PURIFICATION OF TRITIATED GENTAMICIN.

Received on April 3, 1973.

SUMMARY

A simple, high capacity method for the purification of ^3H-gentamicin is described. Confirmation of radiochemical purity using several thin layer chromatography systems is presented. Based on the weight of biologically active material, the specific activity is 1.02 Ci/mM.

Introduction:

Gentamicin is a widely used aminocyclitol aminoglycoside antibiotic (1). The commercially available form consists of about equal proportions of the three members of the gentamicin C complex (2), all of which have similar antibiotic activity (3).

The need for radiochemically pure gentamicin of high specific activity arose from our desire to study its distribution and excretion in humans. ^{14}C-Gentamicin has been used in one in vitro study (4). However, the specific activity reported in that study was too low for our purposes. In the present investigation 100 mg of pure gentamicin sulfate** was submitted to a labelling service(Amersham-Searle, 200 Nuclear Drive, Des Plaines, Ill. 60018). Following a catalytic tritium exchange process, the material was returned to us containing 160 mCi in 25 ml of water. Unfortunately, most of the paper presents the methodology used to purify the ^3H-gentamicin from the material returned to us from Amersham/Searle.

Materials:

Three types of thin layer chromatography plates were used: 1) cellulose 0.1 mm thick, 2) silica gel G 0.25 mm thick, and 3) Kieselguhr 0.25 mm thick. The thin layer chromatography solvent systems were #1, lower phase of chloroform: methanol: 17% aqueous ammonia (2:1:1, v/v); #2, n-butanol:glacial acetic acid:water (75:7.5: 21, v/v). To locate gentamicin on the thin layer chromatography plates a spray reagent composed of 125 mg ninhydrin in 25 ml of acetone and 25 ml of n-butanol was prepared. Gel filtration was performed using a 225 cm X 15 cm column of Sephadex G-10.

Tritium was counted using Spectrofluor Butyl-PBD (Amersham/Searle), prepared to a concentration of 7 g/liter of toluene, as the counting solution (5). A Mark II scintillation spectrometer was used throughout the study.

Methods and Results:

The extent of the radiochemical impurities can be seen from Figure 1. Two ul

of the tritiated solution and two µl of cold gentamicin solution (80 mg/ml) were spotted 3 cm apart on cellulose thin layer chromatography plates and developed in solvent system 1. The cold gentamicin was stained with ninhydrin and developed with gentle heating to dark blue spots. The part of the plate containing radioactive material was divided into 1 cm sections, scraped into liquid scintillation counting vials and mixed with 5 ml of methanol plus 15 ml of the Butyl-PBD solution prior to countin. From this technique it is obvious that the major portion of the radioactivity did not correspond to gentamicin.

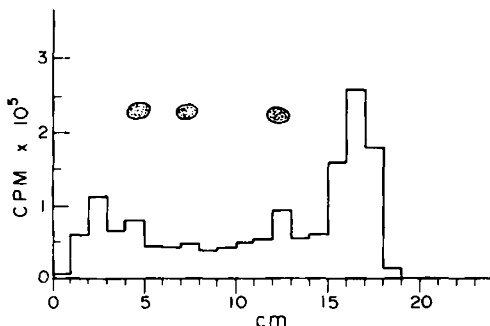
The first stage in purification of the ^{3}H-gentamicin was performed by passing 2 ml of the radioactive solution slowly through the Sephadex column and eluting with 0.02% sodium chloride in water. One ml fractions were collected at a flow rate of 0.5 ml/minute. Twenty µl aliquots of each fraction were counted in 15 mls of the Butyl-PBD solution. Two radioactive peaks were eluted from the Sephadex (Figure 2). The first peak, in fractions 8, 9 and 10, contained the ^{3}H-gentamicin documented by biologic activity. These fractions were lyophilized, redissolved in 1 ml of water and passed through a fresh Sephadex column, run in the manner described before. Again, two peaks were eluted from the Sephadex column (Figure 3). The fractions containing the first peak were again lyophilized and redissolved in 1 ml water. The radiochemical purity of the first peak was established using the two thin layer chromatography solvent systems and the three solid supports (Figure 4). The material contained in the second peak contained negligible biologic activity.

The gentamicin activity of the purified product was analyzed by a well-plate assay (6) as 1000 µg/ml. Based on the weight of the microbiologically active material, the specific activity was calculated as 1.02 Ci/mM.

Sterilization with millipore filtration, dilution with water for injection and storage of the diluted solution for injection did not effect the radiochemical purity. The ^{3}H-gentamicin thus prepared has been administered to humans.

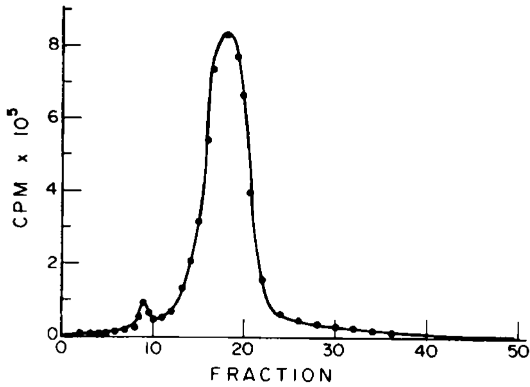
Legend for Figure 1:

Radiochromatogram of crude tritiated gentamicin. The dark spots represent the three gentamicin C components chromatographed on the same plate and stained with ninhydrin. TLC system: 0.1 mm cellulose, solvent system #1.



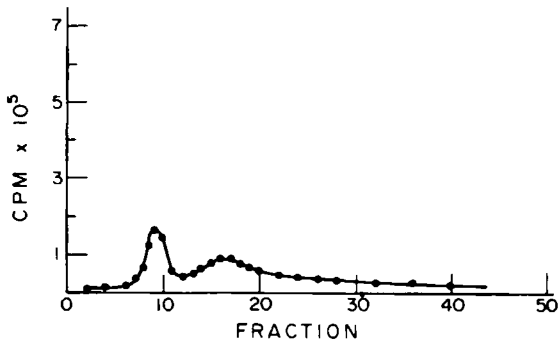
Legend for Figure 2:

Elution pattern of crude tritiated gentamicin from the fi:st Sephadex column.



Legend for Figure 3:

Elution pattern from second Sephadex column.



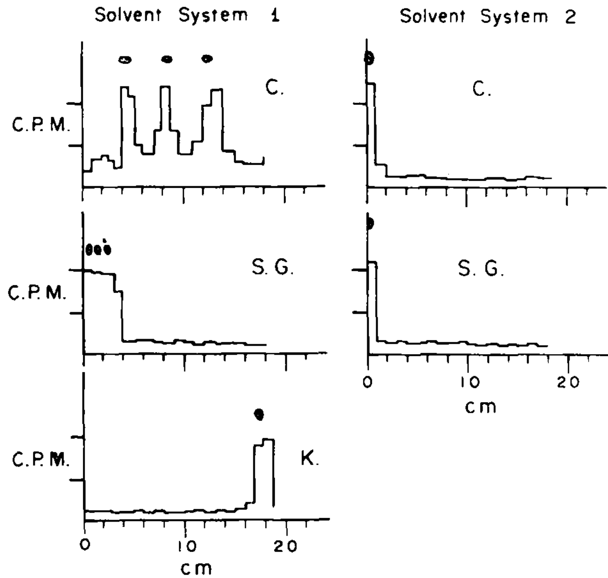
Legend for Figure 4:

Radiochromatograms of purified tritiated gentamicin. Dark spots represent gentamicin C or its components.

Solvent system 1: Chloroform methanol 17 minimum hydroxide 2:1:1 (v/v) - lower phase

Solvent system 2: n-butanol:glacial acetic acid:water 75.7.5:21 (v/v)

C: 0.1 mm cellulose
S.G.: 0.25 mm silica gel
K: 0.25 mm kieselguhr



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+ Dr. Wilson is the recipient of an MRC Canada Fellowship. This work was supported by a grant from Schering Corporation (Canada)

** A generous gift of Dr. J.F. MacDonald, Schering (Canada)

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