk, we determined analogies which were establishable with a commercial rennet. The tests showed a striking relationship, both in manner and degree, of the factors affecting the extract and the commercial rennin; for example, calcium lactate added to sterilized milk rendered the medium readily coagulable by both products;

increasing the acidity of the milk shortened the coagulation time for both preparations, or rendering the milk alkaline prevented clotting, with the amount of extract and rennin regularly employed; adding ammonium oxalate, with resulting precipitation of the calcium together with a slight increase in pH, had an unfavorable effect for both products; and finally, a boiling temperature for 5 minutes rendered each entirely incapable of causing coagulation.

The effect of environmental conditions on the ability of growing organisms to produce clotting was studied. In each case in which an alteration in the milk was made, a decided effect was exerted on the *M. albicans'* ability or time to produce coagulation. Moreover, if the change was such that either rennin or the Monilia extract would be inhibited in its clotting action, then the live inoculum would likewise be affected. Similarly, changes more favorable for rennin action also rendered the milk more coagulable by growing organisms.

pH determinations, made at daily intervals, showed little change in reaction with the *M. albicans* cultures at the time of coagulation. However, at this time the *M. candida* displayed decided increase, a rise from pH 6.05 to 6.50 in 3.5 days, and a pH 7.4 in 8 days. The combination of reaction change together with *candida's* marked deficiency of the coagulating enzyme is considered responsible for its failure to exhibit coagulation. With the *M. albicans* a pH rise begins to occur after coagulation doubtless due to deaminization of protein material. However, even at the tenth day pronounced pH differences still obtain between the two species. That there is a definite biological difference between *Monilia albicans* and *Monilia candida* is shown on the basis of milk coagulation.

8897 P

Effect of 4-6 Dinitro-O-Cresol on Oxidation of d'- and l'-Arabinose by Previously Starved Yeast.*

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It has been shown in previous papers from this laboratory that the respiratory rate of yeast suspended in non-nutrient phosphate

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