

THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE PRODUCTION OF PRECIPITATES IN A SOLUTION OF PEPTONE AND ITS RELATION TO THE NUTRITIVE VALUE OF MEDIA

I. J. KLIGLER

Department of Public Health, American Museum of Natural History, New York

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It is a well-known fact that when media are neutralized to phenolphthalein a precipitate is produced which may be partly redissolved by the addition of acid. If this precipitate is filtered off the nutritive quality of the medium is appreciably lowered. Likewise, experience has taught us that the optimum reaction of a culture medium is +1.0 to phenolphthalein or neutral to litmus. In neither case, however, has an adequate explanation been given for the observed facts.

From previous experiments on synthetic media it became evident that the significant nutritive factor in bacteriological culture media was the peptone and that a careful study of this substance should yield explanations of many puzzling facts. From various analyses it appears that peptone consists of proteoses, amino-acids and salts, the most important of the latter being the phosphates. The proteoses and complex nitrogenous compounds can not be utilized by the common bacteria but they do play an important part as buffers in the medium. The amino-acids are the essential nitrogenous foods upon which the nutritive value of the peptone depends. The phosphates furnish an important salt but are of even greater value as reaction regulators. An ideal peptone would, therefore, be one that contains these ingredients in the right proportions.

Since the reaction of the medium, both in the making and in the finished product, has so great an influence on the nutritive quality, attention was directed to the effect of different reactions,

in terms of hydrogen ion concentration, upon the peptone. Solutions of 1 per cent peptone in distilled water were made up and varying amounts of acid or alkali added to obtain a graded series of hydrogen ion concentrations. Solutions of Witte and Difco peptone were studied in this manner.

The results obtained bear so directly on the problem that even though the procedure was only qualitative they are of sufficient interest to deserve recording. Briefly stated, it was found that peptone has two precipitating zones, one on the acid side, the other on the alkali side, with an intermediate zone of complete solubility. In terms of P_H , peptone (Witte's) is completely soluble between P_H 6.8 and P_H 8.0. At P_H 8.2 precipitation begins and increases progressively to P_H 9.0. Similarly, a precipitation occurs at about 5.4 and increases up to about 5.0, then decreases again to about P_H 4.0 when no precipitate is formed.

The nature of the precipitate in the two zones is suggestive. At the alkali end the precipitate appears to consist largely of phosphates plus some organic constituents. The acid precipitate, on the other hand, is organic and when redissolved gives reactions characteristic of proteoses and peptone.

Both Witte's and Difco peptone behaved in the manner outlined with the following exceptions: Witte's peptone when dissolved in water has a P_H value of 6.5 to 6.8. It dissolves slowly and with a clear solution. On autoclaving only a slight sediment is sometimes obtained. The Difco peptone, on the other hand, has a P_H value of 5.1 to 5.4 when dissolved. It goes into solution readily but gives a heavy precipitate on autoclaving. This is to be expected since its reaction is right at the precipitating zone. Like Witte's peptone it has a clear solubility zone between P_H 7.0 and 8.0 and a precipitating zone beyond 8.0.

The bearing of these facts on the changes obtained in the making of media and on their nutritive quality is obvious. The rôle of peptone in culture media (either infusion or extract) is twofold. It furnishes nitrogenous food in the form of amino-acids and also buffer substance in the form of phosphate salts and higher nitrogen complexes (proteoses, etc.). A peptone

rich in amino-acids will, of course, furnish a basis for abundant growth. Unless, however, there is an effective buffer for regulating (suppressing) the hydrogen or hydroxyl ion, growth will be rapid at first but will soon cease. This is the experience with the Difco peptone. This peptone is richer in amino-acids (Formol titration) than Witte's peptone but is poorer in buffer (as can be seen from the character of the titration curve—small increases in acid or alkali produce appreciable changes in the P_H .) This latter condition is explicable on the basis of the high initial P_H , which may cause a precipitation of the proteoses at some stage of the preparation. This also accounts for the fact that, although richer in amino-acids, this peptone has the same total nitrogen content as Witte's peptone. It is to this difference in the content of the higher nitrogenous complexes that we ought to look for an explanation of the failure to obtain a potent diphtheria toxin with the Difco peptone.¹

Realizing that the precipitate at P_H 8.2+ is mostly phosphates its relation to the character of the medium is quite apparent. Phosphate salts play a very important rôle in regulating bacterial metabolism and act with the proteoses as buffers in the medium. The point at which precipitation occurs is near the turning-point of phenolphthalein, the reaction of all sugar media. This explains the variability obtained in the growth of streptococci on such media, as well as the failure to obtain growth of tubercle bacilli in glycerol media made up in that way, even if the reaction is brought back to +1.0. In both cases undoubtedly the partial removal of the phosphate salt is, in itself, a factor. In the former, however, the suppression of the buffer action may be equally important.

¹ The accepted view is that diphtheria toxin is a secretion by the cell of a complex built up by it. This conception it seems to me is not supported by the facts. The more plausible idea is that it is an excretion of a residue in the cell metabolism just as ammonia or lactic acid is a waste by-product. This residue is only produced from the higher peptone complexes. That is why the Difco peptone, poor in these compounds, is not favorable for toxin production. This view also accounts for the failure on the part of *Corynebacterium diphtheriae* to produce toxin in sugar media. The sparing action of sugar on protein or rather nitrogenous metabolism is well-known.