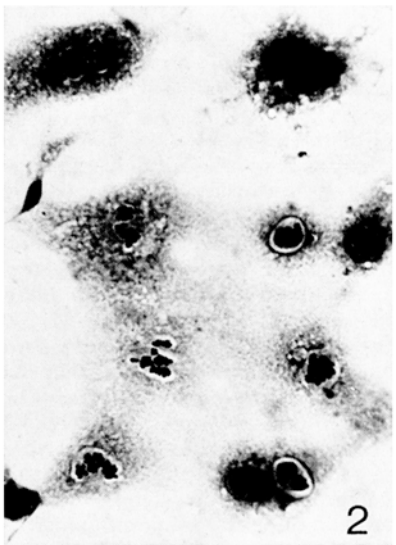

Fig. 2. *Aedes w-albus* cell line (ATC-136). Cells showing mitosis, ×450 (Wright's and Giemsa stained).

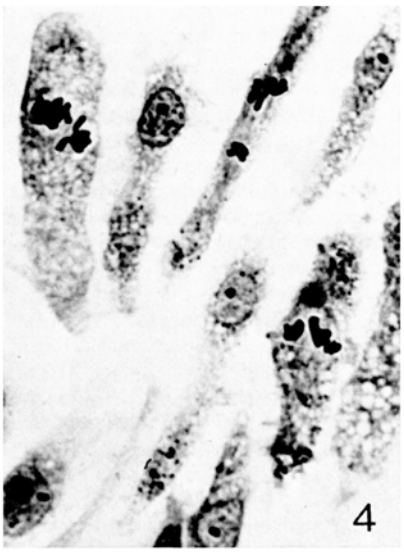

Fig. 4. *Aedes w-albus* cell line (ATC-137). Cells showing mitosis, ×650 (Wright's and Giemsa stained).

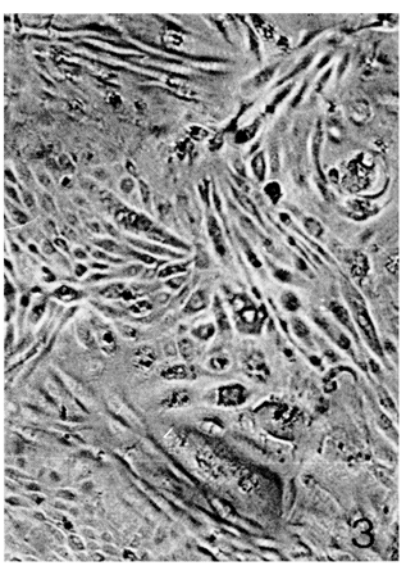

Fig. 3. *Aedes w-albus* cell line (ATC-137). Living culture, ×150.

monolayer had formed. During the first 5 passages the split rate was maintained at 1:2, which was gradually raised to 1:6 by the 10th passage.

The first cell line (ATC-136) was started on 14th July 1969 and the second (ATC-137) on 13th August 1969. So far (June 1970) they have been subcultured 37 and 27 times respectively.

In earlier passages, the cell population in both the lines consisted of multiple cell types similar to those described for *A. albopictus* cell culture[8]. One of the cell line (ATC-136) has a cell population consisting mostly of epithelial-like cells and few fibroblast-like cells (Figures 1 and 2). The other cell line (ATC-137) has a population consisting mostly of fibroblast-like cells and a few epithelial-like cells (Figures 3 and 4). The giant cell population in both the cell lines is almost similar. During the few early passages the cell population in both the cell lines consisted mostly of diploid cells ($2n = 6$). After about ten passages the number of polyploid cells increased noticeably.

The cells from both the lines were stored in liquid nitrogen in the growth medium containing 20% fetal bovine serum and 10% glycerol, and successfully regenerated after 45 days.

Studies on the susceptibility of these cell lines to arboviruses are in progress.

Zusammenfassung. Zwei neue Zellinien von *Aedes w-albus*, die seit der Isolation schon 27 bzw. 37mal kultiviert wurden, sind isoliert worden. Die Zellen konnten 45 Tage in flüssigem Stickstoff aufbewahrt werden, ohne ihre Vermehrungsfähigkeit einzubüssen.

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Virus Research Centre, Poona 1 (India), 29 June 1970.

[8] U. K. M. Bhat and K. R. P. Singh, in press.

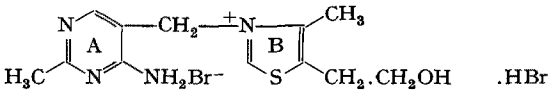
A Thiamine (Vitamin B_1 or Aneurin) Antimetabolite as a Potent Schistosomicidal Agent

Schistosomiasis is a parasitic disease that infects millions of people in the tropical and subtropical regions. There is no ideal treatment for the disease. Till now, there are 2 classes of compounds that are in classical use, compounds of trivalent antimony[1] and those of thiaxanthone derivatives[2]. Recently, the compound, 1-(5′-nitro-2′-thiazolyl)-2-imidazolidinone (Ambilhar), was found to possess potent schistosomicidal activity[3]. But

one was faced with difficulties for its use, due to its insolubility in water and the toxic effects it showed during treatment.

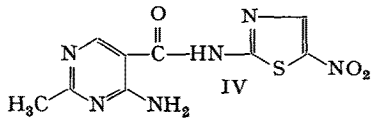
In this work, we aimed to introduce a new water-soluble and tolerable agent, that can be used for treatment of schistosomiasis. Our rational approach to investigating its structure was based upon its close relation to thiamine, in an attempt to profit from the concept of metabolic antagonism or antimetabolites, in this area of chemotherapy. Since, the parasite schistosome seeks its energy for reproduction and living through glycolysis, then interference with an agent that would act as thiamine antimetabolite may be useful for treatment, through interference with the metabolic process, where the vitamin takes part.

Thiamine is a biocatalyst in the glycolytic process. It is an important growth factor for most of the lower organisms, without which they cannot live. Its activity is highly specific to structure, and its effect can be lost by structurally related analogs. Chemically, the vitamin is composed of 2 moieties; a substituted pyrimidine (A) and a substituted thiazole (B).



In this work, we planned to build up a structure keeping the pyrimidine unit (A) without particular changes. For the thiazole part, we used 2-amino-5-nitrothiazole moiety. Both systems were designed to be connected through an amide linkage, as this may be biologically hydrolyzed to free both parts. Thus, the molecule may act as a whole or as separate components in the presence of one another.

The desired pyrimidine moiety was obtained through hydrolysis of 2-methyl-4-amino-5-cyanopyrimidine[4] (I) to give the corresponding acid II, which upon esterification gave 2-methyl-4-amino-5-carbomethoxypyrimidine[5] (III). This was submitted to condensation with 2-amino-5-nitrothiazole, to build up the desired amide linkage, and gave 2-N(2′-methyl-4′-amino-5′-pyrimidoyl)-amino-5-nitrothiazole (IV).



IV

Experimental: 2-Methyl-4-amino-5-cyanopyrimidine (I): This was prepared through condensation of acetamidine hydrochloride and ethoxymethylene-malononitrile in presence of sodium ethoxide, mp 249° (Lit.[4] mp 249°).

2-Methyl-4-amino-5-carboxypyrimidine (II): By hydrolysis of I using 10% potassium hydroxide solution, mp 271° (Lit.[5] mp 270°).

2-Methyl-4-amino-5-carbomethoxypyrimidine (III): Esterification of II with methanol and conc. sulphuric acid, gave III, mp 184° (Lit.[5] mp 184°).

2-N(2′-methyl-4′-amino-5′-pyrimidoyl)-amino-5-nitrothiazole (IV): A mixture of III (1.67 g; 0.01 mole), 2-amino-5-nitrothiazole (1.45 g; 0.01 mole) in the least amount of absolute ethanol, was refluxed together for 6 h. Upon cooling, needle crystals of IV were formed. These were filtered off and crystallized from water. Yield 1.9 g (66%), mp 182°.

Anal. Calcd. for $C_9H_8N_6O_3S.H_2O$: C, 36,04: H, 3.39; N, 28.09; S, 10.80 Found: C, 36.14; H, 3.52; N, 28.12; S, 10.71

The IR-spectrum showed absorption bands at 3350 cm^{-1} for the –OH, at 1715 cm^{-1} for the secondary amide and at 1370 cm^{-1} for the thiazole nitro group.

Biological studies. Groups (a, b, c, d, e) of mice of 5 each, average weight of 30 g, infected with *Schistosoma mansoni*, and showing viable eggs in their stools were used. Group (a) was given daily doses of 60 mg/kg of body weight from compound IV in water solution for 10 consecutive days. Group (b) was similarly treated with the same doses (60 mg/kg of body weight) as group (a) for the same period along with high doses of thiamine hydrochloride (16 mg/kg of body weight). Groups (c, d) were given similar doses of 60 mg/kg of body weight from compounds III and 2-amino-5-nitrothiazole respectively. Group (e) was kept as control. Follow up for ova excretion began after 4 weeks from the end of treatment in all groups. In group (a), a significant decrease in egg count took place, and it completely ceased after 6 weeks from the end of treatment. In groups (b, c, d) there was no significant change in the rate of ova excretion as compared with the control group (e).

Thus, it could be observed, in spite of the schistosomicidal activity shown by compound IV, both of its structural components, III and 2-amino-5-nitrothiazole were devoid of such activity. Also this activity was abolished through parallel administration of high doses of thiamine.

In another experiment a group of 5 infected mice was examined for ova count per microscopic field. The level ranged between 4–6 ova per field, then the animals were fed daily doses of 24 mg/kg of body weight from thiamine hydrochloride in watery solution for 12 consecutive days. Ova count after 3 days from the end of the vitamin hydrochloride administration had shown considerable increase in the rate of their excretion. The levels ranged between 18–21 ova per field. Examination continued for 10 weeks at intervals of 3 days period. Gradual decrease in the rate of ova excretion was observed and by the end of examination period (10 weeks) the levels ranged between 6–8 ova per field.

Zusammenfassung. Die biologische Untersuchung eines neuen, mit Vitamin B_1 (Thiamin, Aneurin) strukturverwandten Vitamins hat eine Wirkung gegen Schistosomiasis gezeigt. Diese wird durch Zugabe des natürlichen Vitamins gehemmt.

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National Research Centre, Dokki (Egypt, U.A.R.), 15 June 1970.

[1] J. CHRISTOPHERSEN, Lancet *195*, 325 (1918).
[2] W. KIKUTH and R. GONNERT, Ann. trop. Med. Parasitol. *42*, 256 (1949).
[3] C. LAMBER and M. WILLHELM, Experientia *20*, 452 (1964).
[4] R. GREWE, Hoppe-Seyler's Z. physiol. Chem. *242*, 89 (1936).
[5] P. PRICE, E. MAY and F. PICKEL, J. Am. chem. Soc. *62*, 2818 (1940).
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