

# A NEW SYNTHETIC MEDIUM FOR DIPLOCOCCUS PNEUMONIAE,

## TRANSFORMATIONS AT THE *amiA* LOCUS<sup>1</sup>

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THE investigation of genetic fine structure has progressed rapidly during the

all mutants were derived. This strain is descended from AVERY's strain R36A (AVERY, MACLEOD

filtration. The filtrate is completed to a volume of 200 ml and sterilized by passage through a membrane filter. This solution is stable for several months. Occasionally a lot number of Difco



crystalline) is added in the place of linoleic acid and spermidine, growth is more rapid, the generation time falling to 36 minutes.

In order to have good growth, L-valine, L-leucine and L-isoleucine must be present in approxi-

strain is transferred to Clone 3, the resulting resistant cells do grow in synthetic

was added at a concentration of 0.02 percent. Cells remain viable for hours in this diluent. Small volumes (0.1 to 0.4 ml) of the appropriate dilution were plated in

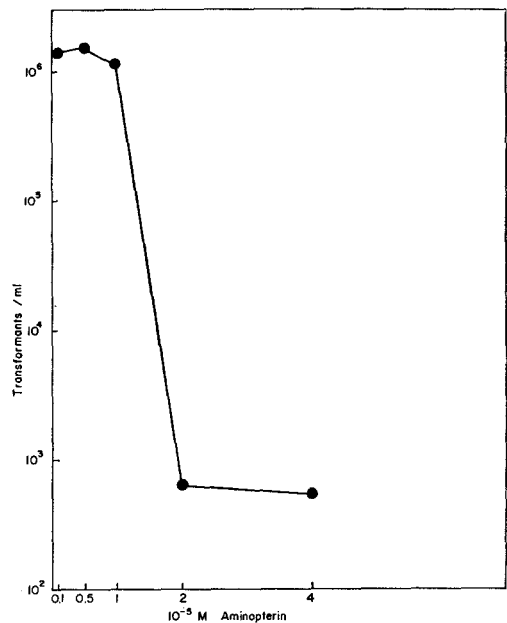


FIGURE 1.—Resistance levels of transformants appearing in cross of Clone 3 by DNA of strain



*Genetic analysis of the remaining aminopterin-resistant strains:* When Clone 3 is transformed by DNA from any one of the remaining aminopterin-resistant strains, a single type of resistant transformant is produced: resistant to  $1 \times 10^{-5}$  M aminopterin, and sensitive to an imbalance of branched amino acids.

thus be confident that all of the wild-type transformants are recovered by the agar overlayer selection technique. Figure 2 presents data of an experiment on

cine means either the *amiA-s* gene is very rapidly expressed, or isoleucine is a

plex recombination behavior. The utility of such crosses (one-point reciprocal

other hand, how histidine and lysine can exert a sparing effect on each other is

TABLE 3

*Summary of results of various crosses designed to test for complex recombination*

Transformation number	Recipient	Donor	<i>amiA-s</i> or <i>r</i>
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*amiA-r*

crossing. A second gene, *amiB*, conferring low-level resistance to aminonpterin.