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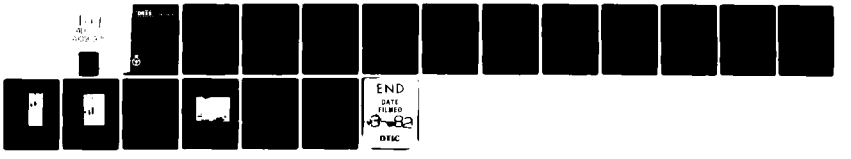

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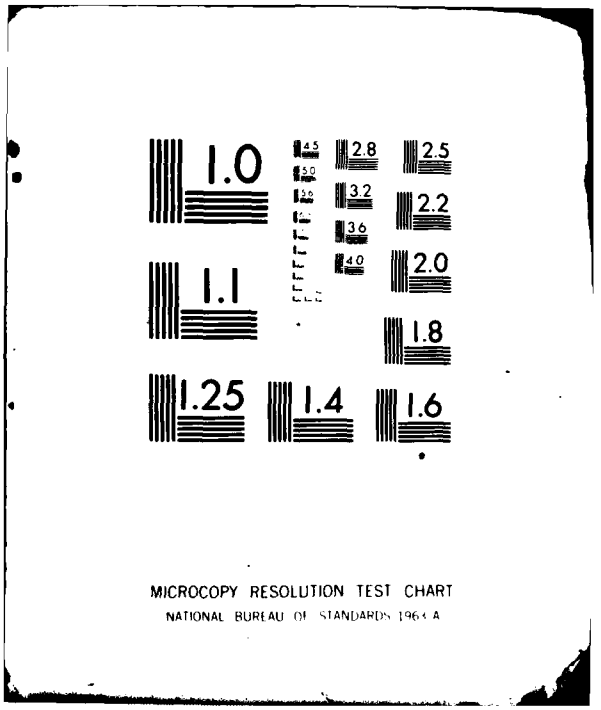
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PARTIAL CHARACTERIZATION OF SMALL PLASMID DEOXYRIBONUCLEIC ACID PRESENT IN *NEISSERIA MENINGITIDIS* (U)

by

A.R. Bhatti, K. O'hara, L.A. White and L.E. Bryan

PCN 16A10

May 1981

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ABSTRACT

The presence of plasmid DNA in *Neisseria meningitidis* was investigated. *N. meningitidis* isolates from clinical meningitis and highly virulent in mice, were found to harbour a small plasmid. Agarose gel electrophoresis of crude lysates from different strains revealed a single plasmid DNA species with the same apparent electrophoretic mobility. Using agarose gel electrophoresis its molecular weight was calculated as 3.4 megadaltons. Electron microscopic observation revealed that plasmid DNA molecules were predominently in the covalently closed form. The estimated contour length of the open circular form was 1.6 μm. The function of the plasmid DNA is presently unknown.

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MILITARY SIGNIFICANCE

The discovery that virulent strains of *N. meningitidis* harbour a plasmid(s) is of great significance to military preventive medicine and biological defence. The possible role of this plasmid in virulence may account for our earlier observations at the Canadian Forces Recruit Schools of lack of correlation between carrier rates and cases of overt meningococcal disease (16). In an epidemiological sense, the fact that important virulence mediating factor(s) may be plasmid-coded could account for the patterns of recent epidemics of meningococcal meningitis in Brazil and Finland. In those outbreaks, there was a rapid increase in overt disease followed by an extended peak and equally abrupt termination of this disease, although *N. meningitidis* of apparently identical antigenic pattern was still present in the community. One can speculate that the plasmid was suddenly lost or some changed environmental factor favoured the maintenance of non-plasmid containing bacteria. Our studies, as yet unpublished, indicate that these plasmids are easily lost on laboratory subculture. Virulence mediating plasmids have also been reported in other bacterial species (4, 17-20), and have been successfully transferred to other strains of the same species (3, 4, 21) or different bacterial species (3, 22, 23) as well. It is apparent, therefore, that increased attention must be paid to the possibility that the development of a new bacterial strain of increased virulence is a distinct possbility.

Comparative studies of plasmid-harbouring and plasmid-less *N. meningitidis* strains will help in elucidating the nature of the factor(s) responsible for the virulence of pathogenic strains of this organism. Such studies will further help in the diagnosis, treatment and control of meningococcal meningitis.

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INTRODUCTION

The presence of extra-chromosomal genetic elements (plasmids) is well documented for the members of Enterobacteriaceae, *Staphylococcus aureus* and several species of Pseudomonas (1-3). In the genus Neisseria, different kinds of plasmids have been reported and characterized from *Neisseria gonorrhoeae* (4), but little is known about the existence of plasmid DNA in *N. meningitidis*.

N. meningitidis continues to be an important etiological agent of bacteremia and meningitis in both the civilian and military population. The organism can be carried in the nasopharynx of apparently healthy individuals and this carrier state may serve as a reservoir of potentially infectious organisms. Lack of immunity usually facilitates the subsequent development of bacteremia which can lead to an acute purulent meningitis. *N. meningitidis* produces several factors, an anti-phagocytic

capsule, lipopolysaccharide and pili, which have been shown to play a role in virulence in the closely-related bacterium, *N. gonorrhoeae* (5, 6). In addition, this organism has been shown to produce different protease activities (7, 8). We have recently reported the presence of plasmid DNA in a virulent strain of *N. meningitidis* (submitted for publication). The use of gel electrophoresis techniques (10) has simplified the detection of plasmids. In this communication, we describe the detection and partial characterization of plasmid DNA present in some virulent strains of *N. meningitidis*.

MATERIALS AND METHODS

Bacterial Strains.

N. meningitidis, strains M1011, DRES 30 and LCDC 604 are clinical disease isolates and DRES 03 is a carrier isolate. *Escherichia coli* K12, SA1306 pro^-, met^-, nal^r (RP4), *Pseudomonas aeruginosa* 130 and *E. coli* strain F-1161 (pAF-6) (kindly supplied by Dr. T. Tamoki, University of Calgary) were used as sources of DNA standards to determine the molecular weight of the plasmid DNA of strain M1011. *N. meningitidis*, strain M1011, was cultivated in Brain Heart Infusion broth (BHI broth) supplemented with 1% (v/v) IsoVitaleX (Baltimore Biological Laboratories, Cockeysville, Md., U.S.A.) for 20 hrs at 35°C in an atmosphere of 5% CO_2 in air (9). Other bacterial strains were grown in BHI broth at 37°C for the same time.

Preparation of Cell Lysates and Agarose Gel Electrophoresis.

Crude cell lysates for plasmid DNA analysis were prepared according to published procedures (10, 11). Agarose gel electrophoresis and detection of plasmid DNA was carried out as described by Hansen and Olsen (10).

Purification of Plasmid DNA.

Purified preparations of plasmid DNA were obtained by CsCl - ethidium bromide - buoyant density centrifugation as described by Grinsted *et al.* (12). Further purification of plasmid DNA was by agarose gel

electrophoresis as described above. Fractions containing plasmid DNA were pooled and dialyzed against the TES buffer (Tris 30 mM, EDTA 5 mM, NaCl 50 mM, pH 8.0) of Meyers *et al.* (11).

Electron Microscopy of Plasmid DNA.

A modification of the aqueous technique described by Davis *et al.* (13) was used for the preparation of plasmid DNA for electron microscope examination as follows:

> A grid with 0.3% formvar was prepared, coated with plasmid DNA, dipped into the cytochrome C solution for 1 to 15 min and stained with 4 times concentrated uranyl acetate solution. The plasmid DNA was examined with a Hitachi S-450 electron microscope operating at an acceleration voltage of 60 kV.

RESULTS AND DISCUSSION

N. meningitidis strain M1011, a clinical isolate has been previously shown to be highly virulent in a mouse model of meningococcal septicemia (Holbein, B.E., Infect. Immun., in press). As some virulence factors are specified by plasmid DNA (1 - 4), an intensive search for such DNA was carried out in *N. meningitidis* strain M1011. Preliminary investigations demonstrated that plasmid DNA was difficult to detect by the commonly used methods of Myers *et al.* (11). It was more readily detected when the plasmid isolation method of Hansen and Olsen (10) was used. Figure 1 shows the results of an agarose gel electrophoresis of plasmid DNA from *N. meningitidis* strain M1011 extracted by the Hansen and Olsen method (10). The plasmid band marked pBO-1 was consistently observed (Fig. 1A). Presence of plasmid DNA was further investigated in other strains of *N. meningitidis*. The results presented in Figure 2 clearly illustrate the presence of plasmid DNA in the virulent strains LCDC 604, M1011 and DRES 30 (Fig. 2; column 1 - 3); whereas, DRES 03 (Fig. 2; column 6), an avirulent strain, did not contain plasmid. These results suggest the presence of plasmid DNA may be a property of virulent strains. Using agarose gel electrophoresis (10) and plasmid DNA standards (Fig. 3) the molecular weight of pBO-1 was calculated to be 3.4

megadaltons. The plasmid DNA from strain M1011 was purified by CsCl - ethidium bromide density centrifugation as described (12). The purified plasmid pBO-1 after agarose gel electrophoresis gave a single DNA species (data not shown) corresponding to that shown in Fig. 1 for the crude cell lysate. Electron microscopic observation revealed that under standard conditions of preparation (13), plasmid pBO-1 was observed in the covalently closed circular form (Fig. 4). However, when the period of cytochrome C treatment was extended to more than 15 min, the open circular plasmid DNA form was obtained. The contour length of plasmid pBO-1 molecules was determined by electron microscopy, and plasmid DNA length with an average value of 1.6 μm was calculated. Using the standard conversion factor of $2.07 \pm 0.04 \times 10^6/\mu m$, the molecular weight was estimated to be 3.3 megadaltons. This value agrees closely with that obtained by agarose gel electrophoresis.

Although plasmid DNA has been detected in *N. gonorrhoea* (4), it has not yet been reported in strains of *N. meningitidis*. Results reported in this study demonstrate the presence of at least one type of plasmid DNA. In some experiments, in addition to pBO-1, two slower moving plasmid DNA bands (not clearly visible in the photograph) were observed after agarose gel electrophoresis (Fig. 1B). These additional bands of plasmid could be larger molecular weight plasmids or conversely might be the open circular and linear form of the 3.4 megadalton plasmid DNA.

The relationship of this plasmid DNA to virulence in *N. meningitidis* strain M1011 remains unknown. Studies are currently in progress comparing nonisogenic virulent and avirulent isolates for the presence of plasmid DNA. As well, we are currently attempting to reduce the virulence of strain M1011 by various techniques. If successful, nonvirulent derivatives and virulent isolates of *N. meningitidis* strain M1011 will be compared for plasmid DNA. At the present time, the function of pBO-1 is cryptic. Cryptic plasmids have been detected in a wide variety of bacteria. Small molecular weight plasmids detected in *N. gonorrhoea* (4) have been shown to code for β-lactamase activity. However, this is not a function of the plasmid in *N. meningitidis*.

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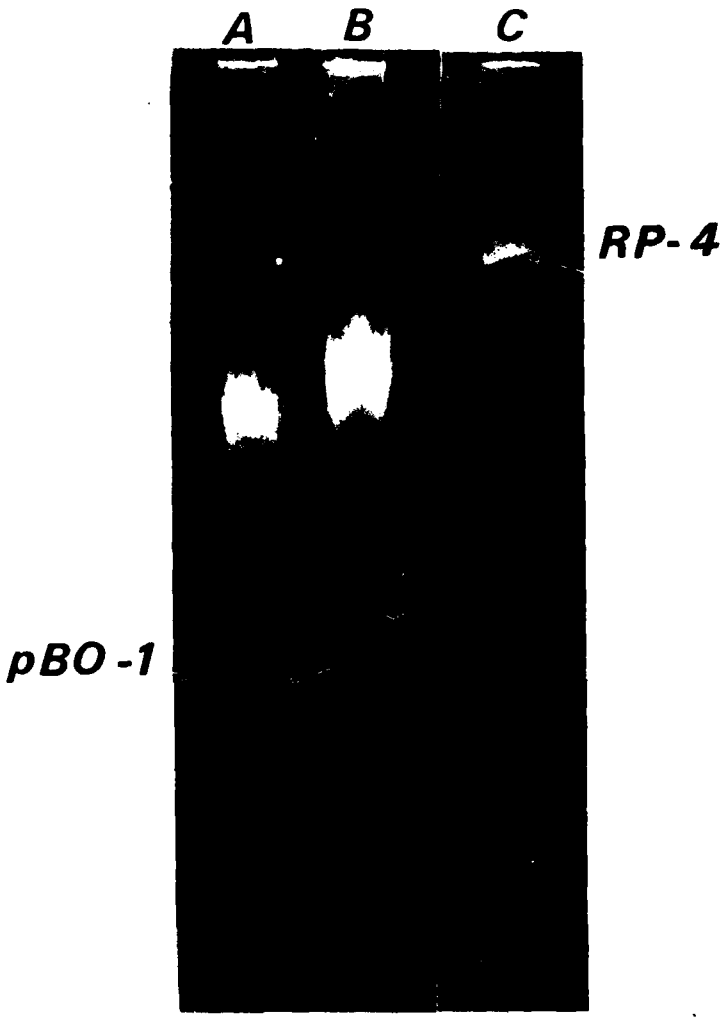

Figure 1. Agarose gel electrophoresis of plasmid DNA from *N. meningitidis* strain M1011.

(A) Ethanol precipitated DNA (10 µL) from cleared lysate of *N. meningitidis* strain M1011.

(B) 20 µL of sample A.

(C) Ethanol precipitated DNA (5 µL) from cleared lysate of *E. coli* 1306 (RP4).

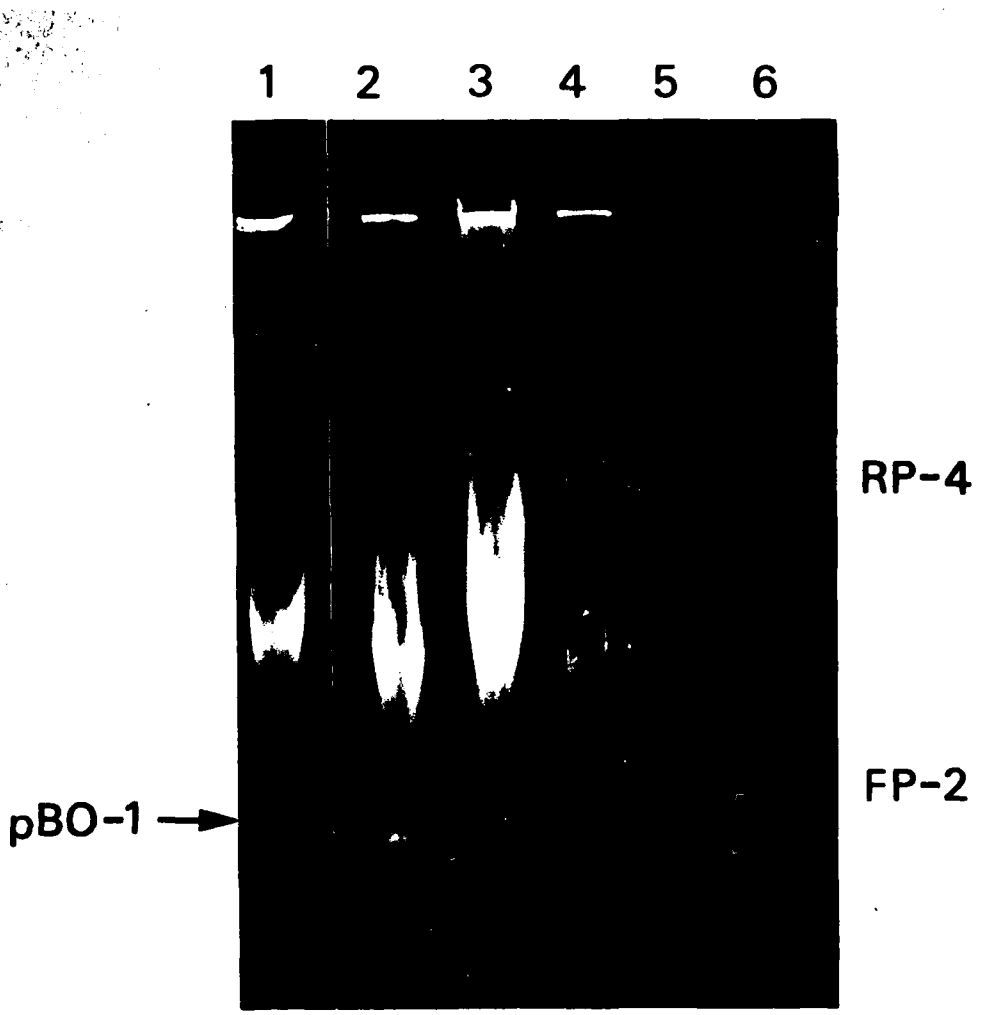

Figure 2. An agarose gel electrophoresis of crude lysate obtained from different strains of *N. meningitidis* and *E. coli*. Columns 1, 2 and 3 represent the presence of plasmid DNA in *N. meningitidis* strains LCDC 604A, M1011, DRES 30 and absence of plasmid DNA in DRES 03, respectively. Whereas, column 4 and 5 gave the results for plasmid DNA present in *E. coli* strains SA 1306 and F-1161 used as for marker plasmid DNA.

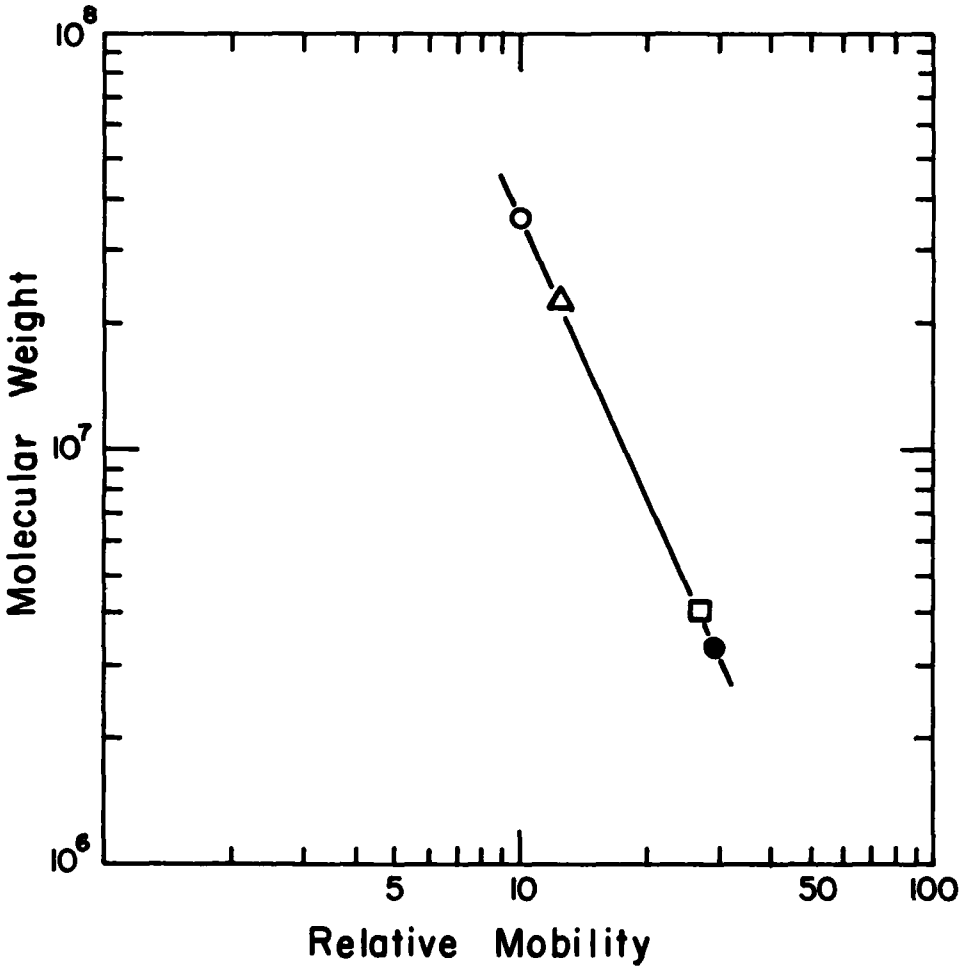

Figure 3. Molecular weight of plasmid DNA from *N. meningitidis* strain M1011. Plasmid RP4 (mol. wt. 36 megadaltons) of *E. coli* (11), R-130a (mol. wt. 23 megadaltons) of *P. aeruginosa* (14) and pAF-6 (mol. wt. 4 megadaltons) of *E. coli* F-1161 (15) were used as standard plasmids DNA for the determination of the mol. wt. of plasmid pBO-1 from *N. meningitidis* strain M1011. Symbols represent ○, RP4; △, R-130A; □, pAF-6 and ●, pBO-1, respectively.

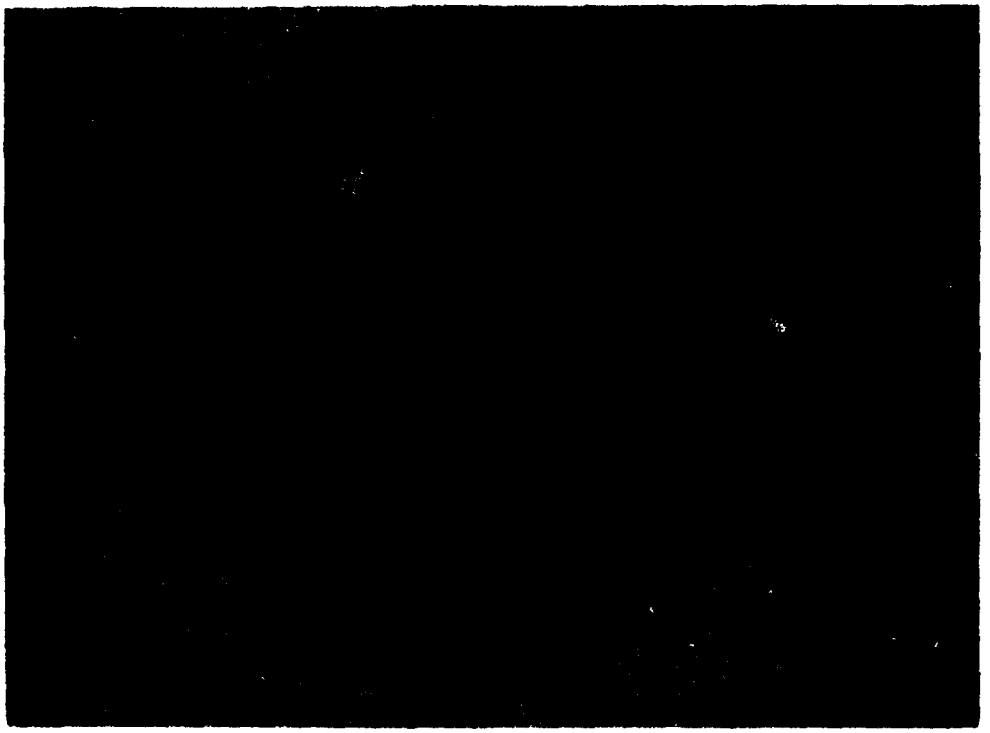

Figure 4. Electron micrograph of covalently closed circular plasmid DNA molecule of *N. meningitidis* M1011. Magnification × 60,400.

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The presence of plasmid DNA in *Neisseria meningitidis* was investigated. *N. meningitidis* isolates from clinical meningitis and highly virulent in mice, were found to harbour a small plasmid. Agarose gel electrophoresis of crude lysates from different strains revealed a single plasmid DNA species with the same apparent electrophoretic mobility. Using agarose gel electrophoresis its molecular weight was calculated as 3.4 megadaltons. Electron microscopic observation revealed that plasmid DNA molecules were predominently in the covalently closed form. The estimated contour length of the open circular form was 1.6 μm. The function of the plasmid DNA is presently unknown.

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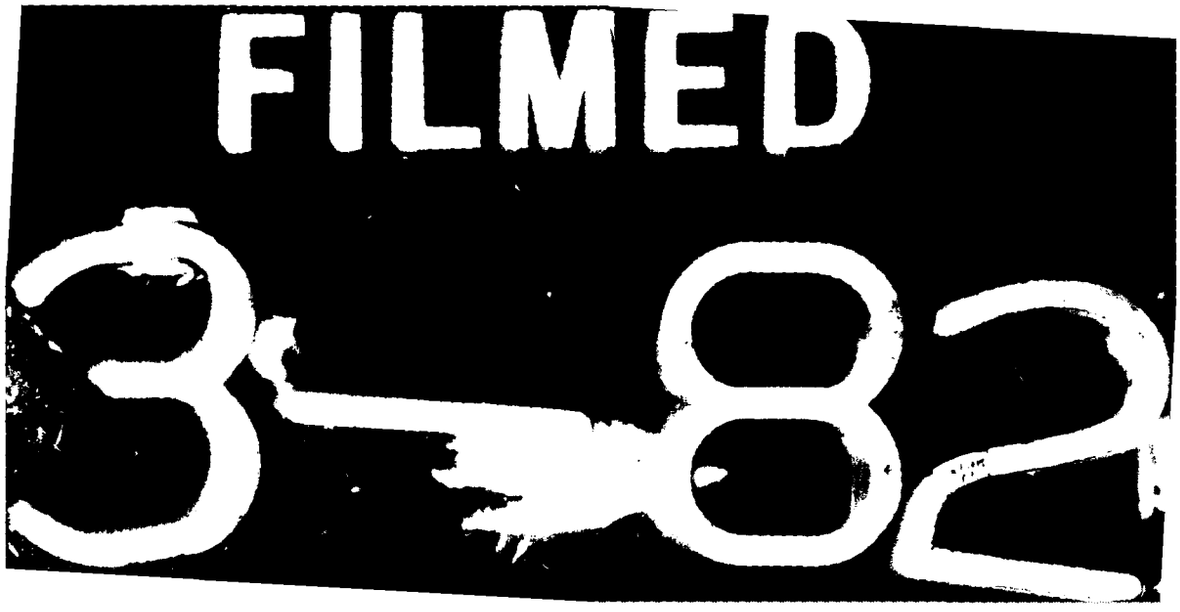
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