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

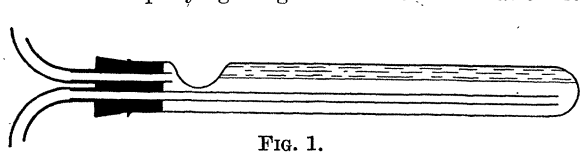

FIG. 1.

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.

clear. When viewed through the substrate, with suitable illumination, the progress of marginal growth can be accurately measured to 1 mm or better. When closed by means of the usual cotton plug this modified culture tube may be more satisfactory for many purposes than is the ordinary test-tube, Petri dish or standard Kolle flask.

In the writer's experiments ten culture tubes of this type, 2.5 cm in diameter and 20 cm long, are joined together and are operated as a single unit. Each tube is provided with a tightly fitted, 2-hole rubber stopper, bearing an inlet and an outlet tube, as shown in the diagram, so that any specified gas mixture may be passed continuously over the culture. The tubes are joined in series by short connections of rubber tubing. To prevent significant exchange of gas between inside and outside of the culture system and to reduce temperature fluctuations, the whole group is inverted and stands vertically in a water bath. The continuous gas stream not only maintains oxygen pressure practically the same for all cultures of a group, but it also insures the continuous removal of the $CO_2$ produced, which may be collected in absorption apparatus and measured from time to time in conjunction with the growth measurements.

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## RAPID WEIGHINGS WITH A TROEMNER SOLUTION BALANCE

THOUSANDS of weighings have been made during the past five years with a 20-kilo capacity Troemner solution balance. To avoid the inconvenient, tiresome and time-consuming method of getting the eye on a level with the pointer-end of the beam in order to make a correct reading, two very simple, but very satisfactory, additions were made to the balance.

The two additions consist of a damper, as shown in Fig. 1, *a*, and two white strips attached to the adjacent projections on the swinging and stationary beams (Fig. 1, *b*). In the center of each of these strips a black line about 1 mm wide was drawn, the two separate black lines appearing as one continuous line when the weighing beam is exactly balanced. Black and white enamel paints could have been used. The parts of the damper are of glass and aluminum to prevent corrosion. The liquid is glycerine and may be diluted or not, as convenience demands. The vessel containing the glycerine is covered to exclude foreign matter, except for a small opening in the center of the cover through which the disk support passes. It does not show in the figure, but the upper

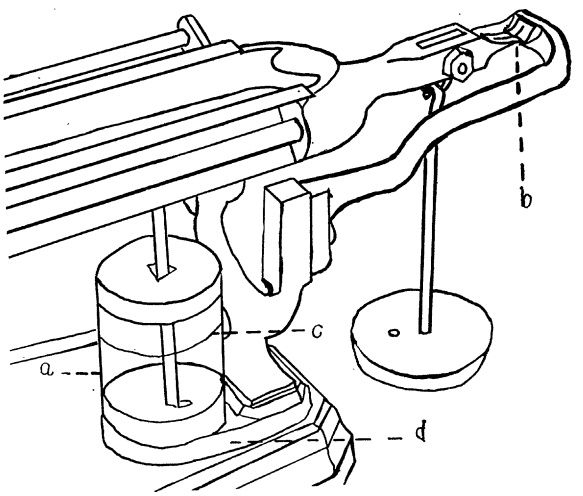


FIG. 1. Aids for reading Troemner solution balance: *a*, damper; *b*, black lines on white background; *c*, level of glycerine; *d*, tape to hold damper vessel in place.

end of the disk support is attached near the distal end of the rod which carries the adjusting rider.

Balance adjustments with objects weighing 15 or more kilos can be rapidly and accurately made to within 1 gram. It has proved helpful in making readings to provide a black background for the outer ends of the beams. This may be done by standing a black card on the table, allowing it to lean at right angles to and against the distal end of the stationary beam.

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