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Growth of *Colpidium* in relation to certain incomplete proteins and amino acids.

By

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(With 5 figures in the text.)

Introduction.

In view of the observations of LWOFF (1932) and ELLIOT (1935), that a medium containing gelatin as the sole source of nitrogen will not support growth of ciliates in bacteria-free cultures, the writers have attempted to determine whether or not the addition of certain amino acids to zein, gliadin and gelatin will overcome such difficulties in the case of the ciliates, *Colpidium campylum* and *Colpidium striatum*. In addition, the accelerating effect of certain amino acids on growth of these two ciliates has been determined.

The bacteria-free strain of *Colpidium campylum* was obtained from Dr. T. L. JAHN and is a strain isolated originally by Dr. ALFORD HETHERINGTON. The strain of *Colpidium campylum* was isolated by ELLIOTT, as described previously (ELLIOTT, 1933). For more than three years, both strains have been maintained in our laboratory in bacteria-free cultures. Methods of culture and general experimental procedures have been described elsewhere (ELLIOTT, 1933, 1935).

Gliadin and zein were prepared in the laboratory from "cream of wheat" and cornmeal, respectively. Tryptophane, gelatin, desiccated liver, yeast extract, tryptophane and asparagin were obtained from the DIFCO Laboratories; lysine-dihydrochloride and silk-peptone, from HOFFMANN-LA ROCHE; and the remaining amino acids, from EASTMAN Kodak Company.

Growth in incomplete proteins.

The results of experiments, using the successive transfer method, showed that *Colpidium striatum* will not grow beyond the second transfer in a medium containing gliadin (gliadin, 1.0 %; KH_2PO_4, 0.2 %). Similar results were obtained with zein. It was observed previously (Elliott, 1935) that gelatin alone does not support growth of *Colpidium striatum* through three successive transfers. Similar negative results were obtained with *Colpidium campylum* in a gelatin medium (gelatin, 1.0 %; KH_2PO_4, 0.2 %). It seems obvious, therefore, that these incomplete proteins will not support continued growth of the ciliates. Silk peptone was likewise tested by the successive transfer method and found to be unsatisfactory as a medium for *Colpidium campylum*. Similar results were reported by Lwoff (1932) for *Glaucoma piriformis*.

Gliadin contains, in sufficient quantities, all the so-called essential amino acids except lysine, which is present only in small amounts. By adding this amino acid to a gliadin medium in a concentration (0.7 %) similar to that found in casein, it was hoped that continuous growth of *Colpidium striatum* might be obtained through successive transfers. The addition of lysine alone failed, however, to support growth beyond the second transfer. Comparable negative results were obtained for zein.

In the case of gelatin, the addition of tryptophane and isoleucine failed to support growth of *Colpidium campylum* through more than three transfers. In gelatin-tryptophane media (0.02—0.0025 % tryptophane) growth was much slower than in gelatin alone, particularly with the higher concentrations of tryptophane. On the other hand, iso-leucine produced a slight acceleration of growth. It was concluded, therefore, that the addition of some of the missing amino acids fails to make possible continued growth of the ciliates in a gelatin medium. The addition of tyrosine likewise was unsuccessful, although there was evidence of acceleration of growth as in the case of iso-leucine.

Acceleration of growth by amino acids.

In view of the observations that growth of the ciliates is somewhat greater in media containing gelatin and lysine, iso-leucine or tyrosine than in gelatin alone, several amino acids and asparagin were compared in regard to their effects on growth of *Colpidium campylum* in gelatin medium. The various substances were added to gelatin medium in concentrations of 0.02 %. With the exception of gelatin-histidine (p_H 5.9), the initial p_H of each medium was 6.1. All tubes were

incubated for 7 days at room temperature and then fixed for counting. In the gelatin controls, x/x_0 (ratio of final to initial concentration of ciliates) was 3.9; in gelatin-valine, 4.7; in gelatin-iso-leucine, 4,6; in gelatin-aspartic acid, 5.9; in gelatin-asparagin, 5.9; in gelatin-taurine, 5.3; in gelatin-histidine, 5.6. From the results, it is obvious that, unlike tryptophane, none of the substances tested in this series inhibits growth of *Colpidium campylum* under the conditions given; on the contrary, acceleration of growth is evident, especially by aspartic acid, histidine and asparagine.

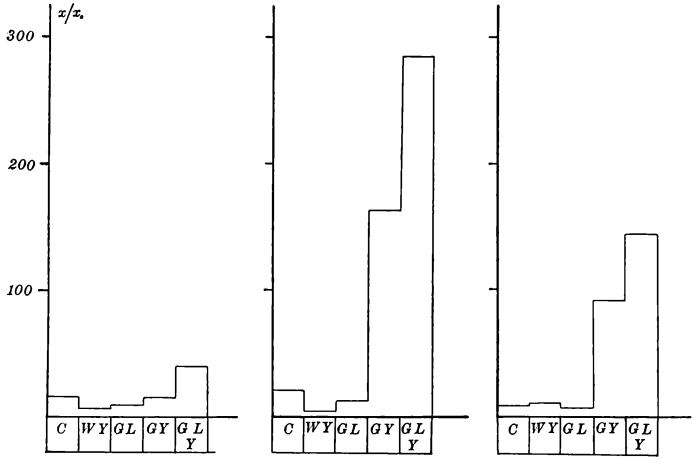

Fig. 1. Growth of *Colpidium striatum* in gliadin media through three successive transfers; growth is expressed as x/x_0 (ratio of final to initial concentration of ciliates). *C*, gliadin controls; *GL*, gliadin-lysine; *GLY*, gliadin-lysine-yeast extract; *GY*, gliadin-yeast extract; *WY*, water-yeast extract.

The acceleration of growth of the ciliates by the addition of single amino acids to a gelatin medium, inadequate for continued growth of *Colpidium*, suggested that attempts be made to determine whether or not a similar accelerating effect would be produced in media satisfactory for growth of the organisms through successive transfers. It was found that the addition of Difco yeast-extract in a concentration of approximately 0.00005 % would support continued growth of *Colpidium* in gelatin, zein and gliadin. Similar results were obtained by the addition of Difco tryptone or Difco liver in approximately the same concentration.

In series I (*Colpidium striatum*, Fig. 1) one drop of 1.0 % Difco yeast-extract was added to each tube of basic gliadin medium both

with and without lysine. The resulting concentration of yeast-extract was approximately 0.00005 %. The 'water controls' contained a drop of yeast-extract and 0.2 % KH_2PO_4. The gliadin medium was tubed in 8.0 cc. amounts and then autoclaved, and the solutions of amino acid, yeast-extract and 10 % dextrose were sterilized separately in the same manner. The p_H of the basic medium and the other solutions, where necessary, was adjusted so that the initial p_H of the experimental media was 5.7 in each case. The water controls were tubed in 8.0 cc. amounts and autoclaved. To each tube of medium and each water control tube, 0.5 cc. of sterile dextrose solution was added aseptically, and the other solutions were added to the appropriate tubes as indicated in figure 1.

All tubes were inoculated with *Colpidium striatum* from a dilution flask (sterile tapwater) seeded with ciliates which had been centrifuged aseptically in sterile tapwater. This procedure reduced to a considerable degree the amount of peptone carried over from the original stock culture into the first transfer series. After inoculation, tubes of each set were used for determination of the initial count and initial p_H, and the remainder were incubated at room temperature for two days. The results are expressed in figure 1 as x/x_0, and indicate that growth of *Colpidium striatum* in the gelatin medium is accelerated by a drop of yeast-extract, while maximum growth occurs in the combination of gliadin, lysine and yeast-extract. It is evident, therefore, that in the presence of a small amount of yeast-extract (0.00005 %, approximately) lysine exerts a decidedly accelerative effect on growth of *Colpidium striatum*. Material from tubes of the first transfer series was used in inoculation of the second transfer series, and material from the second in the inoculation of the third series. The second transfer series was incubated for four days, and the third for three days at room temperature. The same effect of lysine was noted in both the second and third transfers. The amino acid alone exerts no appreciable effect in a gliadin medium, whereas it is distinctly accelerative in the presence of a small amount of yeast-extract.

In series II (*Colpidium striatum*) the same general procedure was followed as in series II; the effects of lysine in gliadin medium were determined in the presence of Difco tryptone, Difco liver, and Difco yeast-extract. Except that the accelerating effect of high dilutions of tryptone and liver extract was slightly greater than that of yeast-extract, the results were much the same as in series I. The combinations, lysine-yeast extract, lysine-tryptone and lysine-liver,

showed in each case a much greater accelerating effect than yeast, tryptone or liver, when added to the gliadin medium, while the addition of only the amino acid exerted little, if any, effect on growth of *Colpidium striatum*.

In series III (Fig. 2) the experimental procedure was the same as in series I, except that both lysine and tryptophane were tested for their effects on growth of *Colpidium striatum* in a zein medium (zein, 1.0 %; KH_2PO_4, 0.2 %). The initial p_H of all media was adjusted to 5.7. The first, second and third transfer series were incubated at room temperature for 4,4 and 5 days, respectively. In

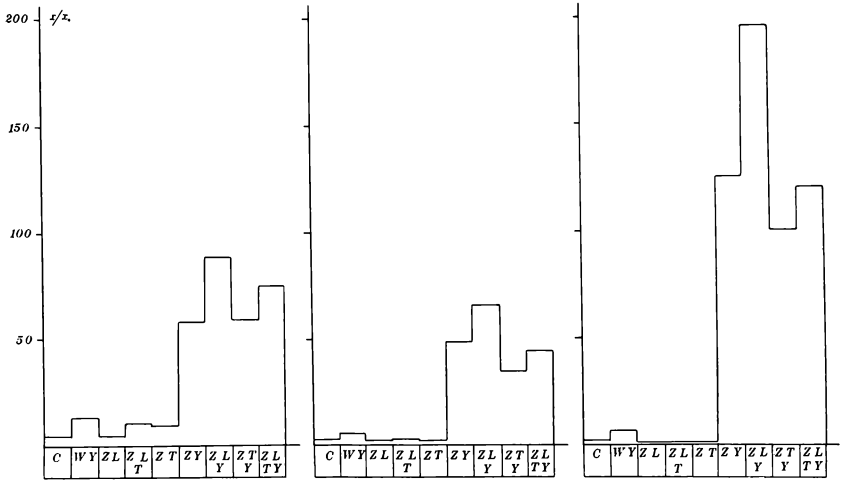

Fig. 2. Growth of *Colpidium striatum* in zein media; growth is expressed as x/x_0. *C*, zein controls; *WY*, water-yeast; *ZL*, zein-lysine; *ZLT*, zein-lysine-tryptophane; *ZLY*, zein-lysine-yeast; *ZLTY*, zein-lysine-tryptophane-yeast; *ZT*, zein-tryptophane; *ZTY*, zein-tryptophane-yeast; *ZY*, zein-yeast.

all three transfers, abundant growth occurred in the zein tubes containing a drop of yeast-extract, while the zein-lysine-yeast tubes showed still greater growth. Lysine, therefore, exerts the same sort of effect in the zein medium as in the gliadin medium. Tryptophane, on the other hand, produced no acceleration in the first transfer, and definite inhibition of growth was noted in the second and third transfers. Even in the medium containing both tryptophane and lysine, in addition to zein and yeast-extract, this effect of tryptophane was noticeable in comparison with the zein-lysine-yeast tubes.

In series IV (Fig. 3) an attempt was made to determine whether lysine would accelerate growth of *Colpidium campylum* in a gelatin

medium, since gelatin already contains a presumably adequate amount of lysine. The technique was similar to that followed previously, except that no dextrose was added to the medium (gelatin, 1.0 %; KH_2PO_4, 0.2 %). The initial p_H was adjusted to 5.9. Tubes were inoculated, incubated for 5 days at room temperature, and then final counts were made. The results, expressed as x/x_0, show (Fig. 3) that in a gelatin medium lysine exerts the same sort of accelerative effect as that observed in zein and gliadin media.

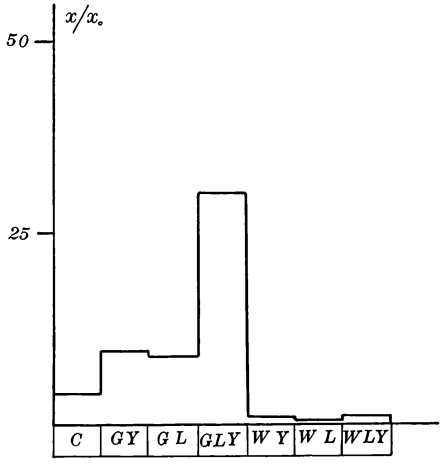

Fig. 3. Growth of *Colpidium campylum* in gelatin media; growth is expressed as x/x_0. *C*, gelatin controls; *GL*, gelatin-lysine; *GLY*, gelatin-lysine-yeast; *GY*, gelatin-yeast; *WL*, water-lysine; *WLY*, water-lysine-yeast; *WY*, water-yeast.

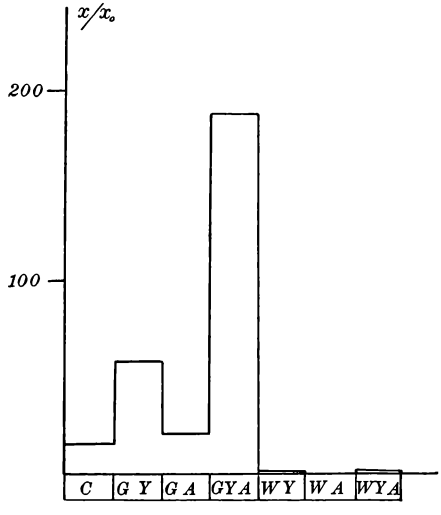

Fig. 4. Growth of *Colpidium campylum* in gelatin media; growth is expressed as x/x_0. *C*, gelatin controls; *GA*, gelatin-asparagin; *GY*, gelatin-yeast; *GYA*, gelatin-yeast-asparagin; *WA*, water-asparagin; *WY*, water-yeast; *WYA*, water-yeast-asparagin.

In series V (*Colpidium campylum*, Fig. 4) the same procedure was followed as in series IV, with the exception that asparagin was substituted for the amino acid, lysine. After inoculation, all tube cultures were incubated for 5 days at room temperature. The results show that the accelerating effect of asparagin is somewhat greater than that of lysine, but are quite similar otherwise to those obtained in figure IV.

Essentially similar results were obtained in a companion series (series VI) in which liver infusion was used instead of yeast-extract.

Series VII (*Colpidium campylum*, Fig. 5) is similar to series IV, except that proline was used instead of lysine. The different media showed an initial p_H of 6.0. Cultures were incubated for 5 days at room temperature, and then fixed for counting. The results (Fig. 5) show that proline, like lysine, exerts an accelerating effect on growth of *Colpidium campylum*, although the acceleration is less marked.

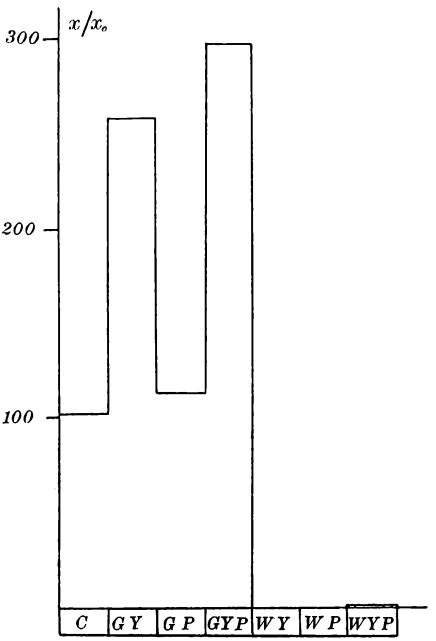

Fig. 5. Growth of *Colpidium campylum* in gelatin media; growth is expressed as x/x_0. *C*, gelatin controls; *GP*, gelatin-proline; *GY*, gelatin-yeast; *GYP*, gelatin-yeast-proline; *WP*, water-proline; *WY*, water-yeast; *WYP*, water-yeast-proline.

Discussion.

The role of amino acids in the nutrition of ciliates is little understood at the present time. Emery (1928), using methods of quantitative analysis, has shown that various single amino acids are absorbed by *Paramecium*, the degree varying with the amino acid. Emery did not imply that growth of the ciliate would be supported by a single amino acid, but he did show that the species is capable of removing the amino acid from solution. Lwoff (1932) found that growth of *Glaucoma piriformis* is not supported by single amino acids or by various artificially prepared mixtures of amino acids. Bond (1933) reported that both asparagin and tyrosine are inadequate for growth of *Colpidium campylum*. Elliott (1935) has drawn similar conclusions for *Colpidium striatum* and *Colpidium campylum* in the case of several amino acids. Lwoff has stressed the view that such ciliates as *Glaucoma piriformis* are dependent upon polypeptids, rather than amino acids; thus, in Lwoff's 'metatrophic' types, "est l'impossibilité d'utiliser un seul acide aminé comme aliment azoté et le besoin d'une peptone" (Lwoff, 1932, p. 147).

Our results demonstrate, however, that growth of *Colpidium* is accelerated by several amino acids and asparagin when each of these

substances is added to a medium which supports relatively slow but steady growth of the ciliates. Tryptophane, on the other hand, inhibited growth of *Colpidium* under the conditions of our experiments. The addition of a series of single amino acids and asparagin to a gelatin medium produced increases in growth ranging from 20 % to more than 50 % that in the gelatin controls. It seems obvious, therefore, that *Colpidium* is able to make use of a number of single amino acids, although none of them alone is adequate for growth of the ciliates.

The results obtained with *Colpidium striatum* and *Colpidium campylum* are in agreement with Lwoff's (1932) findings that gelatin will not support continued growth of *Glaucoma piriformis.* Since *Colpidium* produces a gelatinase (Elliott, 1933), this failure of the ciliates to grow is not due to their inability to hydrolyze the gelatin, but presumably to the absence in gelatin of certain substances essential to growth. The other incomplete proteins, zein and gliadin, were no more satisfactory than gelatin. The addition of certain missing amino acids to zein, gliadin and gelatin failed to compensate for such deficiencies, although the combination of gelatin and a small amount (0.00005 %, approximately) of yeast extract (or liver infusion, or tryptone) proved adequate for growth of the ciliates through a series of successive transfers.

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