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Activation of 2-Aminoanthracene by Cell-Free Microbial Extracts

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ACTIVATION OF 2-AMINOANTHRACENE

BY CELL-FREE MICROBIAL EXTRACTS

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Date 3/15/84

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ACKNOWLEDGMENTS

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The author also wishes to thank his parents for the tireless support they have shown during the years in achieving this and other goals. Last but not least this author wishes to thank his wife for listening, "even when it didn't make sense."

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ACTIVATION OF 2-AMINOANTHRACENE

BY CELL-FREE MICROBIAL EXTRACTS

Thomas S. Hamill May, 1984 35 pages

Directed by: L.P. Elliott, M.R. Houston and T.P. Coohill

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The activation of the precarcinogen 2-aminoanthracene was investigated employing cell-free extracts of Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis, Bacteroides fragilis, Clostridium perfringens and the eukaryote Candida albicans. A modification of the Salmonella typhimurium/mammalian microsomal mutagenicity assay was employed to determine activation. Of the species tested, only cell-free extracts of Escherichia coli and Bacteroides fragilis failed to induce the reversion of the Salmonella typhimurium his- mutants TA-98 and/or TA-100 to his+ revertants.

Separation of the cytosol and membrane pellet enzymes was completed. Of the organisms tested only the membrane pellet enzymes of Clostridium perfringens showed activation of 2-aminoanthracene.

INTRODUCTION

The original Ames assay (Salmonella/microsome mediated mutagenicity test) was developed to screen large numbers of existing and novel compounds for mutagenicity and carcinogenicity in a technically simple, relatively rapid, and cost effective way (Ames et al. 1975b). McCann et al. (1975b) tested 300 chemicals and demonstrated that the Ames assay system was at least 90% effective in screening chemicals correctly for their mutagenic/carcinogenic properties. The Ames mutagenicity test has been well characterized and widely applied to detect environmental mutagens and carcinogens because of its simplicity, sensitivity, and accurary (McCann et al. 1975a; McCann et al. 1975c; DeSeves 1976; Grant et al. 1976; Ashwood-Smith 1977; Neeman et al. 1980; Sinha and Parulekar 1983). There is concern, however, that genetic tests in microorganisms with liver microsomal enzyme preparations may not be sufficient in all situations to accurately predict the potential genetic activity of a compound for humans (Coohill and Moore, [1983]).

An important aspect of the Ames test is that the system incorporates mammalian microsomal enzymes to activate the precarcinogens to their mutagenic potentials thus indicating that similar activation is possible in vivo as well as the in vitro system used. In early testing with the Ames assay, only mammalian enzymes were used because the presence in

bacterial systems of enzymes necessary for the activation of precarcinogens had not been established (Ames et al. 1975b). With the increasing understanding of the metabolic pathways for the activation of certain chemicals, new questions arose concerning the demand for strictly mammalian tissue enzymes. A specifically pertinent question was whether or not the intestinal microflora could indeed contain sufficient enzymes to activate chemicals consumed in a normal diet.

Precarcinogens have been demonstrated that could be activated by mammalian systems to their mutagenic potential. Activated carcinogens were isolated from the urine of mice, and the urine demonstrated a positive response in the Ames assay (Bos et al. 1980), but work was still proceeding in the area of colon enzyme activation. Tamura et al. (1980) clearly showed that "fecalases" (cell-free extract of human feces) could be used to activate dietary glycosides to mutagens. This finding was a step forward in the search for the etiology of colon cancer. The research suggested that glycosides are activated to a mutagenic form by mixed enzymes from fecal bacteria.

The present thesis investigation was conducted to determine if (1) "fecalases" might be enzymes from microorganisms indigenous to the human colon or from pathogens of the colon and (2) if the enzymes isolated were located in the cytosol or membrane of the particular microorganism that had been shown to be active in causing chemicals to produce mutagenic changes in the Ames test.

MATERIALS AND METHODS

Strains and Their Maintenance

Salmonella typhimurium TA-98 and TA-100 were the two bacterial tester strains used in the Ames assay. *S. typhimurium* TA-98 detects frameshift mutagens and TA-100 detects both base substitution mutagens and frameshift mutagens. The cultures were obtained from Dr. L.K. Nakamura, Agricultural Research Center, Peoria, IL 61604. The two strains were received in the lypholized state and were activated by growing in Brain Heart Infusion broth (Difco Laboratories) at 37°C for 18-24 h. The enteric microorganisms used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* (Western Kentucky University stock culture), *Bacteroides fragilis* (isolated from a human abdominal abcess at Greenview Hospital, Ashley Circle, Bowling Green, KY 42101), *Clostridium perfringens* #2 (Western Kentucky University stock culture), and the pathogenic yeast *Candida albicans* (Western Kentucky University stock culture).

Stock cultures of the above aerobic bacteria and *Candida albicans* were made on Nutrient Agar (Difco) slants and grown for 48 h at 37°C before storing as stocks or using as an inoculum in experiments. *Clostridium perfringens* and *Bacteroides fragilis* were grown in Thioglycollate Broth (Difco) at 37°C for 24-48 h before storing as stocks or using

as an inoculum in experiments. All cultures were stored at refrigeration temperature when not in use.

All above mentioned cultures were transferred to fresh media and incubated at 37°C for 24-48 h monthly to maintain viable culture material.

Bacterial Indicator Strains

The genetic properties and spontaneous mutability of the auxotrophic indicator strains of Salmonella typhimurium were routinely checked as described by Ames et al. (1975).

To obtain stock and working cultures of the two indicator strains, the lypholized culture material was inoculated into BHI broth (24 ml) and incubated for 6 h at 37°C. These cultures were then used to inoculated 75 ml of BHI broth contained in a side-arm flask. These flasks were incubated at 37°C for 12 h in a reciprocal shaker by shaking at 100 oscillations per minute in a New Brunswick Scientific Controlled Environmental Incubator Shaker. From these cultures 5 ml were pipetted into each of three sterile screw-cap test tubes for each strain for storage as stock cultures by pipetting 1.1 ml into 5-ml screw-cap vials, and both stock and working cultures were stored at -70°C.

When required, the working cultures were removed from the freezer, warmed to room temperature and allowed to stand at room temperature for 30 to 60 minutes. At that time, 1.0 ml of the warmed culture was inoculated into 25 ml of BHI broth and subsequently incubated at 37°C for 12 to 24 h in a shaker incubator until dense growth was obtained. The

resulting culture was then used as the broth culture in the assay system explained later in this section.

To prepare additional working cultures, the stock culture was removed from the -70°C freezer, and a few ice crystals were removed using a small sterile Pasteur pipette. These ice crystals were transferred into 25 ml of BHI broth and incubated at 37°C until dense growth occurred. For Salmonella typhmurium TA-98 growth usually occurred within 16-18 h and for Salmonella typhmurium TA-100 the incubation period was usually 24 to 30 h. These cultures were then used as an inoculum for 75 ml of BHI broth in a side-arm culture flask. The culture flasks were then incubated at 37°C for 12 h in a shaker incubator. The resulting dense cultures were pipetted in 1.1 ml aliquots into 5.0 ml screw-cap vials and then frozen as previously mentioned.

Liver Homogenates (S-9)

The liver homogenates were prepared from male BALB/c mice stimulated with AROCLOR 1254 (500 mg/kg intraperitoneal injection five days before sacrifice). The livers were placed in preweighed, chilled, glass beakers containing 0.15 M KCl. After weighing, the livers were minced with sterile iris scissors. Next, 3 ml of cold 0.15 M KCl were added to the beaker for each gram of wet liver. This mixture was homogenized in a manual homogenizer maintained at a temperature of 0-4°C in an ice bath.

After the above had been accomplished, the homogenized liquid was decanted into centrifuge tubes that had been previously cooled at 0-4°C. The material was then centrifuged at 9,000 x g for 10 minutes in a Sorvall Automatic RB-2 refrigerated centrifuge at 0-4°C. The resulting supernatant was carefully decanted into sterile 10 ml screw-cap test tubes in 2 ml aliquots which were then stored at -70°C until utilized. The protein concentration of each extract was determined by the Waddel method as described by Murphy and Kies (1960).

Microbial Homogenates (S-13)

Preparation of the bacterial enzyme solution was initiated by inoculating several test tubes containing 25 ml of BHI broth from the working cultures of Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis, and Candida albicans. These were then incubated overnight (16 to 18 h) at 37°C. The incubated cultures were then used to inoculate 250 ml of BHI Broth in 1-L screw-cap flasks. The ratio of inoculum to broth was 1:10.

The above flasks were incubated in a shaker culture for 12 to 18 h at 37°C. At this time, the cultures were removed from the incubator and cooled at 0-4°C. These cooled cultures were then centrifuged at 5,000 x g for 10 minutes. The supernatant was decanted, and the pellet was re-suspended in 30-50 ml of 0.15 M KCl and recentrifuged under the above conditions. This procedure was repeated to ensure that the cells were free of the growth medium. The wet weight of the cells

was then obtained and 3 ml of the 0.15 M KCl solution were used to re-suspend each gram of cells.

The cells were disrupted by sonification. Sonification was performed with a Sonifier Cell Disrupter model W140D (Heat System - Ultrasonics, Inc.). The cell suspension was sonified at 35 to 45 watts for 4 minutes in a 25 ml capacity sonicating flask suspended in an iced brine bath. The sonicated material was then allowed to cool for five minutes after which the procedure was repeated. After the second disruption of the cells, the material was separated into centrifuge tubes and centrifuged at 13,000 x g for 15 minutes at 0-4°C. The supernatant (S-13 fraction) was decanted and stored at -10°C for 1 h and subsequently stored at -70°C until used for experimentation.

The anaerobic bacteria Bacteroides fragilis and Clostridium perfringens were prepared by inoculating 25 ml BHI broth in test tubes from the stock cultures. These tubes were incubated anaerobically in Gas Pak jars (BBL Microbiology Systems, Cockeyeville, MD) at 37°C for 16-18 h. Just prior to removal of the cultures from the anaerobe jar, several 1-L flasks containing 650 ml of BHI broth were heated to 100°C (boiling) to drive off the dissolved oxygen present in the medium and then rapidly cooled to 25-30°C in an ice bath.

Following this procedure, the cultures were removed from the jars, and 50 ml of the broth culture in the tubes were carefully pipetted to the bottom of the 1-L screw-cap

flasks. These were then tightly sealed and placed in 37°C incubator for 18 to 24 h. The flasks were then carefully moved to a 0-4°C refrigerator to cool for several hours. From this point on the procedure for obtaining the S-13 fraction was identical as described for the aerobic bacterial cultures.

Microbial Homogenates (S-100)

The S-100 homogenates were prepared from the S-13 preparations. The S-13 fluid was decanted into centrifuge tubes and centrifuged at 100,000 x g for 1 h at 0-4°C in a IEC preparative ultracentrifuge. Both the supernatant and the resulting pellet were retained. The supernatant was carefully removed with a pipette; the pellet was re-suspended in an equivalent amount of the 0.15 M KCl solution. Both were stored in the same manner as the S-9 and S-13. The S-100 was distinguished from the re-suspended pellet by referring to the pellet as membrane-bound enzyme mixutre.

Chemicals

AROCLOR 1254 was from Aldrich Chemical Co. It was prepared by transferring 1 g to a rubber stoppered vial containing 5 ml of pure olive oil (Pompeli).

The positive control carcinogen, 2-aminoanthracene (Sigma Chemical Co.), was dissolved in dimethylsulfoxide (Sigma Chemical Co.).

Top Agar

The top agar was prepared by dissolving 5 g of NaCl and 6 g of agar (Difco) in 1 ml of distilled water. Before using, molten top agar at 45°C was quickly supplemented with a sterile solution of 0.5 mM L-histidine HCl - 0.5 mM biotin, the contents mixed, and poured on solid minimal agar with 2% glucose.

The minimal-glucose agar consisted of 1.5% agar (Difco) in Vogel-Bonner Medium E with 2% glucose. When properly tempered, 20 ml of the medium were poured into sterile petri plates (100 x 15 mm, Falcon Plastics).

The plate incorporation assays were performed as described by Ames et al. (1975b) with some modifications. Tubes containing 24 ml of BHI broth were inoculated with the tester strains S. typhimurium TA-98 and TA-100 from refrigerator working cultures. The cells were grown in a shaker incubator for 16-18 h (TA-98) and 24-30 h (TA-100) at 37°C. A 0.1 ml aliquot of bacterial tester strain was added to top agar at 50°C followed by 0.1 ml of 2-aminoanthracene and 0.5 ml of the S-9, S-13 or S-100, depending upon the enzyme system being tested. The contents were mixed and poured on minimal glucose agar plates. Duplicate plates were poured for each experiment, and the experiments were repeated at least two times.

After all plates had been properly prepared, they were incubated for 24 h at 37°C with the time starting at the completion of the last assay plate. At this time a few

representative plates were removed from the incubator and examined to assure a confluent background growth. The plates were then returned to the darkened incubator to be incubated for an additional 24 h after which the revertant colonies were counted. Any plate that had a twofold colony count increase over the negative control plates were considered to be positive, indicating that the test chemical was mutagenic and the enzyme activation system was properly functioning.

All plates were then incubated for an additional 24 h, and the control plates and any negative or borderline negative or positive plates were recounted to assure as much accuracy as possible. Never in the experiments did the negative control show a significant variation between the 48 h and 72 h counts.

RESULTS AND DISCUSSION

Testing of Enzyme Preparations from Microorganisms

The plate incorporation assay was performed as previously described using S-9 as a control and testing S-13 enzymatic preparations to determine if they would activate the precarcinogen. Ames et al. (1975) noted that for optimum mutagenesis with a compound the amount of S-9 added per plate is critical. Thus, the optimum amount of S-13 was also tested for optimum mutagenesis from each microorganism. Any of the S-13 preparations that failed to show a twofold increase in reverse mutants over the controls, indicative of the positive response, were termed negative, and testing of that enzyme preparation was discontinued.

Revertant colony numbers for the two tester strains with three different concentrations of S-13 from S. faecalis are presented in Fig. 1. The positive response did occur and the optimal concentration of S-13 was 100 µl. As shown in Table 1, the optimal concentration is equivalent to 0.714 mg of protein per plate.

Revertant colony numbers for the two tester strains with three concentrations of S-13 from E. coli are presented in Fig. 2. The response was negative. It should be noted that the average number of reverse mutants on the control plates was greater as compared to the enzyme preparations

Figure 1. Optimal concentration of the Streptococcus faecalis enzyme preparation.

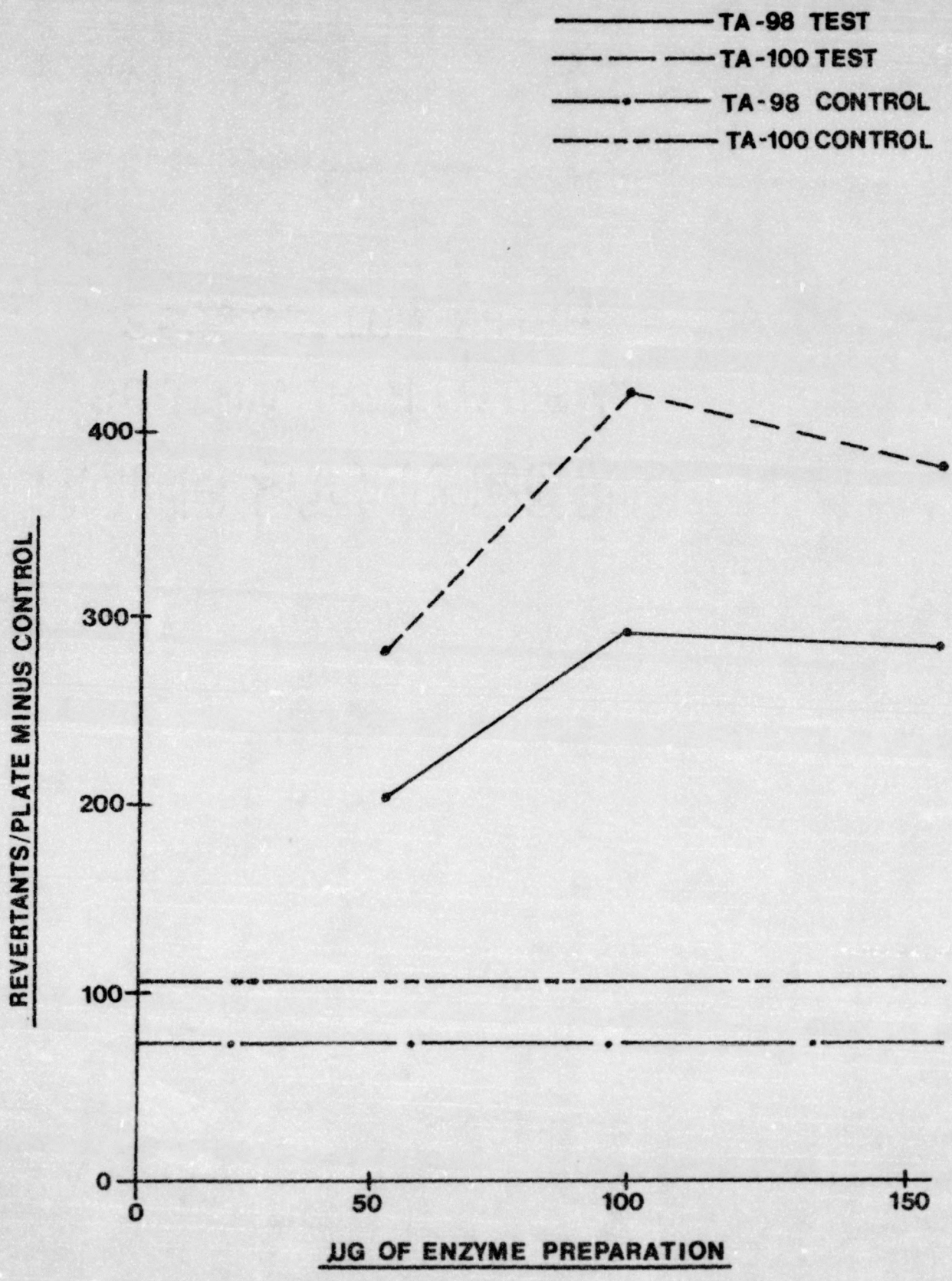

TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
UG OF ENZYME PREPARATION

Figure 2. Optimal concentration of the Escherichia coli enzyme preparation.

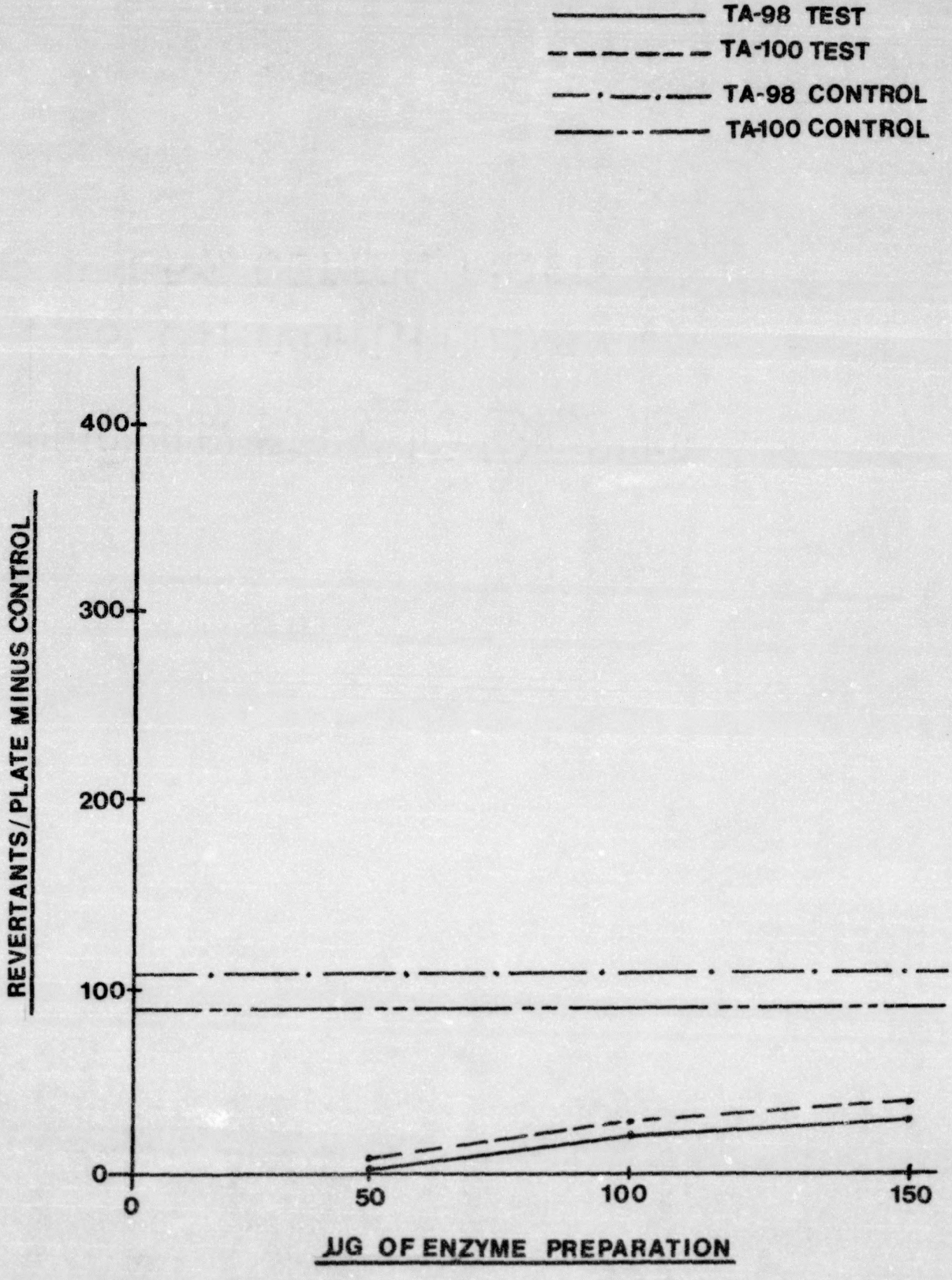

TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
UG OF ENZYME PREPARATION

Table 1. Milligrams of Protein per Assay Plate of Active Microbial S-13 Preparations.

S-13 Preparation*	*Salmonella typhimurium* mutants TA-98	TA-100
Streptococcus faecalis	0.714 mg/plate	0.714 mg/plate
Clostridium perfringens	1.37 mg/plate	1.37 mg/plate
Pseudomonas aeruginosa	2.44 mg/plate	1.63 mg/plate
Candida albicans	0.255 mg/plate	0.255 mg/plate

*Enzyme preparation after centrifugation at 13,000 x g (See Materials and Methods).

from the other microorganisms. This increase in back mutations in the controls could affect the determination of a positive response. This increase in back mutations in the control could make the zero for mutations high thereby causing a false negative.

Revertant colony numbers for the two tester strains with three concentrations of S-13 from P. aeruginosa are shown in Fig. 3. Unlike any of the enzymes from the other microorganisms tested, P. aeruginosa showed different optimal concentrations for each of the tester strains of S. typhimurium. S. typhimurium TA-100 gave the best response. Table 1 shows that the enzymatic preparation from P. aeruginosa required the highest amount of protein per plate for both tester strains of S. typhimurium as compared to enzyme preparations from the other tested microorganisms giving a positive response.

Revertant colony numbers for the two tester strains with three concentrations of S-13 from B. fragilis are presented in Fig. 4. The response demonsrated by this figure was negative. Unlike S-13 prepared from E. coli, B. fragilis S-13 system did not increase the number of back mutations for the tester strains in the control plates. Although the S-13 preparation from this bacterium did demonstrate a peak of 100 µl of enzyme preparation, this peak was well below the twofold increase necessary for designation as a positive response.

Figure 3. Optimal concentration of the *Pseudomonas aeruginosa* enzyme preparation.

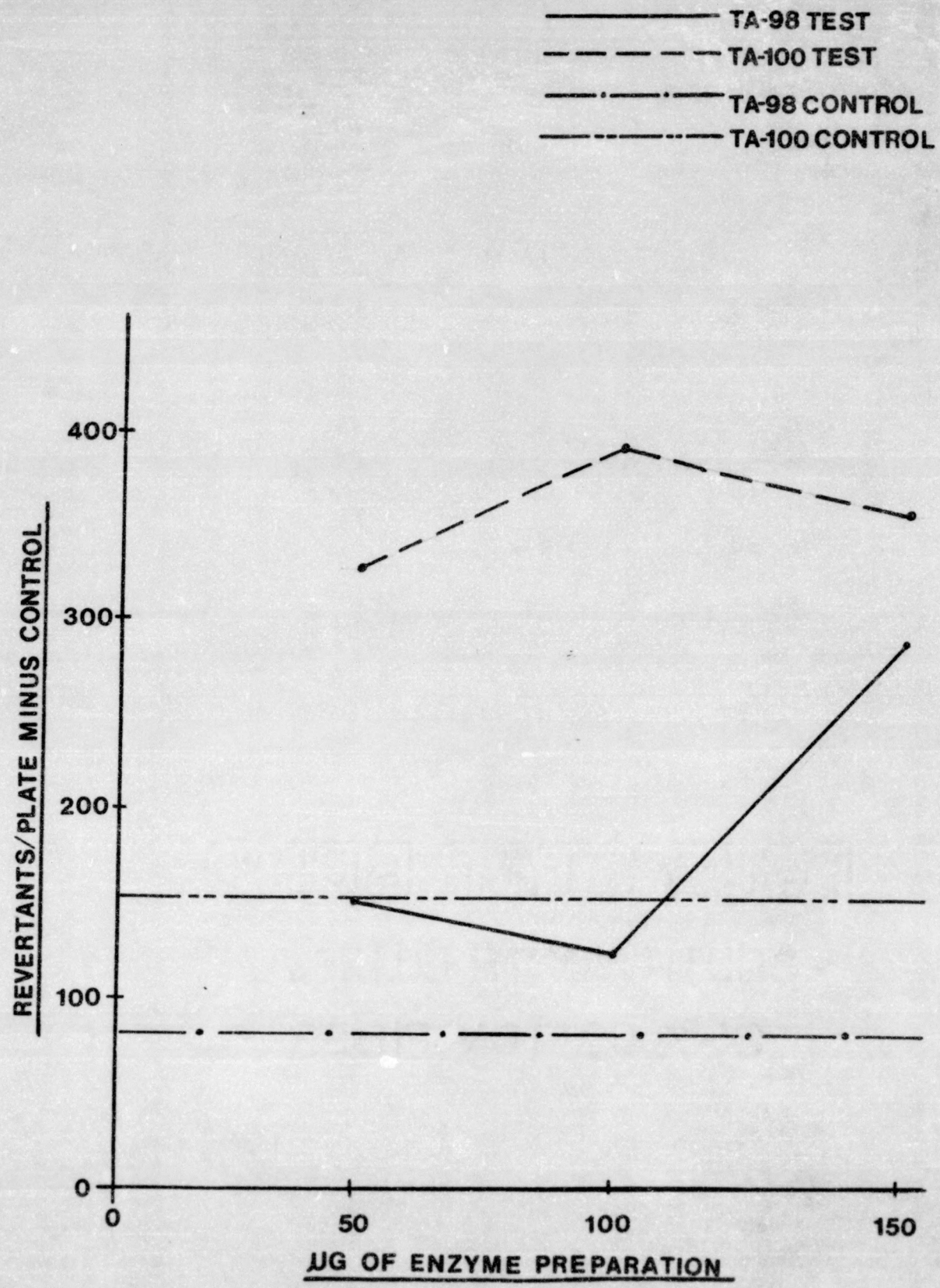
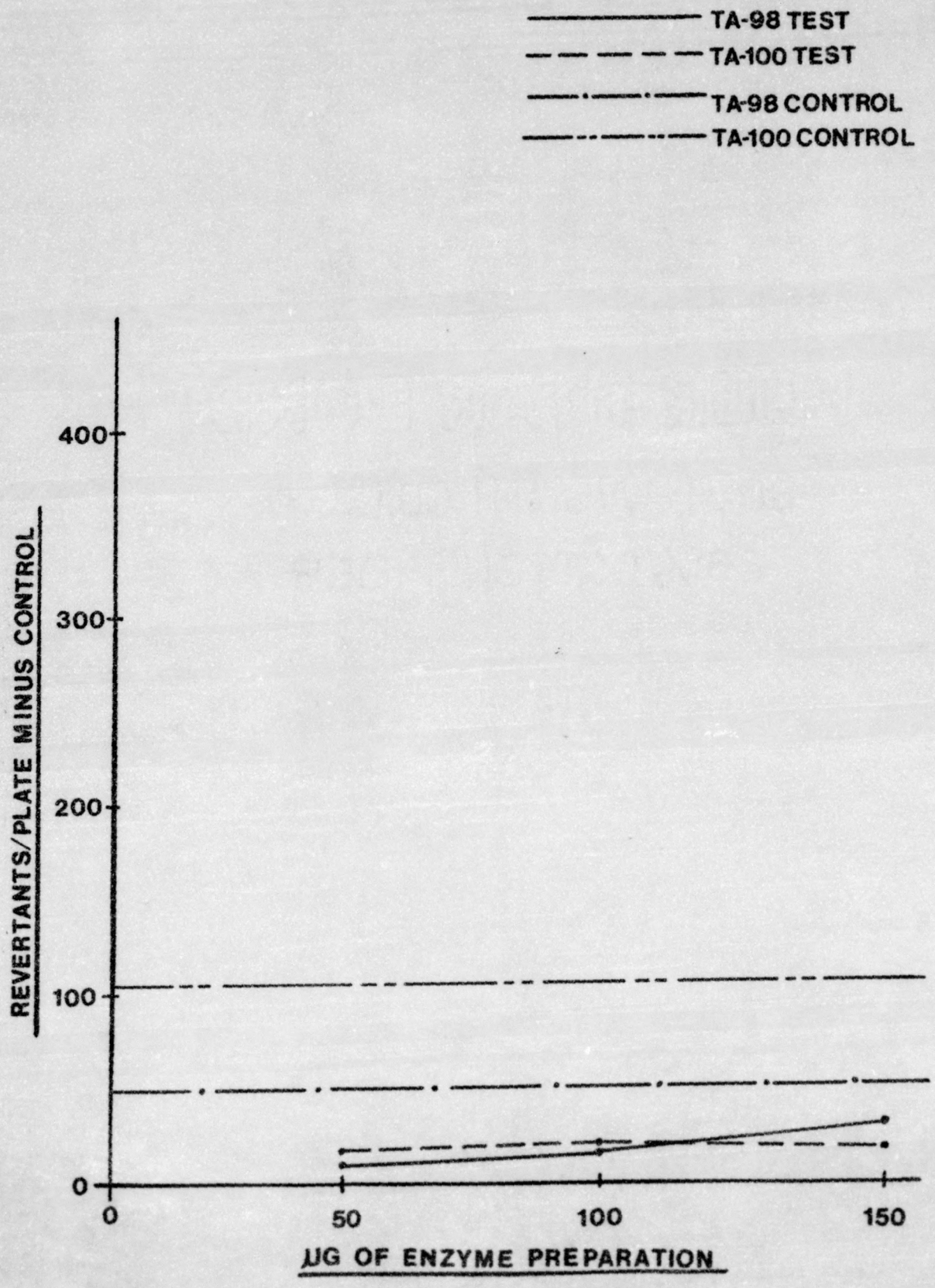

TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
UG OF ENZYME PREPARATION

Figure 4. Optimal concentration of the Bacteroides fragilis enzyme preparation.


TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
UG OF ENZYME PREPARATION

Revertant colony numbers for the two tester strains with three concentrations of S-13 from C. perfringens are presented in Fig. 5. It can be observed that a positive response occurred and that the optimum concentration of the enzyme preparation was 100 µl for both tester strains. As shown in Table 1 the protein concentration necessary for optimal activation of the precarcinogen was approximately twice the amount necessary from S. faecalis and less than the amount necessary for the enzyme preparation from P. aeruginosa.

Revertant colony numbers for the two tester strains with three concentrations of S-13 from the eukaryote C. albicans are presented in Fig. 6. This figure clearly demonstrates that the response was positive with 50 µl of the enzyme preparation. As shown in Table 1 the protein concentration in the enzyme preparation from this yeast was less than one-half the protein needed for activation from the enzymic preparations from the prokaryotic bacteria.

Dose-Response Curves for 2-aminoanthracene Using S-13 Preparations

Revertant colony numbers for the two tester strains tested with the precarcinogen at six different doses using S-13 preparation from C. albicans are presented in Fig. 7. Shown is a response similar to those seen with mammalian enzymes when S. typhimurium TA-98 was used as the tester strain (Ames et al. 1975). As both enzyme preparations are

Figure 5. Optimal concentrations of the Clostridium perfringens enzyme preparation.

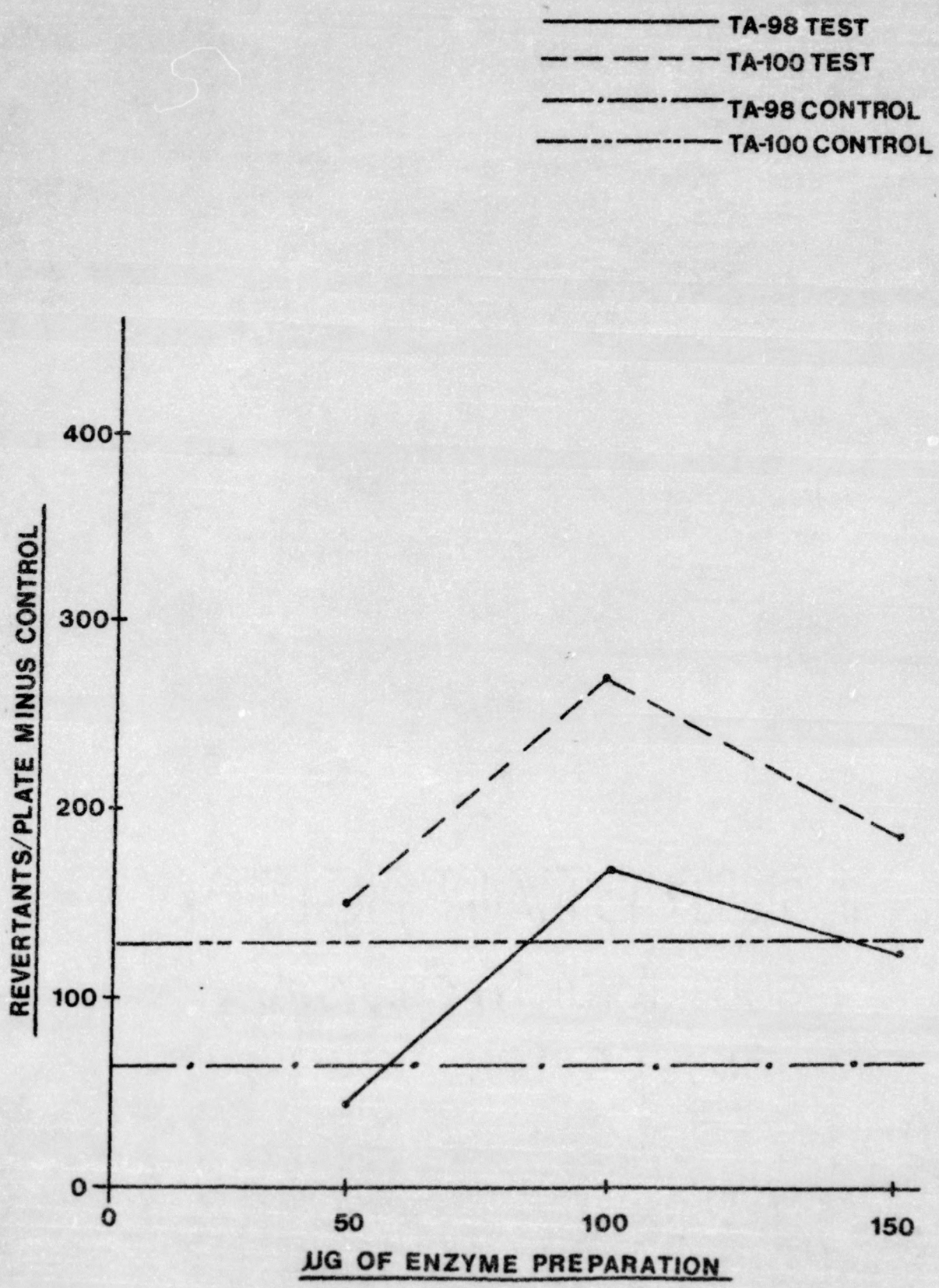

TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
µG OF ENZYME PREPARATION

Figure 6. Optimal concentration of the Candida albicans enzyme preparation.

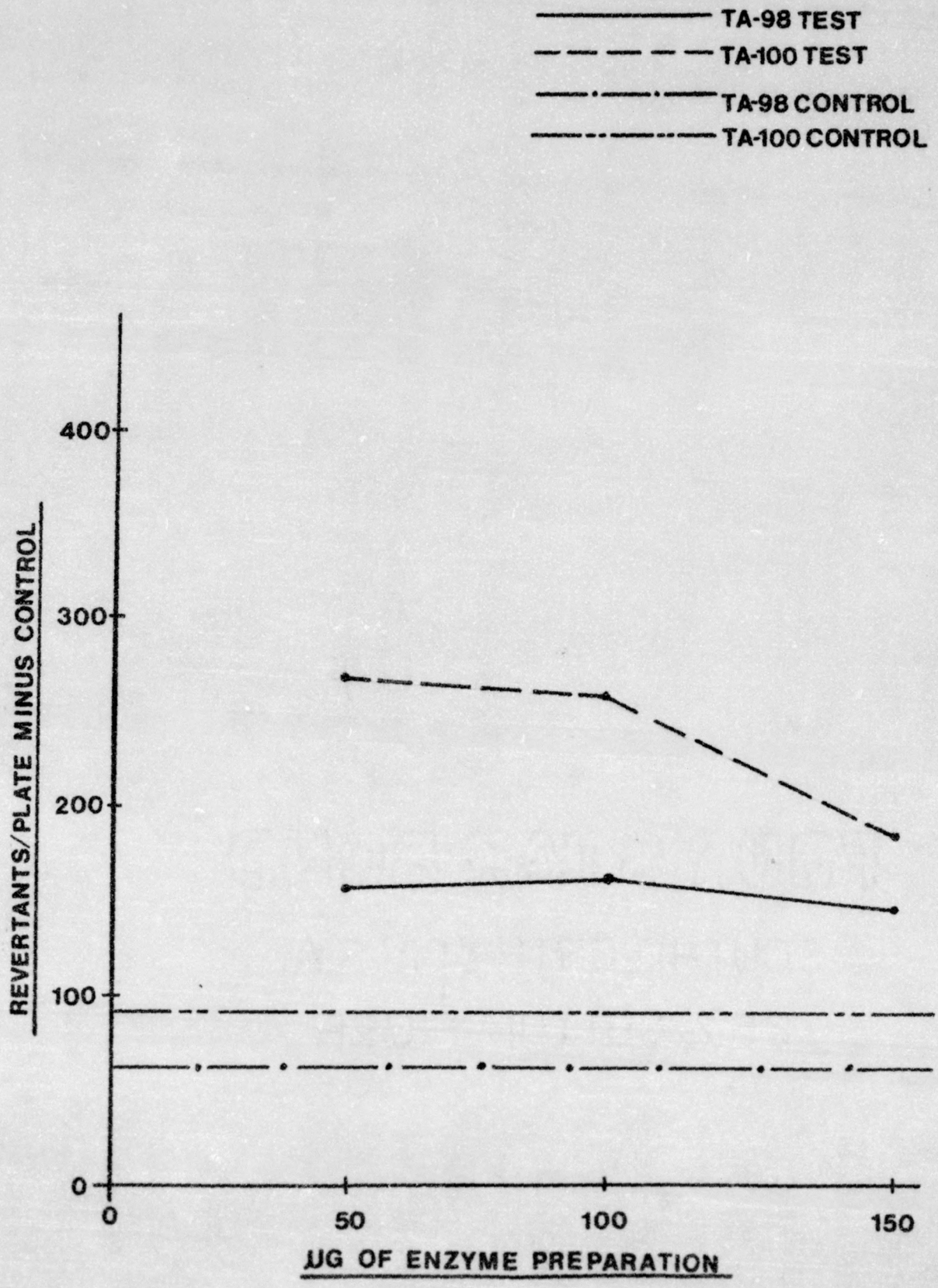
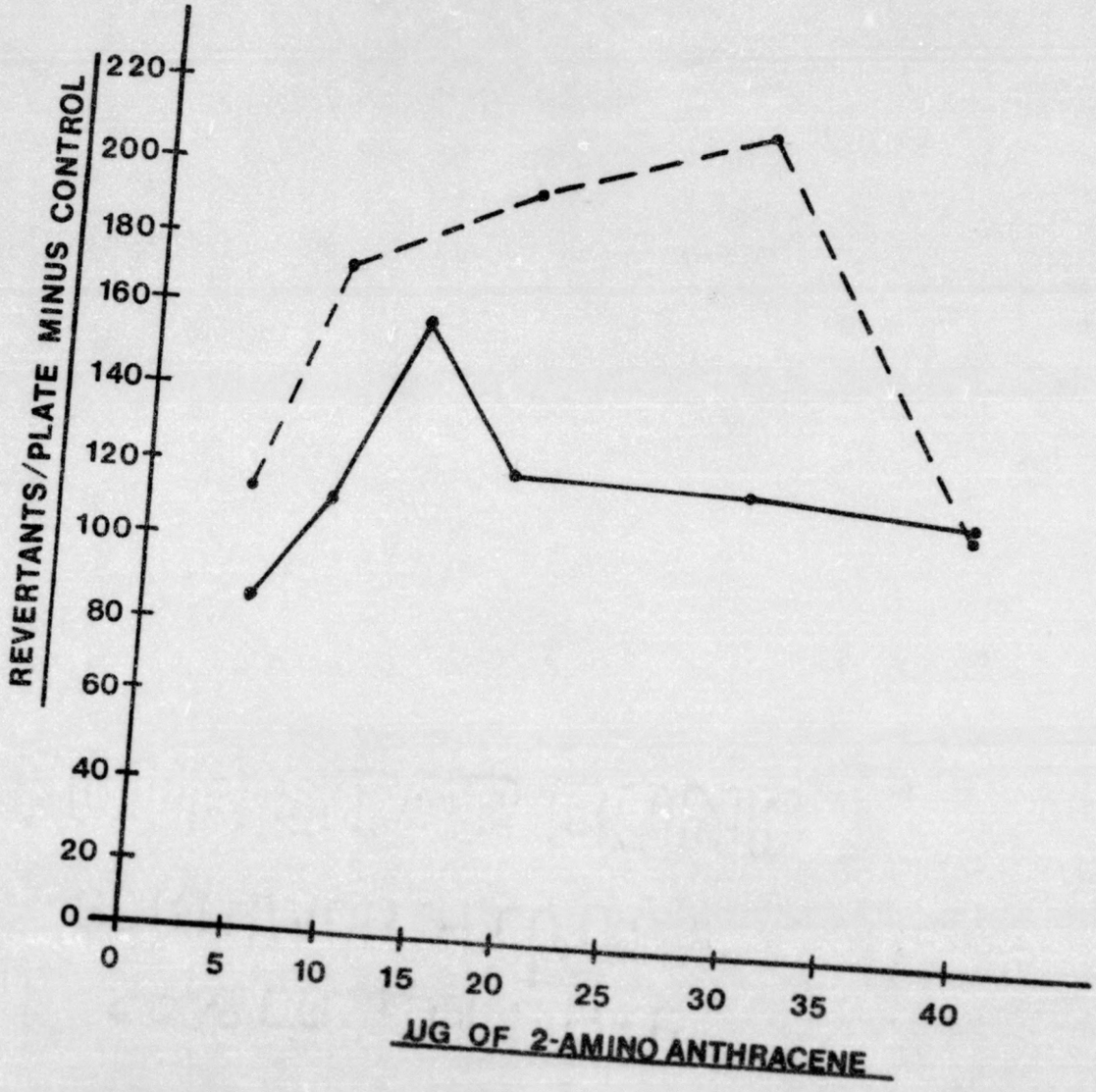

TA-98 TEST
TA-100 TEST
TA-98 CONTROL
TA-100 CONTROL
REVERTANTS/PLATE MINUS CONTROL
400
300
200
100
0
0
50
100
150
UG OF ENZYME PREPARATION

Figure 7. Linear dose-response of 2-aminoanthracene using *Candida albicans* enzymes for activation with Salmonella TA-98 and TA-100.


TA-98 RESPONSE
TA-100 RESPONSE
REVERTANTS/PLATE MINUS CONTROL
220
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
UG OF 2-AMINO ANTHRACENE

from eukaryotic cells, it is not unexpected that these curves would be similar. However, when S. typhimurium tester strain TA-100 was used, the response was not as finely defined as the response demonstrated by TA-98, but it still has the same basic shape when graphed.

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at six different doses using S-13 preparation from S. faecalis are presented in Fig. 8. As noted in Fig. 8, tester strain TA-98 demonstrated a decrease after an initial increase and then a second increase followed by the usual decline in response associated with the higher concentration of a carcinogen/mutagen as it becomes cytotoxic. A possible reason for the unexpected decline in response suggests there are competing enzyme systems present in this S-13 preparation for activation of 2-aminoanthracene. As can be seen in Fig. 8, tester strain TA-100 did not demonstrate this response.

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at six different doses using S-13 preparations from C. perfringens are presented in Fig. 9. Enzymic preparations produced at least two peaks with both tester strains as noted with the enzymic preparation from S. faecalis tested with tester strain TA-98 (Fig. 8). Again, the competition of enzyme systems from C. perfringens could account for the pattern evidenced in the figures.

Figure 8. The linear dose-response of 2-aminoanthracene using Streptococcus faecalis enzymes for activation with Salmonella TA-98 and TA-100.

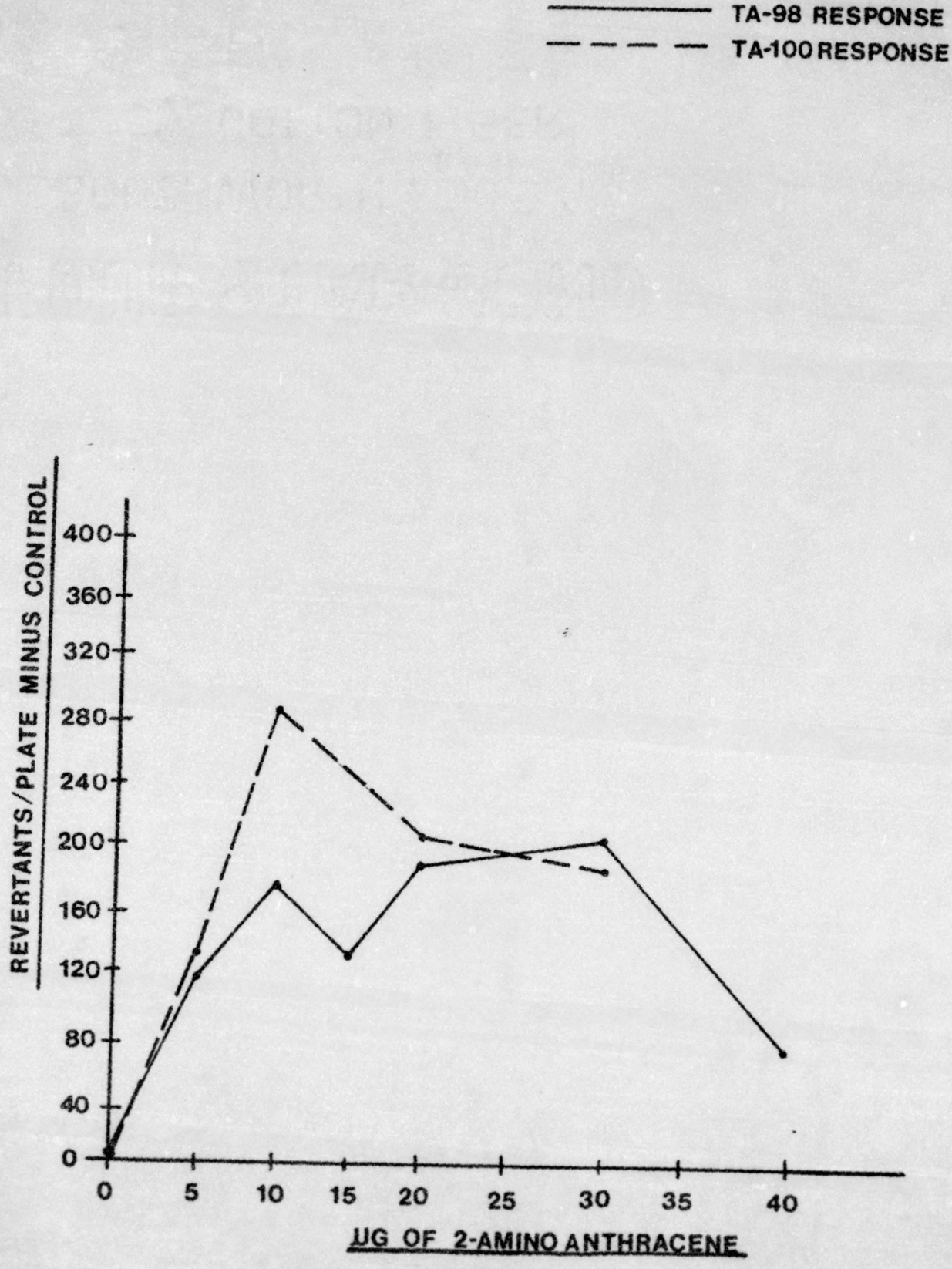
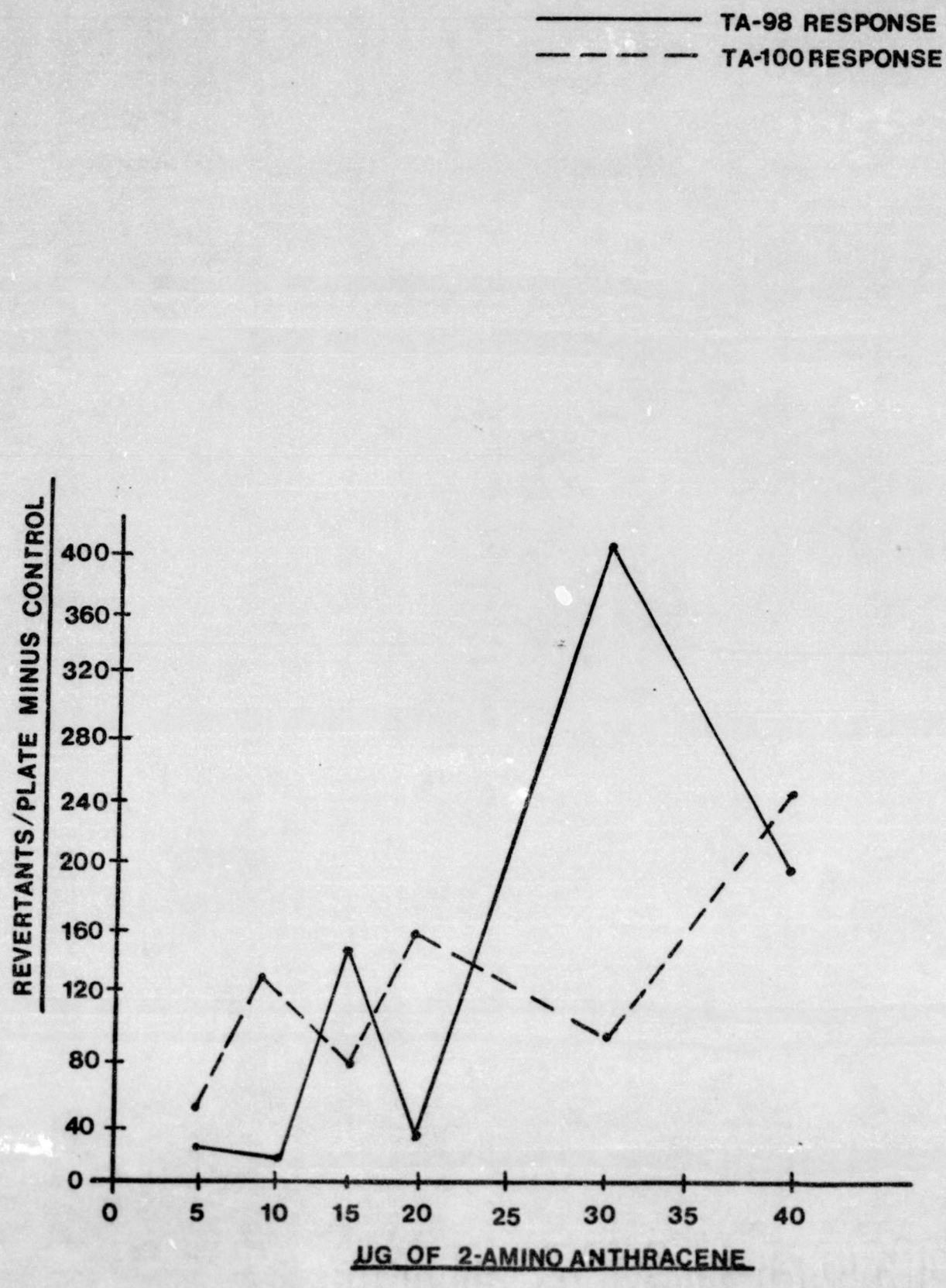

TA-98 RESPONSE
TA-100 RESPONSE
REVERTANTS/PLATE MINUS CONTROL
400
360
320
280
240
200
160
120
80
40
0
0
5
10
15
20
25
30
35
40
UG OF 2-AMINO ANTHRACENE

Figure 9. Linear dose-response of 2-aminoanthracene using Clostridium perfringens for activation with Salmonella TA-98 and TA-100.


TA-98 RESPONSE
TA-100 RESPONSE
REVERTANTS/PLATE MINUS CONTROL
400
360
320
280
240
200
160
120
80
40
0
0
5
10
15
20
25
30
35
40
UG OF 2-AMINO ANTHRACENE

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at six different doses using S-13 preparation from P. aeruginosa are shown in Fig. 10. The S-13 preparation from P. aeruginosa caused the chemical to produce the largest number of reverse mutants as compared to the S-13 preparation from the other microorganisms. It should be noted that at a concentration of 40 µg of 2-aminoanthracene the TA-100 tester strain background "lawn" was reduced, and therefore information is not reported for this point on the figure.

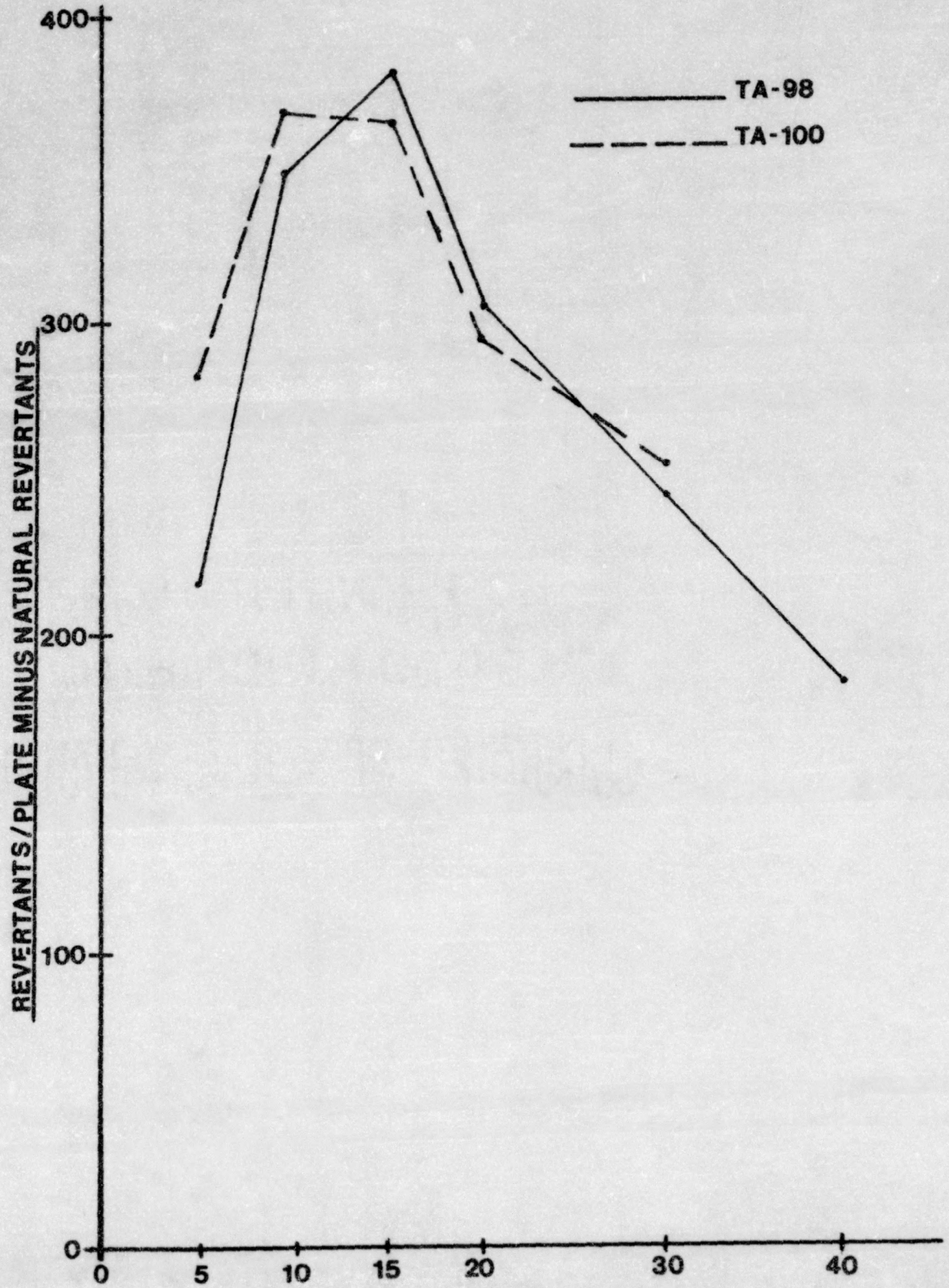
Dose-Response Curves for 2-aminoanthracene Using S-100 Preparation

In an attempt to locate the site of the activating enzymes, activation by S-100 and the membrane pellet obtained during preparation of the S-100 fraction was compared. The comparisons were performed on only those microbial extracts which exhibited activation.

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at three different doses using S-100 (cytosolic) and the membrane pellet from C. albicans are shown in Fig. 11. Since the membrane-bound enzymes demonstrated a negative response, this data is not shown. Thus, it appears that activation is due to the cytosolic enzymes as demonstrated with both S. typhimurium tester strains.

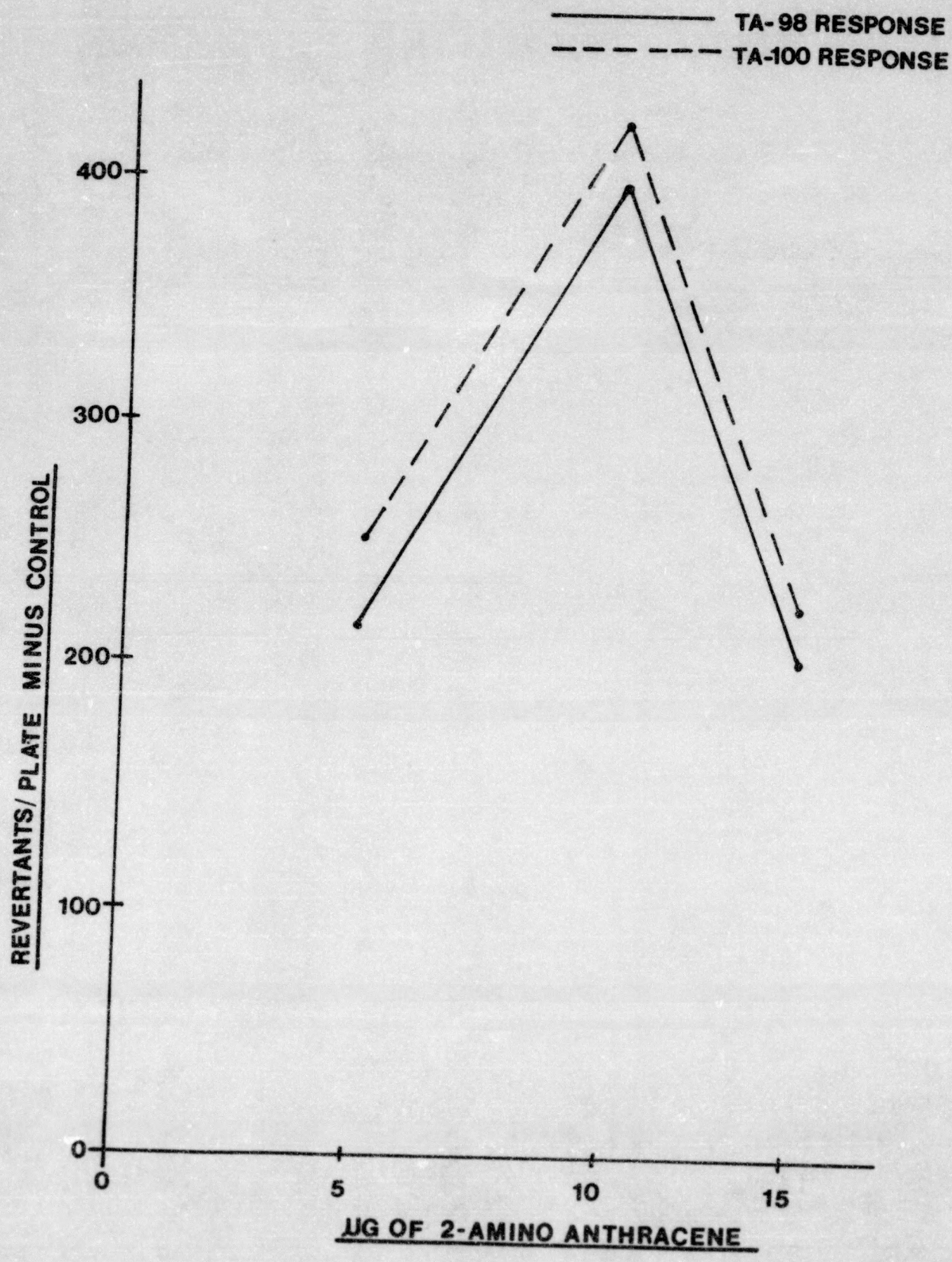
Revertant colony numbers for the two tester strains

Figure 10. Linear dose-response of 2-aminoanthracene using Pseudomonas aeruginosa enzymes for activation with Salmonella TA-98 and TA-100.



LINEAR DOSE RESPONSE OF PSEUDOMONAS AERUGINOSA

Figure 11. The activation of 2-aminoanthracene using Candida albicans cytosolic enzymes for activation with Salmonella TA-98 and TA-100.


TA-98 RESPONSE
TA-100 RESPONSE
400
300
200
100
0
REVERTANTS/ PLATE MINUS CONTROL
0
5
10
15
UG OF 2-AMINO ANTHRACENE

tested with 2-aminoanthracene at three different doses using S-100 and the membrane pellet from S. faecalis are shown in Fig. 12. This data demonstrates that the decline in activation reported previously is not due to competition between membrane-bound enzymes and cytosolic enzymes for activation sites but may be due to some other activity of the enzymes from the cytosol or membranes, since the decline in activation was not observed when the two enzyme systems were separated.

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at three different doses using S-100 and the membrane pellet from C. perfringens are shown in Fig. 13. This data demonstrates that the membrane-bound enzymes of C. perfringens resulted in activation of 2-aminoanthracene and the cytosolic enzymes showed activation.

Revertant colony numbers for the two tester strains tested with 2-aminoanthracene at three different doses using S-100 and the membrane pellet from P. aeruginosa are shown in Fig. 14. Both tester strains showed activation with cytosolic enzymes but failed to show activation with the membrane-bound enzymes.

In conclusion, the results of this research have demonstrated that microbial enzyme systems can replace mammalian enzyme systems in the Ames assay. Of the six organisms that can be isolated from the human intestinal tract and tested, four had sufficient enzyme systems to activate the precarcinogen 2-aminoanthracene to its

Figure 12. The activation of 2-aminoanthracene comparing *Streptococcus faecalis* membrane-bound versus cytosolic enzymes.

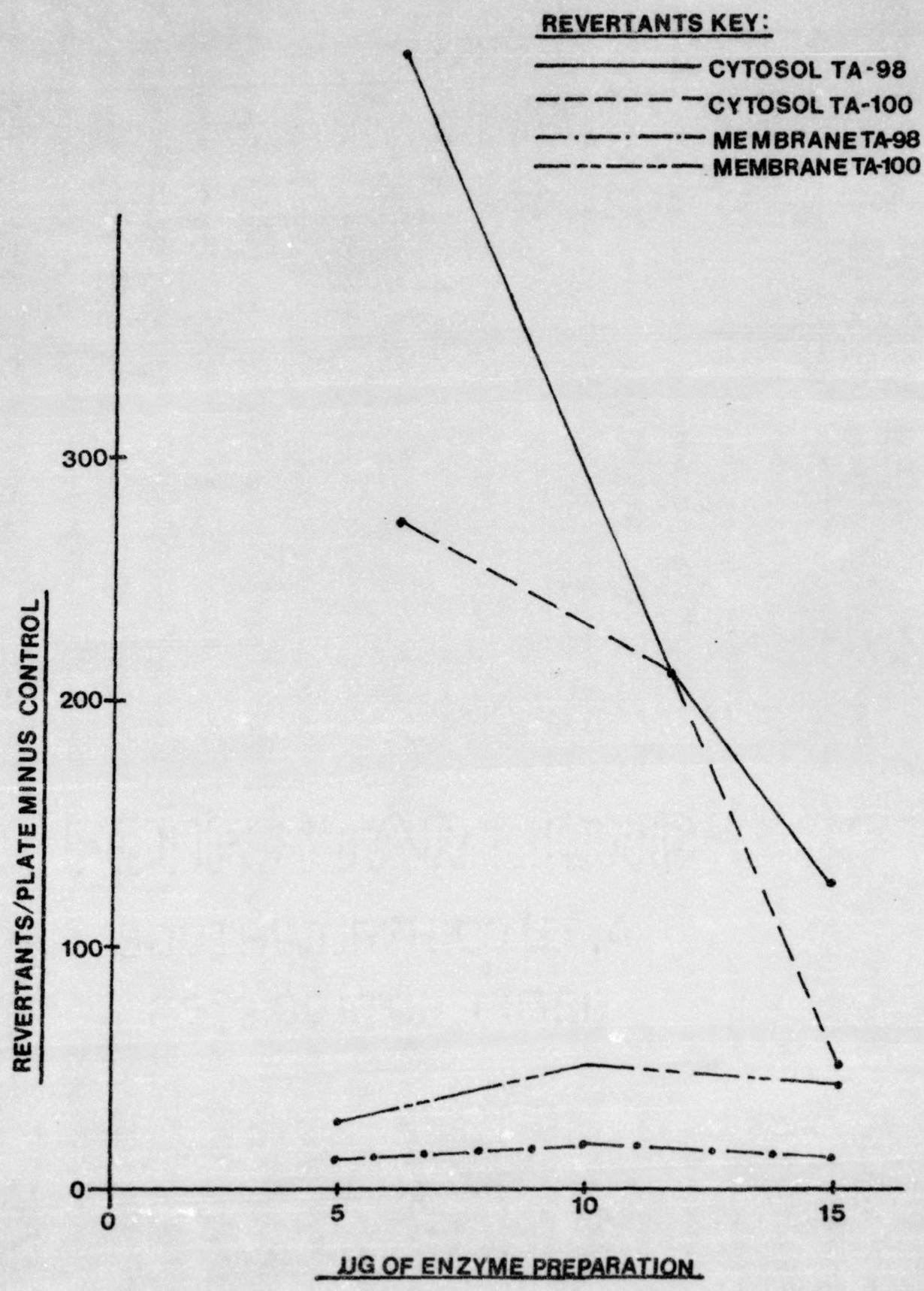

REVERTANTS KEY:
CYTOSOL TA-98
CYTOSOL TA-100
MEMBRANE TA-98
MEMBRANE TA-100
REVERTANTS/PLATE MINUS CONTROL
300
200
100
0
0
5
10
15
UG OF ENZYME PREPARATION

Figure 13. The activation of 2-aminoanthracene comparing membrane bound versus cytosolic enzymes of Clostridium perfringens.

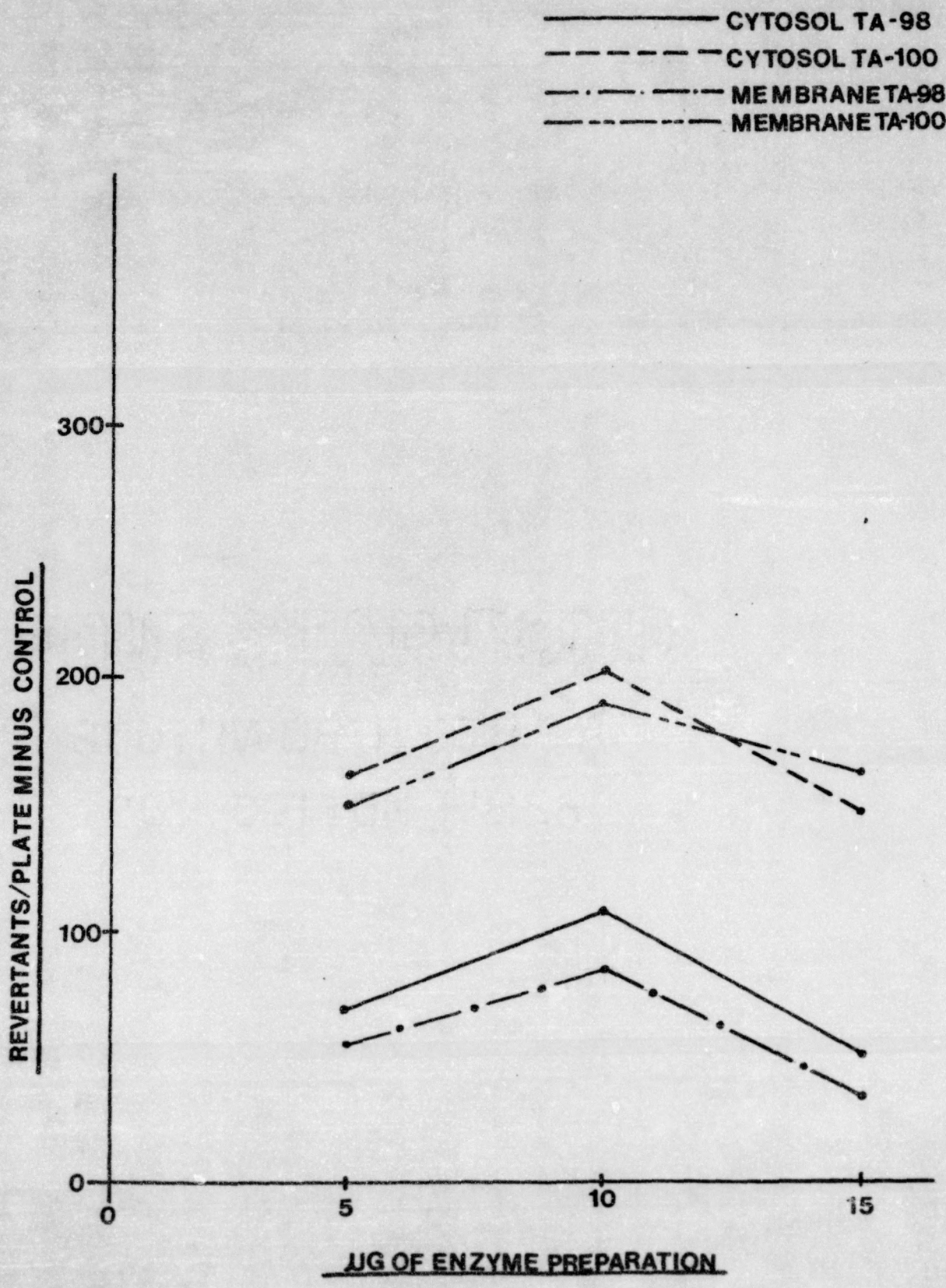
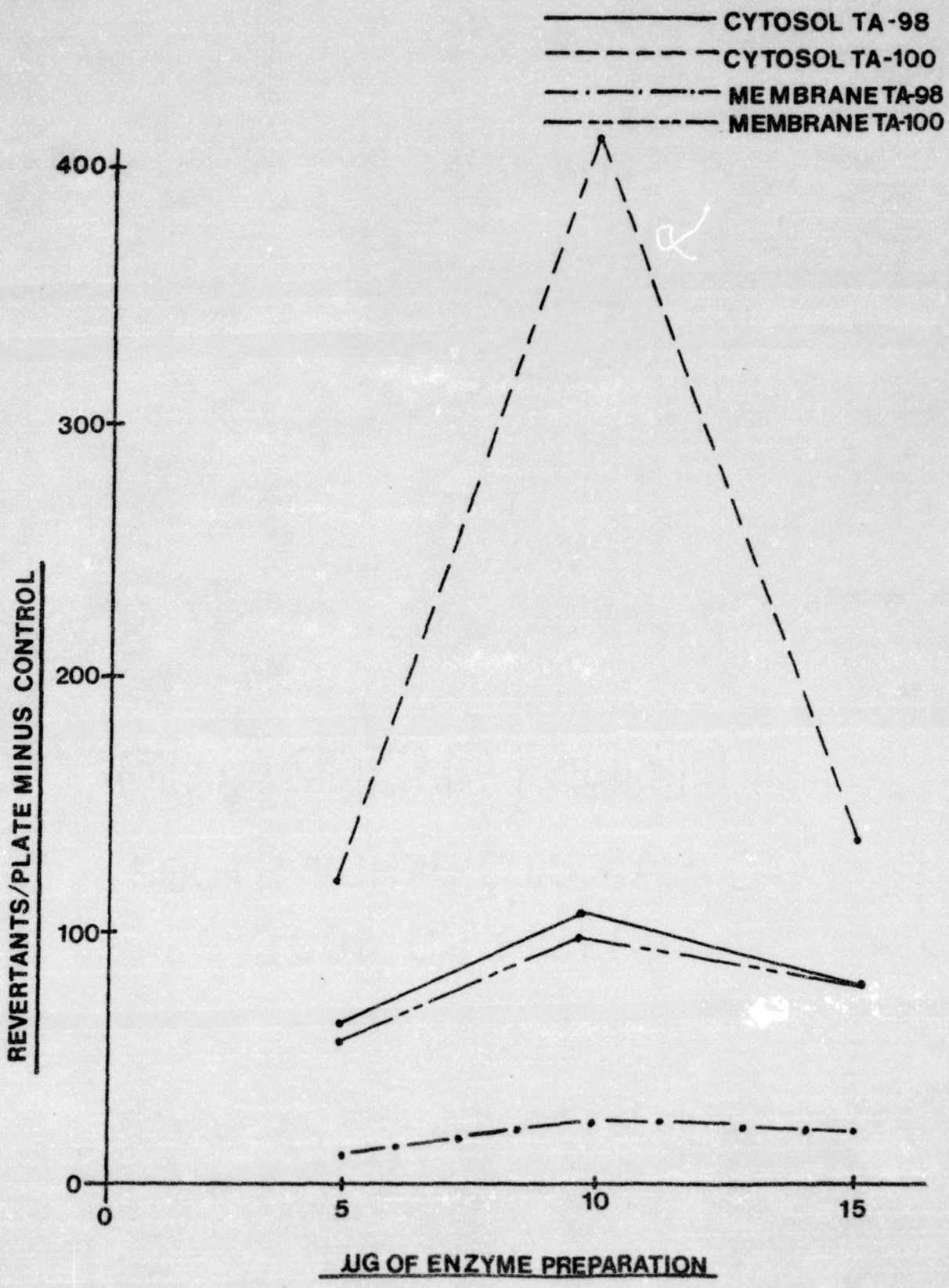

CYTOSOL TA-98
CYTOSOL TA-100
MEMBRANE TA-98
MEMBRANE TA-100
REVERTANTS/PLATE MINUS CONTROL
300
200
100
0
0
5
10
15
UG OF ENZYME PREPARATION

Figure 14. The activation of 2-aminoanthracene comparing membrane-bound versus cytosolic enzymes of Pseudomonas aeruginosa.


CYTOSOL TA-98
CYTOSOL TA-100
MEMBRANE TA-98
MEMBRANE TA-100
400
300
200
100
0
REVERTANTS/PLATE MINUS CONTROL
0
5
10
15
UG OF ENZYME PREPARATION

mutagenic potential. It is interesting that two of the bacteria tested are considered to be indigenous flora of the human intestinal tract did not activate 2-aminoanthracene. It is possible that E. coli and B. fragilis do not possess cytochrome P_{450} or equivalent which is necessary for activation in mammalian systems (Bjorkhem et al. 1976).

Four microorganisms tested, that can be isolated from the human intestinal tract, had proper enzyme systems to activate the precarcinogen 2-aminoanthracene to its mutagenic potential. Of these S. faecalis and C. perfringens are considered indigenous flora of the human intestinal tract. These bacteria certainly might be part of the "fecalases" which Tamura et al. (1980) demonstrated in feces which activated glycosides to mutagens. More significantly is, this research demonstrated that S. faecalis, P. aeruginosa, C. perfringens and Candida albicans activate 2-aminoanthracene to its mutagenic potential without the mammalian liver homogenate. Thus, if this chemical were ingested, intestinal microorganisms could convert it to a mutagen. Whether this reaction would actually happen in vivo has not been tested. Research similar to that presented in this thesis was done by Chug et al. (1981). They demonstrated that Fusobacterium spp. converted methyl yellow to N,N-demethyl-p-phenylenediamine which was metabolically activated to a mutagen.

Epidemiological studies suggest that cancer in human populations results from environmental factors particularly

the diet (Doll, 1977; Hoover and Fraumeni, 1975; and Sinha and Paruleka, 1983). Possibly, certain microflora of the intestinal tract are converting chemicals from the diet into carcinogens. The work presented here could possibly lead to the understanding of the cause of colon cancer and its cure.

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