INTRODUCTION

Recent findings suggest that chemical carcinogens may be formed in certain diseases of the human cervix (1). According to Harington et al. (2), dimethylnitrosamine (DMNA) might be present in the vaginal fluid from cervical and vaginal discharge. The report of these authors, based on the examination of vaginal fluid from a hundred African women, supports the idea that there is a link between the presence of DMNA in the vagina and the development of cancer of the cervix. Although it is generally agreed that DMNA's carcinogenic activity depends on prior conversion to the corresponding diazoalkane (3), a direct transformation of cells exposed to appropriate doses of DMNA has been found in one "in vitro" study (4). DMNA in the vagina could result from synthesis by microbes. It is possible in fact, that a large number of nitrate-reducing microorganisms, including Trichomonas vaginalis, can contribute at least partially to the production of DMNA from secondary amines and nitrates under neutral conditions (5, 6). The pH and temperature found in infected vaginas are thought to be conducive to nitrosamine formation from nitrates present in the urine and dimethylamine (DMA) formed from lecithin present in vaginal epithelium.

Since it has been epidemiologically demonstrated that there is increased risk of cancer of the cervix with Trichomonas vaginalis infection (7, 8), we have examined under conditions as near as possible to those "in vivo", the possibility of a correlation between the production of DMNA and the presence of this Protozoa.
Trichomonas vaginalis was grown for 24 and 36 hours in a modified Diamond's medium (9), with or without sodium nitrate and DMA added. An aliquot of medium without Trichomonas was incubated for the same time, then tested for sterility. The samples were filtered through millipore filters at 2°C to separate medium from cells.

To detect the possible production of DMNA by Trichomonas vaginalis in modified Diamond's culture medium it was necessary to establish a method with a good recovery of very low quantities of DMNA. Known amounts of DMNA (4-8 ng/ml) were added to the medium for this purpose.

DMNA was distilled from the samples at an alkaline pH, then extracted from the distillate with dichloromethane. Interfering amines were removed from the dichloromethane extract by acid washing with a glycine-HCl acid buffer. A special apparatus for concentration of the organic extract was used in order to get a good percentage recovery. DMNA was looked for by gas liquid chromatography (GLC), using a flame ionization detector (10, 11), but the peak attributed to DMNA was poorly resolved. Therefore gas chromatography - mass spectrometry (GC-MS), operating in the single ion monitoring (SIM) mode, was applied (12, 13).

MATERIALS AND METHODS

Reagents. Solvents and reagents were of appropriate purity. 14C-labelled products (DMNA and DMA) were supplied by SORIN, Saluggia Italy.

Sample preparation. To 250 ml of culture medium, filtered through a millipore filter (type XM 10-A, Hamicon Corp.) 30 g of sodium hydroxide were added and the mixture distilled in a glass apparatus, collecting about 150 ml of distillate. Fifteen g of potassium carbonate were dissolved in the distillate and the solution extracted twice with 150 ml portions of dichloromethane (extraction time 10 min). The combined extracts were shaken with 100 ml of glycine-HCl acid buffer, pH 2.1 ± 0.1 and the aqueous layer discarded (14). The dichloromethane layer was washed with 100 ml of 20% aqueous potassium carbonate solution and finally the organic layer was dried over anhydrous sodium sulphate. Half of the anhydrous extract was transferred into a 300 ml pear-shaped flask graduated from the bottom to indicate 15 ml and the chromatographic column, prepared as follows, was fitted. A glass column, 3 cm in diameter and 30-40 cm long, with a teflon stopcock, was used. A 10-15 cm layer of basic alumina (Aluminium oxide basic, activity grade 1 for chromatography) was poured into the column and a glass wool plug was inserted. The plug prevents the loss of alumina during evaporation. The column was prepared just before use.

Vacuum was applied to the column and the flask maintained at 25°C