Compounds possessing the ethylenimine ring are potential bearers of antitumor properties. Thus, the synthesis of these preparations has attracted increasing attention from investigators. In the last few years, a rather large number of preparations have been obtained containing ethylenimine groupings, of which several have been taken as official, antitumor, therapeutic agents (TEF, ThioTEF, DIPIN, et al.).

Obtaining different derivatives of ethylenimine is accompanied by a number of experimental difficulties, involving the exceptional lability of the three-membered ethylenimine ring. Direct alkylation or acylation of ethylenimine, using the usual agents, is often excluded, since in the process of the reaction the ethylenimine ring is broken up. In connection with this, derivatives of ethylenimine are usually obtained by indirect means, through the cyclization reaction of C-substituted β-haloid-ethylamine. Nonetheless, this does not exclude the pathway of direct acylation. There are data in the literature [5] showing that N-acylethylenimine can be obtained by the direct acylation of ethylenimine with ketenes.

Occupying ourselves with a study of the chemistry of carbon suboxide, we obtained [4] N,N1-malonyl-bis-chlor (brom) ethylamine (MEI)—a substance of interest both from the chemical and biological points of view.

Continuing the experiments, we decided to study the reaction of carbon suboxide with ethylenimine. It was shown that gaseous carbon suboxide at -20°C reacts with ethylenimine in a medium of absolute diethyl ether, yielding a quantitative output of MEI according to the schema:

\[
\begin{array}{c}
\text{CH}_2\text{NH} + \text{O} = \text{C} = \text{C} = \text{O} + \text{HN} \\
\text{CH}_2
\end{array}
\rightarrow
\begin{array}{c}
\text{CH}_2\text{N} - \text{C} - \text{CH}_2 - \text{C} - \text{N} \\
\text{CH}
\end{array}
\]

The obtained compound was identified with the aid of chemical and spectral analysis. The infrared spectrum of the material confirmed the presence of the ethylenimine ring (deformation waves of the ring according to our data: 1210 cm\(^{-1}\) and 857 cm\(^{-1}\); according to the data in the literature for ethylenimine [8]: 1218 cm\(^{-1}\) and 852 cm\(^{-1}\)). All this made it possible to attribute to the synthesized compound the molecular structure of MEI. It should be noted that our proposed method of direct malonation of ethylenimine with the help of carbon suboxide is, at present, the only possible technique, and appears to be prospective, since it is possible, following the indicated schema, to malonate other C-substituted ethylenimines. Information on the obtaining and investigation of the latter will be presented in special reports.

**EXPERIMENTAL METHOD AND RESULTS**

Synthesis of MEI. Gaseous carbon suboxide was obtained by the method which we developed [3,4] and was taken directly from the pyrolysis oven and placed in the reaction mixture. Ethylenimine was obtained according to the technique described in the literature, from the chlorhydrate of β-chlorethylamine. For the reaction we used freshly distilled ethylenimine, with a boiling temperature of 56°C. After chilling to minus 20—minus 15°C, a solution of 4 grams of ethylenimine in 11 ml of absolute diethyl ether was passed through a small surplus of dry carbon suboxide. After a certain period of time, fine white crystals began to settle out. Following conclusion of the reaction, the ether was evaporated in a vacuum and the material was washed several times in absolute ether. The melting point was 42—44°C (out of the ether), and the output quantitative.
The material presented as white, acicular or lamellar crystals, soluble in chloroform, dichlorethane, acetone, and less soluble in ethanol, ether and water.

Real (in %): H 6.68; C 54.39; 18.11
Calculated (in %): H 6.54; C 54.53; 18.17

Biological Activity. The biological activity of MEI was studied on different biological subjects. For preliminary appraisal of the antitumor properties of the preparation, we used tissue cultures of tumor and normal cells and a bacteriophage model.

In test tubes containing earlier grown cells from a tissue culture of uterine fibrocarcinoma (strain HeLa) and from a single-layer trypsinized culture of tissue from human embryo, we added various concentrations of MEI.

Dilutions of the preparation were prepared in a medium consisting of Henk's solution plus antibiotics (90%) and aminopeptide (10%).

MEI in a concentration of 60 micrograms/ml and higher caused death of the HeLa cells, which peeled from the walls of the test tube on the following day after addition of the preparation. With use of smaller doses (15–30 micrograms/ml) we observed formation of large cells, with clearly contoured nuclei, which died by the 2nd–3rd day. With still lower concentrations of the preparation (5–10 micrograms/ml) the cells retained their viability, but were significantly larger than in the control. The same changes took place with the action of the preparation on the cells of the tissue culture of human embryo.

The antiphage activity of MEI was studied on bacteriophages previously selected by us as test subjects for a comparative appraisal of derivatives of di-3-chlorethylamine [1]. They were the coli-dysentery phages of the T-system (T1, T2, T3, T6, and T7) and the coli-phages 026 and 0111, lysing enteropathogenic intestinal bacilli of the corresponding serotypes.

In a test tube containing a determined concentration of the preparation in 0.9 ml of physiological saline, we added 10⁷ particles of phage, contained in a volume of 0.1 ml. After a 15 minute exposure we determined the number of active phage particles by the method of agar layers, according to Gratia [7]. In the control, instead of a solution of the preparation we used the same volume of physiological saline, into which we also placed 0.1 ml of phage. The percent activity of the phage was calculated by means of comparing the number of phage colonies in the control (taken as 100%) with the number of colonies obtained after treatment of the phage with the preparation.

### TABLE 1. Antiphage Activity of MEI

<table>
<thead>
<tr>
<th>Concentration of MEI (µg/ml)</th>
<th>T1</th>
<th>T3</th>
<th>T7</th>
<th>026</th>
<th>0111</th>
<th>T2</th>
<th>T6r</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.0007</td>
<td>0.0004</td>
<td>23</td>
<td>0.9</td>
<td>0.003</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.002</td>
<td>0.0007</td>
<td>23</td>
<td>2</td>
<td>0.07</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.03</td>
<td>0.009</td>
<td>25</td>
<td>31</td>
<td>0.009</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>0.09</td>
<td>0.06</td>
<td>32</td>
<td>42</td>
<td>0.1</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 shows that MEI is an inhibitor of even and odd numbered phages of the T-system. Bacteriophages which contain cytosine (T1, T3, and T7) and 5-oxymethylcytosine (T2 and T6) in the composition of their DNA were observed to be highly sensitive to MEI. It should be noted the Embichin 7, in these same concentrations, showed almost no inhibitory action on the phages containing the unusual nitrogenous bases—5-oxymethylcytosine [1].

T3 was comparatively the most resistant of the T-phages, the composition of its DNA differing from the other T-phages in the relationship of the nucleic bases. It is known that for phages T2, T4 and T6 the ratio:

\[
\frac{\text{adenine + thymine}}{\text{guanine + cytosine}}
\]

is equal to 1.79–1.93, for phage T1=1.37, and for phage T3=1 [2]. In connection with this, it is probable that the equal index of the relationship of nucleic bases in phage T3 and its host gives the former a higher resistance than the other phages against MEI, which, like other ethylenimine derivatives, possesses DNA-tropic activity.