XIII. THE INFLUENCE OF REACTION ON COLOUR CHANGES IN TYROSINE SOLUTIONS.

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(Received February 24th, 1920.)

THE work which is described in this paper is a continuation of that which was previously carried out on colour changes produced by two groups of bacteria on caseinogen and certain amino-acids [Cornish and Williams, 1917]. In that paper it was demonstrated that certain groups of bacteria which had been isolated from discoloured Stilton cheese were capable of producing changes of colour on certain media, consisting of simple suspensions or solutions in water of caseinogen and certain amino-acids, which are among its degradation products and many of which are known to be present in ripe Stilton cheese. In the course of that work it had been frequently found that the constitution and reaction of the media upon which the organisms were grown appeared to exercise a very considerable influence upon the degree of discoloration, which tended to vary with the nature of the amino-acids present and to diminish with increased acidity.

Further tests were therefore carried out to study the influence of these two factors. Water solutions or suspensions which were either neutral or slightly acid were made of caseinogen, tyrosine, tryptophan, histidine, alanine and cystine and, in addition, agar media containing these substances were prepared and standardised to a reaction of -1 and -3 according to Eyre's method. The growths upon these media produced some striking colours which are recorded in Cornish and Williams' paper.

The present paper is concerned with further investigations designed to discover the effect of varying degrees of acidity and alkalinity upon the colours produced by one strain upon a single amino-acid. For this purpose a member of the Gram-negative Alk. group [Cornish and Williams, 1917] $C_{22} 4^B$ was chosen, and its colour-producing effects upon tyrosine were studied. This strain $C_{22} 4^B$ had been found capable of producing colour in histidine water, alanine water, caseinogen water, tyrosine water, tryptophan water and upon agar media containing respectively these amino-acids. Tyrosine was selected as the amino-acid partly because it is present in very considerable quantities in ripe cheese and is prepared with relative ease, and partly because it is a medium upon which colour changes are easily produced.

As a preliminary experiment a solution of 0.1 % tyrosine was made, and from this solutions of varying reaction from approximately + 15 to - 35 (Eyre's standard) were prepared by the addition of the requisite quantities of sodium hydroxide or hydrochloric acid. After inoculation, colours were produced in those media, of which the reaction lay between + 5 and - 35. In the latter case the colour was black, and in the former there was only slight darkening of the solution. The tubes which contained solutions of

Fig. 1.

a = Glass tubing.
b = Platinum wire.
c = Rubber band.
g = Wire leading to potentiometer.

d = Platinum foil.
e = Glass cover.
f = Mercury cup.

greater acidity than + 5 showed no change in colour and the controls remained unchanged. In view of these observations the series was then extended and solutions were made up with reactions which varied between - 35 and - 85. At the end of 27 days no colour change was found in those solutions with a greater alkalinity than - 45. These results seemed to indicate that there was a certain range of reaction within which this strain of bacilli was capable of acting upon tyrosine with production of colour, but beyond which colour changes were not produced.

In order to obtain a more accurate measure of the range within which colour changes may be produced, it was decided to carry out electromotive force determinations on a further series of inoculated and control tyrosine solutions.

The apparatus used in these investigations was a Tinsleys's universal potentiometer, a very sensitive d'Arsonval galvanometer, and as a calomel half electrode the saturated cell described by Barendrecht [1915].

For some time an endeavour was made to use the small hydrogen electrode also described by Barendrecht, but it was found very difficult to obtain consistent results with this type of electrode, owing no doubt to the nature of the solutions under investigation and the difficulty of cleaning the electrode. The simple hydrogen electrode described below was therefore devised, and for this particular piece of work has been found to give consistently satisfactory results.

The hydrogen electrode is shown slightly larger than actual size in the accompanying diagram (Fig. 1). (a) is a piece of glass tubing bent at right angles and slightly thickened as shown. (b) is a length of platinum wire, one end being fused through the glass and the other one carrying a platinum plate (d). The outer glass covering (e) is held in position by the rubber band (c) and the space above is filled with mercury forming the cup (f) by which contact is made to the potentiometer by wire (g) by simply inserting the wire into the mercury.

The advantages of this simple electrode are two. There is no dead space or side tube, and it is very easily and quickly cleaned and platinised. The disadvantage is the use of rubber tubing, but if this ring is placed in the position shown, the liquid under investigation need never come into contact with the rubber. The electrode is used in the same way as that of Barendrecht [1915] and shares its advantage of being small and therefore needing less time for saturation than a larger one. When a reading has been completed, the mercury is tipped out, the outer tube and rubber ring are taken off, and the electrode is cleaned and replatinised. This is done between each determination. It is found that there is no danger of leakage if the sizes of the glass and rubber tubings are carefully adjusted.

The results obtained by this method are given in the following table from which it appears that there is a definite range of reaction over which the organism studied is capable of producing darkening in tyrosine solutions, and beyond which such discoloration fails to appear.

In all cases except Nos. 30 and 31, the P_H was determined within a month of the date of inoculation of the solution, and a control uninoculated solution was also tested in 31 cases. Solutions Nos. 30 and 31 were kept for 14 months after inoculation before the P_H was determined, but at the end of that time no colour had developed and the P_H values were those recorded.

No explanation is offered of the fact that the inoculated portion of solution No. 29 with a P_H of 1.95 shows a slight colour. Apart from this single instance there appears to be a gradual increase of colour from P_H 3.23 passing through a maximum and gradually disappearing as the P_H approaches a value of about 9.7. The gradations in colour are not a perfect sequence, but if the whole series be considered it is found that, except in the case of No. 29, no colour

was produced at a P_H below 2.28; at 3.23 a lightish brown colour appeared which became a deep brown or reddish brown from 5.83 to 9.47. Above this point the colours are for the most part of a yellowish type until the limit is reached at 9.71.

Table of colour reactions produced in 0.1 % tyrosine solutions of varying P_H .

No.	Solution	P_H	Colour	No.	Solution	P_H	Colour
1	Inoculated	9.67	No colour	17	Inoculated	8.68	D.B.
	Control	9.71			Control	8.74	
2	Inoculated	9.63	V.Sl.Y. with	18	Inoculated	8.43	V.D.B.
	Control	9.59	V.Sl.P.		Control	8.57	
3	Inoculated	9.61	V.Sl.Y.	19	Inoculated	8.32	D.B.
	Control	9.63			Control	8.38	
4	Inoculated	9.61	V.Sl.Y.	20	Inoculated	8.20	D.P.B.
	Control	9.63			Control	8.60	
5	Inoculated	9.61	Cinnamon	21	Inoculated	7.51	D.B.
	Control	9.63			Control	7.63	
6	Inoculated	9.55	Sl.P.Y.	22	Inoculated	6.69	D.B.
	Control	9.60			Control	7.15	
7	Inoculated	9.55	D.Y. with	23	Inoculated	5.83	V.D.B. with
	Control	9.58	P. tinge				Sl.P.
8	Inoculated	9.54	V.V.V.Sl.P.	24	Inoculated	3.23	L.B.
	Control	9.56			Control	(?) 2.30	
9	Inoculated	9.54	V.Sl.Y.	25	Inoculated	2.59	No colour
	Control	9.53			Control	2.35	
10	Inoculated	9.50	P.B.	26	Inoculated	2.28	No colour
	Control	9.45			Control	2.47	
11	Inoculated	9.40	R.B.	27	Inoculated	2.26	No colour
	Control	9.40			Control	2.05	
12	Inoculated	9.39	V.V.Sl.	28	Inoculated	2.09	No colour
	Control	9.45			Control	2.05	
13	Inoculated	9.14	V.D.B.	29	Inoculated	1.95	L.P.B.
	Control	8.87			Control	1.93	
14	Inoculated	8.91	D.R.B.	30	Inoculated	1.72	No colour
	Control	9.20		31	Inoculated	1.71	No colour
15	Inoculated	8.87	R.B.	32	Inoculated	1.46	No colour
	Control	8.68			Control	1.63	
16	Inoculated	8.75	P.V.Sl.B.	33	Inoculated	1.32	No colour
	Control	8.43			Control	1.30	

Abbreviations: B=Brown; D=Dark; L=Light; P=Pink; R=Red; Sl=Slight; V=Very; Y=Yellow.

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