streptococcus viridans, while the growth of Pneumococcus type 2 started to show the acceleration only at pH 7.8 and that of *B. Abortus* at pH 7.0.

Since the observations described above demonstrate that the food accessory substances of tomato show their optimum growth accelerating effect on certain bacteria at Hydrogen ion concentrations which seem to be absolutely independent of the optimum pH for their growth in ordinary media, an explanation of this phenomenon was sought. See 18 (2541).

6 (2529)

The action of pepsin on insulin.

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Because of the practical advantages that would accrue from the oral administration of insulin in the treatment of diabetes, an exact knowledge of the mode of action of the digestive ferments upon it would be valuable. The various attempts made to procure physiological effects from insulin given by mouth have so far proved futile, and this failure has been ascribed to the destructive action of the gastro-intestinal ferments upon it. Recorded studies on the action of the individual proteolytic ferments on insulin agree that they 'destroy' insulin by proteolysis. These investigations assume that insulin is a 'protein' body, and that the loss of its physiological potentialities is the result of cleavage.

Certain considerations prompted a reexamination of this matter. In work recently reported,[1, 2] it was found that trypsin does not actually destroy insulin, but that it merely renders it inactive

[1] Epstein, Albert A., and Rosenthal, Nathan, *J. Am. Med. Assn.,* 1924, lxxxii, 1990.

[2] Epstein, Albert A., Rosenthal, Nathan, and collaborators, *Am. J. Phys.,* 1924, lxx, 225 (in press).

by combining with it. The reaction which takes place between the two substances is in the nature of a chemical combination similar to that now known to occur between trypsin and safranin.[3] The experiments referred to give the conditions under which trypsin inactivates insulin. Briefly, this inactivation takes place instantly when the pH of the medium is to the alkaline side of 4.6. Inactivation of insulin does not occur if the pH is below 4.6. From then on, dissociation or reactivation of insulin can be brought about even after many hours of contact with trypsin, by adjusting the pH of the medium to a point below 4.6.

Similar studies were undertaken with pepsin and insulin. The method of preparation of pepsin was as follows: Pepsin (Fairchild) was extracted with 50 per cent alcohol; the extract filtered and treated with 8 volumes of pure acetone. The precipitate was dissolved in water and passed through a Berkefeld filter. The solution was kept in the ice-box under sterile precautions. Such a solution of pepsin remains active for a long time and thus has the advantage that the same preparation can be used in a large series of experiments.

Pepsin and insulin mixtures were acidified (pH below 3.0) and portions of the mixtures were injected at different intervals into suitable test animals. It was thus found that under the conditions described, inactivation of insulin takes place instantly. The amount of insulin contained in each portion of the mixture used for injection was at least 15 units and frequently much more. The results have been uniform in that none produced the physiological effects of insulin. The type of acid used for acidification is apparently of no great consequence. The addition of buffered solutions to the pepsin-insulin mixtures yields the same result. Neutralization or alkalinization of the mixure prevents the inactivation.

The brief contact necessary for the inactivation of the insulin suggests that the process underlying it is not one of proteolysis. This supposition is supported by the fact that liberation or reactivation of the insulin can be effected by neutralizing or alkalinizing the pepsin-insulin mixture even after contact under conditions favorable for digestion (thermostat at 37.5° C.) for as long a period as 4 days.

While the exact range of the pH necessary for the inactivation

[3] Marston, Hedley R., *J. Biol. Chem.*, 1923, xvii, 851.

of insulin by pepsin and its reactivation has not as yet been determined, it may be stated that the inactivation of insulin by pepsin and its reactivation, occur under conditions diametrically opposite, as far as the pH is concerned, to those necessary for a similar action of trypsin on insulin.

SUMMARY.

1. Pepsin 'inactivates' insulin but does not digest it.
2. Liberation or dissociation of insulin from pepsin takes place, even after prolonged contact, at a properly adjusted pH.

7 (2530)

Diphtheria toxin-antitoxin titration by Ramon method for practical application.

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The Ramon test was evolved from the works of Calmette and Massol,[1] who in 1909 applied the flocculation test for titration in vitro of antivenom serum against Cobra venom. Nicolle, Cesari and Debains[2] in 1919 applied the same principle for titration of diphtheria and tetanus toxin-antitoxin by the method of Ascoli.[3] The reaction consisted in the formation of an opalescent ring in contact with a concentrated toxin and gelatinized antitoxin. Georgi[4] added a suspension of cholesterolized heart extract to the mixtures of toxin-antitoxin to obtain flocculation.

Ramon[5] in 1922 found that diphtheria and tetanus toxin and antitoxin alone, when mixed in certain proportions, will bring about flocculation. The mixtures with a deficiency or excess of either toxin or antitoxin will fail to flocculate. The first precipitate to appear in the mixtures Ramon calls the "precipitate indi-

[1] *Ann. de L'Inst. Pasteur,* 1909, xxiii, 155.
[2] *Compt. Rend. Acad. des. Sci.,* 1919, clxix, 1433.
[3] *Berl. Tieraerzol. Wochenschr.,* 1911, No. 22, 389.
[4] *Medizinische Klinic,* 1920, xvi, 1053.
[5] *Compt. Rend. Soc. de Biol.,* 1922, lxxxvi, 711.