Kleinere Mitteilungen.

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Effect of certain 'peptone' media and carbohydrates on the growth of *Paramecium bursaria.*

By

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The use of bacteria-free cultures of protozoa for classroom and laboratory study has necessitated the selection of suitable, conveniently prepared media for their growth and maintenance. Since successful culture depends largely upon the synthetic medium used, it seemed highly desirable to test certain commercial preparations for their effectiveness in promoting growth. A number of 'peptones' and carbohydrates were used for a series of growth experiments with *Paramecium bursaria* under bacteria-free conditions. Preliminary investigations indicated that this form is not as readily cultured in synthetic media as are *Glaucoma*, *Colpidium*, *Chilomonas* or certain of the phytomonad and euglenoid species. Since *P. bursaria* represents a common genus and since its nutritional requirements appear to be somewhat restricted, any information gained from its study may prove to be of importance for the culture of other protozoa. The strain which was used has been maintained bacteria-free for more than two years by subculturing at regular intervals (Loefer, 1934). The results outlined here and elsewhere (Loefer, 1936) indicate that not only the medium is of great importance for obtaining best

growth, but the concentration of organic and inorganic constituents as well.

The following dessicated 'peptones' obtained from several laboratories [1]) were made up at 0.5 and 1.0 % concentrations in a mineral medium consisting of $Ca(NO_3)_2$, 0.1 gm; NaCl, 0.1 gm; K_2HPO_4, 0.01 gm; $MgSO_4 \cdot 7H_2O$, 0.01 gm; $FeCl_3$, trace; and distilled water, 1 liter: Bacto-tryptone (DIFCO), Proteose-peptone (DIFCO), Protone (DIFCO), Bacto-peptone (DIFCO), Neopeptone (DIFCO), Seidenpepton (HOFFMANN-LA ROCHE), Pepton "Roche" (HOFFMANN-LA ROCHE), Pepton "Witte" (WITTE), Seidenfibroin (HOFFMANN-LA ROCHE), Bacto-veal (DIFCO), Bacto-liver (DIFCO), Blood fibrin (DIFCO), Bacto-gelatine (DIFCO), Bacto-yeast extract (DIFCO), Bacto-hemoglobin (DIFCO), Casein (DIFCO), Bacto-blood serum (DIFCO), Bacto-beef blood (DIFCO), Bacto-beef extract (DIFCO) and Bacto-egg-meat medium (DIFCO). The p_H was adjusted to 6.7—6.8 with normal NaOH, colorimetrically determined with a La Motte Comparator. Infusions were made from those media which did not readily go into solution.

After autoclaving six tubes of each medium along with six control tubes containing only the basic mineral medium, they were inoculated from a tryptone stock. The average initial count of *P. bursaria* was 24 per cc (x_0) in each of the tubes. Following a 15-day period of growth at room temperature (19—26° C.) at a north window the final concentration of ciliates per cc (x) was determined with a modified Sedgwick-Rafter counting chamber adapted for use with a dissecting binocular (LOEFER, 1936). Increased growth over the controls was evident in many cultures in both the 0.5 and 1.0 % series, but in every case the lesser of the two concentrations was equally and often more favorable than the higher concentration of the same medium. In the liver infusion the p_H had dropped to 6.2, although in the other media it had not changed appreciably.

Another series was made up (0.5 % concentrations) of those media which, in the preceding series, showed growth equal to or better than the controls. The purpose of this series was to determine whether these media would support growth indefinitely or through at least four subcultures. The procedure was similar to that above. Inoculations were made from a tryptone-mineral 12-day stock and the initial count of all tubes was 39 per cc (x_0). In table 1 are the results of four subcultures in each medium, extending over a total period of 41 days, expressed in terms of growth increase (x/x_0,

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Table 1.

Growth of *Paramecium bursaria* in various 'peptone' media.

Medium	x/x_0 of cultures			
	1st (8 days)	2nd (10 days)	3rd (14 days)	4th (9 days)
Bacto-tryptone	4.0 (39)	8.8 (12)	14.5 (12)	7.0 (20)
Proteose-peptone	9.9 (39)	14.6 (40)	14.5 (60)	10.9 (88)
Bacto-veal	4.5 (39)	12.6 (19)	16.5 (25)	8.4 (38)
Seidenpepton	4.0 (39)	6.6 (6.3)	5.7 (5.4)	4.8 (3.3)
Bacto-liver	2.2 (39)	2.0 (9.2)	1.9 (2.7)	0.0 (0.5)
Blood fibrin	2.3 (39)	2.6 (10)	2.8 (2.6)	1.8 (0.7)
Neopeptone	2.5 (39)	1.0 (10)	0.0 (1.7)	0.0
Bacto-peptone	1.2 (39)	1.2 (5.0)	0.0 (0.6)	0.0
Mineral solution control	1.2 (39)	1.2 (6.9)	0.0 (0.8)	0.0

Numbers = x/x_0 values (ratio of final to initial concentration of organisms per cc). Figures in parentheses designate x_0 of respective cultures. See text for explanation.

where x represents the final concentration of ciliates per cc). Thus, the first figure in the first column signifies that after eight days the original 39 organisms per cc (x_0) had increased four times, i. e., there was an average of 156 ciliates per cc in the six tubes of which samples were counted. One of these tubes, which contained only 120 ciliates per cc was used as a stock for the second transfer, 1 cc having been transferred to 9 cc of medium in each tube of the second subcultures. This accounts for the x_0 of 12 in parentheses in the second column. Organisms in the second subculture in tryptone increased 8.8 times after 10 days, etc. Growth in each of the other media is followed in the same way.

There is an increase of more than four times at the end of the fourth subculture period in tryptone, proteose-peptone, veal and seidenpepton. Although there was growth with blood fibrin, it was very slow as compared with the media just mentioned, and no growth followed a fifth transfer to fresh fibrin solution. Cultures with liver infusion changed to p_H 6.2 in this series also, which fact may account for the absence of growth in the fourth subculture. Neither Neopeptone nor Bacto-peptone supports growth. Since proteose-

peptone and tryptone were found to be very good for *P. bursaria* and since they are easily prepared, a mixture of both is being used as a stock medium.

The effect of certain carbohydrates was determined in the following manner. A 0.5 % tryptone solution was made up with the mineral solution mentioned above. It was adjusted to p_H 6.7—6.8, tubed in 8 cc amounts and autoclaved. One cc of a 5.0 % sterile solution of the respective carbohydrate to be tested was added aseptically to the tryptone in each tube. The concentration of the carbohydrate became 0.5 % when a one cc inoculum of organisms was added as described below. The list of carbohydrates (DIFCO) used is shown in table 2.

Table 2.

Growth of *Paramecium bursaria* in certain carbohydrate media.

Medium	x/x_0 (average of 6 cultures)
Tryptone controls	13.4
Arabinose .	—
Xylose .	—
Rhamnose .	—
Dextrose	34.0
Levulose	12.4
Galactose	12.0
Mannose	24.0
Mannitol	10.4
Lactose .	10.0
Maltose .	21.1
Sucrose .	17.3
Melezitose .	22.0
Dextrin .	23.8
Inulin . . .	—
Soluble starch	16.4
Salacin	17.7

x/x_0 = relation of final to initial concentration of ciliates per cc. A dash indicates that the organisms were dead at the end of the culture period.

Control tubes, to which one cc of sterile distilled water had been added, were then inoculated along with the tubes containing carbohydrates from a 5-day tryptone stock flask which contained 110 organisms per cc. All cultures were incubated for 25 days. Growth determinations indicated results as shown in table 2.

These results show that dextrose is most favorable to growth, the number of ciliates having increased 34 times compared to 13.4 times in the controls. Cultures with mannose, maltose, dextrin and mele-

zitose also show an increase in number of organisms, although levulose, galactose, mannitol, lactose, sucrose, starch and salacin are without much effect. The pentoses arabinose, xylose and rhamnose are apparently toxic, as is also inulin. Addition of 0.5 % dextrose to proteose-peptone also resulted in better growth, although concentrations above 0.5 % were less favorable. No p_H changes greater than the normal $\pm$ 0.2 were observed.

Discussion.

Results of these experiments indicate that proteose-peptone is the best of a series of 'peptone' preparations tested for growth of *P. bursaria*. Bacto-tryptone is distinctly less favorable, since the cultures approximate only one-half the density normally attained in proteose-peptone. These findings are interesting in view of the fact that Bacto-tryptone has frequently been used to advantage for the cultivation of other bacteria-free protozoa by different investigators (JAHN, 1931; HALL, 1933; ELLIOTT, 1933). LOEFER's (1935) experiments with *Chilomonas paramecium* resulted in the selection of Bacto-tryptone as a stock medium, inasmuch as it supported better growth than did proteose-peptone or any of the other media tested. ELLIOTT's (1935) results on *Colpidium* indicate that tryptone is somewhat better than proteose-peptone, neopeptone, Bacto-peptone or protone for growth of this form. He correlates growth with the relative abundance of Van Slyke and free amino nitrogen. The same correlation is hardly applicable to *P. bursaria*, since proteose-peptone contains less Van Slyke and amino nitrogen than does tryptone. It would seem that *P. bursaria* is more of a 'haplometatrophic' organism (utilizing a complex nitrogen which also serves as a carbon source) and therefore resembles *Glaucoma pyriformis*, according to LWOFF's (1932) classification. However, such a conclusion cannot be drawn since the exact role of the zoochlorellae in the nutrition of *P. bursaria* is still unknown.

The favorable results obtained with Bacto-veal indicate that perhaps this medium may prove to be even more successful than Bacto-tryptone for the cultivation of other forms. It is seen from table 1 that growth of *P. bursaria* was consistently better in veal infusion than in tryptone, although neither was as good as proteose-peptone. The unfavorable results obtained with the liver infusion are probably in a large measure due to the increased hydrogen-ion concentration (from p_H 6.7 to 6.2), since the optimum for growth of this form is near p_H 6.7 (LOEFER, 1936 a). Moreover, CAILLEAU (1933)

finds a beef-liver medium quite suitable for the growth of *Acanthamoeba castellanii*, and GLASER and CORIA (1935) have successfully cultured *Paramecium caudatum* and *P. multimicronucleatum* bacteria-free in a medium consisting in part of a liver infusion base.

Although Seidenpepton supports growth of *P. bursaria*, the organisms are less abundant than in proteose-peptone, veal or tryptone cultures. DIFCO yeast-extract has been found to be good for other forms (*Chilomonas* LOEFER, 1935a; *Colpidium* ELLIOTT, 1935; *Glaucoma pyriformis* PHELPS, 1935; *G. ficaria* JOHNSON, 1936), although it was rather unfavorable to *P. bursaria* and supported growth only when used in very dilute concentrations. The findings of CHATTON and TELLIER (1927) on *Glaucoma* and of BERGER (1929) on *Colpidium* indicate that these forms are much more resistant to toxic factors than is *Paramecium caudatum* (CHEJFEC, 1933). Both ELLIOTT and JOHNSON found yeast-extract in concentrations as high as 0.5 % favorable for growth of *Colpidium* and *Glaucoma*, respectively. The findings indicate that *P. bursaria* is susceptible to some factor in the medium to which these other forms are resistant.

Although cultures with dextrose were considerably better than the controls (table 2), there was no appreciable drop in p_H during the culture period. This absence of evident fermentation may be due to concomitant alkali formation (cf. certain bacteria, JONES, ORCUTT and LITTLE, 1931) and insert this line between or to the strong buffering action of tryptone. Dextrose, when added to a tryptone medium, produced the greatest increase in growth, but mannose, maltose, dextrin and melezitose were also favorable. Levulose, galactose, mannitol, lactose, sucrose, starch and salacin are without much effect as compared with growth in the controls. Arabinose, xylose, rhamnose and inulin were toxic. The relative growth-promoting effect of these carbohydrates is different in *P. bursaria* than in *Chlorogonium* and *Chilomonas* (LOEFER, 1935) for which forms other sugars were more favorable than dextrose. The difference might be accounted for by the fact that in the present experiments the sugars were added aseptically to the sterile tryptone solution, instead of mixing the carbohydrate and tryptone before autoclaving.

In their findings on *Paramecium caudatum* and *P. multimicronucleatum* GLASER and CORIA (1935) reported no change in p_H following a period of growth in glucose, lactose, sucrose or maltose. Starch, however, was converted to dextrose, and cellulose was digested. But whether there was any increased growth of organisms over

controls without carbohydrate was not stated. PRINGSHEIM (1928), from studies on bacterized cultures, concluded that starch was probably utilized by *P. bursaria.* However, in the present experiments with bacteria-free cultures no marked growth acceleration was observed in cultures containing soluble starch.

CAILLEAU'S (1933) analyses by the Folin and Wu method of sugar-containing cultures in which growth had occurred, showed that the more common sugars are not used by *Acanthamoeba castellanii.* The effect on the protozoan was not determined. The results of REICH (1934) on a bacteria-free strain of *Mayorella palestinensis* are interesting in this connection, since dextrose (or levulose, sucrose or lactose) is indispensable for good growth of this form. However, no acid is produced. Consequently, acid formation cannot be considered as a criterion of growth. It is quite possible to conceive of accelerated growth of protozoa without direct utilization of the sugar in the medium, in view of the changes in oxidation-reduction potential effected by certain sugars (MICHAELIS, 1930). That such physico-chemical phenomena do affect growth has been shown by JAHN (1933) for *Chilomonas paramecium.* Since no p_H change was observed in *P. bursaria* cultures containing dextrose in which there was increased growth, a similar cause may underlie the observed results.

LWOFF and DUSI (1934) reported that of a series of carbohydrates, only starch accelerated the growth of *Polytoma uvella, Euglena gracilis* (Colorless race), *Astasia chattoni* and *Chilomonas paramecium.* Quantitative results obtained for *Chilomonas* by LOEFER (1935) however, indicated that arabinose, dextrose, levulose, galactose, maltose, sucrose and dextrin were effective in promoting growth, while soluble starch was not. Whether these differences are due to technique, materials or to the particular strain of *Chilomonas* remains to be determined. The literature dealing with the effect of carbohydrates on other bacteria-free protozoa is discussed in the paper cited above.

In his studies on trypanosomes VON BRAND (1933) found that *T. brucei* utilized glucose, mannose, maltose, fructose and galactose in the respective ratios, 100:86:50:21:9. Arabinose, xylose, lactose and sucrose were not utilized. These results are similar to the growth results obtained with *P. bursaria,* inasmuch as glucose, mannose and maltose were effective in the order named and in that the effect of sucrose was not marked, while that of lactose and the pentoses was unfavorable.

Summary.

Of a series of 'peptone' media tested for their effect in promoting growth of a bacteria-free strain of *P. bursaria*, the following were found to be most suitable, in the order named: Proteose-peptone (Difco), Bacto-tryptone (Difco), Bacto-veal (Difco) and Seidenpepton (Hoffmann-la Roche). A dilute mineral solution containing 0.5 % concentrations of the above-mentioned substances supported growth through four or more subcultures. Growth of *P. bursaria* is compared with growth of other protozoa in similar media.

Of a series of carbohydrates tested, dextrose, mannose, maltose, dextrin and melezitose were best in the order named. Levulose, galactose, mannitol, lactose, sucrose, soluble starch and salacin appear to be without much effect. Arabinose, xylose, rhamnose and inulin are unfavorable. It is pointed out that growth of protozoa in carbohydrate media does not preclude utilization of the carbohydrate.

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