NOTE ON THE INFLUENCE OF "PEPTONE" ON THE CLOTTING OF MILK BY RENNET.

By F. S. LOCKE, M. A. (Cantab.), M. B. (Lond.).

E. Gley* and A. Edmunds† have recently independently shown that Witte's "peptone" has a distinct effect in delaying the clotting of milk by rennet. An abstract of Gley's paper in the *Centralblatt für Physiologie* has made me aware (January, 1897) of the results of these workers. In October and November, 1894, I made experiments to determine the effect of "peptone" on the action of rennet on milk, and will shortly state the results of them here, as they differ in some not unimportant respects from those already published.

Gley and Edmunds both used Witte's "peptone" in their experiments.‡ I, at first, also made use of this, and obtained with it results exactly comparable with those of these observers. On testing the reaction of aqueous solution of Witte's "peptone," however, I found it markedly alkaline to red litmus paper. As alkalies have themselves a marked influence in delaying and even preventing the action of rennet on milk,§ the results I had obtained appeared to me to be valueless, and it became a question to obtain a "peptone" neutral or faintly acid in reaction.

Schuchardt's "peptone" I found to resemble Witte's in its action on red litmus. It was, therefore, useless for my purpose.

* E. Gley, *Compt. rend. de la Soc. de Biol.*, 1896, 591.

† A. Edmunds, *Journ. of Physiol.*, xix, 474, 1896.

‡ With the exception of one experiment reported by Gley in which 5 cc. of milk mixed with 3 cc. of a 10 per cent aqueous solution of "propeptone," made in accordance with the description of Grosjean, were acted on by rennet. The reaction of the propeptone is not mentioned. Judging from a statement of Spitzer's (*Arch. f. d. ges. Physiol.*, 1, 573, 1891) it would probably have been alkaline to lacmoid.

§ See p. 496 of this paper.

Gehe's "peptone," however, I found to be amphoteric in reaction, turning red litmus paper faintly blue and blue litmus paper faintly red. Its aqueous solution gave almost immediately a very distinct precipitate when an alkaline oxalate was added, pointing to the presence of soluble calcium compounds. It had the same action as Witte's "peptone" in delaying the action of rennet.*

Grübler's "peptone" was next made use of. Its aqueous solution was markedly acid to blue litmus paper and did not turn red litmus paper blue. It gave no precipitate in some minutes with ammonium oxalate and ammonium hydrate; only traces of calcium could therefore be present in it. Its effect in delaying the action of rennet was quite

* Experiment, November 3, 1894. The rennet used was, as in all my experiments, Crosse and Blackwell's, and was neutral in reaction to litmus. The "peptone-milk" was made by dissolving 1 gramme of Gehe's "peptone" in milk, and diluting with milk to 100 cc.

Time p. m.	Remarks.	10 cc. milk + 0.1 cc. 10% CaCl ₂ .	10 cc. milk.	10 cc. 1% peptone- milk + 0.1 cc. 10% CaCl ₂ .	10 cc. 1% peptone- milk.
3.21-22	0.3 cc. rennet added to each. The four tubes placed in water- bath at 39° C.	Thickening. Pours with difficulty. Clotting complete.	Pours with difficulty. Clotting complete.	Thickening. Pours with difficulty. Clotting complete.	Thickening. Pours with difficulty. Clotting complete.
3.22					
3.25					
3.25¼					
3.26					
3.28½					
3.28¾					
3.30					
3.30½					
3.31½					
3.35					
3.37					
3.40					

marked,* although not so much so as that of Gehe's "peptone," a difference very likely due to their difference in reaction, since acidity favors the action of rennet.

After these results it might appear that the reaction of "peptone" in delaying the clotting of milk by rennet was sufficiently established as being independent of any change in reaction caused by its addition, more especially since Gehe's "peptone" contained easily detectable soluble calcium compounds, which would themselves tend to hasten the action of rennet.† This is not, however, I think, the case. Our conceptions of "neutrality," "acidity" and "alkalinity" have, in recent years, undergone very great modifications, owing to the discovery of new indicators which differ in their behavior with the same chemical compounds.‡ Grüber's "peptone" affords an example of this, for

* Experiment, November 6, 1894.

Time p. m.	Remarks.	9 cc. milk + 1 cc. H ₂ O.	9 cc. milk + 1 cc. H ₂ O.	9 cc. milk + 1 cc. 10% H ₂ O solution of Grüber's "peptone."	9 cc. milk + 1 cc. 10% H ₂ O solution of Grüber's "peptone."
3.35-37	0.5 cc. rennet added to each. The four tubes placed in water- bath at 41° C.	Flakes. Thickening. Pours with difficulty. Clotting complete.	Flakes. Thickening. Pours with difficulty. Clotting complete.	Thickening. Pours with difficulty. Clotting complete.	Thickening. Pours with difficulty. Clotting complete.
3.38½					
3.40					
3.43					
3.43½					
3.44					
3.44½					
3.46½					
3.47					
3.48					
3.50					

† See experiment in foot-note * on p. 494. This effect of calcium salts has been observed by Hammarsten and many other workers.

‡ See R. M. Thomson, *Chem. News*, xlvii, 123 and 184, 1883; xlix, 32, 38, and 119, 1884; lii, 18 and 29, 1885. A. H. Allen, *Pharm. Journ. and Trans.*, xxii (3rd series), 752 and 772, 1892, gives a good general account of the subject. G. Courant's valuable paper, über die Reaction der Kuh- und Frauenmilch (*Arch. f. d. ges. Physiol.*, 1, 108, 1891), treats of it especially in relation to milk.

although markedly acid to litmus, it was markedly alkaline to lacmoid, as was also Gehe's "peptone," which to litmus reacted amphotERICALLY. These substances must, therefore, contain salts of "weak" acids, or bodies acting as such, which are possibly the cause of their power to delay clotting. This possibility prevented the publication of my experiments.

That increase in the alkalinity of milk hinders its clotting by rennet was pointed out in 1872 by Soxhlet,* Heintz† and Hammarsten.‡ Soxhlet and Heintz found that small additions of sodium carbonate, Hammarsten§ of sodium hydrate, had this effect.

Duclaux|| has investigated the influence of various substances on the action of rennet and finds that sodium carbonate, sodium borate, borax, and even boric acid, delay it. He observed also that sodium and calcium hydrates have the same effect.

Söldner¶ found that the coagulability of milk by rennet disappeared when it was rendered no longer acid to phenolphthalein by sodium hydrate. Courant** found that lime water exerts a similar action.

Ringer†† observed that a small percentage of sodium bicarbonate prevents the action of rennet. Arthus and Pagès‡‡ found that while sodium carbonate had this effect, sodium bicarbonate apparently had not.

* F. Soxhlet, zur physiologischen Chemie der Milch. Dissertation, Leipzig, 1872 (*Journ. f. prakt. Chemie*, N. F., vi, i, 1872).

† W. Heintz, *Journ. f. prakt. Chemie*, vi (N. F.), 374, 1872.

‡ O. Hammarsten, *Upsala Läkareförenings Förhandlingar*, viii, 63, 1872. This paper I have been unable to consult in the original. It is abstracted by Hammarsten himself in *Jahresber. u. d. Fortschr. d. Thierchemie*, ii, 118, 1874, and by Panum in *Jahresber. u. d. Fortschr. d. Anat. u. Physiol.*, ii, 392, 1875.

§ Hammarsten found that "certain salts, especially sodium phosphate (not only neutral, but also an amphotERICALLY reacting mixture of neutral and acid sodium phosphate), delay or prevent coagulation by rennet." (*Jahresber. u. d. Fortschr. d. Anat. u. Physiol.*, ii, 392, 1875.) What percentage of these salts Hammarsten worked with I have not been able to find.

|| E. Duclaux, *Annales de l'Institut Agronomique*, iv, 1880 (this paper I have been unable to consult), *Encyclopédie Chimique*, ix (1), 177, Paris, 1883, and *Le Lait*, Paris, 1887, p. 109.

¶ F. Söldner, die Salze der Milch. Dissertation, Erlangen, 1888 (*die landwirtschaftliche Versuchstationen*, xxxv, 351, 1888), pp. 78 and 84.

** G. Courant, op. cit., p. 146.

†† S. Ringer, *Journ. of Physiol.*, xi, 474, 1890.

‡‡ M. Arthus and C. Pagès, *Arch. de Physiol.*, xxii, 336, 1890.

Söldner endeavored to account for the favorable influence of acids for rennet and the unfavorable influence of alkalies by their action on the calcium salts in milk, acids increasing the amount of soluble calcium salts at the expense of the insoluble suspended tricalcic phosphate normally present, and alkalies having the reverse effect. If alkalies act only in this way it is difficult to see how Gehe's "peptone," containing as it does soluble calcium salts, can act as an alkali when added to milk and precipitate these. Söldner himself, however, refuses to deny that the degree of alkalinity may exercise a specific influence apart from its effect on the amount of calcium salts in solution, and from the evidence hitherto obtained I hesitate to draw any conclusion as to whether "peptone" has any specific effect on the coagulation of milk by rennet.

Grosjean,* who investigated in Fredericq's laboratory the influence of peptone and propeptone on the coagulation of the blood both when injected intravascularly and when mixed with it shed, declares that propeptone must be added in so great a proportion to shed blood to delay or suppress coagulation that its action cannot be regarded as different from that of strong solutions of neutral salts, of sugar, and of other indifferent substances.

Such an explanation of the action of "peptone" is, however, justifiable neither in the case of blood nor of milk. Grosjean† in one experiment lengthened the time before coagulation began from three to fourteen minutes, and the time before complete coagulation took place from eight to thirty-four minutes, by the addition of 2.5 per cent of "propeptone." Pollitzer‡ before him had found 2 per cent of heteroalbumose to increase the time necessary for coagulation from between 3 and 5 minutes to between 15 minutes and 24 hours. Edmunds and I observed, respectively, a distinct effect in delaying the clotting of

* A. Grosjean, *Memoires couronnes et autres Memoires publiés par l'Académie royale de Belgique*, xlii, 1-80, 1892 (somewhat condensed in *Arch. de Biol.*, xii, 381, 1892, and *Travaux du Laboratoire de L. Fredericq*, iv, 45, 1892).

† Op. cit., p. 28.

‡ S. Pollitzer, *Journ. of Physiol.*, vii, 288, 1886.

milk of 0.625 per cent and 1 per cent of "peptone." Now Hammarsten* found that 0.25 per cent of sodium chloride and of potassium chloride had no effect on the "Gerinnungszeit" of milk, and that 1 per cent of sodium chloride from three and a half minutes only increased it to four minutes, and 1 per cent of potassium chloride from two minutes only increased it to two and a half minutes. When the high molecular weights of albumoses† (relatively to these salts) is taken into consideration, it is impossible to assume that their action is analogous to that of neutral salts.

Gley, Edmunds and myself noticed that the effect of "peptone" in delaying the action of rennet can be antagonized by calcium chloride. I found afterwards, as had already been established by several observers, that calcium chloride added to milk in the absence of "peptone" increased the activity of rennet. Its action, therefore, in the presence of peptone may consist not in directly antagonizing an unfavorable influence, but in exercising independently a favorable one, and can be in no wise regarded as a support of the view that the action of "peptone" consists essentially in an attraction for calcium, which has been insisted on especially by Pekelharing‡ as the explanation of the action of "peptone" on the coagulation of the blood. Such a power to fix calcium in solution is very difficult, if not impossible, to conceive, and Pekelharing himself states that "peptone" gives no precipitate with soluble calcium salts. Just too as calcium chloride added to normal milk favors its coagulation by rennet, so does the intravenous injection of calcium chloride into the normal blood hasten its coagulation when shed, as Wright§ and others have found, a result which I also obtained without knowledge of their work in unpublished experiments on cats made in consequence of the experiments I had

* O. Hammarsten, zur Kenntniss des Caseins und der Wirkung des Labferments, Upsala, 1877, pp. 70 and 71.

† See Hammarsten, Lehrbuch der physiologischen Chemie, 3te Auflage, Wiesbaden, 1895, pp. 32 and 634.

‡ C. A. Pekelharing, Virchow's Festschrift, i, 433, 1891.

§ A. E. Wright, *Brit. Med. Journ.*, 1891 (ii), 1306.

made on milk. In the case, then, of the coagulation of the blood also, the annulling of the effect of "peptone" by calcium chloride may be due not to its directly undoing the work of "peptone," but to the same action favorable to extravascular coagulation which it exerts on unpeptonized intravascular blood.

It may be added that Halliburton and Brodie concluded merely from the fact that calcium chloride injected intravenously annulled the well-known effect of Haycraft's leech-extract in doing away with the coagulability of the blood, that this substance owes its power, like "peptone," to an "affinity" for calcium.*

HARVARD MEDICAL SCHOOL, BOSTON, MASS., *April*, 1897.

* W. D. Halliburton and T. G. Brodie, *Journ. of Physiol.*, xvii, 147 and 160, 1894.