

A COMPARATIVE STUDY OF THE CONVERSION  
OF ADENOSINE DEAMINASE C TO A

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PURPOSE:

This experiment was designed to fulfill two functions. A conversion factor would be reacted with C form adenosine deaminase (to be further referred to as AD-C) obtained from various sources. An attempt would also be made to isolate the conversion factor (referred to as C.F.) from a cancerous tissue.

Nishihara, et.al., isolated the protein C.F. which catalyzes the reaction  $2 \text{ AD-C} + \text{ C.F.} = \text{ AD-A}$  (A form).<sup>1</sup> This reaction is the basis for this study.

METHODS AND PROCEDURES:

Isolation of C.F. and AD-C

50 grams of human liver was homogenized with 250 ml double distilled water in an ice bath. The sample was then centrifuged at 39000 g's for 40 min. Supernatant was saved and a 40% solution of ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  was reached by adding while stirring and refrigerating (0-4°C). The salt solution was allowed to stir for a minimum of 24 hrs. It was then centrifuged again. Supernatant was salted to 60% solution, allowed to stir, centrifuged, supernatant again salted (to 80%), allowed to stir, and centrifuged. The resulting precipitate was then dissolved in a minimum amount phosphate buffer (0.05 M, pH 7). The sample was then frozen for storage.

A sample of cancerous liver was prepared in the same manner.

Samples of human stomach, rabbit intestine, and rat intestine were chosen for sources of AD-C due to the GI tract's general high activity for this enzyme. Sample treatment was

similar to that described above. The salt percentages were slightly altered. The rabbit sample was the result of a 45-55-65% cut, the 55-65% cut being the AD-C source. The rat source was a 45-65% cut. Human stomach was a 40-60% cut, but the sample had to be further processed due to a presence of AD-A. Several 50 ul portions were passed through a Bio-Gel column. Fractions collected showing AD-C activity were pooled, ultrafiltrated (40 psi, 150 ml to appr. 5 ml), and stored. A commercial grade calf AD-C, obtained from Sigma Chem. Co., was also used.

#### Column Preparation

Bio-Gel A (pre-swelled) for a 50 x 0.9 cm column was supplied by Bio-Rad Laboratories. Sephadex 200 G for a 30 x 0.6 cm column was obtained from Pharmacia. Both columns were equilibrated with 0.05 M phosphate buffer for a minimum of 24 hrs. The columns were calibrated with Blue Dextran and Cytochrome C protein markers. The Bio-Gel column showed peak Dextran absorbance, as well as peak AD-A activity at fraction 22. AD-C and Cytochrome C peaked at fractions 34 and 36 respectively. In general, AD-C fractions collected were between 31-48. The sephadex column was used for the actual assay plots. Markers peaked as shown (Fig. #1).

#### Assay Method

Activity assays were executed in the manner described by Ma.<sup>3</sup> Instruments involved were a Gilford 240 spectrophotometer with accompanying 6050 chart recorder. All readings were made at 265 nm with a chart speed of 30 in/hr and a temperature of 40°C.

#### Reaction Conditions

Reaction of C.F. with the various samples of AD-C was carried out in a test tube-water bath system. Bath temperature

was maintained at 25°C. C.F. and AD-C source were pipetted into the test tube and left in the bath for one (1) hour. Incubations were also carried out with phosphate buffer and each source of AD-C or C.F. as controls.

#### RESULTS:

Numerical values for activity are direct readings from assay chart. Each fraction was allowed to react for three minutes, thus all values equal the change in units optical density over a three minute period. This effectively standardizes measurements for a qualitative comparison.

Normal liver C.F., incubated with buffer, showed little activity over the first eight to ten fractions and none in the final five (Fig. #2). Human stomach AD-C with buffer displayed peak activity at fraction 11 with no AD-A activity present (Fig. #3). Reaction of the C.F. with stomach AD-C yielded an apparently complete conversion of the C form enzyme to the A (Fig. #3). It was this result that confirmed the presence of C.F. in the liver sample.

The C.F. was then reacted with the calf AD-C. An incomplete conversion resulted (Fig. #4). Another incubation was performed, this time with a 2::1 volumetric ratio of C.F. to the AD-C. Conversion was much more complete (Fig. #4).

Incubation of the C.F. with the rabbit AD-C, like the human stomach, showed complete conversion with a 1::1 ratio (Fig. #5).

The rat AD-C was active with very small amounts of sample (10 ul portions). For ease of handling, 40 ul of buffer was incubated with the 10 ul sample. This small amount still displayed high AD-C activity. As a result, a 4::1 ratio of C.F. to AD-C was incubated. The resulting assay showed no conversion at all (Fig. #6).

The cancerous liver-buffer incubation yielded a generally

consistent, low activity (Fig. #7). Incubation with human stomach AD-C showed a possible partial conversion, although results may be more confidently explained as a simple additive effect (Fig. #8).

#### DISCUSSION:

There is no doubt that C.F. is present and readily obtainable from tissues other than human lung, the source of Nishihara's C.F.<sup>1</sup> Although in cruder form, the liver C.F. was very active and not limited to reaction with human sources of AD-C.

Due to the lack of conversion of the rat AD-C, however, it must be theorized that the rat intestinal enzyme is a different protein of the same general size and function as that found in the other sources tested. If this is the case, it is possible that one sample source may contain more than one form of AD-C. This would explain the incompleteness of conversion of the calf enzyme (incubation of a 3::1 ratio of C.F. to AD-C yielded results similar to that of the 2::1 incubation, incomplete conversion).

The results of the cancerous liver study indicate an apparent lack of C.F. in such tissues, provided it may be accepted that the incubation curve is simply an additive one, composed of the two samples involved. This is certainly a more appropriate interpretation due to peak placement and separation more closely matching the liver-buffer incubation than other AD-A/AD-C peaks. The lack of C.F. in this cancerous tissue may explain the difference in the ratio of AD-A to AD-C found between normal and cancerous tissues in a previous study.<sup>4</sup>

Several questions are now raised. Is there a second form of C.F.? Can one form of AD-C be converted to the other? Is the lack of C.F. in cancerous tissues a symptom or possible

cause of the cancer? Answers will be obtained through further experimentation.

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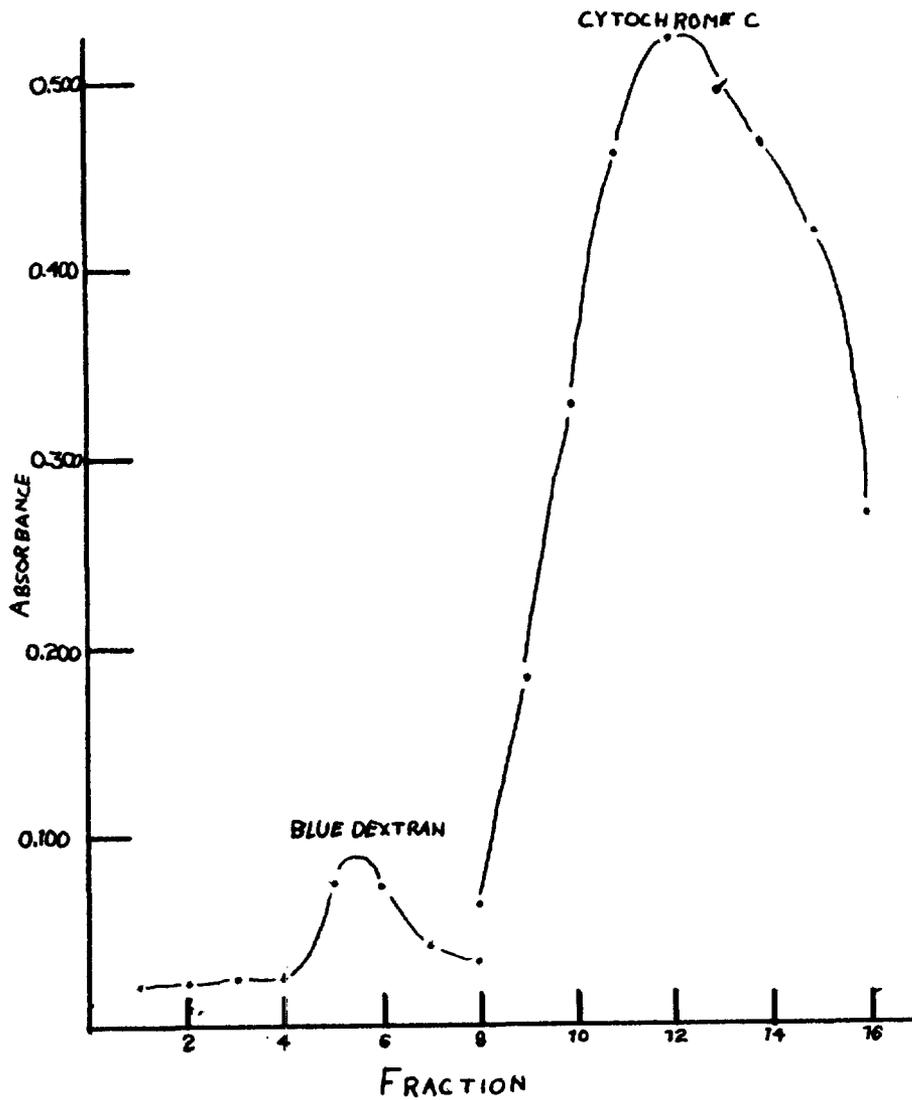


Fig. #1 A mixture of 75 ul of each marker protein was added to the column. Absorbance readings for Blue Dextran were made at a wavelength of 625-nm. Cytochrome C readings were made at 410. Due to placement of the Blue Dextran peak, all subsequent assays began with fraction 4.

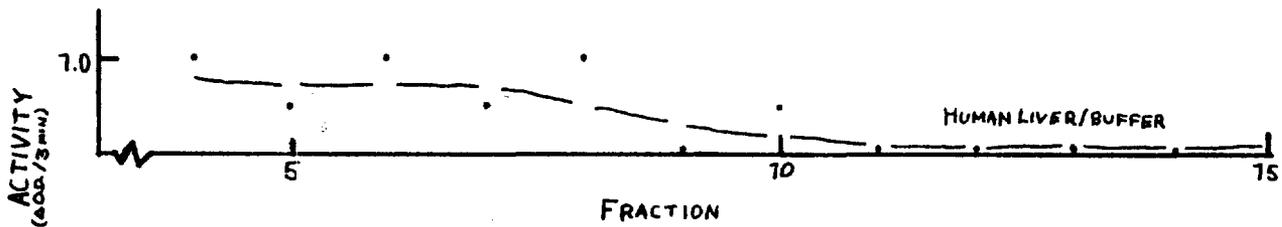


Fig. #2 Incubation of 50 ul of human liver with 50 ul phosphate buffer. This and all following plots were run on a Gilford 240 spectrophotometer and 6050 chart recorder. The following conditions were employed through the study: Wavelength 265 nm, Chart Speed 30 in/hr, Sensitivity Ratio 0.50, 100 ul fraction sample reacted with 3.0 ml Adenosine, Temperature 40°C.

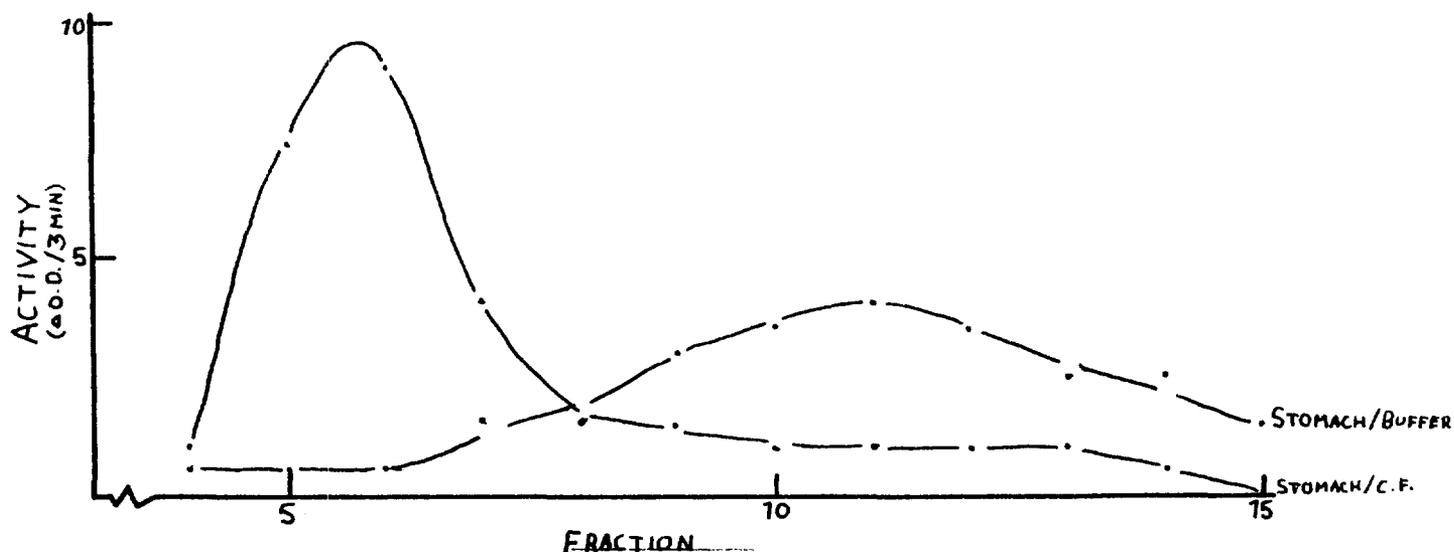


Fig. #3 Incubation of 50 ul human stomach / 50 ul phosphate buffer and 50 ul stomach / 50 ul human liver.(C.F.).

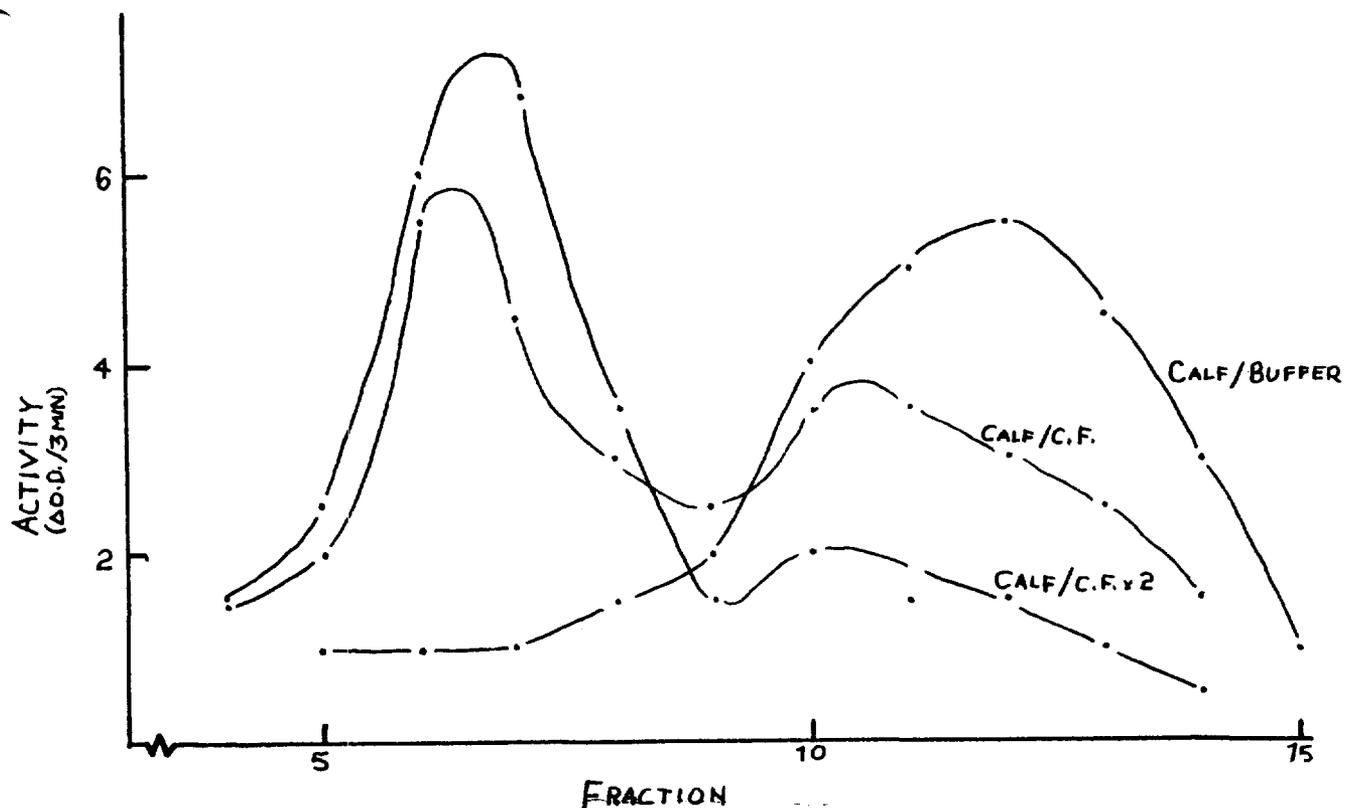


Fig. #4 Incubation of 50 ul calf / 50 ul buffer of which only 75 ul was added to the column (instead of total), 50 ul calf / 50 ul C.F. (75ul added to column), and 50 ul calf / 100 ul C.F. Note the incomplete conversion in both C.F. incubations.

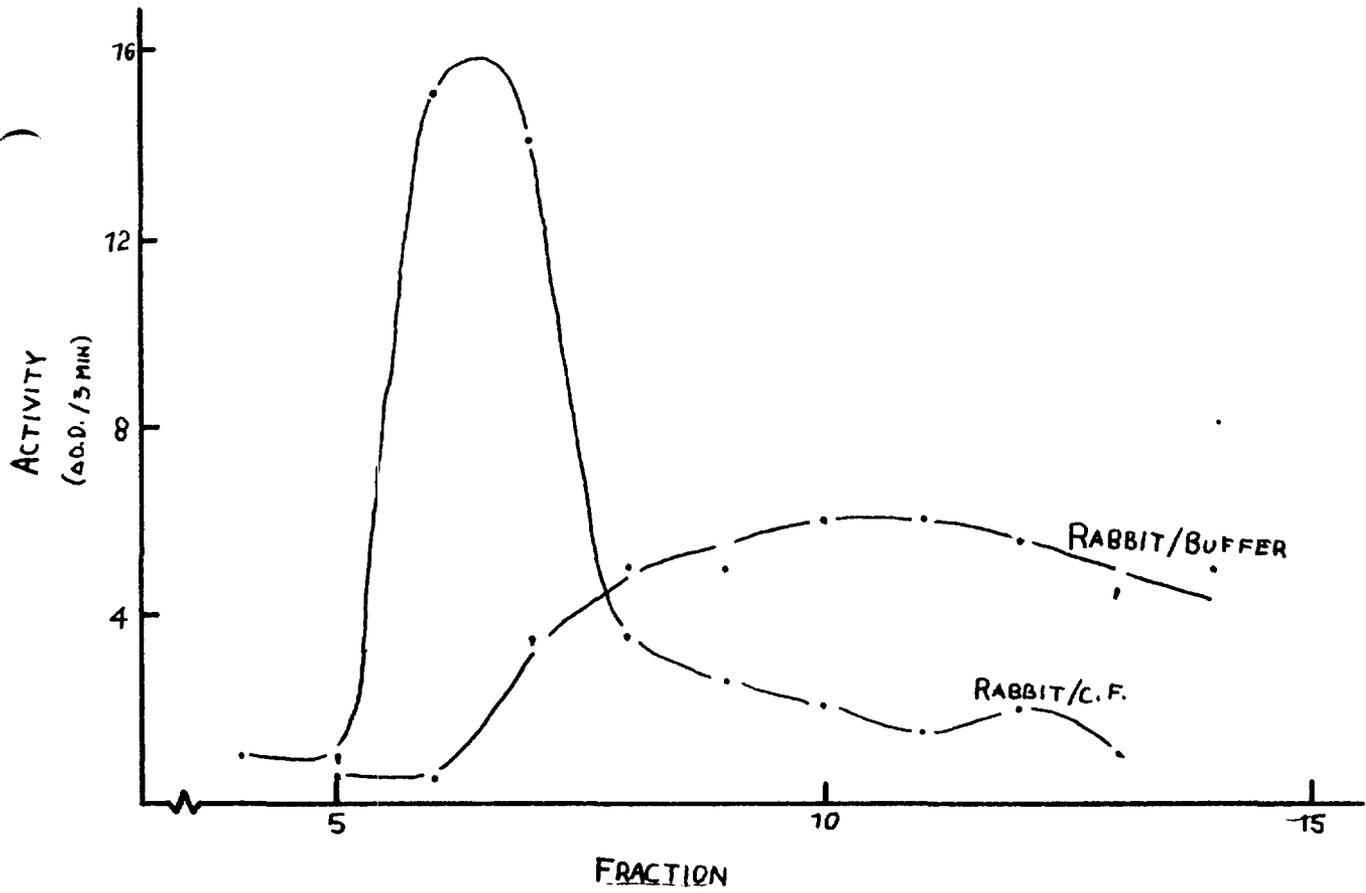


Fig. #5 Incubation of 50 ul rabbit intestine / 50 ul buffer (75 ul added to column) and 50 ul rabbit / 50 ul C.F. (75 ul added to column).

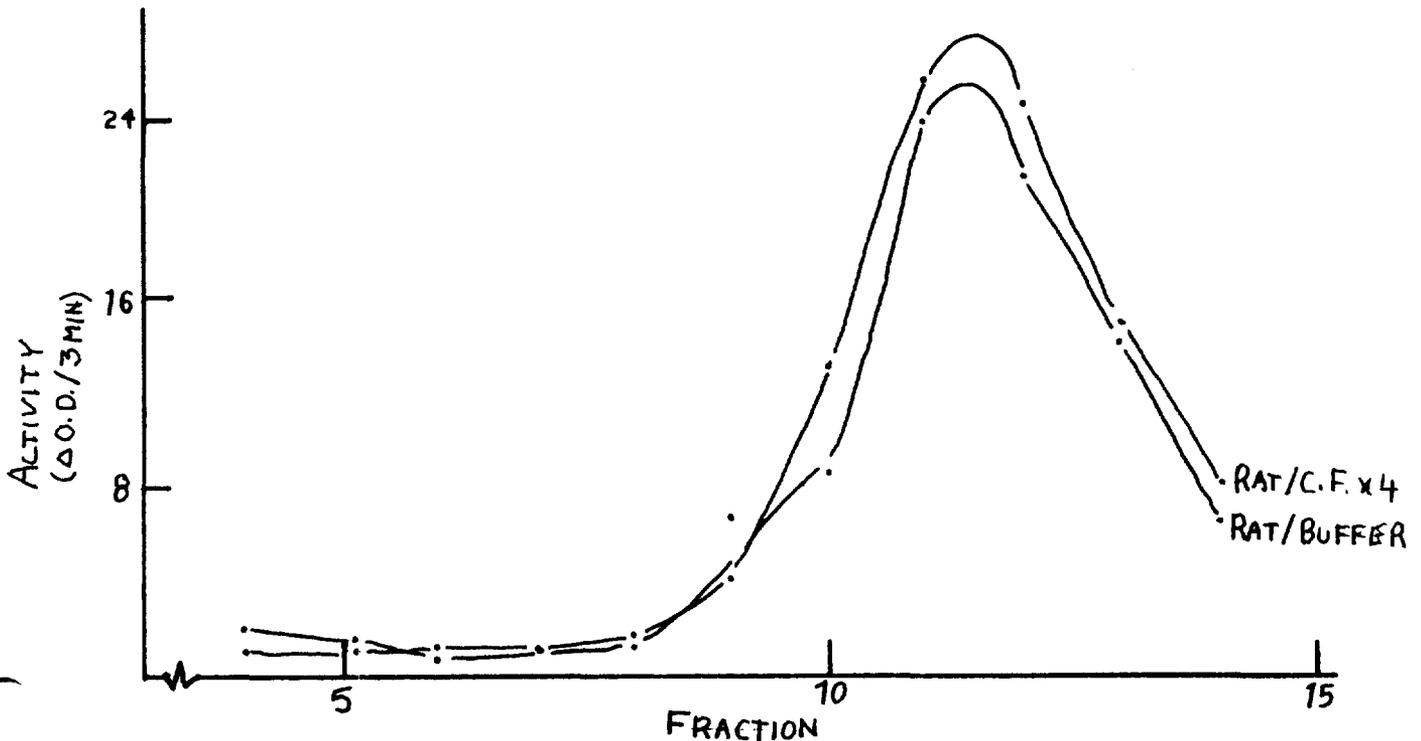


Fig. #6 Incubation of 10 ul rat intestine / 40 ul buffer and 10 ul rat / 40 ul C.F. Note complete absence of conversion.

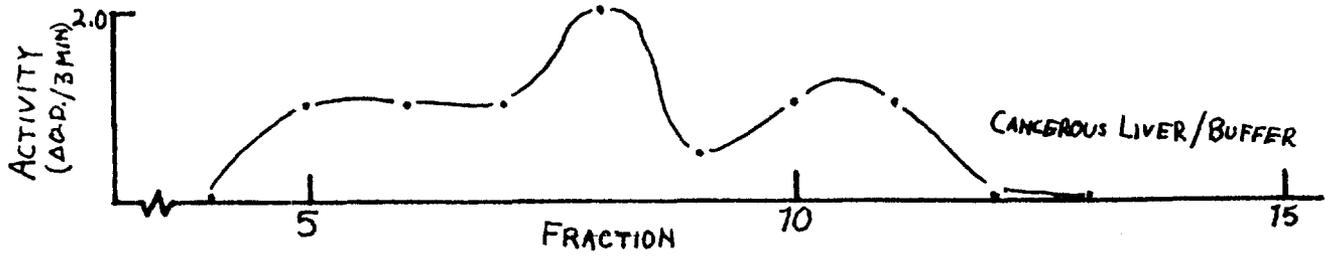


Fig. #7 Incubation of 50 ul cancerous human liver / 50 ul buffer.  
General activity is low.

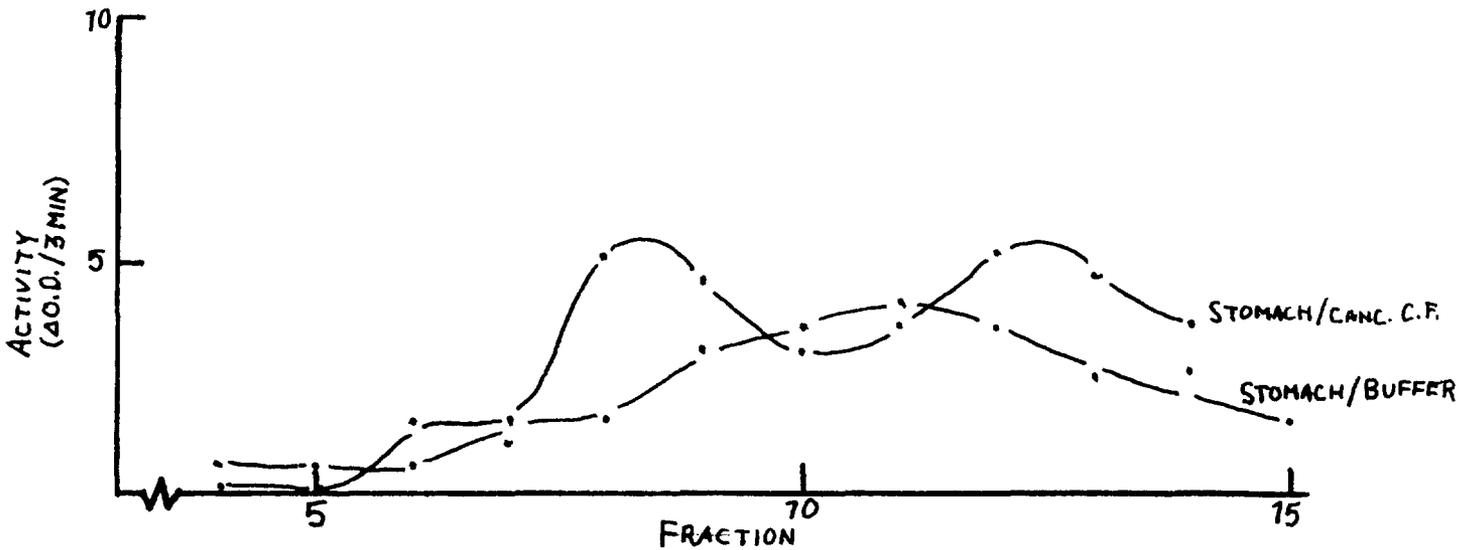


Fig. #8 Incubation of 50 ul human stomach / 50 ul buffer and  
50 ul stomach / 50 ul cancerous liver. Note lack of AD-A  
activity and maintained AD-C activity in stomach / liver plot.