The Effect of Emetine on Macromolecular Synthesis in Synchronized *Tetrahymena pyriformis*

Emetine has been known as the active agent of Ipeccacuanha in treating amebiasis since 1912[1]. However its mechanism of action remained obscure until GROLLMAN[2,3] showed that it inhibited protein synthesis in mammalian, plant and yeast cells. Emetine also inhibits incorporation of ^{3}H-leucine into rat heart cells[4] and incorporation of L-amino acids into rat liver protein[5]. It inhibits energy metabolism in perfused rat heart[6] and in embryonic chick heart cells.[7] Chronic dosing of rats with emetine inhibits glycose metabolism, oxygen uptake and carbon dioxide production in their heart homogenates[8].

The primary mechanism of action of emetine in protozoans has not been worked out. The present study demonstrates the effect of emetine on macromolecular synthesis in *Tetrahymena pyriformis*, a ciliated protozoan.

Materials and methods. *Tetrahymena pyriformis* GL were grown in a medium containing 0.1% bactodextrose, 1.0% proteose peptone, 0.1% sodium acetate and 0.1% dibasic potassium phosphate. When the cells reached a population of 80,000 to 120,000 per ml they were synchronized, resuspended in an inorganic medium and counted by a previously described method[9]. Emetine dihydrochloride[10] was added at the end of the last heat treatment (EHT) and the cells were incubated at 28 °C on a rotator at 100 c/sec for the duration of the experiment.

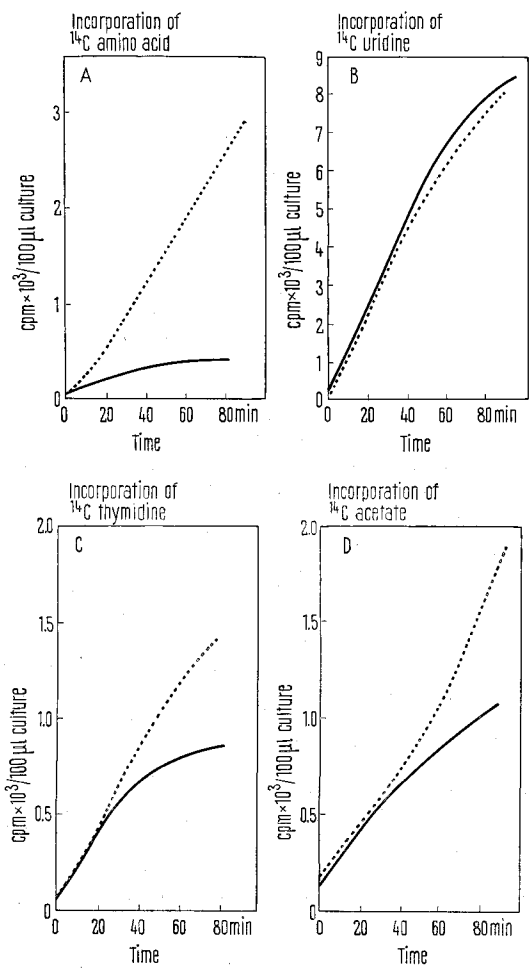
Incorporation of uniformly labeled ^{14}C-amino acids (algal profile), ^{14}C-uridine (sp. act. 53.1 mc/mmole), ^{14}C-thymidine (sp. act, 35 mc/mmole) and ^{14}C-acetate (sp. act. 40 mc/mmole) was assayed by adding 10 ml aliquots of cells into 50 ml Erlenmeyer flasks containing a measured amount of emetine and the radiolabeled precursor sufficient to attain the following concentrations: ^{14}C-amino acids 0,25 μc/ml, ^{14}C-uridine 0.25 μc/ml, ^{14}C-thymidine 2μc/ml, and ^{14}C-acetate 0.5 μc/ml. Controls contained precursor alone. The rest of the procedure was carried out and counted exactly as described by CONKLIN et al[9].

Results. Emetine at $4 \times 10^{-6} M$ blocked synchronized division completely for 240 min after EHT. Cells were counted at EHT and every 30 min from 60 until 240 min and no decrease in cell population was noted.

The effect of emetine on the incorporation of amino acids, uridine, thymidine and acetate is shown in the Figure. In the treated cells protein snythesis, as measured by incorporation of amino acids, showed some inhibition of incorporation during the first 20 min (51.2% ± 2.5% $n = 5$). After 20 min there was hardly any further incorporation and at 80 min inhibition was 75% (Table). In the control cells, incorporation of amino acids was linear throughout the experiment. There was no effect on uridine incorporation, which measures RNA synthesis. No effect on thymidine incorporation was detected during the first 20 min, but the inhibition of DNA synthesis was noted as a later effect and 53.8% inhibition was recorded at 80 min (Table and Figure). Measuring acetate incorporation demonstrated little effect for the first 40 to 60 min after which significant inhibition, 46.8%, could be seen (Table and Figure).

Discussion. These studies indicate that emetine blocks protein synthesis directly, and not as a result of altered nucleic acid function. This is evidenced by the normal incorporation of uridine in the cells. Dependence of DNA synthesis on protein synthesis has been demonstrated in both bacterial[11] and mammalian cells[12]. This would explain the delayed inhibition of thymidine incorporation.

Acetate is incorporated into lipids[13] and glycogen[14] in *Tetrahymena*. Probably some of it finds its way into proteins and part or all of its inhibition could reflect protein synthesis inhibition. The delayed block of acetate incorporation could also be due to deceased synthesis of fatty acids because of disruption of the mitochondria. These



Incorporation of ^{14}C-amino acids (0.25 μc/ml), ^{14}C-uridine (0.25 μc/ml), ^{14}C-thymidine (2 μc/ml) and ^{14}C-acetate (0.5 μc/ml) with and without emetine. Protein and DNA synthesis and acetate incorporation are reduced in the presence of emetine while RNA synthesis was not affected.

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Effect of emetine $4 \times 10^{-6} M$ on incorporation of ^{14}C-labeled precursors

^{14}C Precursor	Inhibition of incorporation at EHT + 80 min (%)
Amino Acids	75.2±2.1 ($n = 5$)
Acetate	46.8±0.2 ($n = 4$)
Thymidine	53.8±2.1 ($n = 6$)
Uridine	4.7±3.1 ($n = 3$)

Percentages represent the mean and standard error.

organelles depend on microsomal and their own protein synthesis for assembly of their functional units[15] and both are inhibited by emetine[16]. This agent has also been noted to selectively damage mitochondria in dog heart[17].

The possibility that emetine is acting by blocking the uptake of precursors seems unlikely in light of the normal incorporation of uridine and the normal incorporation of thymidine and acetate in the early part of the experiment. RASMUSSEN and ZEUTHEN[18] have demonstrated in *Tetrahymena* that cell division is blocked by inhibition of protein synthesis and this seems the major reason why emetine is inhibiting division.

Comparing this system to GROLLMAN'S[3] work in Hela cells demonstrates a parallel effect in protein and nucleic acid synthesis. He noted that HeLa cells concentrated the drug and that higher concentrations of emetine were required to inhibit protein synthesis in cell-free preparations. This does not seem to be the case in *Tetrahymena* as $10^{-6} M$, a concentration which does not inhibit the in vivo system (results not shown) inhibits protein synthesis in cell-free preparations of *Tetrahymena*[19].

Zusammenfassung. Emetin hemmt die synchronisierte Teilung von *Tetrahymena pyriformis*, verhindert die rasche Aufnahme von Aminosäuren und verursacht offenbar eine Zellteilungshemmung.

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Ionically Induced Volume Changes of the Smooth Muscle of the Guinea-Pig *Taenia coli*

If the osmolarity of Krebs solution is doubled by addition of potassium salts of permeant anions (KCl, KNO_3), pieces of guinea-pig taenia coli muscle exposed to these solutions shrink, but fail to recover weight, in contradiction to the predictions of the Gibbs Donnan equilibrium. If the NaCl in the Krebs solution is replaced by equivalent amounts of permeant potassium salts (isosmotic potassium solution) the tissues gain little if any weight, again not obeying the predictions of the Gibbs Donnan equilibrium. Similar results have been reported by several authors[1–3].

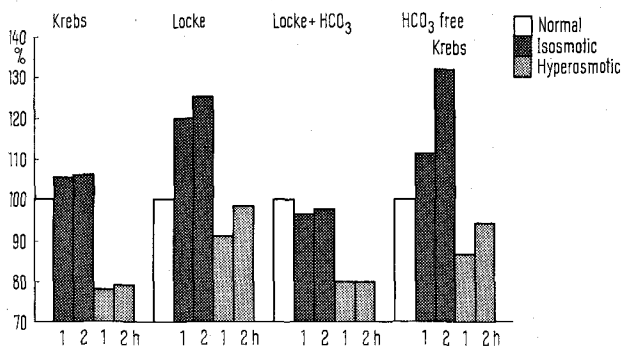

Fig. 1. The importance of HCO_3. Weight changes after exposure for 1 and 2 h to high potassium solutions. The isomotic solutions were made by replacing the NaCl in the normal solution with KNO_3, and the hyperosmotic solutions by adding 154 mM KNO_3 to the normal solutions. The results are expressed as a percentage of the weight of control pieces exposed to the normal solution. The columns are each the mean results of between 6 and 25 tissues, with standard errors of between ± 0.5 and ± 2.0.

The lack of recovery from shrinkage in hypertonic KCl or KNO_3 solution, and the lack of swelling in isosmotic K solution may suggest that the smooth muscle membrane has very low permeability to K, Cl and NO_3 ions. However, estimates of the membrane permeability to K and Cl ions from flux experiments in Krebs solution[4,5] indicate that, even assuming that high concentrations of K^+ do not increase membrane permeability, the failure of penetration of net amounts of KCl is not due to the low permeability of the membrane. Rough calculations, even using the least advantageous figures, show that sufficient ions should be able to penetrate into the cells for them to double their volume within an hour.

In order to obtain more information on the factors controlling the volume changes of the taenia, the effects of changes in the external medium were investigated. In the present set of experiments, KNO_3 has been used as the permeant potassium salt, since it has been shown[3] that NO_3 is more permeant than Cl in this tissue. Tissue pieces

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