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Coreopsis palmata Nutt. 10576, Freeborn County.
Erigeron canadensis L. 10320, Dakota County.
Erigeron ramosus (Walt.) B. S. P. 10187, Dakota County.
Eupatorium altissimum L. 10326, Scott County.
Gnaphalium obtusifolium L. 10612, Washington County.
Helianthus rigidus (Cass.) Desf. 10523, Lac Qui Parle County.
Helenium autumnale L. 10427, Washington County.
Helianthus tuberosus L. 10457, Traverse County.
Lactuca spicata (Lam.) Hitchc. 10565, Jackson County.
Rudbeckia hirta L. 10192, Dakota County.
Solidago altissima L. 10455, Wilkin County.
Solidago graminifolia (L.) Salisb. var. *Nuttallii* (Greene) Fernald 10540, Lincoln County; 10575, Freeborn County.
Solidago gymnospermoides (Greene) Fernald 10561, Rock County.
Solidago hispida Muhl. 10592, Chisago County.
Solidago rigidiuscula (T. and G.) Porter 10577, Freeborn County; 10607, Washington County.
Solidago serotina Ait. 10458, Traverse County.
Solidago ulmifolia Muhl. 10588, Chisago County; 10615, Washington County.
Vernonia fasciculata Michx. 10610, Washington County.
Xanthium echinatum Murr. 10488, Big Stone County.

GROWTH SUBSTANCES AND NUTRITION OF PROTOZOA *

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For many years investigators have concerned themselves with the isolation, synthesis, and subsequent influence on plants and animals of many divers substances referred to by the non-committal term of "growth substances." The major portion of this research has been confined to the higher plants and animals; however, some attention has been given to bacteria, yeasts, and molds. Very little work has been reported on the role of growth substances in the nutrition of protozoa. This was due primarily to the fact that protozoa have usually been studied in culture media contaminated with microorganisms other than the one in question, a situation which made any results obtained difficult to interpret. Although certain protozoa, namely the plant-like chlorophyll-bearing forms, have been successively cultured bacteria-free for some time, it is only recently that the animal-like ciliates have been grown in artificial media free from other living microorganisms. Facts concerning the nutrition of protozoa are somewhat obscured by the presence of chlorophyll; hence in order to obtain a true picture of the nutrition of both green and colorless protozoa it is essential to study both types in bacteria-free media. Until this was accomplished it was impossible to determine with any degree of accuracy the nutritional

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requirements of single-celled forms. The writer was successful in isolating a ciliate, *Colpidium striatum,* which has been cultured in artificial media for the past seven years with no loss of vigor. Much of the work presented in this discussion involves this protozoan as the experimental organism.

The term "growth substances" includes a variety of compounds but in this discussion it shall refer to certain animal hormones, auxins, and vitamins.

Several earlier attempts were made to determine the effect of certain animal organ extracts on the growth of *Paramecium,* but since the experiments were performed with contaminated cultures interpretation of the results was rather difficult. In all cases the extracts were found to accelerate the growth rate of *Paramecium,* a statement which later was shown by Woodruff and Swingle (1924)[1] to be incorrect. Furthermore, Lwoff (1925)[2] working with *Glaucoma piriformis* in bacteria-free cultures, studied the effects of extracts of the thyroid, ovary, testes, suprenal, pituitary, and thymus and demonstrated that the accelerated growth observed was due entirely to the supplemented food contained in the extracts rather than to any growth substance. Burge and Williams (1927)[3] found that thyroxin depressed the rate of sugar utilization by *Paramecium* while insulin accelerated the use of several sugars. The relative rates which *Paramecium* consumed sugar led these investigators to believe that the sugar metabolism of this ciliate was similar to that of mammals. The writer was able to demonstrate that insulin caused no increase in sugar metabolism in *C. striatum* (Elliott, 1935)[4] as indicated by unaccelerated growth. In so far as the author has been able to determine animal hormones have no effect on the growth rate of protozoa in bacteria-free media.

While it is agreed that auxins in general have little or nothing to do with the substances stimulating growth in bacteria, yeasts, and molds, e.g., bios, vitamins, etc., it has been demonstrated by Popoff (1933)[5] that very dilute extracts from various parts of *Zea* seedlings when added to cultures of *Euglena gracilis* caused marked increase in cell division as well as augmenting the rate of excystment. Yin (1936)[6] and Brannon (1937)[7] working with various species of *Chlorella* found that the growth rate was increased considerably when certain purified auxins (indoleacetic acid, indolepropionic acid, phenylacetic acid, and a-naphthaleneacetic acid) were added to the culture medium. The writer (Elliott, 1938)[8] was able to confirm the work of Popoff employing crystalline phytohormones

[1] Woodruff, L. L. and Swingle, W. W., 1924. Am. Jour. Physiol. 69:21.
[2] Lwoff, A., 1925. C. R. Soc. Biol. 93:1925.
[3] Burge, W. E. and Williams, Maud, 1927. Am. Jour. Physiol. 81:307.
[4] Elliott, A. M., 1935. Archive F. Protisenk. 84:156.
[5] Popoff, M. 1933. Biol. Zentralbl. 53:661.
[6] Yin, H. C., 1937. Natl. Acad. Sci. 23:174.
[7] Brannon, M. A., 1937. Science 86:353.
[8] Elliott, A. M., 1938. Physiol. Zool. 11:31.

instead of the extracts. The growth substances employed were heteroauxin, indolepropionic acid, and indolebutyric acid. In concentrations of 1:10,000 indolebutyric acid produced marked acceleration of growth; moderate acceleration with indolepropionic acid was noted, while indoleacetic acid was slightly toxic. However, with greater dilutions heteroaxin brought about an acceleration of growth also.

It was also shown that the plant hormones were effective only in the presence of adequate illumination (Elliott, 1939).[9] Even though growth was abundant in darkness still the hormones had no accelerating effect on the growth rate and at certain concentrations they proved to be actually toxic.

For comparative purposes a flagellate, *Khawkinea halli,* identical with *E. gracilis* in all respects except for the lack of chlorophyll, was tested along with the green form and shown to be uneffected by the phytohormones. Considerable research on animals reveal a similar situation, that is, plant hormones seem to have no nutritional effects on animal cells. For example, results with *C. striatum* when grown in cultures containing the same hormones as employed with *E. gracilis* and *K. halli* indicated no accelerating effect on the growth rate. In so far as these experiments go they seem to demonstrate that plant hormones stimulate growth of only those protozoa containing chlorophyll in their cytoplasm and are effective only in the presence of an adequate source of illumination. The next series of substances tested were certain members of the vitamin B complex. In an earlier paper with Professor R. P. Hall (Hall and Elliott, 1935)[10] it was reported that some substance or substances present in yeast extract, liver infusion, and partially hydrolized casein were responsible for the continued growth of *C. striatum* and *C. campylum.* Some members of the vitamin B complex were suspected as being the active agent. It was thought that perhaps "pantothenic acid," a universally occurring substance closely resembling vitamin B_2, might be the growth substance in question. Its effects were tested (Elliott, 1935)[11] and found to double the growth of *C. striatum* in media already adequate to support good growth. It was concluded that pantothenic acid, either was the growth substance sought or contained a substance which was essential for the successful culture of this ciliate.

Since pantothenic acid has never been identified as a specific vitamin it was decided to test certain purified members of the vitamin B complex in an effort to determine the role, if any, they might play in the nutrition of *C. striatum.* The basic medium in which the stocks have been cultured for the past seven years contains Difco tryptone, a casein enzymatic digest, as the sole source of carbon and nitrogen. There has been no apparent loss in vigor

[9] Elliott, A. M., 1939. Trans. Am. Mic. Soc. (in Press).
[10] Hall, R. P. and Elliott, A. M. 1935. Archive F. Protistenk. 85:443.
[11] Elliott, A. M. 1935. Biol. Bull. 68:82.

during this period which indicates that the diet is adequate in all respects. In this group of experiments the vitamin B complex or some components of it, was destroyed by heating the basic tryptone medium to 120° C. for one hour at pH 9.6. When the pH was readjusted to 6.0 this medium would not support the growth of *C. striatum*. However, when thiamin chloride was added in concentrations as low as 1:10,000,000 growth was equal to that of the untreated control. Increased concentrations of thiamin up to 1:2000 produced no better effects although no harmful results were noted. These experiments demonstrated conclusively that thiamin chloride was an essential nutritional factor in the diet of this protozoan.

The fact that thiamin was an essential growth factor for *C. striatum* led the writer to speculate as to whether or not other members of the vitamin B complex might not also be important. Such investigation is under way at present and so far it has been shown that neither riboflavin nor a vitamin B_6 concentrate is able to supplant thiamin. Whether or not these substances are essential for the continued existence of this ciliate has not yet been determined.

THE GROWTH RATE OF WALL-EYED PIKE (*STIZOSTEDION VITREUM* (MITCHILL) IN VARIOUS LAKES OF MINNESOTA *

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One of the important guides for modern fish propagation and management is the growth rate of fishes. In some lakes, the fishes caught by fishermen are smaller than those caught in other lakes and this raises the question of whether fish grow at the same rate in all lakes. The fish culturist must know the rate of growth and the length of time necessary for various species of game fish to reach adult size. The study of the rate of the growth of fishes was undertaken at the University of Minnesota several years ago and has been continued with the cooperation of the Department of Conservation, the N.Y.A., the W.P.A., the C.C.C., the United States Forest Service, and other agencies. Species of all common fishes have been studied, but emphasis has been placed on the study of important game fishes, such as the wall-eyed pike.

Each scale of a fish has characteristic marks from which the age, growth rate, and other details of the life history may be ascertained.

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