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EFFECT OF BILHARCID AND TARTAR-EMETIC ON DNA, RNA AND PROTEIN SYNTHESIS IN *ESCHERICHIA COLI* B

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Abstract—The bacteriostatic concentration of piperazine diantimony tartrate (bilharcid) and potassium antimony tartrate (tartar-emetic) for *Escherichia coli* B was determined and the reversibility of the bacteriostasis established. The influence of each of bilharcid and tartar-emetic on the synthesis of DNA, RNA and protein by *E. coli* B was studied. Each of these drugs caused complete inhibition of the synthesis of DNA, RNA and protein when present at its bacteriostatic concentration. When the inhibitor was removed, these biosynthetic processes, as well as cell division, were reinitiated and resumed, rates parallel to those characteristic of the control, uninhibited culture.

ANTIMONIALS have been used as medicines and cosmetics since 4000 B.C. They were the basis for much of the fame of Paracelsus (1493–1541). Christopherson[1] discovered the effectiveness of tartar-emetic in schistosomiasis and so introduced the use of antimonials in tropical diseases.

The antiparasitic action probably is similar to that of arsenicals, residing in sulfhydryl inhibition. Other possibilities have been suggested, however, including inhibition of enzymes involved in glycolysis, e.g. the phospho-fructokinase[2,3] of the parasite.

As far as we are aware, the effect of antimonials on the synthesis of DNA and RNA which are the control mechanism for the synthesis of cell proteins, including enzymes, has not been fully investigated. A new synthetic drug, namely, piperazine diantimony tartrate (bilharcid) has recently been produced in the Laboratories of Chemical Industries Development "CID" Cairo, Arab Egyptian Republic with encouraging therapeutic effect. It is thus felt to be of great value to study the effect of this drug on DNA, RNA and protein synthesis. Tartar-emetic was also studied for comparison.

MATERIALS AND METHODS

Viable-cell count vs concentration of bilharcid. The organism used was *Escherichia coli* B, and the medium used was Difco nutrient broth NaCl.† The medium for the agar plates was prepared from the liquid medium by the addition of 15 g of agar to 1 l. of the medium.

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† Purchased from Merck, Germany.

All incubations were carried out in a shaker at room temperature (30°).

About the method employed, an inoculum from an overnight culture was added to nutrient broth–NaCl media, and the content incubated until viable-cell counts had reached almost 1×10^8 cells/ml.

A series of culture flasks, were prepared by the addition of graded increments of bilharcid and these were incubated for a period of 4 hr. Samples for viable cell counts were removed from the control flask at zero time, and from all flasks at the end of each hour of incubation. The viable cell counts were determined by the usual plate-out procedure, the counts being the average of three plates, following a 12–18 hr incubation period at 30°. This experiment was repeated three times using higher concentrations of bilharcid, aiming to reach the bacteriostatic concentration of the drug.

Viable-cell count vs concentration of antimony potassium tartrate (tartar-emetic). This was done by the same procedure used for bilharcid.

Reversal of the bacteriostatic action of bilharcid and tartar-emetic. The reversibility of the inhibition of cell division caused by the bacteriostatic concentration was determined for bilharcid and tartar-emetic. Three flasks were prepared for each exercise by the same procedure as that used above for a determination of viable cell count vs concentration. One flask contained no inhibitor, and the other two contained the bacteriostatic concentration of the drug tested. Viable-cell counts were made as

TABLE 1. INHIBITION OF SYNTHESIS OF MACROMOLECULES IN *E. coli* B BY BILHARCID AND TARTAR-EMETIC

Flask	Time (min)	μg* of macromolecules/ml culture		
		DNA synthesis	RNA synthesis	Protein synthesis
Control	0	0·29	2·86	9·70
	60	0·58	3·81	11·13
	120	0·82	7·62	14·06
	180	2·38	16·07	27·99
	240	3·70	22·18	46·88
Inhibited by bilharcid†	0	0·29	2·86	9·70
	60	0·49	3·33	8·73
	120	0·48	4·08	8·07
	180	0·53	3·83	13·67
	240	0·49	4·00	14·17
Inhibited (W)‡	180§	1·56	12·55	20·70
	240	3·27	16·72	28·65
Inhibited by tartar-emetic†	0	0·31	2·90	10·00
	60	0·38	3·55	10·75
	120	0·38	3·86	10·90
	180	0·39	3·86	12·51
	240	0·38	4·05	13·75
Inhibited (W)‡	180§	1·25	13·10	22·60
	240	2·50	17·25	31·00

* Data given are the average of three successive experiments.

† The concentration of the inhibitor was at its bacteriostatic concentration.

‡ A single, inhibited culture was used for the 240-min test period. At the end of 120 min of incubation, 60 ml was removed from this culture and the inhibitor was removed. The cells were then resuspended in the same volume of new medium. This culture was then designated inhibited (W) for "inhibited, washed".

§ This interval of 180 min is to relate the culture age to the others. The 180-min time indication is only 60 min after the completion of the removal of the inhibitor and the resumption of incubation at the end of 120 min.

before but at the end of the second hour of incubation, the inhibitor was removed from one of the experimental flasks by centrifugation and washing with medium. The cells from this flask were resuspended in the original volume of medium, and incubation was resumed; viable-cell counts were determined for each flask for the next 2 hr. Figures 3 and 4 illustrate the bacteriostatic action of bilharcid and tartar-emetic respectively and the reversibility of this inhibition by removal of the inhibitor.

Synthesis of macromolecules. To study the influence of bilharcid and tartar-emetic on DNA, RNA, and protein synthesis, essentially the same procedure was used as that outlined for the reversibility studies above. Larger volumes of culture were used so that 30-ml samples could be removed from the control flask and the experimental flask at the end of each hourly interval. The cells were collected by centrifugation. DNA was determined by Burton's procedure,[4] calf thymus DNA being used as a standard. RNA was determined by Mejbaum's procedure,[5] yeast RNA being used as a standard. Protein was determined by Lowry's procedure,[6] crystalline bovine albumin being used as a standard. Table 1 summarizes the results.

RESULTS

The bacteriostatic concentration for *E. coli* B was determined for both bilharcid and tartar-emetic (Figs. 1 and 2). It was found that $4{\cdot}244 \times 10^{-6}$ moles/ml and $19{\cdot}510 \times 10^{-6}$ moles/ml are the bacteriostatic concentrations of bilharcid and tartar-emetic respectively. From these results, it is evident that bilharcid is 4·5 times more potent than tartar-emetic.

The reversibility of the bacteriostasis caused by each of the two drugs was shown by the removal of the inhibitor from a culture in which it had been present for 2 hr. Once the inhibitor was removed from the culture, cell division was re-initiated, and

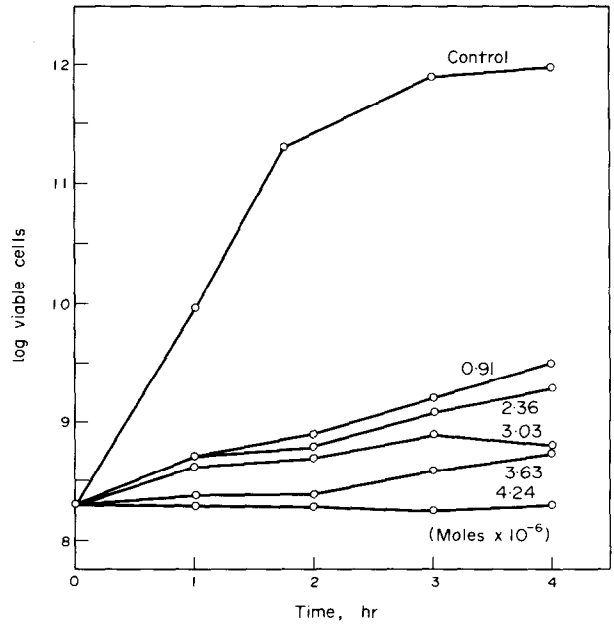

FIG. 1. Viable cell counts vs concentration of bilharcid.

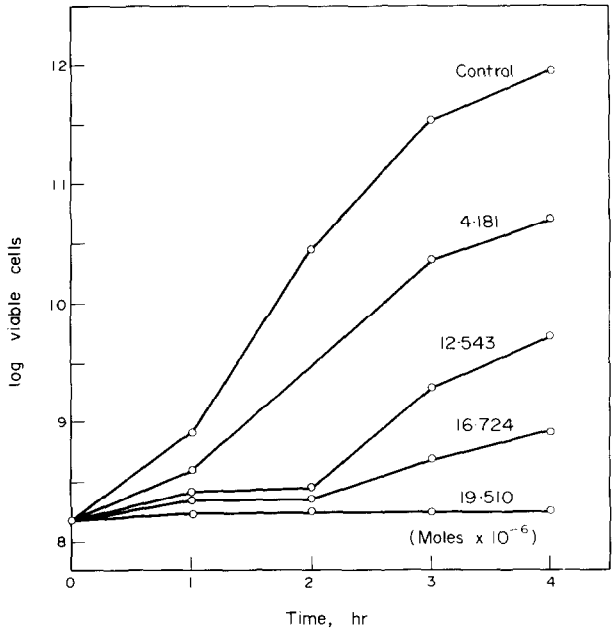

FIG. 2. Viable cell counts vs concentrations of tartar-emetic.

it then proceeded at a rate which paralleled that of the control culture. Figures 3 and 4 illustrate the reversibility of the bacteriostasis produced by bilharcid and tartar-emetic.

The influence of bilharcid and tartar-emetic on the synthesis of DNA, RNA and protein by *E. coli* B were also studied. In the case of each of the above two drugs,

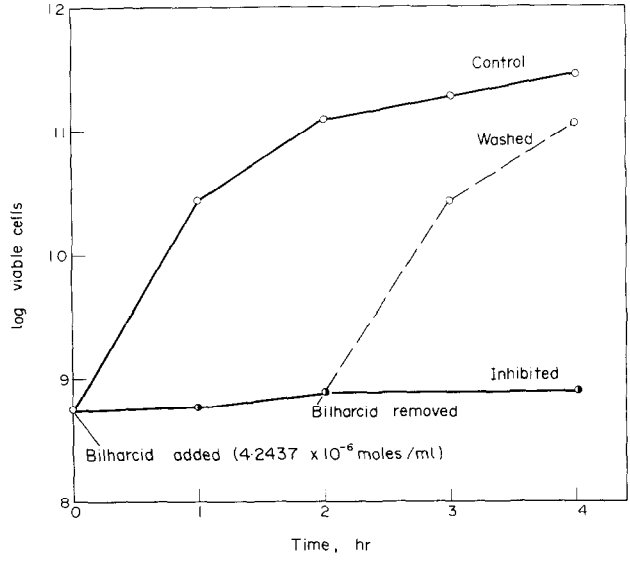

FIG. 3. Reversal of the bacteriostatic action of bilharcid.

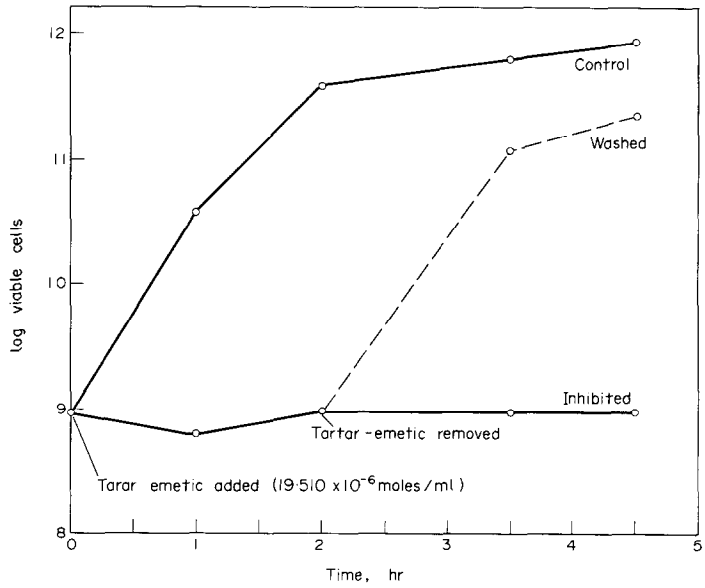

FIG. 4. Reversal of the bacteriostatic action of tartar-emetic.

it was found that, when it was present in a culture at its specific bacteriostatic concentration, it caused almost complete inhibition of the synthesis of DNA and RNA. Protein synthesis was completely inhibited for the first 2 hr, followed by a low rate production for the next 2 hr when compared to the control as indicated in Table 1. When the inhibitor was removed, these biosynthetic processes were resumed and maintained rates parallel to those of control, uninhibited cultures.

DISCUSSION

From our results it is evident that bilharcid is 4·5 times more potent than tartar-emetic with respect to its effect on cell division in *E. coli* B. The mechanism of the inhibition by bilharcid and tartar-emetic was not limited to the cessation of DNA synthesis, but RNA and protein synthesis were also inhibited. When the inhibitor was removed these biosynthetic processes were resumed and maintained rates parallel to those of the control, uninhibited culture, again indicating the reversibility nature of the effect of these two drugs. The bacteriostatic activity of these drugs for *E. coli* B certainly cannot be attributed to a specific inhibition of DNA synthesis.

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