NOTES ON THE MODE OF ACTION OF RENNIN AND FIBRIN-FERMENT.

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IN a recent paper[2], Fick has put forward the view that the mode of action of the clotting ferments is fundamentally different from that of the ordinary digestive enzymes. He bases his views entirely upon a consideration of the phenomena of milk-clotting during the manufacture of cheese on the large scale, and upon an experiment made with a glycerin extract of rennin[3]. The conclusion to which he comes is that unlike the true enzymes, the molecules of the clotting ferments do not require to come into intimate relationship with the molecules of the substance which is undergoing the change, but that when once the change has been set up by the ferment in any one portion of the substance, this change is propagated from particle to particle of the same without the further necessary intervention of the ferment. If this view were correct it would imply that after the change is once started, each portion of the already changed substance acts like the original ferment to the still unchanged part, and indeed the various conclusions which would follow from the establishment of Fick's view make it important to investigate it rather more fully than he has done.

[1] In view of the recent publication by Latschenberger (*Centralb. f. Physiol.* Bd. IV. No. 1, April 12, 1890) of a paper in which he has arrived at the same result as ourselves it seems necessary to state that the MS. of this communication was completed for press on Feb. 26 and that the experiments described below were demonstrated to the Cambridge Philosophical Society on March 10. (*Camb. University Reporter*, March 18, 1890, p. 562.)

[2] *Pflüger's Arch.*, Bd. XLV. (1889), S. 293.

[3] We propose to use the name 'rennin' for the milk-clotting ferment of 'rennet' as being more convenient than the more usually employed phrases 'the rennet ferment' &c. and analogous to the ordinary names of other enzymes.

Fick's experiment was as follows. He placed on the bottom of a test-tube a few drops of a glycerin extract of the gastric mucous membrane of a calf; on to this he poured with all possible care some milk, previously warmed to 40° C., so as to completely fill up the tube, while avoiding as far as possible any mixing of the two fluids. He then placed the tube in a water-bath at 40° and observed that the milk was constantly clotted up to the very top of the tube in about a minute or less. On this experiment he bases his view and applied it to the action of the fibrin-ferment, without however testing it by a similar experiment with a diluted plasma and ferment solution. The evidence was thus somewhat scanty and the method of experiment was also not quite satisfactory or conclusive. Rennin is a ferment which is readily obtained in solution in such amounts that it is possessed of extraordinary activity[1]. It is thus quite conceivable that Fick's result arose from the slight mixing which must inevitably occur however carefully the milk was poured on to the ferment extract. We therefore proceeded to repeat the experiment, using every precaution to avoid mixing of the two fluids, and carrying out the experiments in tubes of varying diameter, so as to obtain contact-surfaces of varying extent between the milk and the ferment solution[2]. By this latter means it was also possible to obtain an idea of the influence exerted by any convection currents in effecting a mixture of the ferment and supernatant milk.

The following is typical of our experiments and their results. Three pairs of tubes were taken, whose diameters were respectively 1·5, 2·5 and 5 cm. Into each of the first pair 10 c.c. of fresh milk were introduced, into each of the second pair 20 c.c., and into the third 40 c.c. The six tubes were then placed in a water-bath at 40° and the milk raised to this temperature. We now introduced the rennin solution, previously warmed to 40°, into the bottom of the tubes so that it formed a clear layer below the milk, with a perfectly sharp line of demarcation between the two fluids. This was done by means of a glass tube drawn out to a long and very fine capillary ending, so fine that the ferment would pass through it in the slowest and most regular stream, and thus ensure that there should be the least possible mixing.

[1] One part of purified rennin is stated to be able to effect the clotting of 400,000—800,000 parts of casein. Hammarsten. See *Maly's Jahresbericht*, Bd. VII. (1877), S. 166.

[2] The rennin used was the ordinary commercial preparation, made presumably by extracting the mucous membrane with sodium chloride, of which it contained a considerable quantity.

The amount of ferment added to the smallest tubes was 2 c.c., to the next 4 c.c., to the largest 8 c.c., so that the proportion of ferment to milk was the same in each. The contents of one of each of the pairs of tubes were now mixed by a short but violent agitation and the times of clotting observed. The tubes whose contents were mixed, clotted in 3, 4 and 4 minutes respectively. With the tubes whose contents were unmixed, the results were as follows:

Tube 1·5 *cm. wide.* After 20 minutes' digestion at 40° there was a clot at the junction of the ferment and milk ·5 c.m. deep, the rest of the milk being at this stage perfectly fluid and remaining so for 3 hours. The supernatant milk was not completely clotted to the top until 5 hours 4 minutes after the commencement of the experiment.

Tube 2·5 *cm. wide.* After 20 minutes' digestion the clot at the junction of the ferment and milk was ·75 cm. deep: the milk did not clot to the surface until 4 hours and 39 minutes from the beginning of the digestion.

Tube 5 *cm. wide.* After 5 hours and 24 minutes the milk had clotted half way up to its surface; it had not clotted completely up to the top at the end of 7·5 hours, at which period we had to leave the laboratory for that day.

In another experiment with the same tubes, the times required for the complete clotting of the milk in the narrower tubes was much less than the above, but was still 1 hour and 1 hour 10 minutes respectively. In the wide tube the clotting was not complete even after the digestion had been carried on for 2 hours and 15 minutes.

The results thus obtained were strikingly at variance with those observed by Fick and lend no support to his view as to the mode of action of the ferment. Indeed they point rather to the conclusion that the clotting is finally due to the gradual dissemination of the ferment by convection currents. When 20 c.c. of water to which a little finely powdered charcoal had been added, were placed in the 2 cm. tube and immersed in the water-bath at 40° we were always able to observe the existence of convection currents, even when the temperature of the water in the tube, as taken by a thermometer, was the same as that of the bath. The currents appeared to flow up the sides of the tube and down the centre. On enquiry of a professed physicist as to the probable relative extent and activity of these currents in the tubes of varying width, he expressed his opinion that they would be most marked in the narrower tubes; this statement corresponds to the result which we generally obtained that the clotting was progressively

slower in the wider tubes. We further determined how small a quantity of the rennin was required to clot 10 c.c. of milk and found that one minute drop of the ferment solution sufficed to clot the milk in 32 minutes. From this it was obvious that a mere trace of the ferment distributed by the inevitable convection currents would suffice to bring about the results we obtained.

The above experiments with milk were repeated with a dilute salted blood-plasma and an active solution of fibrin-ferment, prepared by extracting fibrin with 10 p.c. sodium chloride. The results of these experiments were the same as of those made with rennin and milk, but were if anything more striking. It will suffice to describe one experiment. 20 c.c. of the dilute salt-plasma were placed in each of the 2 cm. tubes: 2 c.c. of the fibrin-ferment solution were carefully introduced as before beneath the plasma in one tube, while 2 c.c. of the same ferment were mixed by agitation with the contents of the other tube. The latter clotted in 11 minutes. After 6 hours' digestion in the other, the condition of its contents was as follows; there was a clear layer of ferment solution 1·5 cm. deep at the bottom, then a clot of fibrin ·75 cm. deep above this, and the rest of the plasma was as fluid and clear as at the outset of the experiment. The clot of fibrin was so firm that the tube could be inverted, the supernatant fluid plasma poured away, and the fluid ferment-solution was then found to be completely imprisoned in the upturned closed end of the tube. In another experiment similarly conducted to the above, the general results were the same. But in this case instead of pouring away the supernatant plasma above the first formed clot, the tube was shaken so as to break up this clot, liberate the imprisoned ferment-solution and thus mix the ferment with the still unclotted plasma. On now digesting the contents of the tube again at 40°, this plasma was solidly clotted after the lapse of 20 minutes. In this way the necessity of a contact between the ferment molecules and the fibrin-factors in the plasma seemed to be most conclusively shown.

Before concluding we must now describe an experiment, several times repeated, which though it yielded negative results was to a large extent satisfactory, since our previous experiments had led us to expect this negative result. Some milk was placed in a narrow (2 cm. wide) porous battery-cell made of extremely thin earthenware; the cell was then immersed in a beaker of rennin solution so that the level was the same inside and outside the cell, and digested at 40° for 17 hours. At the end of this time there was not a trace of clot in

the milk or on the inner surface of the porous cell. The experiment was repeated with the same cell for a period of 27 hours, with a similar result. It appeared to us that if Fick's view is correct, then a clot might have been expected to form in the above experiment, if not throughout the whole mass of milk, at least along the inner walls of the cell, but no trace of clot was observed. It would however be perhaps unwise to lay too much stress on this experiment, for we know but little of what is happening in the pores of the cell's wall when it is used as a septum between two different fluids. But it is scarcely conceivable that the rennin should not have come in contact with the milk, bearing in mind that such a cell is permeated by pores through which a filtration of fluids can be carried on under pressure. Had the septum been composed of parchment paper, no conclusions could have been drawn from the experiment.

On the basis of these observations we feel ourselves compelled to question the validity of Fick's views, and to see in the results we obtained nothing but a confirmation of what had previously been believed as to the mode of action of rennin and fibrin-ferment being essentially similar to that of other well characterized enzymes, as far as contact between the ferment and the alterable substance is concerned. The mode of action of enzymes is of such absorbing interest, that no statements which seem to indicate a possible difference in the way they produce their effects should be allowed to pass without careful examination and experimental confirmation.

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