

observed took place 32 to 35 days after tumor cell implantation. The mechanism responsible for these regressions has not been determined and is the subject of further study.

Summary. Under certain conditions C3H mice implanted with lymphosarcoma 6-C3H-ED were protected either by injection of ZBC spleen tissue alone or by A-methopterin treatment alone. A more successful treatment consisted of a single subcutaneous injection of ZBC spleen tissue with daily doses of A-methopterin. Mice which had received tumor implantations 24, 48 or 96 hours preceding therapy were treated with this combination. Some were not protected, but in others tumors either failed to become palpable, or having become palpable appeared to have regressed completely.

ADDENDUM. The mice enumerated in the column, "Number of Mice Protected," shown

in Table I, were challenged for immunity by injection of 5,000,000 lymphosarcoma cells subcutaneously into the left flank. The mice in Exp. 1 and 2 were challenged on the 42nd, and those in Exp. 3 on the 40th day of the experiment. Immune mice either developed small tumors which regressed, or no tumors at all; non-immune mice died within 30 days with progressive lymphosarcoma. Of the 14 mice which had been treated with A-methopterin alone in the three experiments, 6 were immune; of 11 which had been treated with ZBC spleen alone all were immune, while of the 24 which had received the combination treatment (A-methopterin and ZBC spleen) 20 were immune.

1. Foley, E. J., PROC. SOC. EXP. BIOL. AND MED., 1951, in press.

Received December 13, 1951. P.S.E.B.M., 1952, v79.

Replacement of Protogen by Lipic Acid in the Growth of *Tetrahymena*. (19306)

GERALD R. SEAMAN.

From the Carter Physiology Laboratory, University of Texas Medical Branch, Galveston.

The ciliate *Tetrahymena geleii* is readily grown in synthetic medium comprising, with only one exception, known chemicals. The one unknown factor is the liver fraction concentrate, protogen(1). Protogen has been identified(2) with the acetate(3) and the pyruvic oxidation(4) factors. Kidder and Dewey(5) found, as would thus be expected, that in *T. geleii*, protogen serves at least one other function than the replacement of acetate.

Reed and co-workers have recently(6,7) purified a series of factors from liver residues which show high activity in replacing both the acetate and pyruvic oxidation factors. One of these factors has been crystallized(8) and named, tentatively at least, lipic acid. It was thus of interest to ascertain if this compound may replace protogen in the growth of *Tetrahymena geleii*.

Methods. *Tetrahymena geleii* S(9) was grown in the synthetic medium described

by Elliott(10). Protogen was omitted from the medium and was replaced by varying amounts of lipic acid.* Inoculations were made in all cases by loop transfer. Cells for inoculation into the first experimental culture were washed with 500 volumes of distilled water before transfer, to assure against a carry over of material. The data presented was obtained from cultures which had gone through three sub-cultures in the experimental medium. Growth was measured turbidimetrically after 96 hours growth at 25°C and is expressed as optical density.

Results and discussion. Fig. 1 shows that lipic acid in concentrations of 1 unit[†]/ml and higher is capable of maintaining growth

* Protogen was generously supplied by Dr. E. L. R. Stokstad. Concentrates of lipic acid were kindly supplied by Dr. L. J. Reed.

† One unit is equivalent to the manometric pyruvate oxidase response produced by 1 mg of yeast extract.

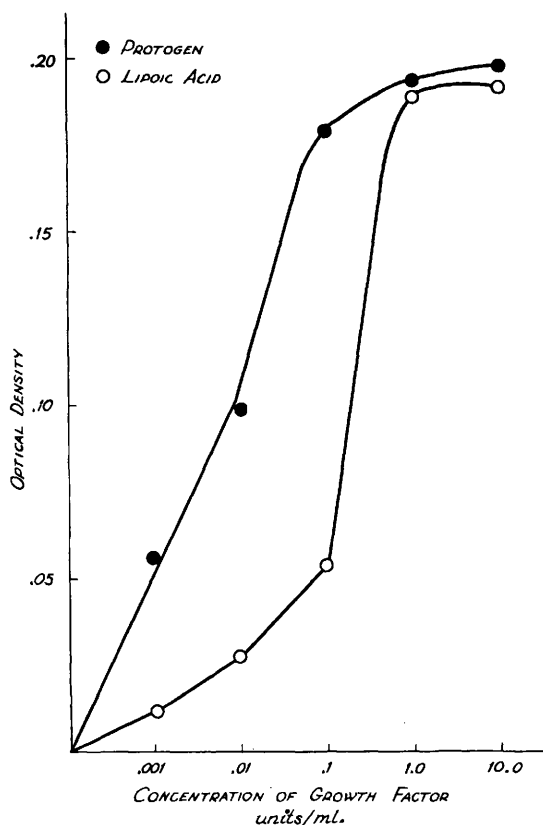


FIG. 1. Growth of *Tetrahymena geleii* S in response to various concentrations of protogen and of lipoic acid.

equal to that obtained in the presence of protogen. Since crystalline samples of neither protogen nor lipoic acid are available at present for investigation, it is of course not

possible to designate one factor as more active than the other.

It has been suggested that lipoic acid may be a new B vitamin. It has been shown that there occur a variety of active forms of this factor in nature(6,7); this had been previously suggested by Snell and Broquist(2). Thus lipoic acid appears to resemble such vitamins as pyridoxine and niacin which also occur in nature in a variety of chemical forms. In fact, since lipoic acid replaces protogen, it appears that protogen may actually be either lipoic acid or one of its chemical derivatives.

Summary. Lipoic acid is capable of completely replacing protogen in the growth of *Tetrahymena geleii*.

1. Stokstad, E. L. R., Hoffman, C. E., Regan, M. A., Fordham, D., and Jukes, T. H., *Arch. Biochem.*, 1949, v20, 75.
2. Snell, E. E., and Broquist, H. P., *Arch. Biochem.*, 1949, v23, 326.
3. Guirard, B. M., Snell, E. E., and Williams, R. J., *Arch. Biochem.*, 1946, v9, 381.
4. O'Kane, D. J., and Gunsalus, I. C., *J. Bact.*, 1948, v56, 499.
5. Kidder, G. W., and Dewey, V. C., *Arch. Biochem.*, 1950, v27, 463.
6. Reed, L. J., DeBusk, B. G., Johnston, P. M., and Getzendaner, M. E., *J. Biol. Chem.*, 1951, v192, 851.
7. ———, *J. Biol. Chem.*, 1951, v192, 859.
8. Reed, L. J., DeBusk, B. G., Gunsalus, I. C., and Hornberger, C. S., *Science*, 1951, v114, 93.
9. Seaman, G. R., *J. Biol. Chem.*, 1950, v23, 85.
10. Elliott, A. M., *Physiol. Zool.*, 1949, v22, 337.

Received September 25, 1951. P.S.E.B.M., 1952, v79.

Use of Perchloric Acid for Nucleic Acid Histochemistry in Mammalian Nerve and Liver Cells.* (19307)

HAROLD KOENIG AND HELMUTH STAHLCKER. (Introduced by P. P. Foa.)

From the Department of Anatomy, The Chicago Medical School, Chicago.

Following the report of Ogur and Rosen(1) on the differential extraction of pentose nucleic (PNA) and desoxypentose nucleic acid

* Partial accounts were presented at the Annual Meetings of the Histochemical Society March 1950, and 1951.

(DNA) from plant and animal tissues with perchloric acid, there have been several attempts to apply this procedure to the histochemical demonstration of nucleic acid. Ogur and Rosen's procedure has been employed for this purpose on plant tissue(2), a proto-