the first few days of the experiment while the animals fed 2-methyl-1,4-naphthoquinone died on various days over the 30-day feeding period. A marked fall of the erythrocyte count and hemoglobin was observed in rats fed doses of 0.1 g per kg of Phthiocol, and 0.35 g per kg of 2-methyl-1,4-naphthoquinone while vitamin K_1 failed to produce such an effect.

Grateful acknowledgment is made to Mr. O. Graessle and Mr. J. Mayner for valuable technical assistance.

Summary. The acute and chronic toxicity of Phthiocol, 2-methyl-1,4-naphthoquinone and vitamin K_1 was studied in mice, rats, and chicks. The oral L.D. 50 in mice was found to be approximately 0.2 g per kg for Phthiocol and 0.5 g per kg for 2-methyl-1,4-naphthoquinone; no lethal effect could be produced by doses up to 25 g per kg of vitamin K_1. In the chronic experiments in rats, daily feeding over a period of 30 consecutive days of 0.35 g per kg of Phthiocol, and 0.5 g per kg of 2-methyl-1,4-naphthoquinone was toxic; doses of 0.1 g per kg of Phthiocol and 0.35 g per kg of 2-methyl-1,4-naphthoquinone produced a marked fall of the erythrocyte count and hemoglobin. No such effects were observed following vitamin K_1 administration. In the abdominal cavity of animals sacrificed 10 days after an intraperitoneal injection of vitamin K_1 considerable amounts of an oily suspension could be observed, indicating an extremely slow rate of absorption of vitamin K_1.

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Effects of Culture Filtrates and Old Medium on Growth of the Ciliate, *Colpidium campylum.*

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Effects of protozoan metabolic products on growth of homologous species have been investigated by several workers. One view, based originally upon Woodruff's[1] findings, predicates that waste products of a given species exert an inhibitory effect on growth of that species. The opposite view is represented by Dimitrowa's[2] conclusion that growth of *Paramecium caudatum* is accelerated by small amounts of old culture fluid added to fresh cultures. Johnson and Hardin[3]

1 Woodruff, L. L., *J. Exp. Zool.*, 1911, **10**, 557.

2 Dimitrowa, A., *Zool. Anz.*, 1932, **100**, 127.

3 Johnson, W. H., and Hardin, G., *Physiol. Zool.*, 1938, **11**, 333.

have observed no significant effects of old culture fluid on growth of *P. multimicronucleata.* Mast and Pace,[4] however, have reported that old culture fluid in high concentration inhibits, whereas, in low concentration, it accelerates growth of *Chilomonas paramecium.* Preliminary observations, reported by Hall and Loefer,[5] indicated that growth of *Colpidium campylum* in bacteria-free cultures is significantly accelerated by the addition of old culture filtrates to a peptone medium. Kidder[6] has recently confirmed this accelerating effect of old culture fluid on growth of *C. campylum,* and has attributed it to a "biological conditioning" of the medium.

In further study of this problem, the acceleration of growth by old culture filtrates has been compared with the effects produced by aged sterile medium added to fresh peptone solution. The results obtained in 8 experimental series are described graphically in Figs. 1 and 2.

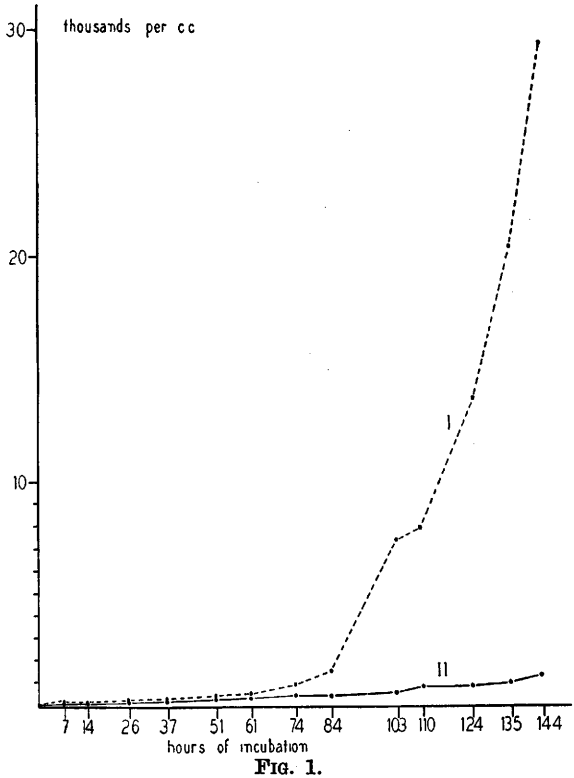

FIG. 1.

[4] Mast, S. O., and Pace, D. M., *Physiol. Zool.,* 1938, **11**, 359.

[5] Hall, R. P., and Loefer, J. B., *Anat. Rec.,* 1938, **72**, 50 (abstract).

[6] Kidder, G. W., *Science,* 1939, **90**, 405 (abstract).

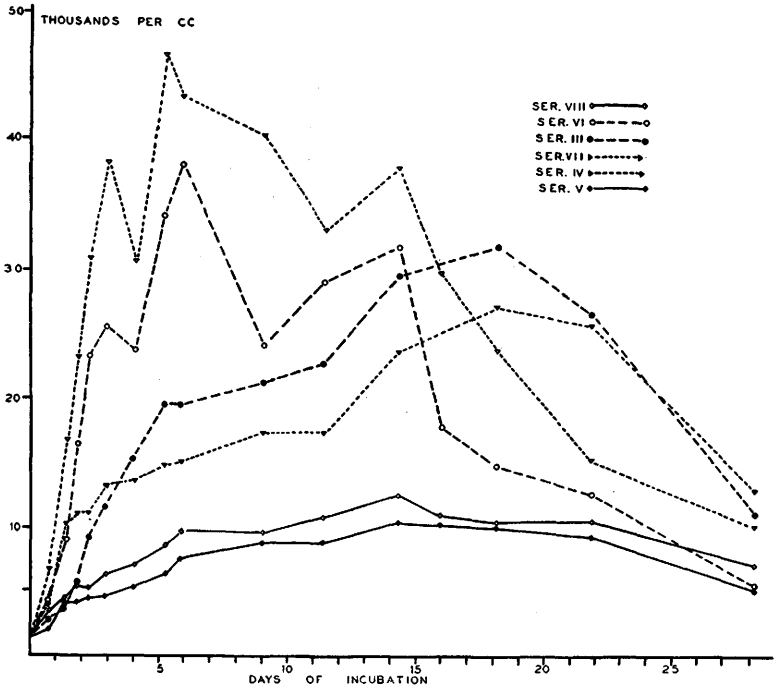

FIG. 2.

The following basic medium was used in all 8 series: casein-peptone (Difco "tryptone"), 10.0 g; KH_2PO_4, 2.0 g; glass-distilled water, 1.0 liter. However, 2 samples of tryptone were tested, one in Series I and II and the other in Series III-VIII. As indicated specifically for different series, old culture filtrate, filtered fresh medium, or filtered aged medium (uninoculated) was added to tubes containing fresh unfiltered peptone medium. In each case, Whatman No. 12 paper was used in filtration. All tubes were sterilized in the autoclave for 20 minutes at 122°C before inoculation. After inoculation, several tubes were set aside for determination of initial count (number of ciliates per cc), one tube in each series was used for initial pH readings, and the remainder were incubated as described below. Counts were made from 4 tube cultures fixed at each of the intervals indicated in Figs. 1 and 2. Our method of counting has been described elsewhere.[7] Final pH readings were taken at the end of incubation by means of a LaMotte roulette comparator.

Series I and II. In Series I, each tube contained 8.0 cc of fresh unfiltered medium and 2.0 cc of a filtrate from pooled cultures with

[7] Hall, R. P., Johnson, D. F., and Loefer, J. B., *Trans. Am. Micr. Soc.*, 1935, **54**, 298.

an average age of about 7 weeks. In Series II each tube contained 10.0 cc of unfiltered fresh medium from the same source as Series I. After sterilization, the tubes in both series received 1.0 cc inocula from the same 48-hour stock culture of *C. campylum* in peptone medium. Initial count (67 ciliates per cc), initial pH (6.1) and final pH (6.3) were the same in the two series. In both cases, cultures were incubated in darkness at room temperature.

During the first 74 hours of incubation the increase in population (Fig. 1) was somewhat more rapid in Series I. Thereafter, the differences were progressively greater until, at the end of 144 hours, the population in Series I had reached 29,353 per cc, while that in Series II was only 1,327. These results indicate that addition of old culture filtrate to fresh medium, in a dilution of 1 :5, definitely accelerates growth of *C. campylum* during the first 6 days of incubation. However, these two series were not carried long enough to determine any possible difference in maximal density of population. Furthermore, none of the medium in Series II had been passed through filter paper, and it was necessary to consider this factor in later series.

Series III-V differed in the following respects. In Series III the tubes contained 9.0 cc of fresh unfiltered medium and 1.0 cc of a 3-month-old culture filtrate; in Series IV, 9.0 cc of fresh medium and 1.0 cc of filtered, 3-month-old uninoculated medium; in the control (Series V), 9.0 cc of fresh medium (unfiltered) and 1.0 cc of filtered fresh medium. In all 3 series the tubes received 0.5 cc inocula from the same 48-hour flask culture of *C. campylum,* and the initial pH (6.3) and initial count (1219 per cc) were the same in the 3 cases. Final pH ranged from 6.9 in the controls to 7.1 in the other series. All cultures were incubated in darkness at room temperature, and counts were made at the intervals indicated.

The population curves (Fig. 2) show that, after the second day, growth was much more rapid in Series III and IV than in the control. In Series V, a rather low maximal density of population was reached about the fourteenth day, while in Series III and IV much higher maxima were reached in 18 days. Hence, it is obvious that both old culture filtrate and aged sterile medium accelerated growth of *C. campylum* and increased the density of population.

In Series VI-VIII, all tubes contained 5.0 cc of unfiltered fresh medium. In addition, the tubes in Series VI received 5.0 cc of a 3-month-old culture filtrate; those in Series VII, 5.0 cc of filtered uninoculated medium of the same age; the tubes of Series VIII (control), 5.0 cc of filtered fresh medium. Initial pH, inoculum, initial count and conditions of incubation were the same as in Series

III-V, with the exception that initial pH in Series VI was 6.5 instead of 6.3. The results are similar to those obtained in Series III-V, although growth was more rapid and maximal densities of population were higher in VI and VII than in Series III and IV. In addition, maxima were reached in 5-6 days, instead of 18 as in Series III and IV. Growth in the control (Series VIII) was similar to that in Series V. In fact, the apparent differences between the 2 controls are not statistically significant in most cases and are of doubtful significance in others. These data suggest that passage of fresh medium through filter paper added little or nothing of value to the ciliates, since the effects produced by 1.0 cc and 5.0 cc volumes are practically the same. A comparison of Series II, containing unfiltered medium only, with the other controls is not altogether valid, since initial counts were different and different samples of peptone were used in the 2 cases. While growth of the ciliates in Series II represents an increase of approximately 20 times at the end of 6 days and the corresponding increase was less than 8 times in Series V and VIII, our previous experience with large and small inocula has shown that a difference of this order would be expected even if the same sample of peptone had been used in all three series. Hence, there is no basis for assuming that mere filtration of the medium results in acceleration of growth, or that filtration removes any essential constituent of the medium.

It is interesting to note that, in spite of the difference in initial counts, the density of population in Series I after 6 days was intermediate between the corresponding densities in Series III and VI; furthermore, that the various densities were somewhat proportional to the amounts of old culture filtrate added to the medium. Thus, Series III, with 1.0 cc of filtrate, showed approximately 20,000 ciliates per cc; Series I, with 2.0 cc of filtrate, approximately 29,000; Series VI, with 5.0 cc of filtrate, approximately 38,000. A similar relationship seems to hold with respect to maximal density of population in Series III and VI; the population density showed a greater increase in the larger volume of old culture filtrate.

The effect produced by aged sterile medium was surprising. In Series IV, which received aged medium in a dilution of 1:10, growth was almost as heavy as in Series III with old culture filtrate, while the population level in Series VII was even higher than in Series VI. This is particularly interesting in view of the obvious differences between aged medium and old culture fluid. The latter had been subjected to the action of digestive enzymes and contained metabolic products liberated by the ciliates, while the changes in uninoculated

medium were undoubtedly much less extensive. Nevertheless, the mere aging of sterile medium changed it in such a way that it acquired the property of accelerating growth of *C. campylum* when added to equal or larger volumes of fresh peptone solution.

These observations have a bearing upon the question of "biologically conditioned" medium, and upon the "allelocatalytic effect" described by Robertson and others. The results obtained in Series I, III and VI might reasonably be attributed to a "biological conditioning" brought about during growth of the ciliates, if it were not for the fact that aged sterile medium produced comparable effects. Our findings do not demonstrate that the factors producing acceleration of growth are identical in old culture filtrates and in aged sterile medium, and it is possible that they are not the same. On the other hand, it is equally obvious that a "biological conditioning" cannot be invoked as the sole explanation for the accelerating effects of old culture filtrates.

Our results show further that, for studies on the "allelocatalytic effect" in ciliates, old culture fluid transferred to experimental cultures may accelerate growth in proportion to the amount of fluid carried over. Hence, in such investigations on growth in relation to initial density of population, it is imperative that the organisms in the inocula should be washed thoroughly in order to remove old culture fluid. Failure to observe such a precaution may well be responsible, at least in part, for some of the apparent conflicts in current views regarding the "allelocatalytic effect".

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Histological Demonstration of Vitamin A in Rats by Means of Fluorescence Microscopy.

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Vitamin A reveals a green fluorescence in ultraviolet light which disappears rapidly during irradiation.[1] This was observed under the fluorescence microscope using dilute aqueous emulsions of vitamin A concentrates. The striking green of the droplets fades during the

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1 Peacock, P. R., *Lancet*, 1926, **2**, 328.