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Vitamin Deficiency as One Explanation for Inhibition of Protozoan Growth by Conditioned Medium.

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Accelerative effects of biologically conditioned casein-peptone medium, when added to fresh medium in proportions of 1-5 parts in 10, have been reported previously for *Colpidium campylum.*[1] Subsequently, it has been found that an increase in the proportion of conditioned medium induces inhibitory effects on the growth of *Colpidium campylum* and *Glaucoma piriformis.** Observations on the latter are presented below.

Series I and II. A culture filtrate was prepared from 96-day flask-cultures of *G. piriformis* in casein-peptone (Difco "tryptone") medium containing KH_2PO_4 (1.0 g per 1). The cultures were filtered through paper (Whatman No. 12), as previously described by Hall and Loefer,[1] and the filtrate was divided into two portions. One portion (Series I) served as the control medium. To the other (Series II), the following growth-factors were added: Merck's thiamine hydrochloride (1×10^{-6} g per cc), Merck's riboflavin (1×10^{-7} g per cc), and S. M. A. Corporation's nicotinic acid amide (1×10^{-7} g per cc). Each medium was tubed in 10 cc amounts and sterilized in the autoclave at 122° C for 20 minutes. All tubes received 0.5 cc inocula from a 3-day culture of *G. piriformis* in the basic medium. In both series, the initial pH was 6.9; initial count, 4,000 ciliates per cc. All cultures were incubated in darkness at 24°C, and counts were made at the indicated intervals (Fig, 1, A).

Series III and IV. The procedure was the same as above, except that 109-day flask-cultures were used as the source of the filtrate, and a 21-day stock culture was used for inoculation. Series III served as the control; in series IV, the medium received the 3 growth-factors. In both series, the initial count was 6,400 ciliates per cc; initial pH, 6.9. The results are described in Fig. 1, B.

In both Fig. 1, A and 1, B the dotted lines represent growth-curves for *G. piriformis* in fresh medium prepared from the same sample of casein-peptone. These series were not run in parallel with Series

[1] Hall, R. P., and Loefer, J. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 128.

* In view of the present confusion in nomenclature of these ciliates, it may be pointed out that these 2 strains are known to be antigenically distinct. Consequently, the writer is retaining the older designations for the present.

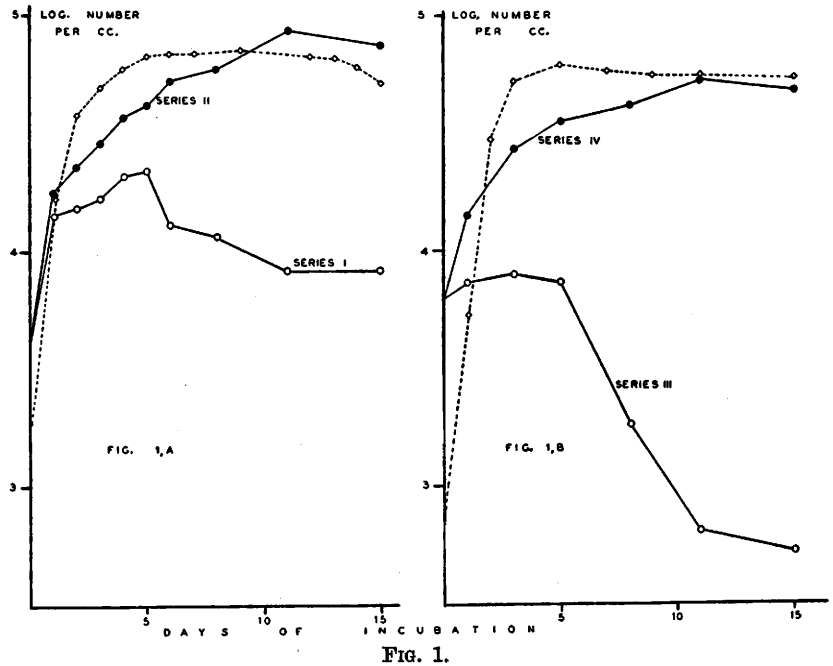

FIG. 1.

I and II, or with III and IV; furthermore, the initial pH was 6.3 instead of 6.9, and the initial counts were lower. However, these series do afford valid comparisons with respect to population yield.

In interpreting the results, it may be pointed out that in all 4 series the medium contained old culture filtrate ("biologically conditioned" medium) in the proportion of approximately 19 parts in 20. In Series I and II, inoculated from a 3-day stock culture, the concentration of any essential materials introduced with the inocula might approach that of fresh peptone medium. For the inocula in Series III and IV, this probably would not be true, since the supply should be depleted to some extent in a 21-day culture. Hence, it might be expected that the differential effect of added vitamins would be greater in Series IV than in Series II. This was actually the case. In both series, however, it is obvious that the addition of 3 growth-factors restored an old culture filtrate to approximately the condition of fresh peptone medium, so far as population yield was concerned.

Comparison of Series I and III suggests that exhaustion of the vitamin supply in cultures of *G. piriformis* is well under way after 21 days, and that inocula from such old cultures carry over no significant amounts of these essential substances. Furthermore, the

results obtained in Series II and IV demonstrate that the concentration of certain vitamins is reduced below the minimum long before the available food supply is exhausted.

These results supplement the earlier report of an accelerating effect produced by old culture filtrates in lower concentrations (Hall and Loefer, *loc. cit.*) The present findings demonstrate that old culture filtrate ("biologically conditioned" medium), in excessive concentration, tends to inhibit growth of *G. piriformis,* with a consequent reduction in population yield. Since this inhibitory effect disappears upon the addition of three growth-factors to the conditioned medium, it may be concluded that the effect actually represents a vitamin deficiency.

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Use of Sulfaguanidine in Nutrition Experiments.*

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The variable results obtained in studies on the newer members of the vitamin B complex have led us to suspect that intestinal bacteria may synthesize certain unidentified factors essential in the nutrition of the rat. Sulfaguanidine (sulfanilylguanidine), an antibacterial agent which is poorly absorbed from the intestine,[1] appeared to be a useful tool in attacking this problem. The following basal ration was used in our experiments: sucrose 76, purified casein 18, salts 4, corn oil 2, choline hydrochloride 200 mg, nicotinic acid 2.5 mg, calcium pantothenate 2 mg, and .3 mg each of thiamin, pyridoxine and riboflavin. Two drops of haliver oil containing 1 mg of *dl*

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1 Marshall, E. K., Jr., Bratton, A. Calvin, White, H. J., and Litchfield, J. T., Jr., *Bull. Johns Hopkins Hosp.*, 1940, **67**, 163.