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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.*

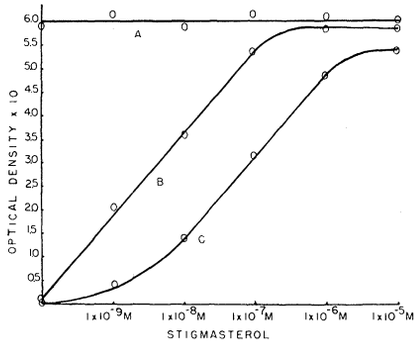

Fig. 1. Effect of DNP and stigmasterol on the growth of *Tetrahymena piriformis, W. Tetrahymena* were cultured in Kidder's medium A by means of the techniques and procedures developed by him for axenic culture (*11*). All points represent the optical density (×10) of cultures grown at 25°C for 96 hours, 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.

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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.

All the samples of tissue contained *trans* fatty acids. Adipose tissue contained from 2.4 to 12.2 percent, liver, 4.0 to 14.4 percent, heart, 4.6 to 9.3, percent, aortic tissue, 2.3 to 8.8 percent, and atheroma from subjects who had died of atherosclerosis, 2.3 to 8.8 percent of *trans* fatty acids. It has been pointed out that *trans* linoleic acid does not function efficiently as an essential fatty acid (*9*), although *trans* fatty acids seem to be metabolized (*10*). Furthermore, it has recently been reported that *trans* crotonyl CoA is the preferred substrate for the unsaturated acyl CoA hydrase from beef liver (*11*). Presumably, therefore, long-chain *trans* fatty acids may be metabolized as readily as the *cis* fatty acids. However, in view of the current controversy on the relationship of "hard" vs. "soft" fats (*12*), it would seem necessary to determine what effect, if any, *trans* fatty acids have on the normal metabolic process.

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8 August 1957

Uptake of Calcium-45 and Strontium-90 from Water by Fresh-Water Fishes

In conjunction with our studies concerning the uptake and accumulation of calcium-45 and strontium-90 into the body and tissues of the guppy (*Lebistes*) (*1, 2*), I observed that zebra fish (*Danio*) and white cloud mountain fish (*Tanichthys*) take up both isotopes from water in a similar manner. It is well known that strontium, when fed or injected into animals, accumulates in bone and tissues of high calcium content and appears to be related to the metabolism of calcium. However, Alexander *et al.* (*3*), Norris and Kisieleski (*4*), and Comar *et al.* (*5*) have shown that small laboratory mammals discriminate against strontium relative to calcium. More recently, Boroughs *et al.* (*6*) have shown that marine fishes discriminate against strontium relative to calcium, when strontium-89 is added to the sea water in which they swim. The present report (*7*) gives data comparing the uptake of calcium-45 and strontium-90 by three species of fresh-water fishes.

Table 1. Rate of uptake of calcium-45 from water by various fishes.

Species	Body calcium ± S.D.*(*A*) (percentage of body wt.)	Rate of uptake† ± S.D.* (*B*)	Ratio *B/A*
Guppy	1.14 ± 0.09 (23)‡	0.81 ± 0.01 (38)§	0.71
Zebra fish	1.13 ± 0.12 (22)‡	0.84 ± 0.02 (27)§	0.74
White cloud mountain fish	1.01 ± 0.13 (28)‡	0.78 ± 0.02 (27)§	0.77

* Standard deviation = $(\Sigma d^2/n)^{1/2}$.
† $\frac{\text{Log count/min/100 mg/day}}{\text{Log water activity count/min ml}}$ The water contained 10^5 counts/min ml.
‡ The number in parentheses indicates average values obtained on pools of two to three fishes.
§ The number in parentheses indicates number of fishes derived from experiments shown in Fig. 1.

Adult fishes, obtained from commercial sources, were of mixed sexes for the zebra and white cloud mountain fish, but the guppies were all sexually mature males. The guppies and white cloud mountain fishes averaged about 125 mg, while the zebra fish were each about 225 mg in weight. The experimental design and the assay of radioactivity have previously been reported in detail (*1, 2*). The samples were counted to within a 5-percent statistical error with a windowless gas flow counter, with counting efficiencies for strontium-90 (*8*) and calcium-45 of 1.5×10^{10} and 1.0×10^9 count/min mc, respectively. Self-absorption and decay corrections have been made when necessary. Calcium analyses were made by permanganate titration following precipitation of the oxalate, as has been previously described (*9*).

The uptake of calcium-45 and strontium-90 from the water in which the fishes swim is linear with respect to time for the three species at all water isotope activities studied. Representative data of some of our experiments with both isotopes are shown in Figs. 1 and 2. The linear uptake of these isotopes may be interpreted to indicate the continual formation or exchange of the mineral component of bone, with sequestration of the isotopes into bone matrix.

Previous investigators have indicated that the rate of uptake of strontium-89 from water by goldfish (*10*) and marine *Tilapia* (*11*) decreased with time. An explanation for the discrepancy between my results and those of other investigators is not readily apparent. However, at the isotopic water activities used in the present work, it was found that strontium-90 activity in water could not be maintained at a constant level but began to decrease appreciably when the experiments were extended beyond 20 days. For this reason, experiments longer than 14 to 18 days were not performed. It is presumed that excess accumulation of metabolic products and feces may have removed some of the strontium-90 from solution by adsorption. Similar difficulties in keeping strontium-89 in

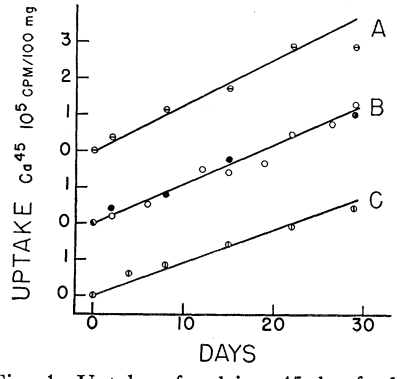

Fig. 1. Uptake of calcium-45 by fresh-water fishes versus days in water containing about 10^5 count/min ml. (*A*) Zebra fish; (*B*) guppy (two experiments); (*C*) white cloud mountain fish. Each point represents an average of two to six fish.

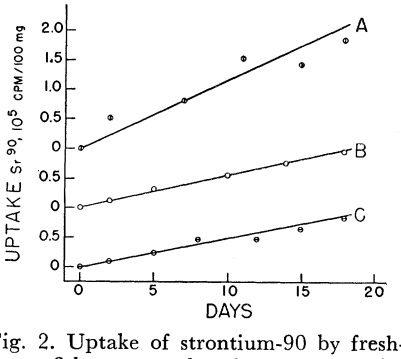

Fig. 2. Uptake of strontium-90 by fresh-water fishes versus days in water containing about 10^5 count/min ml. (*A*) Zebra fish; (*B*) guppy; and (*C*) white cloud mountain fish.