XXV. THE INFLUENCE OF REACTION ON COLOUR CHANGES IN TRYPTOPHAN SOLUTIONS.

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(Received February 18th, 1921.)

THE work which is outlined below is a continuation of that already described [Cornish and Williams, 1917; Venn, 1920].

In the first paper a study was made of the colour changes produced by a number of strains of two groups of organisms, gram-negative alkaline and *B. proteus vulgaris*, upon caseinogen and upon some of its degradation products. In the course of this work it was noticed that the gram-negative alkaline organisms produced very marked colours on tyrosine, and only slight colours on the other amino-acids used, while the strains of *B. proteus vulgaris* produced definite colours on tryptophan and only slight colours, or none at all, on the others.

For these reasons the amino-acid tyrosine and one strain of the group of bacilli called gram-negative alkaline were chosen for an investigation into the influence of the reaction upon the colour production of this group of organisms, and the results of these experiments were given in the second paper previously mentioned.

The subject of the present paper is the influence of the reaction upon colour production in tryptophan solutions by *B. proteus vulgaris*. One strain (C₂₀2B₂) of this organism which was used in the original work was chosen, and after replating, was tested upon sugars, etc. with results which corresponded with those previously described [Cornish and Williams, 1917]. The technique of the P_H determinations was similar to that adopted for the tyrosine solutions [Venn, 1920].

The tryptophan was prepared by Hopkins and Cole's method and a solution of 0.05 % was made and steamed for half an hour on each of three successive days. It was then filled out into Erlenmeyer flasks in quantities of 90 cc. and caustic soda (concentration before sterilisation 0.004556 g./cc.) was added in varying quantities to eleven, and hydrochloric acid (concentration before sterilisation 0.02421 g./cc.) to two of the solutions in these flasks. At a later date hydrochloric acid of 1/10 of this concentration was added to four

others in an endeavour to obtain P_H values between 8.3 and 2.5 but the table shows that this attempt was unsuccessful. After the addition of caustic soda or hydrochloric acid the experimental solutions were kept for about two and a half months before the inoculations were made. All the work was carried out under sterile conditions.

Before this series of solutions was made preliminary experiments had been carried out over a range corresponding approximately to reactions of -80 to $+20$ Eyre's standard and colours had been produced from -30 to $+2$. The tryptophan itself was found to be approximately $+2.5$ (Eyre's standard). The quantities and concentrations of the caustic soda and hydrochloric acid used in the later experiments were calculated to give reactions within which this colour producing range would be included.

At the time of the inoculation each solution was tested for sterility and no evidence of contamination was ever found. Each solution was then divided into three parts; the P_H of one was determined, the second was inoculated with *B. proteus vulgaris*, and the third was kept as a control. The control and the inoculated solutions were incubated at 30° for seven days, when they were removed from the incubator and kept at laboratory temperature for another seven days, at the end of which time the P_H of both portions was determined. Tests for growth in the inoculated solutions and for sterility of controls were made. Again, no evidence of contamination in the controls was found.

Frequent examinations for colour production were made by comparison with the controls.

The table shows that the organisms did not grow or produce colour when inoculated into tryptophan alone (solution 6), and a similar negative result was obtained with acidities varying from P_H 1.8 to 2.5 (solutions 1 to 5 to which hydrochloric acid had been added). In those solutions to which caustic soda had been added, growth was found, accompanied by colour production, in solutions nos. 7 to 13 inclusive; growth was demonstrated but no colour was produced in solutions nos. 14, 15 and 16, while solution no. 17 was found to be sterile and no colour was produced. This shows that the range of reaction within which colour production was found in this series was between P_H 8.9 and P_H 9.4. The colours produced in the different solutions showed a definite sequence from deep orange in solution no. 7, passing through orange and deep yellow, to a light yellow until it disappeared at no. 14. These results confirmed those already obtained in the preliminary series in which colours were found over a range of -30 to $+2$ but were entirely absent at -40 and $+4.5$ (Eyre's standard).

The table given below is a summary of the results obtained.

Original solutions. Each contains 90 cc. of 0.05 % tryptophan solution. Varying amounts of NaOH (1 cc. = 0.004556 g.) or HCl (1 cc. = 0.02421 gr.) or HCl (1 cc. = 0.002421 gr.)						
No. of solution	No. of cc. NaOH or HCl	P _H at time of Inoculation	Control solutions P _H	Inoculated solutions		
				Growth	Colour	P _H
1 cc. = 0.02421 g. HCl						
1	2.05	1.73	1.73	—	—	1.68
2	1.3	2.12	1.86	—	—	1.86
1 cc. = 0.002421 g. HCl						
3	10	2.10	2.16	—	—	2.18
4	5	2.32	2.24	—	—	2.23
5	3	2.36	2.53	—	—	2.55
6	Tryptophan only	8.24	8.12	—	—	8.19
1 cc. = 0.004556 g. NaOH						
7	0.75	8.95	8.63	+	Deep orange	8.95
8	1.25	8.89	9.03	+	Deep orange	8.95
9	2.25	9.19	9.07	+	Orange	9.07
10	3.25	9.27	9.21	+	Deep yellow	8.98
11	4.25	9.32	9.27	+	Greenish yellow	9.35
12	4.85	9.36	9.37	+	Yellow	9.36
13	5.6	9.44	9.44	+	Light yellow	9.41
14	7.0	9.47	9.51	+	—	9.50
15	8.0	9.51	9.50	+	—	9.46
16	9.0	9.47	9.60	+	—	9.62
17	11.0	9.58	9.57	—	—	9.57

REFERENCES.

- Cornish and Williams (1917). *Biochem. J.* 11, 180.
Venn (1920). *Biochem. J.* 14, 99.