Further work is in progress, and the entire investigations will be reported in detail elsewhere.

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THE DIPHASIC ASPECT OF THE CURDLING OF MILK BY RENNIN

STUDIES of rennin action are all based upon the physical changes which it induces in milk or casein solutions. The end-point of the process is the appearance of a flocculent precipitate, the formation of a solid gel or the rise in viscosity immediately preceding these gross evidences of rennin activity. Added ions or compounds are found to accelerate or retard the process as a whole, and this is usually interpreted to indicate acceleration or inhibition of the enzyme. As a matter of fact, we may have marked change in curdling time without any change in the activity of the enzyme. It is clear that the curdling of milk involves two distinct phases. One is the enzymic phase, in which, according to Bosworth, casein is split into two molecules of paracasein. The other is the phase of aggregation, in which paracasein separates out as a precipitate or forms a gel. The two phases may be separated for the purpose of study in the following way.

To a standard milk preparation a small amount of rennin is added. Samples are removed at intervals; treated with formaldehyde to check the enzyme, and with sufficient calcium chloride to precipitate paracasein only. The volume of the paracasein is measured in a graduated centrifuge tube after whirling at a given speed for a given time. Nitrogen determined on the supernatant indicates accurately the residual casein left in the digest at the time of sampling. When plotted, the curves of paracasein increase and casein decrease are reciprocal. When paracasein reaches a constant and maximum volume, the supernatant liquid is perfectly clear and shows no casein N. This marks the end of the enzymic phase. The phase of aggregation ends when the paracasein precipitates or curds. The enzymic phase occupies less than 60 per cent. of the time required for the appearance of curd. From the data obtained, therefore, we may conclude that the digestion phase of curdling is over in about half the time required to develop the actual curd.

If calcium chloride is added to the milk, and a similar digest started, with accurate control of pH, the process as a whole is accelerated. When studied by the method outlined, it is found that the calcium salt has no effect upon the enzymic activity of rennin, but markedly shortens the time required for the precipitate or curd to form. On the other hand, an increase of the H ion accelerates both phases.

We expect to report later in more detail studies of the two phases involved in curdling of milk by rennin and other proteases.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TUBE FOR CULTURING FUNGI

IN connection with experimentation on the temperature and oxygen relations of growth and respiration in some wood-inhabiting fungi cultured on solid nutrient agar, a new form of culture tube has proved very satisfactory. It consists of the ordinary test-tube, modified by a rounded indentation or invagination of the wall on one side near the mouth, as shown in the accompanying diagram. This modification is

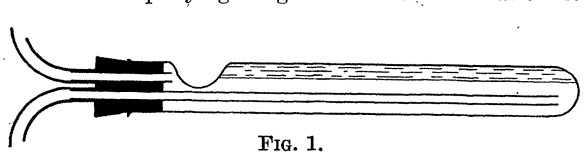

FIG. 1.

easily accomplished by holding the test-tube at right angles to the flame of a wing-top Bunsen burner until the portion of the wall which is to be altered is sufficiently softened, when gentle mouth suction is applied internally by means of rubber tubing attached to a bit of glass tubing that leads through a cork stopper set tightly into the test-tube opening. Finally, the heated portion should be subjected to a yellow flame to insure adequate annealing. With a suitable flame tubes of hard or other chemically resistant glass may be employed.

Such a modified tube is charged with agar substrate in the usual way, excepting that it is not slanted but is kept horizontal, with the indentation below, while the agar solidifies. Since the indentation prevents the escape of the agar while still liquid, there results a uniform narrow strip of solidified substrate (shaded in the diagram) lying along one side of the tube. The tube when thus prepared can be used for short-term growth studies with advantages noted by Fawcett in connection with his long-tube cultures of parasitic fungi.[1] Visible characteristics of growth may be studied by means of surface observations, or inspection through the substrate if the latter is reasonably

[1] H. S. Fawcett, *Ann. Appl. Biol.*, 12: 191–198. 1925.