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* This work was done during the tenure of an Established Investigatorship of the American Heart Association.

26 July 1957

Interaction of Stigmasterol and 2,4-Dinitrophenol in the Growth of Tetrahymena piriformis

Stigmasterol has been reported as a growth factor for several organisms, including the guinea pig (*1*), *Paramecium aurelia* (*2*), *Paramecium multimicronucleatum* (*3*), *and Stylonychia* (*4*). Extensive studies have been carried out on the mode of action of stigmasterol (antistiffness factor) in the metabolism of the guinea pig (*1*). These investigations revealed a marked reduction of anaerobic glycolysis in the tissues of deficient animals—a condition corrected by the addition of the antistiffness factor or adenosine triphosphate (ATP). Van Wagtendonk concluded that the steroid deficiency led to an altered phosphate metabolism, possibly a defect in the mechanism for the generation or transport of high-energy phosphate groupings. We believe that this hypothesis is strengthened by studies on the ciliated protozoan, *Tetrahymena piriformis.*

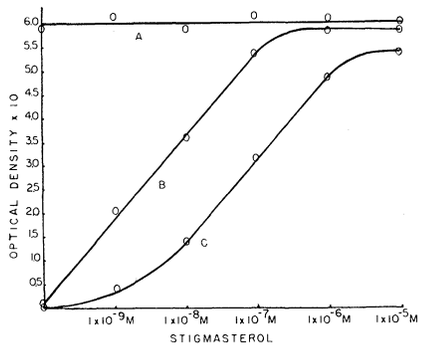

Fig. 1. Effect of DNP and stigmasterol on the growth of *Tetrahymena piriformis, W. Tetrahymena* were cultured in Kidder's medium A by means of the techniques and procedures developed by him for axenic culture (*11*). All points represent the optical density (×10) of cultures grown at 25°C for 96 hours, as measured in a Lumetron colorimeter at 650 mμ. Each experiment was repeated 5 times. Curve *A* represents stigmasterol; curve *B,* stigmasterol + DNP ($5 \times 10^{-5} M$); and curve *C,* stigmasterol + DNP ($1 \times 10^{-4} M$).

Tetrahymena has no exogenous nutritional steroid requirement but synthesizes a steroidlike compound, the configuration of which is not as yet known (*5*). However, the presence of this compound is capable of maintaining the growth of another protozoan, *Paramecium aurelia* (*6*), which has an absolute steroid growth requirement. The molecular configuration necessary for biological activity has been well established for *Paramecium,* and stigmasterol is one of the most effective sterols in promoting the growth of this organism (*7*). The *Tetrahymena* steroid is equivalent to stigmasterol in growth-promoting activity (*6*); thus, it seems possible that the *Tetrahymena* steroid is a member of the stigmasterol group.

In *Tetrahymena,* certain growth inhibitors, both steroidal and nonsteroidal in nature, induce a steroid requirement, which can be satisfied by stigmasterol (*6*)—further evidence of a possible relationship between the *Tetrahymena* steroid and stigmasterol.

Among the nonsteroid growth inhibitors, the most interesting is 2,4-dinitrophenol (DNP). The reversal of DNP growth inhibition in *Tetrahymena* in the presence of stigmasterol is shown in Fig. 1.

These growth studies indicate a close relationship between DNP and stigmasterol in the metabolism of *Tetrahymena.* The linearity of the reversal of DNP growth inhibition may indicate a competitive phenomenon. Further, since DNP is known to be an effective uncoupling agent of oxidation and phosphorylation (*8*) and an activator of adenosine triphosphatase (*9*), we believe that this study in *Tetrahymena* (*10*) not only indicates a mode of action of stigmasterol similar to that suggested for the guinea pig but greatly strengthens such an interpretation. While the studies with the antistiffness factor in the guinea pig indicated an influence of this compound on anaerobic glycolysis, this interpretation may be too limited and perhaps should be enlarged to include aerobic mechanisms. Further *in vivo* and *in vitro* experiments are being conducted to test this hypothesis.

ROBERT L. CONNER
Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania

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22 July 1957

Occurrence of trans Fatty Acids in Human Tissue

Except for small amounts of *trans* fatty acids in animal fats, dietary fats are composed of unsaturated fatty acids of *cis* geometric configuration. In 1928, Bertram (*1*) found small amounts of *trans* Δ 11-octadecenoic acid in ox, sheep, and butterfat; more recently (*2*), the presence of 4- to 11-percent *trans* fatty acids has been reported in deer, ox, and sheep depot fats. Although *trans* fatty acids do not seem to be normally present in nonruminants, they are found in the depot fats of rats which have been fed *trans* fatty acids (*3*).

Considerable amounts of *trans* fatty acids are formed during the commercial hydrogenation of vegetable oils (*4*); the shortenings and margarines which include these hydrogenated oils have been reported to contain as much as 23 to 42 percent of *trans* fatty acids (*5*). Furthermore, the isomers formed during selective hydrogenation are composed of a complex mixture of both geometric and positional isomers (*6*). The consumption of such fats would presumably lead to the deposition of *trans* fatty acids in depot fats.

In the present study, autopsy and biopsy material from 24 human subjects (*7*) was examined for the presence of *trans* fatty acids. The tissues were extracted in a Soxhlet apparatus for 24 hours with acetone and petroleum ether (Skellysolve F) as solvents, the extracts were dried over anhydrous sodium sulfate and filtered, and the solvent was removed under vacuum. The amounts of *trans* isomers in the lipid extracts were determined by the Jackson and Callen baseline method (*8*), in which a Beckman IR-2A spectrophotometer was used.