STUDY OF BACTERIAL FLUORESCENCE IN VARIOUS MEDIA

II. THE PRODUCTION OF FLUORESCENCE IN MEDIA MADE FROM PEPTONES

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INTRODUCTION AND HISTORICAL

In a previous communication the authors (1931) have shown that sulphates, phosphates, and magnesium must be present, in addition to an organic constituent, for the production of bacterial fluorescence in synthetic media by members of the *Pseudomonas fluorescens* group of bacteria. A number of peptones were used for making nutrient broth for the study of these organisms. The media made from some peptones produced fluorescence, whereas those made from other lots of peptones did not show pigment production.

Gessard (1892) studied the production of pigment in peptones using *B. pyocyaneus*. He concluded that a peptone, in order to produce fluorescence, must contain decomposed lecithine. Lepierre (1895) studied a pathogenic fluorescing bacillus in peptones made from various protein materials. He stated that fluorescence was due to meat extractives, such as xanthine and creatinine, plus soluble albuminoids.

The object of the research reported in this communication was to determine which peptones were suitable for use in media for the production of fluorescence; and also to endeavor to find what materials, necessary for pigment production, were lacking in the peptones which did not produce fluorescence.

TABLE 1

List of peptones used

NUMBER OF PEPTONES	MANUFACTURERS	KIND OF PEPTONE	pH VALUE
1	Arlington Chemical Company	Aminoids peptone (Beef)	5.70
2	Arlington Chemical Company	Aminoids peptone (Milk)	6.70
3	Armour and Company	Peptonum Siccum	5.50
4	Armour and Company	Peptonum Siccum	6.10
5	Armour and Company	Peptonum Siccum	6.05
6	Armour and Company	Peptonum Siccum	6.30
7	Baker, J. T.	Peptone, Bacto "Baker's"	6.95
8	Digestive Ferments Company	Bacto-peptone	7.00
9	Digestive Ferments Company	Bacto-peptone	6.45
10	Digestive Ferments Company	Bacto-peptone	6.90
11	Digestive Ferments Company	Bacto-peptone	6.90
12	Digestive Ferments Company	Bacto-peptone	6.52
13	Digestive Ferments Company	Bacto-peptone	6.91
14	Digestive Ferments Company	Proteose peptone	7.05
15	Digestive Ferments Company	Proteose peptone	6.92
16	Digestive Ferments Company	Proteose peptone	6.77
17	Digestive Ferments Company	Proteose peptone	6.90
18	Digestive Ferments Company	Proteose peptone	6.65
19	Digestive Ferments Company	Proteose peptone	7.05
20	Eimer and Amend	Peptone from albumin	6.21
21	Eimer and Amend	Peptone from beef	4.40
22	Fairchilds Bros. and Foster	Peptone	5.10
23	Fairchilds Bros. and Foster	Peptone	4.88
24	Fairchilds Bros. and Foster	Peptone	5.15
25	Merck and Company	Peptone Merck meat	4.50
26	Merck and Company	Peptone Merck meat	4.63
27	Parke, Davis and Company	Bacteriologic peptone	6.45
28	Parke, Davis and Company	Peptone	5.82
29	Parke, Davis and Company	Bacteriologic peptone	5.85
30	Parke, Davis and Company	Bacteriologic peptone	5.85
31	Special Chemical Company	Peptone Pfanstiehl	7.00
32	Squibb and Sons	Meat peptone	6.82
33	Squibb and Sons	Meat peptone	6.73
34	Squibb and Sons	Meat peptone	6.83
35	Stearns and Company	Peptonum Siccum	4.90
36	Stearns and Company	Peptonum Siccum	4.85
37	Stearns and Company	Peptonum Siccum	6.00
38	Witte, Rostock	Peptonum Siccum	6.95
39	Witte, Rostock	Peptonum Siccum	7.00
40	Witte, Rostock	Peptonum Siccum	7.18
41	Witte, Rostock	Peptonum Siccum	7.05
42	Witte, Roqtock	Peptonum Siccum	6.92

METHODS AND MATERIALS

The methods used were similar to the ones previously used by the authors (1931). The peptone broth was made with 0.5 per cent peptone and 0.5 per cent potassium chloride. This was sterilized by the discontinuous method for thirty minutes each day for three successive days. The organisms used in this study were *Pseudomonas fluorescens* or closely allied species. A large number of peptones were used during the course of this research. These are listed in table 1, together with the pH of the unadjusted medium made from each peptone. Where there is more than one sample from a given manufacturer, each sample was of a different lot number.

TABLE 2

The effect of pH on pigment production in peptone broth

pH VALUES	1 DAY	2 DAYS	3 DAYS	4 DAYS
6.1	−	−	++	+++
6.5	−	−	++	+++
6.8	−	++	++++	++++
7.1	−	++	++++	++++
7.3	−	++	+++	++++
7.7	−	++	++	+++
8.1	−	+	++	+++

EXPERIMENTAL

In order to determine the effect of the pH on the production of fluorescence a series of media, having pH values ranging from 6.1 to 8.9, were prepared from one of the peptones which showed good pigment production. The results for one of the organisms used are shown in table 2. In this table, as well as those which follow, the relative amount of pigment produced is indicated by means of plus signs, four plus signs being the maximum. A negative mark indicates no fluorescence. In every case where there was lack of pigment production, the growth was good. Lack of fluorescence could not, therefore, be charged to absence of growth. The figures in parentheses indicate the time in hours required for the production of the maximum fluorescence.

TABLE 3

Fluorescence produced in media made from different peptones

NUMBER OF PEPTONES	ORGANISM A	ORGANISM B	ORGANISM C	ORGANISM D
1	++(60)	+++(24)	+++(24)	+(84)
2	−	−	−	−
3	−	−	−	−
4	−	−	−	−
5	−	−	−	−
6	−	−	−	−
7	−	−	−	−
8	−	−	−	−
9	−	−	−	−
10	++(84)	+++(48)	++++(36)	+(84)
11	−	+++(36)	+++(24)	−
12	++(96)	+(48)	++(108)	+(60)
13	+(84)	++(48)	−	+(60)
14	+++(24)	++++(24)	++++(36)	++++(24)
15	++++(24)	++++(24)	+++(36)	+++(24)
16	+++(24)	++++(36)	+++(36)	+++(24)
17	+++(24)	++++(36)	+++(24)	++++(24)
18	++++(24)	++++(36)	+++(24)	++++(24)
19	++++(24)	++++(36)	+++(24)	+++(24)
20	+(108)	+(96)	−	−
21	+++(24)	+++(36)	+++(24)	++++(24)
22	+++(24)	++++(24)	+++(24)	++++(24)
23	+(60)	−	−	+(84)
24	+++(96)	+++(48)	+(48)	++(108)
25	−	−	−	−
26	−	−	−	−
27	−	−	−	−
28	++++(48)	++++(24)	+++(36)	+++(24)
29	+++(60)	−	+(48)	++(84)
30	++(72)	−	−	++(84)
31	−	−	−	−
32	−	−	−	−
33	−	−	−	−
34	++(96)	+(48)	−	+(108)
35	++(48)	++++(24)	+(48)	++++(24)
36	++(60)	+++(24)	+(84)	+(84)
37	+++(24)	++(36)	+++(24)	+++(24)
38	−	−	−	−
39	−	−	−	−
40	−	−	−	−
41	++(48)	−	−	−
42	+(144)	−	−	−

In table 2, we find that very good pigment formation was produced in media having a pH value of 6.1 to 8.1. The best production was, however, for the values between 6.8 and 7.3. In all of the media used, hereafter, the reaction was adjusted to a value between 6.9 and 7.1.

Each of the different peptones, as given in table 1, was used to prepare samples of media. Each medium was tested for ability to produce fluorescence with different organisms. The results for four of these organisms are recorded in table 3.

From the results given in table 3, we find seventeen peptones which produce no fluorescence with any of the four organisms. Slight fluorescence was produced by one or more of the organisms in ten peptones, whereas satisfactory fluorescence was produced in five peptones by two of the four organisms. Satisfactory fluorescence was produced in ten peptones by all four organisms. It will be noted that different strains will vary in their pigment production in the same medium.

A number of peptones whose media showed no pigment production were selected for further experimentation. Media were prepared from these peptones and various amounts of dipotassium phosphate added in order to determine whether this constituent was the one which was lacking for the production of pigment. Another set of media, containing varying amounts of magnesium sulphate, was prepared. A third set containing different amounts of these two constituents was also prepared. The results of these experiments for one of the six organisms used are listed in tables 4, 5, and 6.

In studying these three tables, one will notice that most of the peptones gave a good pigment production when phosphate was added. This was true with peptones numbers 7, 8, 9, 20, 23, 25, 27, 32, 33, 38, 39, and 40. A few of the peptones lacked both phosphates and magnesium sulphate. These included numbers 2, 4, 26, 31, and 42. Peptones numbers 2 and 4, however, did not give a very satisfactory fluorescence even when both constituents were added. Four of the peptones which produced a fairly good pigment on the addition of phosphates were improved somewhat by the addition of a small amount of magnesium sulphate. These

were numbers 7, 27, 38, and 40. A number of the peptones would produce a good fluorescence in three or four days, but the pigment would fade in a day or two thereafter.

Peptones numbers 3, 5, and 6 showed practically no fluorescence when both phosphates and magnesium sulphate were added. It was thought that possibly these peptones lacked sufficient organic constituents with the nitrogen in an available form. Therefore,

TABLE 4

Pigment production in peptones with added phosphate—organism A

NUMBER OF PEPTONES	K_2HPO_4				
	0.001 per cent	0.01 per cent	0.025 per cent	0.05 per cent	0.1 per cent
2	−	−	−	−	++(72)
3	−	−	−	−	+(48)
4	−	−	−	−	+(48)
5	−	−	−	−	−
6	−	−	−	−	−
7	−	−	+++(72)	++(72)	+++(60)
8	+(108)	++(36)	+++(48)	+++(36)	+++(24)
9	−	++(60)	+++(36)	++++(36)	++++(24)
20	++(96)	+++(48)	+++(48)	+++(48)	+++(48)
23	−	++(48)	++++(24)	+++(48)	++++(48)
25	−	+++(36)	+++(24)	++++(24)	++++(12)
26	−	−	−	++(48)	++(48)
27	−	−	++(60)	+++(36)	+++(60)
31	−	−	−	−	+++(48)
32	−	++(60)	+++(24)	++++(24)	+++(24)
33	−	++(60)	+++(48)	+++(24)	+++(36)
38	−	+++(48)	++(60)	++(60)	++(48)
39	−	++(60)	+++(48)	+++(48)	+++(60)
40	−	+(60)	++(60)	+++(48)	+++(48)
42	−	++(72)	−	++(60)	++(48)

varying amounts of asparagine were added to media made from these peptones. No improvement, however, was noted. Several different amounts of asparagine were also added to the media made from each of the other peptones studied. Additions of this constituent were also made to the media prepared from each peptone containing added phosphates, magnesium sulphate, and the combination of the two. No improvement was noted in any of the above media.

According to Lepierre (1895) fluorescence is caused, in part, by meat extractives. A number of these extractives, and other closely related compounds, were added to the media made from the different peptones which showed no pigment production. Media were prepared containing various concentrations (0.001, 0.01, and 0.1 per cent) of these compounds. These media were tested for pigment production alone, and with the addition of

TABLE 5

Pigment production in peptones with added magnesium sulphate—organism A

NUMBER OF PEPTONES	$MgSO_4$				
	0.001 per cent	0.01 per cent	0.025 per cent	0.05 per cent	0.1 per cent
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	+(120)	+(96)
9	–	–	–	–	–
20	–	–	–	–	++(72)
23	–	–	–	–	++(48)
25	–	–	–	+(60)	++(48)
26	–	–	–	–	–
27	–	–	+(60)	++(72)	++(60)
31	–	–	–	–	–
32	–	–	–	++(108)	++(96)
33	–	–	–	–	–
38	–	–	–	–	–
39	–	–	–	+(108)	+(72)
40	–	–	–	–	++(108)
42	–	–	–	++(96)	++(96)

phosphates and magnesium separately, and in various combinations. Sixty-six different media from each peptone were tested with four different organisms, but no marked improvement in fluorescence could be detected over a corresponding medium minus the special compound. The meat extractives and other compounds used were: caffeine, creatine, creatinine, guanine, theobromine, urea, uric acid, and xanthine.

The effect of heating, under different amounts of pressure, was

next tried. Lepierre (1895) stated that heating under pressure destroyed the power of the medium to produce fluorescence. Media made from a number of peptones which produced no pigment alone were first tried. A small amount of phosphate and a large amount of magnesium sulphate were added to part of the media. In the other part the amounts of these constituents were reversed. These media were divided into different batches and

TABLE 6

Pigment production in peptones with added phosphate and magnesium sulphate—organism A

NUMBER OF PEPTONES	0.001 PER CENT $MgSO_4$ 0.01 PER CENT K_2HPO_4	0.01 PER CENT $MgSO_4$ 0.01 PER CENT K_2HPO_4	0.05 PER CENT $MgSO_4$ 0.05 PER CENT K_2HPO_4	0.1 PER CENT $MgSO_4$ 0.1 PER CENT K_2HPO_4
2	−	−	++(48)	+(48)
3	−	+(60)	−	+(48)
4	−	+(120)	++(48)	++(48)
5	−	−	−	−
6	−	−	−	−
7	−	+++(48)	+++(72)	+++(48)
8	+++(24)	+++(24)	++++(48)	++++(48)
9	++(60)	++++(36)	+++(48)	++++(48)
20	+++(72)	+++(24)	+++(48)	+++(24)
23	+++(48)	+++(24)	++++(48)	++++(48)
25	+++(24)	++++(12)	++++(24)	+++(24)
26	−	+++(48)	+++(24)	+++(48)
27	++(60)	++++(48)	++++(48)	++++(48)
31	−	++(60)	++++(48)	++++(48)
32	+++(36)	+++(48)	++++(48)	++++(48)
33	++(69)	+++(48)	++++(48)	++++(48)
38	+++(48)	++++(48)	+++(48)	++++(48)
39	+++(36)	+++(48)	+++(48)	+++(48)
40	+++(24)	++++(24)	+++(48)	++++(48)
42	−	++(48)	++++(48)	+++(48)

heated under different pressures for varying lengths of time. The results are given in table 7. It was thought that the phosphate and magnesium might be rendered ineffective when magnesium ammonium phosphate was precipitated as the media were heated under pressure. However, the fluorescence in the media heated under pressure was nearly as good as in the media sterilized with live steam, except that the fluorescence was somewhat

diminished in the media heated for thirty minutes under 25 pounds pressure. The fluorescence was exceedingly good in the media containing 0.1 per cent magnesium sulphate and 0.01 per cent phosphate when heated for twenty minutes under 20 pounds pressure.

TABLE 7

Effect on pigment production of heating under pressure of media made from peptones, magnesium sulphate and phosphate—organism A

NUMBER OF PEPTONE	0.1 PER CENT K_2HPO_4 + 0.01 PER CENT $MgSO_4$ ADDED			0.1 PER CENT $MgSO_4$ + 0.01 PER CENT K_2HPO_4 ADDED		
	Time and pressure used					
	15 pounds, 15 minutes	20 pounds, 20 minutes	25 pounds, 30 minutes	15 pounds, 15 minutes	20 pounds, 20 minutes	25 pounds, 30 minutes
3	+(48)	+(48)	−	+(48)	+(60)	−
9	++++(48)	++++(48)	+++(48)	++++(48)	++++(36)	++(48)
23	++++(48)	++++(48)	++++(48)	++++(36)	++++(36)	++++(48)
27	+++(48)	+++(48)	++++(48)	++++(36)	++++(36)	++(48)
33	++++(48)	++++(48)	++++(48)	++++(36)	++++(48)	+++(48)

TABLE 8

Effect on pigment production of heating under pressure of media made from peptones —organism A

NUMBER OF PEPTONE	TIME AND PRESSURE USED				
	Original*	15 pounds, 15 minutes	20 pounds, 20 minutes	25 pounds, 30 minutes	15 pounds, 60 minutes
16	+++(48)	+++(48)	++(48)	+(60)	++(48)
17	++++(24)	+++(24)	+++(48)	+++(48)	+++(48)
18	++++(48)	++++(24)	++++(48)	+++(48)	++++(48)
19	++++(48)	+++(36)	+++(36)	++(48)	+++(48)
21	+++(48)	++(48)	+++(48)	−	++(48)
28	+++(48)	+++(48)	++(48)	++(48)	+++(48)
35	+++(48)	+++(48)	+++(48)	+(72)	+++(48)
37	+++(48)	++(48)	+(48)	−	+(60)

* Sterilized in live steam, thirty minutes for three consecutive days.

Media were also prepared from several peptones which gave good fluorescence without the addition of other substances. These were sterilized as above. The results for one of the organisms with these media are reported in table 8. In general the pig-

ment production was equally good in all media, with the exception that there was somewhat of a diminution at 25 pounds pressure.

In all media used up to this point the peptone content was 0.5 per cent. It was thought, perhaps, that this medium did not furnish enough food material in some of the peptones for pigment production. Media were prepared with each peptone using 3 per cent peptone and 0.5 per cent potassium chloride. These were tested as before. It was found that all of the peptones which failed to produce fluorescence with 0.5 per cent peptone also failed in the 3 per cent concentration. Also, in a number of the other peptones the pigment production was not as good as when there was less peptone, although the growth was as good or even better. Different concentrations of phosphate and magnesium sulphate, and combinations of these two, were added to the different media. The restoration of fluorescence, however, was not as good as with the media containing the lesser amount of peptone. Also, the addition of meat extractive and other substances did not increase pigment production in the media with the increased amount of peptone. It is thought that the greater concentration of food material promotes growth at the sacrifice of pigment production.

CONCLUSIONS

1. Peptones vary greatly in their composition and in their suitability for pigment production.

2. Some peptones lack the necessary amount of the different constituents which are necessary for pigment production. One may lack phosphate, another magnesium, etc.

3. On account of the variable composition it is necessary to test each lot of peptone for pigment production and then run a series of experiments to determine the constituent or constituents lacking so that these may be supplied to the medium.

4. A too concentrated medium will not satisfactorily support fluorescence even if the necessary constituents are present. A 0.5 per cent peptone broth is more satisfactory than a 3 per cent broth.

5. Purines, meat bases, and asparagine do not stimulate, to any great degree, the power of pigment production in peptones.

6. Heating peptone broth for a reasonable period of time under pressure, does not destroy the power to produce fluorescence.

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