

similar type of exchange reaction with sulphhydryl groups essential for phosphorylation<sup>9</sup>.

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### A Factor in Agar which reverses the Antibacterial Activity of 1-Methyl-3-nitro-1-nitrosoguanidine

WHILE working with 1-methyl-3-nitro-1-nitrosoguanidine, an anticancer agent in mice<sup>1,2</sup> and an antibacterial agent *in vitro*, we observed that Difco agar contained a factor which inhibited the activity of 1-methyl-3-nitro-1-nitrosoguanidine against *Escherichia coli* strain S. The activity of this compound was tested by the filter paper disk-assay method on minimal<sup>3</sup> medium pH 6.8, containing 2 per cent agar. The zones of inhibition were smaller when the medium was sterilized by steaming for 20 min. than when sterilized by autoclaving (15 lb./sq. in.) for the same length of time (Table 1). Several lots of Difco agar had the same property, but other commercially available agars did not. These latter agars are: *BBL* (Baltimore Biological Laboratory), *NBC* (Nutritional Biochemical Co.), 'Ion Agar No. 2' (Oxo, Ltd., London), and Difco 'Noble Agar'.

Difco agar was freed of the factor by extraction with water or methyl alcohol, but not with ethyl alcohol, acetone, or butanol. The aqueous or methanol extracts (vacuum dried and reconstituted in water) reversed the effect of 1-methyl-3-nitro-1-nitrosoguanidine when added to liquid cultures and assayed

turbidimetrically. Aqueous extracts lost all activity within 24 hr. at room temperature, but were stable for at least four weeks at  $-10^{\circ}\text{C}$ .

The activity of 1-methyl-3-nitro-1-nitrosoguanidine is reversed by amino-acids, —SH compounds, methylamine and ascorbic acid (personal observation). The active extracts were ninhydrin-negative. Polarographic analysis revealed no —SH or S—S compounds. There was no detectable amount of ascorbic acid. The factor did not absorb ultra-violet light.

Compounds with biological properties similar to 1-methyl-3-nitro-1-nitrosoguanidine were tested for their response to the agar factor. The antibacterial activity of 11 nitrosoguanidines, differing only in the alkyl substituent on N-1, was reversed. Only 1-chloroethyl-3-nitro-1-nitrosoguanidine was not reversed even though the analogous compounds, 1-ethyl and 1-chloropropyl-3-nitro-1-nitrosoguanidines, were reversed. The activity of 1-nitroso-2-nitramino-2-imidazoline, a condensed nitrosoguanidine, and of 5-diazouracil was reversed, but that of nitrogen mustard, azaserine, 'Mitomycin C',  $\beta$ -propiolactone, 4-nitroquinoline-N-oxide was not. The agar factor had no effect on the antibacterial activity of penicillin, streptomycin, or aureomycin.

There is in Difco agar a water- and methanol-soluble, heat-unstable substance which reverses the antibacterial activity of the anticancer agents, 1-alkyl-3-nitro-1-nitrosoguanidines and 5-diazouracil. The isolation and identification of this factor might be helpful in elucidating the mechanism of action of these compounds.

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### A New Approach to the Study of Thiamine Deficiency

IT is well known that dietary deficiency of thiamine impairs normal carbohydrate metabolism because of the role of cocarboxylase as coenzyme in the oxidative decarboxylation of pyruvate and  $\alpha$ -ketoglutarate<sup>1</sup>, and the transaldolation and transketolation<sup>2</sup> of aldoses and ketoses, which inhibit the liberation of the energy necessary to maintain tissue in its living state. Also, it has been shown that fat has a sparing action on the thiamine<sup>3</sup>; but little is known concerning protein metabolism in this form of avitaminosis.

Re-investigation<sup>4</sup> of this problem has shown that glyoxylic acid, a metabolic intermediate, arises from excessive breakdown of tissue protein; by radioactive isotopic tracer study, the origin of this acid was proved to be glycine. The presence of glyoxylic acid in normal animals is transient, since it can be reconverted into glycine or further metabolized into formate and then into carbon dioxide and water, or it can be excreted in the urine.

Table 1. EFFECT OF AGAR ON THE ACTIVITY OF 1-METHYL-3-NITRO-1-NITROSOGUANIDINE AGAINST *Escherichia coli*

Agar	Preparation	Diameter of zone of inhibition (mm.) <sup>a</sup>		
		40 $\mu\text{gm.}/\text{disk}$	100 $\mu\text{gm.}/\text{disk}$	200 $\mu\text{gm.}/\text{disk}$
<i>NBC</i> †	Steamed	27	33	36
<i>NBC</i> †	Autoclaved	28	34	39
<i>BBL</i> ‡	Steamed	25	31	35
<i>BBL</i> ‡	Autoclaved	26	33	35
'Difco' Lot No. 616	Steamed	12	15	16
'Difco' Lot No. 616	Autoclaved	26	30	38
'Difco' Lot No. 188	Steamed	12	14	16
'Difco' Lot No. 188	Autoclaved	26	33	36

<sup>a</sup> 12-mm. filter paper disk. Incubation, 18 hr.;  $37^{\circ}\text{C}$ .

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