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MASTER

Inducible Error-prone Repair in B. subtilis

Progress Report
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Progress Report

Table of Contents

Research Report

Abstract

The grant application "Inducible Error-prone repair in B. subtilis proposed to investigate the mechanism of activation and the mode of action of the 'SOS' system in the bacterium Bacillus subtilis. Interesting aspects of the 'SOS' system in B. subtilis are: 1) the differences between 'SOS' functions in this bacterium and in the enteric bacteria (Yasbin, R.E., 1977, Mol. Gen. Genet. 153,211); 2) the spontaneous activation of 'SOS' functions in competent cells (Yasbin, R. E., 1977, Mol. Gen. Genet. 153,219); 3) the difficulty in obtaining consistent results for UV mutation studies in this bacterium (see grant renewal proposal; also unpublished observations from our laboratory and from the laboratory of Dr. Herbert Kubitschek). In order to characterize the 'SOS system of B. subtilis, we proposed to: 1) isolate bacteria mutated in genes controlling various repair function; 2) investigate inducible repair; 3) determine the role of endogenous Bacillus prophages in 'SOS' functions, and 4) develop a tester system for potential carcinogens from competent Bacillus subtilis cells. In the data and in the appendices which follow, we have been able to: 1) isolate strains of B. subtilis in which the endogenous prophages have been removed or neutralized; 2) demonstrate the association of one 'SOS' function with prophage SPβ; 3) demonstrate that the survival of UV irradiated B. subtilis is not significantly altered by the removal and neutralization of the endogenous prophages; 4) develop competent B. subtilis into a tester system; and 5) show that DNA polymerase III is absolutely necessary for 'W' reactivation. In addition, we have isolated UV and mitomycin C resistant mutants and we have looked at inducible post-replication repair in excision-repair deficient mutants of B. subtilis. The last two results are somewhat confusing but highly exciting in regards to DNA repair mechanism in B. subtilis.

Isolation of Prophage Cured and Prophage Neutralized Strains of B. subtilis

As described in the original grant proposal, we felt it was absolutely essential to remove the known endogenous defective prophages of B. subtilis. The removal of these prophages would permit the studying of inducible 'SOS' functions without having to worry about the bacteria being lysed. Prophage SPβ was removed from B. subtilis as described in Appendix I. We were unable to remove prophage PBSX and therefore choose to introduce the xin-1 mutation into our strains. This mutation prevents the induction of prophage PBSX. Thus, we were able to construct strains of B. subtilis in which prophage SPβ had been removed and in which prophage PBSX had been neutralized (Appendix I). These cured strains were tested for their UV sensitivity. The results (figure 1) indicate that the two prophages have little if any effect on the survival of UV irradiated bacteria. We can not rule out the possibility that additional prophages may be present in the Bacillus chromosome. However, prophages PBSX and SPβ are definitely the major defective prophages of B. subtilis 168 and its derivatives (Appendix I).

The Development of Competent B. subtilis into a Tester System

The spontaneous activation of 'SOS' functions in competent B. subtilis presented the possibility that these cells could be developed into a sensitive tester for potentially carcinogenic agents. The development of this tester system was based on the assumption that as the cells became competent they also became more sensitive to 'SOS' inducing agent. This enhanced sensitivity to 'SOS' inducing agents would be the result of bacterial death due to the

induction of the endogenous prophages. Thus competent cells would have a greater number of prophages induced at a specific dose of an agent than the non-competent cells. More prophages would be induced because the competent cells would already have had their 'SOS' system "precociously activated". In Appendix I, the development of this tester system and the rationale behind this system are more specifically explained. Significantly, competent B. subtilis cells have been made into a tester for 'SOS' inducing agents. In addition, this system apparently is able to discriminate between non-carcinogenic mutagens (or weakly carcinogenic mutagens) and carcinogenic mutagens. Also the 'SOS'-inducing antibiotic Nalidixic acid does not give a positive result in the "Comptest". Thus the Comptest which may became an effective screen for carcinogenic agents, could be used in addition to other bacterial tester systems. The differences and the similarities between the "Comptest" the Ames Assay and The Inductest are discussed in Appendix I. We have already used this test in conjunction with the Ames Assay to investigate the potentially harmful effects of disel particulates (Appendix II).

The Role of DNA polymerase III in Inducible-Repair

The investigation of essential enzymes in inducible repair processes can be accomplished at both the molecular and the whole organism level. However, in order to look at inducible repair on the macromolecular level one must have a system where the organism can survive even if the repair processes are inhibited. DNA polymerase III or its associated exonuclease activities have been suggested as an important constituent of inducible repair (Vellani et al., 1978, Proc. Nat. Acad. Sci. 75:3037; Devoret, R. 1978 Biochimie, 60:1135). The investigation of the role of DNA polyermase III in inducible repair is made difficult since this enzyme is absolutely essential for bacterial DNA replication. Also, an experimental procedure must be designed which would permit one to distinguish between the actual involvement of DNA polyermase III in inducible repair as compared to the possibility that this enzyme must be functional in order to induce the 'SOS' system. We feel that we have developed a system which permits the analysis of the role of DNA polymerase III in 'SOS' repair.

Our system involves the 'W'-reactivation of bacteriophage SP02 (see grant proposal). In Fig. 2 survival causes for UV irradiated bacteriophages SP02 and ϕ105 are shown. These bacteriophages are of the same molecular weight and have been shown to have approximately the same number of essential genes (complementation mapping). The greater UV resistance of SP02 as compared to ϕ105 has not been explained. However, we feel that this enhanced survival is due to the fact that bacteriophage SP02 is resistant to the drug 6-hydroxyphenylazo uracil (HPura; a known inhibitor of DNA polymerase III activity; Brown, N.C., 1970, Proc. Nat. Acad. Sci. 67:1454; Goss, et al., 1973 Proc. Nat. Acad. Sci. 70:103). Bacteriophage ϕ105 is sensitive to this antibiotic and thus uses the bacterial DNA polymerase III while bacteriophage SP02 makes its own DNA polymerase. We had previously shown that bacteriophage ϕ105 could be 'W'-reactivated and that 'W' reactivation in B. subtilis was part of the 'SOS' system (Yasbin, 1977, Mol. Gen. Genet. 153,211). The data in fig. 4 demonstrates that 'W'-reactivation also occurs for bacteriophage SP02. However, the levels of 'W'-reactivation achievable for SP02 are about 1/5 of these obtained for bacteriophage ϕ105. This difference can also be attributed to the SP02 DNA polymerase. When, HPura is added to the bacterial

culture immediately following bacteriophage infection, 'W'-reactivation can no longer be demonstrated (Fig. 4). Also, the addition of HPura 30 minutes after the UV irradiation of the bacterial culture (30 minutes after the induction of the 'SOS' system) also abolished 'W'-reactivation (Fig. 5). HPura does not inhibit burst size or burst time for bacteriophages which make their own DNA polymerase (Yasbin et al., 1975, J. Bacterial 121,305, unpublished data). Therefore DNA polymerase III is essential for at least one type of inducible repair ('W'-reactivation) in B. subtilis. Furthermore, the essential activity is apparently that of the polymerase and not of the associated exonucleases. Also DNA polymerase III is definitely involved in the mechanism of repair and not just in the induction of the system (Fig. 5). It has previously been established that HPura induces prophage SP02 (Arwert, F. and Rutherg, L., 1974. J. of Virol. 14:147). This datum also suggests that DNA polymerase III activity is not necessary for the induction of 'SOS' functions. Further analysis of the role of DNA polymerase III is 'SOS' functions will be outlined in the renewal proposal.

Induced DNA Modification is Controlled by Bacteriophage SPβ

In an attempt to determine if other 'SOS' functions were inhibited by HPura, induced DNA modification studies were done (see initial grant proposal). Induced DNA modification had been shown to be part of the 'SOS' system in B. subtilis. However, we were unable to repeat our earlier experiments. The only difference between the two series of experiments was the bacterial strains utilized. In our recent experiments the prophage cured strain was being employed. Therefore, we suspected that the induced methylation enzyme might either be part of, or controlled by one of the endogenous prophages. The results in tabel 1 demonstrate that induced methylation is indeed regulated by bacteriophage SPβ. However, we have not yet determined whether the bacteriophage carries the gene for the methylation enzyme or just controls its activation. We also have not yet determined if HPura blocks the inducible methylation system.

Isolation of UV Resistant Mutants

B. subtilis strain 886 (a prophage cured strain) was treated with ethylmethane sulfonate and colonies resistant to mitomycin C were isolated. These mutants were checked for their UV sensitivity (Fig. 6) for their ability to support the replication of UV irradiated bacteriophage (Fig. 7) for their ability to be lysogenized and for their ability to demonstrate 'W'-reactivation. Our original intent was to isolate bacteria constitutive for 'SOS' functions. While these mutants have enhanced resistance to UV and mitomycin C, they do not demonstrate enhanced survival for irradiated bacteriophage, nor do they have trouble being lysogenized by temperate bacteriophage. In addition, these mutants still demonstrate inducible 'W'- reactivation. Thus these mutants are not constitutive for 'SOS'-functions nor do they have increased excision-repair (host cell reactivation) capacity.

Inducible Post-Replication Repair

We have also studied post-replication repair and inducible post-replication repair in B. subtilis (see appendix in initial grant proposal). We are investigating this type of repair after first eliminating excision-repair by introducing the uvs-1 and uvr-1 mutation into our bacterial strain. Both of these mutants abolish excision-repair. However, we have found that the uvs-1 mutation apparently also abolsihes inducible post-replication repair (but not constitutive post-replication repair). This is a highly exciting result which fits in well with some recent findings in Escherichiu coli (see DNA Repair Mechanisms, P.C. Hanawalt, E.C. Friedberg, C.F. Fox, (eds), Academic Press, N.Y. 1978, pg. 247-260). We are presently investigating these results and examining the

effects of the uvr-1 mutation as well as the uvs-1 mutation on inducible post replication repair.

In summary, during the past year we have:

1) developed competent B. subtilis into a tester for 'SOS'-inducing agents and possibly into a tester for carcinogenic agents;
2) demonstrated the importance of DNA polymerase III in 'W'-reactivation;
3) identified the inducible methylation enzyme (inducible modification) as a phage controlled system;
4) isolated UV and mitomycin C resistant mutants which presently do not appear to have altered 'SOS' functions or altered excision-repair functions (mutants are not constitutive for 'SOS'-system nor do they demonstrate greater host-cell reactivation); and
5) we have some preliminary results which may link some excision-repair processes with inducible post-replication repair processes.

Publications (published in press or submitted for publication):

1. Yasbin, R.E., J.D. Ferwalt and P.I. Fields (1979) DNA Repair in Bacillus subtilis: Excision Repair Capacity of Competent Cells. J. Bacteriol. 137, 391-396

2. Yasbin, R.E., R. Miehl, and C.R. Matthews (DNA Repair in Bacillus subtilis: The development of competent cells into a tester for carcinogens. (Appendix I, to be submitted to Environmental Mutagenesis)

3. Streips, U.N., A.D. Laumbach, and R.E. Yasbin, Bacillus subtilis assays for mutation and DNA repair, and Bacterial Mutation Monitors for active metabolites of Chemical Carcinogens, C.I. Felkner ed., book in press (Appendix III)

Talks Presented at meetings, seminars:

1. Fields, P.I. and R. E. Yasbin. The Role of DNA polymerase III in "W"-Reactivation. Wind River Conference on Genetic Exchange. June, 1979.

2. Campbell, L.A., J.M. Musser, and R.E. Yasbin. The DNA repair capacity of Neisseria gonorrhoeae. Wind River Conference on Genetic Exchange. June, 1979.

3. Dukovich, M., S. Lestz, T. Ryan, and R.E. Yasbin. Biological effects of Disel Particulates. Argonne Conference on Environmental Mutagens and Carcinogens. May, 1979.

4. Yergey, J., S. Lestz, T. Ryan, T.H. Rishy, R.E. Yasbin, S.R. Prescott, M. Dukovich. The Sampling, Chemical Characterization and Biological Assay of Diesel Exhaust Particulate Matter From a Light Duty Diesel Engine. Spring Meeting Central States Section, The Combustion Institutes. April, 1979. (Appendix II)

Personell on the Project

R.E. Yasbin - principal investigator - (25% of time)

Barbara Andersen - research technician - (100%, 12 months DOE)

Mitchell Dukovith - laboratory assistant - (100%, 9 months DOE)

Jane Matsko - laboratory assistant - (100%, 3 months DOE)

Patricia Fields - graduate student (other funds)

Michael Weiner - undergraduate (not supported)

Sara Weinstock - undergraduate (not supported)

Rosemarie Miehl - undergraduate (other funds)

Lee Ann Campbell - graduate student (other funds)

Table #1: Inducible Modification System

Bacterial[a] Strain	UV Fluence J/m^2	Plaque forming Units/ml BR151	YB880	Restriction[b] Ratio	Modification[c] Ratio
YB886	0	2.0×10^6	9.6×10^2	5×10^{-4}	1.0
	20	2.6×10^6	9.3×10^2	4×10^{-4}	.8
	50	1.5×10^6	6.8×10^2	5×10^{-4}	1.0
BR151	0	2.0×10^6	3.5×10^1	2.0×10^{-5}	1.
	20	2.0×10^6	2.0×10^2	1.0×10^{-4}	5.
	50	1.5×10^6	1.0×10^3	7×10^{-4}	35.0
YB950[d]	0	1.0×10^6	2.5×10^3	3.0×10^{-3}	1.0
	20	7.1×10^5	1.6×10^4	2.0×10^{-2}	6.7
	50	5.4×10^5	2.6×10^4	5.0×10^{-2}	16.7

a/ The bacteriophage were grown in the irradiated (or unirradiated) bacteria at a MOI of 1.0. After one burst, the bacteriophage were diluted and plated on the permissive host (BR151) and on the restricting host (YB880)

b/ The restriction ratio was equal to the number of bacteriophage which gave plaques on strain YB880 divided by the number of plaques on strain BR151.

c/ Modification ratio was equal to the Restriction Ratio at a given UV dose divided by the Restriction Ratio at 0 UV dose.

d/ Strain YB950 was constructed by reinfecting strain YB886 (prophage cured) with a temperature inducible mutant of bacteriophage SPβ.

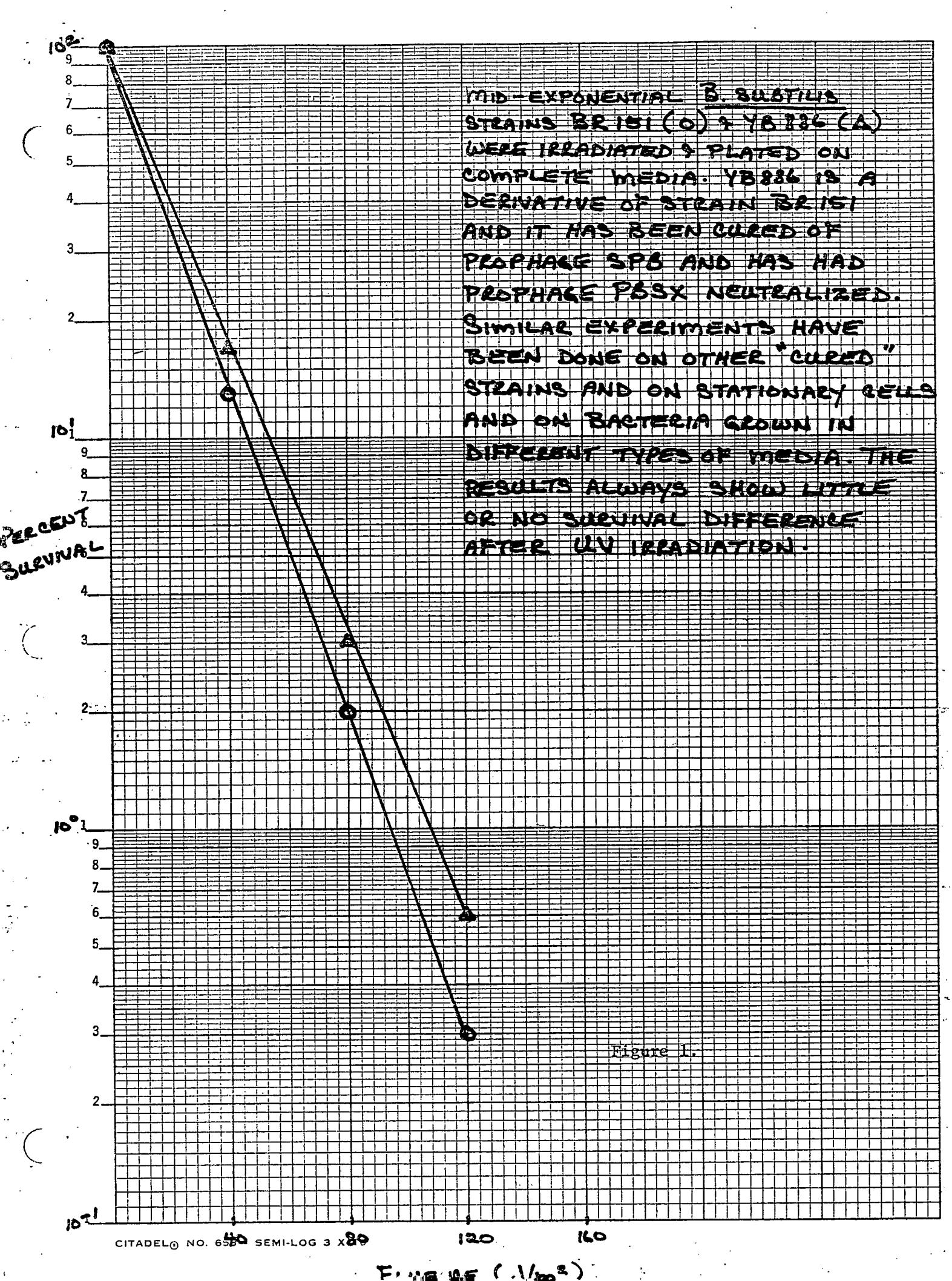
MID-EXPONENTIAL B. SUBTILIS STRAINS BR 151 (O) & YB 886 (A) WERE IRRADIATED & PLATED ON COMPLETE MEDIA. YB 886 IS A DERIVATIVE OF STRAIN BR 151 AND IT HAS BEEN CURED OF PROPHAGE SP8 AND HAS HAD PROPHAGE P8SX NEUTRALIZED. SIMILAR EXPERIMENTS HAVE BEEN DONE ON OTHER "CURED" STRAINS AND ON STATIONARY CELLS AND ON BACTERIA GROWN IN DIFFERENT TYPES OF MEDIA. THE RESULTS ALWAYS SHOW LITTLE OR NO SURVIVAL DIFFERENCE AFTER UV IRRADIATION.
PERCENT SURVIVAL
10^2
10^1
10^0
10^{-1}
40
80
120
160
CITADEL® NO. 658 SEMI-LOG 3 X 80

Figure 1.

SURVIVAL OF BACTERIOPHAGES ϕ105 AND SPO_2 AFTER IRRADIATION.

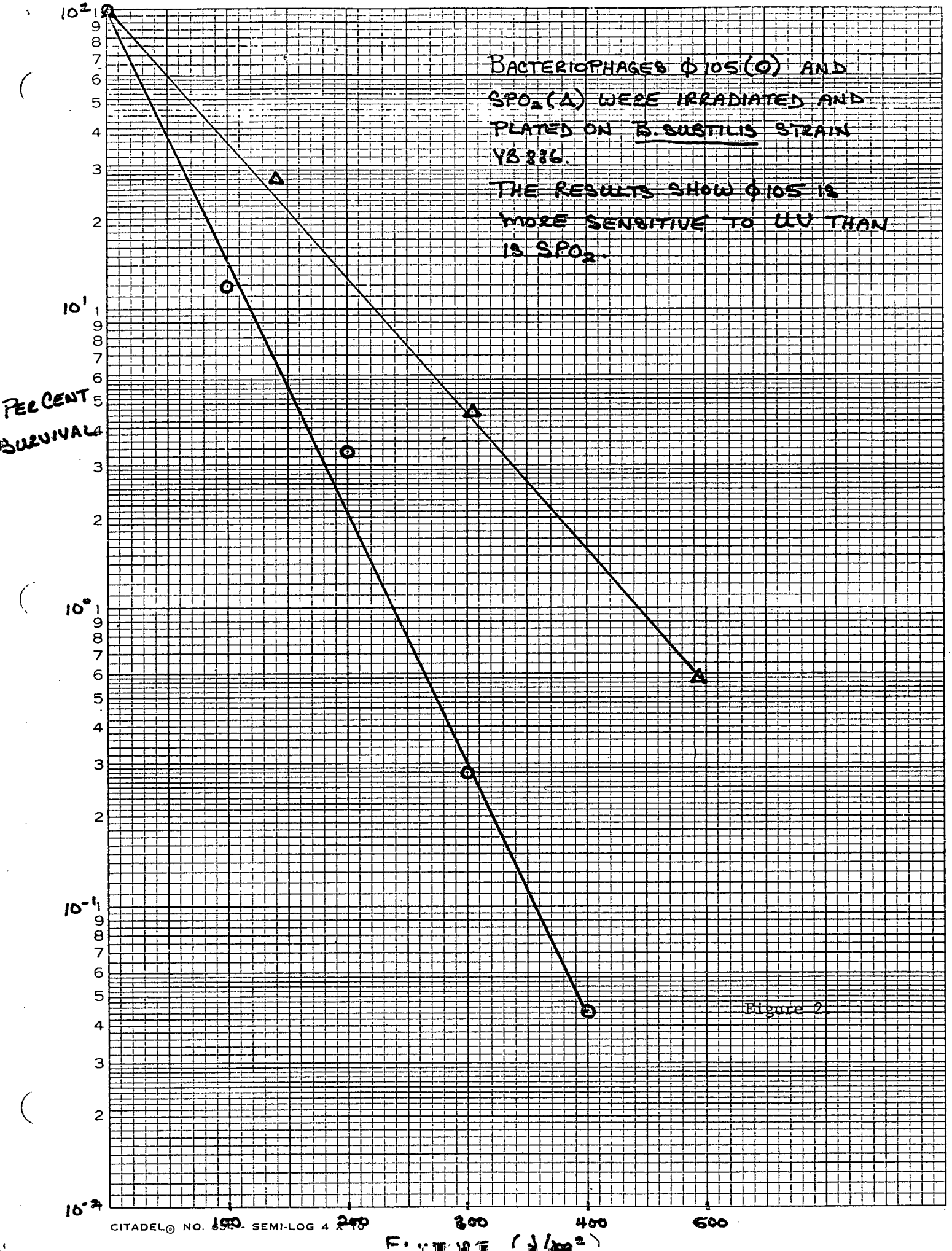

Figure 2.

'W'-REACTIVATION OF BACTERIOPHAGE SPO_2 IMMEDIATELY AFTER BACTERIAL IRRADIATION.

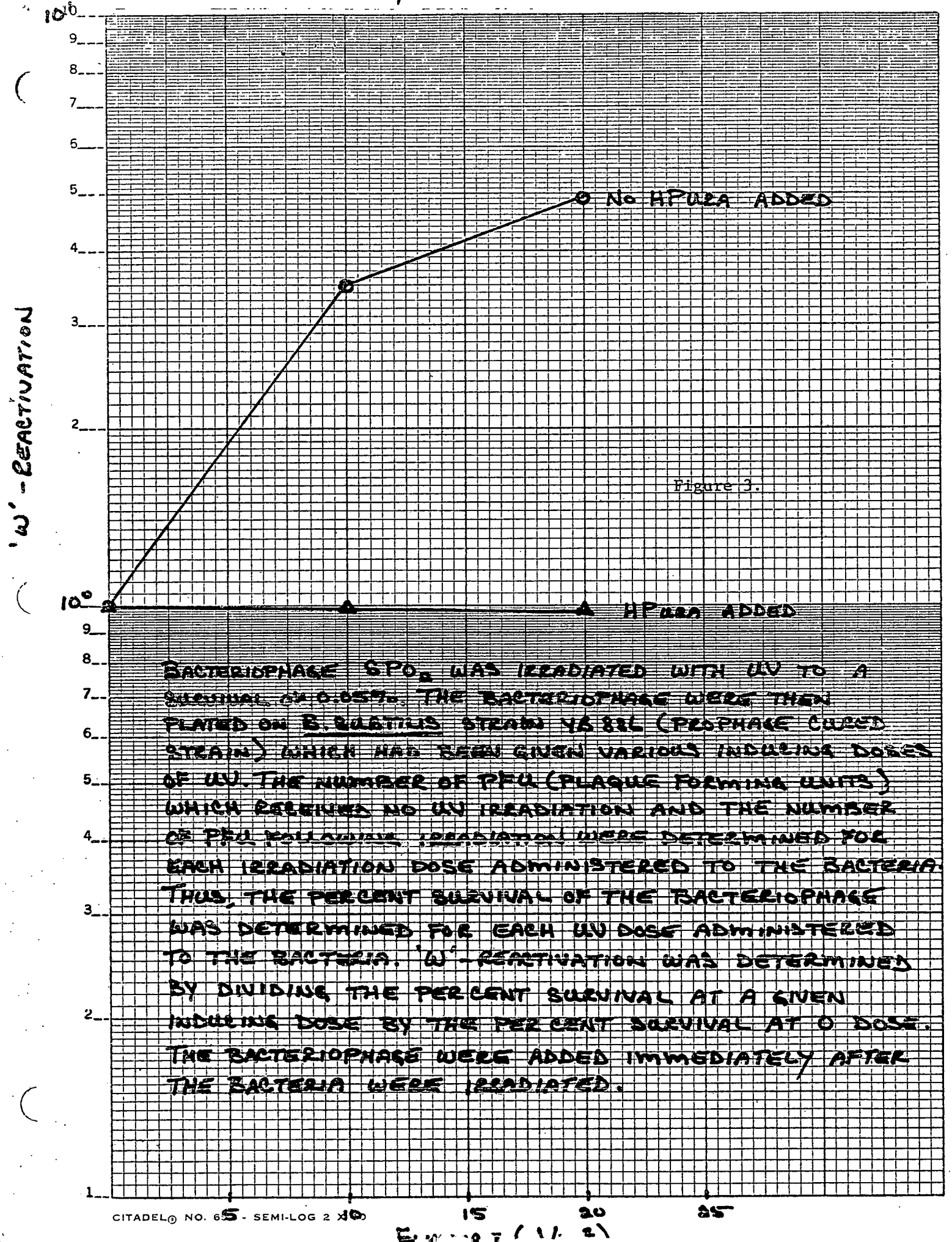

Figure 3.

BACTERIOPHAGE SPO_2 WAS IRRADIATED WITH UV TO A SURVIVAL OF 0.05%. THE BACTERIOPHAGE WERE THEN PLATED ON B. SUBTILIS STRAIN 168 (PROPHAGE CURED STRAIN) WHICH HAD BEEN GIVEN VARIOUS INDUCING DOSES OF UV. THE NUMBER OF PFU (PLAQUE FORMING UNITS) WHICH RECEIVED NO UV IRRADIATION AND THE NUMBER OF PFU FOLLOWING IRRADIATION WERE DETERMINED FOR EACH IRRADIATION DOSE ADMINISTERED TO THE BACTERIA. THUS, THE PERCENT SURVIVAL OF THE BACTERIOPHAGE WAS DETERMINED FOR EACH UV DOSE ADMINISTERED TO THE BACTERIA. 'W'-REACTIVATION WAS DETERMINED BY DIVIDING THE PERCENT SURVIVAL AT A GIVEN INDUCING DOSE BY THE PERCENT SURVIVAL AT 0 DOSE. THE BACTERIOPHAGE WERE ADDED IMMEDIATELY AFTER THE BACTERIA WERE IRRADIATED.

W - REACTIVATION OF BACTERIOPHAGE $SP0_2$ AFTER 30 MINUTE INCUBATION OF IRRADIATED BACTERIA.

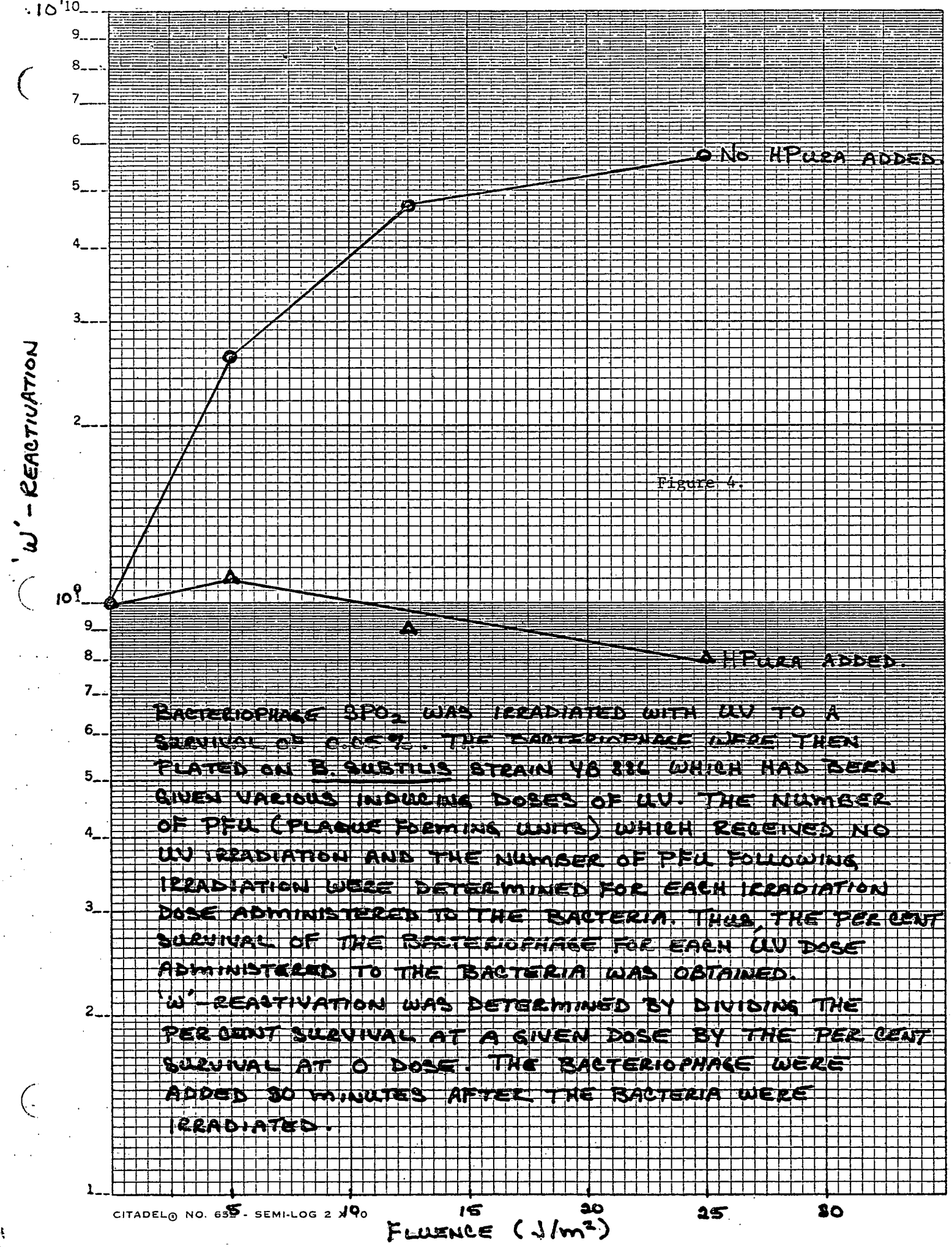

Figure 4.

BACTERIOPHAGE $SP0_2$ WAS IRRADIATED WITH UV TO A SURVIVAL OF 0.05%. THE BACTERIOPHAGE WERE THEN PLATED ON B. SUBTILIS STRAIN YB 116 WHICH HAD BEEN GIVEN VARIOUS INDURING DOSES OF UV. THE NUMBER OF PFU (PLAQUE FORMING UNITS) WHICH RECEIVED NO UV IRRADIATION AND THE NUMBER OF PFU FOLLOWING IRRADIATION WERE DETERMINED FOR EACH IRRADIATION DOSE ADMINISTERED TO THE BACTERIA. THUS THE PERCENT SURVIVAL OF THE BACTERIOPHAGE FOR EACH UV DOSE ADMINISTERED TO THE BACTERIA WAS OBTAINED. 'W'-REACTIVATION WAS DETERMINED BY DIVIDING THE PERCENT SURVIVAL AT A GIVEN DOSE BY THE PERCENT SURVIVAL AT 0 DOSE. THE BACTERIOPHAGE WERE ADDED 30 MINUTES AFTER THE BACTERIA WERE IRRADIATED.

UV SURVIVAL OF MITOMYCIN C RESISTANT STRAINS.

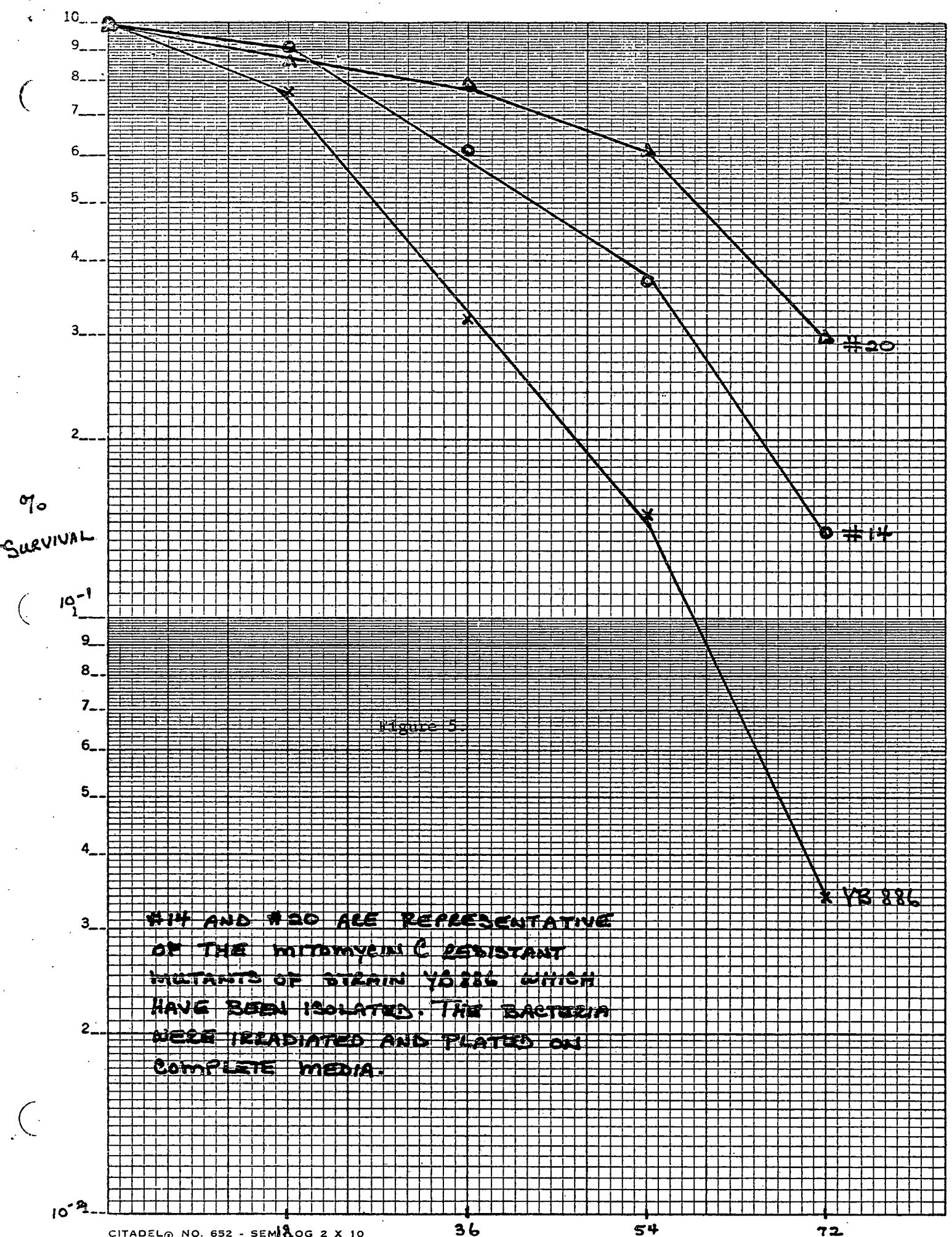

Figure 5.

#14 AND #20 ARE REPRESENTATIVE OF THE MITOMYCIN C RESISTANT MUTANTS OF STRAIN YB886 WHICH HAVE BEEN ISOLATED. THE BACTERIA WERE IRRADIATED AND PLATED ON COMPLETE MEDIA.

CITADEL® NO. 652 - SEMILOG 2 X 10

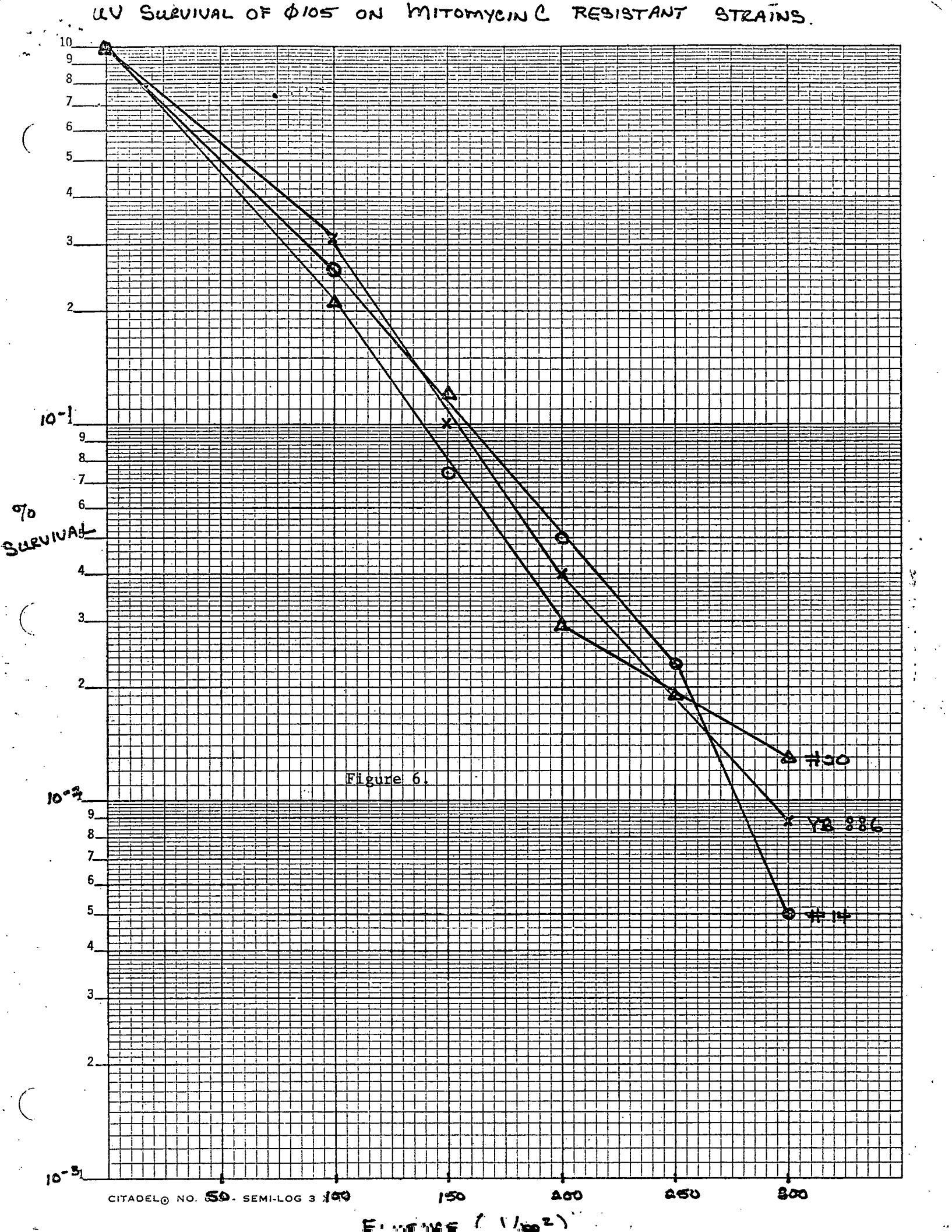
UV SURVIVAL OF ϕ105 ON MITOMYCIN C RESISTANT STRAINS.
10
9
8
7
6
5
4
3
2
10⁻¹
9
8
7
6
%
SURVIVAL
4
3
2
10⁻²
9
8
7
6
5
4
3
2
10⁻³
#30
YB 886
#14
100
150
200
250
300
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Figure 6.