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.

cateur" and this corresponds to a nearly neutral mixture. From the latter the values of toxin or antitoxin are calculated.

Scholtz[6] in 1923 found that it was not necessary to employ for this test the enormous quantities of toxin used by Ramon (20 cc. for each tube). He used instead 2 cc. of a stable titrated toxin and corresponding diiutions of serum. Glenny and Okell[7] in 1924 also used from 2 to 5 cc. of a stable titrated toxin and minute quantities of undiluted serum. All workers agree that the Ramon titrations agree closely with guinea pig tests and are therefore applicable for practical routine estimation of values of toxin and antitoxin.

Povitzky and Banzhaf working on this test since early spring of 1924 arrived at the same conclusions. They found that the most reliable and constant results are obtained by the use of a potent stable toxin titrated for its L+ and flocculation values and undiluted serum or (if the latter is sufficiently strong) diluted 1:1, that is, with an equal amount of physiological salt solution. The amount of toxin was usually 2.5 cc. per tube. They also tried to work with only 1 cc. of toxin, diluting the serum correspondingly. The results, however, with a serum diluted 1:5 were variable (delayed) in comparison with parallel tests with undiluted serum and larger amounts of toxin (2.5 to 5 cc.). It is very important to read the results on the same day the tests are made, since the next day more than two tubes may show flocculation and it would be impossible to tell which tubes precipitated first. With a stronger toxin, however, (this toxin is about 4 L + per cc.) the dilutions of the serum need not be so high and the test should be perfectly workable with 1 cc. of toxin.

In testing the serum and plasmas of thirty-five horses at different times the above authors found only a very few discrepancies between the results of guinea pig tests and Ramon flocculation method.

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[6] *Centralbl. f. Bact. Orig.*, 1923, xci, 72.

[7] *J. Path. and Bact.*, 1924, xxvii, No. 2, 187.

Diphtheria Toxin-Antitoxin Titration by Ramon Tests.

| Horse No. | | Amts. of serum per 2.5 cc. toxin* | | | | Time of† flocculation | Units of flocculation | Units by guinea pig test |
|---|---|---|---|---|---|---|---|---|
| 28 | P | .08 | .07 | .06 | .05 | 2.20 hrs. | 400+ | 375-400 |
| | dil. 1:1 | — | — | + | (+) | | | |
| 33 | P | .07 | .06(\).05 | | .04 | 2½ hrs. | 350 about | 350-375 |
| | dil. 1:1 | — | + | ± | — | | | |
| 34 | P | .08 | .07 | .06 (\).05 | | 3¾ hrs. | 175+ | 175-200 |
| | | — | — | + | ± | | | |
| 40 | P | .06 | .05 | .045 | .04 | 3¼ hrs. | 175 about | 200-210 |
| | | (+) | — | — | — | | | |
| 42 | P | .04 | .035 | .03 | .025 | 3 hrs. | 250+ | 325-350 |
| | | (+) | — | — | — | | | |
| 43 | P | .04 | .035 | .03 | .025 | 2¾ hrs. | 250+ | 275-290 |
| | | (+) | — | — | — | | | |
| 44 | P | .045(\).04 | | .035 | .03 | overnight | 225+ | 300-310 |
| | | + | ± | — | — | | | |
| 45 | P | .045 | .04 | .035 | .03 | 3¾ hrs. | 300— | 275-280 |
| | | — | + | (+) | — | | | |
| 46 | P | .06 | .05 | .045 | .04 | 5 hrs. | 175+ | 200-210 |
| | | (+) | — | — | — | | | |
| 49 | P | .06 | .05 | .045 | .04 | 4 hrs. | 200+ | 200-220 |
| | | + | (+) | — | — | | | |
| 60 | P | .15 | .13 | .12(\).10 | | overnight | 100 about | 175 |
| | | — | — | + | + | | | |
| 62 | P | .05 | .045 | .04 | | 2 hrs. | 225+ | 225 about |
| | | + | (+) | — | | | | |
| 63 | S | .095 | .09 | .085 | .08 | overnight | 100+ | 200 minus |
| | | + | (+) | — | — | | | |
| 66 | P | .08 | .06 | .05 | .045 | 1¾ hrs. | 200 | 175-180 |
| | | — | + | (+) | — | | | |
| 67 | P | .15 | .13 | .12 | .10 | overnight | 75 | 175 |
| | | (+) | — | — | — | | | |
| 68 | S | .09 | .085 | .08 | | 4¼ hrs. | 100+ | 200 much less |
| | | (+) | — | — | | | | |
| 69 | P | .04 | .035 | .03 | .025 | 4¾ hrs. | 250+ | 250-260 |
| | | (+) | — | — | — | | | |
| 72 | S | .06 | .05 | | | overnight | 175 | 175-200 |
| | | (+) | — | | | | | |
| 73 | S | .125 | .12 (\).115 | | .10 | 4 hrs. | 100— | 100-110 |
| | | — | + | + | — | | | |

P = Plasma. S = Serum.

* 1 cc. toxin = about 4 L+. The flocculating value of the indicating mixture contains 4 units of antitoxin per 1 cc. of toxin. Units of antitoxin per cc. can be calculated from amounts used for flocculation.

† Tubes placed in water bath at 50° C. Tubes not showing reaction on same day were placed in incubator overnight.

(+) show "indicating mixture". (\) between two numbers, take number between the two, or average number.