light yellowish white amorphous powder melting at 170–172°. It yielded an acetyl derivative which was a dark brown mass melting below 100°. On hydrolysis this saponin was resolved into a pentose and a sapogenin which was of a darker color than the original saponin melting at 188–191°.

It has been mentioned above that this saponin is not toxic to animals or fish and has no haemolytic action. This conclusion was indicated by the following experiments:

(A) 0.5 Gm. of the saponin was dissolved in 25 Cc. of physiological salt solution, thus making it 2% in strength. Ten test tubes each containing $^1/_2$ Cc. of a 2.5% three-times-washed sheep red corpuscles were prepared, and physiological salt solution was added to each to make the final volumes 3.5 Cc. Into these tubes were pipetted 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 Cc. respectively of the above prepared saponin solution. The tubes were then shaken and allowed to stand at room temperature. In 24 hours the corpuscles had settled down, leaving a colorless liquid at the top in all the tubes. The tubes were allowed to stand for one week, but no change was observed.

Another set of experiments was carried out as above except the tubes were kept in the incubator at 37° instead of at room temperature. The results were the same.

(B) A 10% solution of this saponin was prepared, 2 Cc. was injected to each of two rabbits and one guinea pig intravenously and another 2 Cc. to another guinea pig subcutaneously. All these animals were examined every day, which resulted in no symptoms of physiological activity.

(C) A 1–2000 solution of the saponin was prepared in a large glass jar and a gold fish weighing 28 Gm. put in. The jar was placed outside of a window and the fish fed with fish food. For two days, it was just as healthy as it was at the beginning. At the end of four days no change was shown, it was taken out and its weight noted, which showed neither increase nor decrease.

## THE RELATION OF THE DISSOCIATION OF HYDROGEN TO ENZYMIC ACTIVITY.* PEPSIN STUDIES.

BY HOWARD T. GRABER.

Sörensen demonstrated that the measure of the reaction of hydrolysis by Pepsin, and also Catalase, is not dependent upon the titrable acidity but rather upon the "H" ion concentration.

He showed that the action of these enzymes has an optimum at a definite "H" ion concentration and that the presence of other ions exerts an influence which, while not measureable, should not be neglected. He pointed out that he could produce one and the same "H" ion concentrations by means of either of two methods:

1—By the use of what he termed regulators or buffers, or

2—By the dilution of the acid.

It was also shown that regardless of what buffer controls the acidity, and irrespective of the concentration of the other ions, the enzyme exerts the same activity if the concentration of the "H" ions is the same.

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The experiments here recorded were made with the idea of corroborating the above findings with special regard to buffer action in the case of various acids, and peptic digestion.

It is universally recognized that the pepsin digest, when egg white is used as the proteid under digestion, takes place best in a medium of .3% HCl and at a temperature of 52°C. If the peptic activity were dependent upon the titrable acidity alone, it would only be necessary to adjust other acids, both organic and mineral, to this same .3% strength and get the same activity, assuming that the acid employed does not directly inhibit peptic digestion.

The following experiment proved that this theory is fallacious.

U. S. P. test for pepsin with the use of various acids.

| Acid. | Titration. | *p*H. | Residue. |
|---|---|---|---|
| Hydrochloric | .30% | 1.25 | 1.5 Cc. |
| Acetic | .30% | 3.30 | 17.0 Cc. |
| Citric | .31% | 2.40 | 14.0 Cc. |
| Lactic | .32% | 2.38 | 20.5 Cc. |
| Nitric | .31% | 1.25 | 19.5 Cc. |
| Phosphoric | .32% | 2.11 | 22.0 Cc. |
| Sulphuric | .31% | 1.31 | 20.0 Cc. |

If the titrable acidity were the controlling factor we should expect the same digestion in all of the above. The amount of titrable acid is practically the same in all.

An electrometric measurement of the dissociated hydrogen in each, at a temperature of 16° C., shows that there is quite a difference in the concentration of "H" ions. Here, however, it is evident that the three mineral acids used were of similar "H" ion concentration yet the results showed a great difference in degree of digestion. Apparently there may be an error in measuring "H" ion concentration at 16° and carrying on digestion at 52° since temperature has an appreciable effect on the dissociation of acids.

In the next set of experiments the acids were heated to 50° C., quickly measured against a calomel cell at 16° C. and the "H" ion concentration calculated as of 16°. This gave a figure which was more nearly that of the true dissociation at the higher temperature used in digestion. The results of the experiments are recorded in the following table:

| Acid. | Titrable acidity. | *p*H at 52° C. Cell at 15° C. | Residue after digestion on 10 Gm. egg at 52° C. for 2½ hours. |
|---|---|---|---|
| Acetic | 40.30% | 1.16 | 30.00 Cc. |
| Citric | 44.60% | .92 | 21.00 Cc. |
| Lactic | 20.40% | .87 | 30.00 Cc. |
| Nitric | .55% | 1.16 | 16.00 Cc. |
| Phosphoric | 38.30% | .85 | 19.00 Cc. |
| Sulphuric | 4.10% | 1.14 | 19.50 Cc. |
| Hydrochloric | .30% | 1.18 | 1.75 Cc. |

As a result of the above test it was concluded to adjust the "H" ion concentration of the various acids at the digestion temperature, 52° C., and also consider the buffer effect of the egg white when added to the acid.

This test is most encouraging and shows that under the proper conditions, with an "H" ion concentration equal to that of .3% HCl, the phosphoric acid of 49.00 titrable acidity digested equally as well as the hydrochloric acid with a titrable acidity of .3%.

| Acid. | Titrable acidity. | $p$H at 52° C. Cell at 15° C. | Residue after digestion as above. |
|---|---|---|---|
| Hydrochloric | .30% | .82 | .80 Cc. |
| Lactic | 30.50% | .87 | 35.00 Cc. |
| Nitric | .37% | .79 | 19.50 Cc. |
| Sulphuric | .98% | .79 | 23.00 Cc. |
| Citric | 22.00% | .82 | 10.00 Cc. |
| Acetic | 45.30% | .92 | 35.00 Cc. |
| Phosphoric | 49.00% | .82 | .80 Cc. |

To test out the buffer effect of the albumen upon the .3% HCl, the $p$H reading was taken before adding the egg white and after adding the egg, but before adding the pepsin, and the addition of the egg white buffered the concentration from $p$H .82 to $p$H 1.19. It was also determined after digesting the egg for one, two, and two and one-half hours but no appreciable change in the concentration of the "H" ion was noted during digestion.

The results, so far compiled, led to a last and final experiment in which the "H" ion concentrations were brought to comparable values.

The last question to be settled concerns the presence of HCl as an activator of the pepsin. To determine whether the presence of HCl is necessary as an activator of the pepsin in the presence of other acids, we dissolved the pepsin in .3% HCl in four of the experiments and in phosphoric acid in the other two experiments.

| Acid. | Titrable acidity. | $p$H at 50° C. Cell at 15° C. | Residue after digestion as above. |
|---|---|---|---|
| Hydrochloric | .30% | .64 | .40 Cc. Pepsin phosphate |
| Hydrochloric | .30% | .64 | .40 Cc. Pepsin hydrochloride |
| *Phosphoric | 49.00% | .68 | .60 Cc. Pepsin phosphate |
| Phosphoric | | .68 | .60 Cc. Pepsin hydrochloride |
| Acetic | 50.20% | .68 | 35.00 Cc. Pepsin hydrochloride |
| Citric | 25.90% | .63 | 10.00 Cc. Pepsin hydrochloride |

* Same phosphoric acid used in this as in previous tests.

These results show that phosphoric acid brought to the same "H" ion concentration as .3% HCl at 52° C. before adding egg white is just as efficient as the HCl in activating peptic digestion. Also it is shown that the slight amount of HCl in which the pepsin is ordinarily dissolved has no appreciable effect on this digest, since the solution of pepsin in phosphoric acid of the same "H" ion concentration is equally successful.

It is also shown that acetic and citric acids brought to the proper "H" ion concentration at 52° C. before adding egg white are not efficient activators of pepsin digestion after the egg white is added.

This is perhaps due to the strong depressant action of the egg white which acts as a buffer toward these weakly dissociated acids. This will be investigated in later experiments.

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