HOST-CONTROLLED MODIFICATION OF AN INTERMEDIATE SEX FACTOR IN BACTERIAL CONJUGATION

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ABSTRACT

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by Barry Alan Friedman

Bacterial conjugation was performed via three different methods: millipore, centrifuge, and flask, to determine the efficiency of transfer of a F-lac$^+$ particle by each method and to observe the occurrence of host-controlled modification.

The efficiency of transfer was found to vary with the method as well as with the organisms. The superior method was the millipore method, while the centrifuge and flask methods usually produced similar results.

Restriction was found when Salmonella pullorum was used as both a donor and recipient; a greater restriction was noted in the donor state of interstrain crosses. Escherichia coli BB also donated the F-lac$^+$ without difficulty to other K-12 and BB recipients, but was restricted by S. pullorum.

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By

Barry Alan Friedman

A THESIS

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To

Mom, Dad,

Jan, and Brian

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I wish to thank Dr. Delbert E. Schoenhard for his patience, understanding, and consideration throughout the course of this study. The interest he has shown through continuous discussions have helped to give meaning to this thesis.

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B.A.F.

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INTRODUCTION

The study of host-controlled modification has of late gained momentum due to the emphasis placed on molecular biology. Because both phage and bacterial deoxyribonucleic acid (DNA) have been found to gain new nonheritable properties without altering their observed genetic content, the basis of the phenomenon seems to lie on a molecular level.

Host-controlled modification has two components: modification and restriction. Modification is controlled by the host cell; it acts directly on the DNA and alters the base sequence or more likely certain portions of the base sequence. When the phage or chromosomal material of the bacteria is then transferred to another cell, it can be identified either as "self" or "nonself." If it is identified as "nonself," restriction occurs and the incoming DNA is degraded. If the material is recognized as "self," no breakdown occurs and the DNA can successfully develop within the cell.

The study involving host-controlled modification was undertaken to determine whether these properties might be extended to various strains of Escherichia coli and Salmonella pullorum. F-lac$^+$ was used to test for host-controlled modification and three different methods were employed to analyze the efficiency of transfer.

LITERATURE REVIEW

Conjugation

The process of bacterial conjugation is a unidirectional transfer of genetic material from donor to recipient cells in which contact is required (Hayes, 1964). The discovery of this kind of chromosomal transfer was made by Lederberg and Tatum (1946, a,b).

The donor state of a cell is imparted by genetic elements known alternately as fertility factors, sex factors, or F-factors and these elements are known collectively as F^+ (Hayes, 1953 a, b). The F^+ is one example of an episome (Jacob and Wollman, 1961); it may exist autonomously in the cytoplasm and replicate independently of the chromosome, or it may be integrated on the bacterial chromosome and replicate with it. While in the cytoplasm, replication occurs faster than that of the bacterial chromosome (de Haan and Stouthamer, 1963), but appears to stabilize with time.

The integration of the F^+ on the chromosome gives rise to what is known as high frequency of recombination (Hfr) donor cells (Hayes, 1953 b). Genetic markers transfer at a rate 1000 times greater in the Hfr than in the F^+ state. The Hfr also determines the location of the origin and the resultant order in which the chromosome will transfer

(Jacob and Wollman, 1958). Rarely is the Hfr itself transferred. The F^+, in contrast, promotes its own transfer, but not that of the chromosome. The sex factor is not removed by treatment with acridine dyes (curing) from Hfr strains, but has been removed from strains harboring the F^+ (Hirota, 1960; Watanabe and Fukasawa, 1961).

In addition to the F^+ and Hfr strains of bacteria there exists a third type that is of an intermediate nature (Adelberg and Burns, 1959, 1960). In this case the sex factor is attached to a fragment of the bacterial chromosome--the length varying from one to several markers. The resultant is known as a F-merogenote (Clark and Adelberg, 1962) or F-prime (F'), and transfer has been referred to as sexduction, F-duction (Jacob and Wollman, 1961), or F-mediated transduction.

The F-prime fragment is similar to the F^+ in that it behaves as an episome. The F-prime may recombine with the bacterial chromosome if homology is present; otherwise it will only multiply in the cytoplasm.

Host-controlled Modification

Host-controlled modification was discovered early in the 1950's when it was found that certain phages could gain new nonheritable properties without altering their genetic content when passed through a host bacterial strain (Luria and Human, 1952; Bertani and Weigle, 1953). Subsequent passage in the same strain resulted in only a minor, if any,

decrease in efficiency of plating. However, passage into a second host strain resulted in symmetry or asymmetry. Symmetry refers to the restriction of a phage propagated in one strain from multiplying in a second, and those phage propagated in the second from multiplying in the first. Asymmetry refers to the restriction of a phage propagated in one strain from multiplying in a second, but phage propagated in the second are capable of multiplying in both. In neither case must the results be quantitative (Arber and Dussoix, 1962).

Work by Arber and Dussoix (1962) has shown that DNA carries the host specificity. Bacteria infected with labelled, restricted phage were found to degrade the phage as observed by the appearance of radioactive breakdown products. Experiments conducted with conserved, semiconserved, and newly synthesized DNA gave evidence that only the newly sythesized DNA of the phage carried no host specificity for its former bacterial host.

Host-controlled modification appears to be under genetic control. Bacterial mutants have been isolated that while no longer restrictive (r^-), still carry out modification (m^+); others have been isolated that do not restrict or modify (r^- m^-). Both types are in contrast to the wild type (r^+ m^+) (Glover et al., 1963). Some have been found that show intermediate modification activity. These include

temperature-sensitive mutants giving good modification at low temperatures but little or no modification at high temperatures, and streptomycin mutants (Lederberg, 1957) that affect both restriction and modification.

The role of methylation is being investigated as the biological mechanism for host-controlled modification. Arber (1965) deprived methionine-requiring auxotrophs of E. coli K-12 of methionine while the vegetative phage were being replicated. Methionine was then added to permit phage synthesis and maturation. The early, mature phage were found lacking in host specificity. Klein and Sauerbier (1965) found that host-controlled modification of T1 DNA by lysogenic host bacteria involves methylation of the DNA which can be suppressed by simultaneous infection with T3.

Direct evidence is lacking for host-controlled modification at the present time. Gold and Hurwitz (1964 a, b) have isolated a number of methylating enzymes and have measured the uptake of labelled methyl groups using both enzymes and DNA from the same and different strains. Ledinko (1964) found that phage lambda contained equal amounts of 5-methylcytosine when propagated in strains of E. coli B, C, K, or K(P1). Thus, if host-controlled modification involves methylation, it appears to be determined by only a fraction of the bases methylated, presumably in a few specific sequences (Stacey, 1965).

Direct evidence has been obtained with the T-even phages regarding the role of uridine diphosphoglucose (UDPG). Phage released from mutants of salmonellae and E. coli B/4 deficient for the capacity to synthesize UDPG were found to be restricted in E. coli B, but not in shigellae. These experiments suggest the presence of a nuclease in E. coli B, but absence in shigellae which can prevent the development of phage not carrying the prescribed amount of glucose.

Host-controlled modification may be demonstrated via conjugation involving chromosomal DNA, as well as F^+ and F-prime episomes. With chromosomal DNA the linkage between chromosomal markers was found to be reduced in restrictive crosses (Boyer, 1964; Pittard, 1964; Colson et al., 1965; Hoekstra and de Haan, 1965). They also reported that the locus of restriction was closely linked to the threonine locus in E. coli K-12 (Boyer, 1964; Pittard, 1964; Colson et al., 1965) and E. coli B (Hoekstra and de Haan, 1965). The results of restriction have also been reported with F-gal (Hoekstra and de Haan, 1965), F-lac, F^+ (Boyer, 1964), and RTF (Arber and Morse, 1965)--the efficiency depending upon the system and episome utilized.

The passage of bacterial DNA may be hindered by inefficient copulation. It may also be hampered by the prevailing physiological conditions. In addition, nonhomology of donor and recipient DNA may be a reason for unsuccessful

exchange of genetic material between bacterial strains which are not closely related. However, transfer of episomes, such as F-prime, which can express themselves without integration into the chromosome should not be greatly affected by nonhomology, thus leaving the task to host-controlled modification (Arber and Morse, 1965).

MATERIAL AND METHODS

Cultures

The bacterial strains utilized and those genetic characteristics which are pertinent to this study are listed in Table I. Table II lists all variations of the above strains and includes the mode of production.

Media

The media used during the course of this study are listed in Table III.

Triphenyl tetrazolium chloride agar (TTZ agar), a medium discovered by Lederberg (1948), was prepared by adding 23 g of nutrient agar (Difco) to 1000 ml of water and steaming until dissolved. To this was then added 50 mg/liter of 2,3,5 -triphenyl-2H-tetrazolium chloride (Eastman Organic Chemicals) and 1.0% (10 g) lactose (Pfanstiehl). The medium was autoclaved at 121 C for 15 minutes and then supplemented with dihydro-streptomycin sulfate (Squibb) to give a final concentration of 200 µg /ml.

Triphenyl tetrazolium chloride synthetic agar was prepared in the same manner as a modification of eosin methylene blue synthetic agar (Lederberg, 1950)--the dye constituting the only change. Supplements for the growth of *E. coli* AB266 were also added.

TABLE 1.--Bacterial strains.*

Strain		Mating type	Auxotrophic characters	Carbohydrate utilization					Ind	Mot	Response to str
				lac	gal	ara	xyl	mtl			
Escherichia coli	AB785	F-lac$^+$	thi$^-$	+	+	+	+	+	+	+	s
	AB113	F$^-$	his$^-$ leu$^-$ met$^-$ thi$^-$ thr$^-$	-	-	+	-	-	+	+	r
	AB266	F$^-$	leu$^-$ pro$^-$ thi$^-$ thr$^-$	-	-	-	-	-	+	+	r
	BB	F$^-$		+					+	+	s
Salmonella pullorum	35	F$^-$	cys$^-$ leu$^-$	-	+	+	+	+	-	-	s

*Abbreviations and symbols: cys = cysteine; his = histidine; leu = leucine; met = methionine; pro = proline; thi = thiamine; thr = threonine; ara = arabinose; gal = galactose; lac = lactose; mtl = mannitol; xyl = xylose; ind = indole production; mot = motility; str = streptomycin; + = utilized or produced; - = not utilized or produced; s = sensitive; r = resistant.

TABLE 2.--Variations of strains and mode of production.

Strain		Variation	Mode of production
Escherichia coli	AB113	F-lac$^+$	Conjugation
	AB266	F-lac$^+$	Conjugation
	BB-1	lac$^-$	Spontaneous or UV*
	BB-2	str^r	UV
	BB-3	F-lac$^+$ str^r	UV + conjugation
	BB-4	F-lac$^+$ str^s	UV + conjugation
Salmonella pullorum	35-1	F-lac$^+$	Conjugation
	35-2	str^r	UV
	35-3	F-lac$^+$ str^r	UV + conjugation

*UV = ultraviolet light.

TABLE 3.--Media used and purpose of each.*

Medium	Source	Purpose	Supplements
Levine's EMB Agar (EMB-lac)	Difco	Detection of recombinants: lactose utilization	0.8% casamino acids
Nutrient Agar	Difco	Growth medium	
Nutrient Soft Agar	Difco	Growth medium for conjugation on millipore filter	0.7% Bacto-Agar (Difco) 0.5% NaCl
Penassay Broth	Difco	Growth medium	
Phenol Red Broth Base (PR)	Difco	Detection of recombinants: lactose utilization	1.0% carbohydrate 1.5% Bacto-Agar (Difco)
SIM	Difco	Differential medium	
Triphenyl Tetrazolium Chloride Agar (TTZ)		Detection of recombinants: lactose utilization	
Triphenyl Tetrazolium Chloride Synthetic Agar (TTZ Syn)		Selective medium Detection of recombinants: lactose utilization	amino acids and vitamins as required

*Streptomycin was added as necessary to the above media to give a concentration of 200µg/ml.

Sodium Succinate	5.0 g
NaCl	1.0 g
$(NH_4)_2SO_4$	5.0 g
K_2HPO_4	2.0 g
Lactose (Pfanstiehl)	10.0 g
Bacto-agar (Difco)	15.0 g
2,3,5-triphenyl-2H-tetrazolium chloride	50.0 mg
Distilled water	1000.0 ml

The amino acid supplments were added to give a final concentration of 20 µg/ml; they include leucine, proline, and threonine. Thiamine HCl was added to give a final concentration of 5 µg/ml.

Reagents

The production of indole was detected in SIM agar stabs after 24 hours of incubation by overlaying the medium with 0.5 ml chloroform followed by 0.5 ml Kovac's reagent. A deep red hue occurred in the chloroform layer when indole was present.

Kovac's Reagent

Amyl alcohol	75.0 ml
Hydrochloric acid (12 N)	25.0 ml
p-Dimethylaminobenzaldehyde	5.0 g

Mutation Procedure

E. coli BB, taken from a nutrient agar slant, was inoculated into 10 ml of penassay broth (Difco) and incubated at 37 C until the culture was in the logarithmic phase (10 hours). One ml of the culture was then transferred to 9 ml of fresh medium and incubated for an additional 2 hours. After the 2 hour period the cells were pelleted by centrifugation and resuspended to a concentration of approximately 1 x 10^8 cells/ml in penassay broth. Five ml were removed and irradiated for 80 seconds at a distance of 13 3/4" in a Petri dish that was placed upon a Mag-Mix (Precision Scientific) and rotated with a magnetic stirrer. This irradiation produced a 99% kill. A 30 watt, 35 inch long General Electric Germicidal Lamp (G 30T8) was employed for the purpose of irradiation. The bacteria were then incubated in the dark for 6 hours.

After the 6 hour incubation period, the culture was plated either on nutrient agar or nutrient agar gradient plates supplemented with 200 µg/ml streptomycin. Two to eleven colonies were found on each plate. Selected colonies were subcultured four times on TTZ-str to confirm the resistance to streptomycin and to determine if the ultraviolet treatment had affected the lactose phenotype.

Mating Procedure

Three different methods were utilized to test for host-controlled modification. In each case the bacteria were

taken from a nutrient agar slant, inoculated into 10 ml of penassay broth, and grown until in the logarithmic phase, i.e. 11 hours for E. coli and 10 hours for S. pullorum. A 10 ml amount of each suspension was added to 90 ml of penassay broth and incubated for an additional 2 hours (E. coli) or 3 hours (S. pullorum) to insure logarithmic growth. The cells were then spun on a centrifuge (Servall) at 12,100 x g for 15 minutes and resuspended in penassay broth to give 10 males to 1 female or approximately 1 x 10^9 males/ml to 1 x 10^8 females/ml (Echols, 1963). (See individual tables and graphs for exact ratios as well as variations in procedure).

Flask Method--The cell suspensions were prewarmed for 10 minutes with a temperature block (Chemical Rubber Co.) at 37 C and 4 ml of each were placed in a prewarmed 125 ml Erlenmeyer flask. The flask was rotated for 5 minutes to insure maximum contact and then allowed to remain motionless until each sample was drawn.

Samples were drawn at 15, 30, 60, and 120 minutes. One ml was added to a 20 x 150 mm test tube and agitated on a Vortex Junior (Scientific Industries, Inc.) mixer for one minute (Pittard and Adelberg, 1964). Immediately following this, the sample was diluted 10^4 and placed in an ice bath until usage. Dilutions were then prepared to give 100 to 1000 colonies per plate and were plated on a medium to counter select and/or differentiate male and female colonies.

Centrifuge Method--The cell suspensions were warmed for 10 minutes on a temperature block at 37 C and 4 ml of each were placed in prewarmed centrifuge tubes. The tubes were placed in a centrifuge equilibrated at 37 C and spun for a period of 5 minutes at 12,100 x g. The total run required 16 1/2 minutes; the raising of the powerstat from a setting of 0 to 50 required 105 seconds. The cells were agitated on a Vortex Junior for 2 minutes, diluted 10^4, and placed in ice until usage. The remaining procedure followed as above (flask method).

Millipore Method--The cell suspensions were not prewarmed, but placed in ice until usage. One ml of the male and one ml of the female were placed upon a 0.45 μHA millipore filter (Millipore Filter Corp.) without a supporting pad and rotated gently for 60 seconds. Vacuum was then applied, impinging the bacteria to the millipore (Matney and Achenbach, 1962). The filters were then transferred to prewarmed nutrient soft agar and placed at 37 C for the desired period of time (15, 30, 60, or 120 minutes). Zero time did not begin until the filters were on the agar. The samples were then either removed immediately, placed in a 50 ml beaker containing 10 ml of saline, and diluted 10^5 before placing in an ice bath, or transferred to a cold nutrient soft agar plate and placed in the refrigerator until usage. The remaining procedure followed as above (flask method).

RESULTS

Three methods were utilized to examine the frequency of transfer of the F-lac$^+$ by conjugation. In each case frequency of transfer and host-controlled modification were specifically sought.

In each series of crosses *E. coli* AB785 F-lac$^+$ served as the initial donor of the genetic material. Thereafter, the infected recipients were used as donors in their respective crosses.

Series I

In the first series of crosses *E. coli* AB785 F-lac$^+$ was mated with a homologous *E. coli* K-12 recipient (AB113 lac$^-$). *E. coli* AB113 F-lac$^+$ was then mated with *E. coli* AB266 lac$^-$. These tranfers were initiated to fulfill the requirements governing host modification and restriction and also to serve as a control for subsequent experiments.

The following graphs (Figures 2 and 3) indicate the rate of transfer of genetic material during a two hour period. It should be noted that the frequency of transfer with the millipore method was much greater than with either the centrifuge or flask method. In addition restriction occurred in the *E. coli* AB113 F-lac$^+$ X *E. coli* AB266 lac$^-$ cross as observed by the low frequency of transfer. Table IV lists the frequency of transfer and Figure 1 summarizes the indicated crosses.

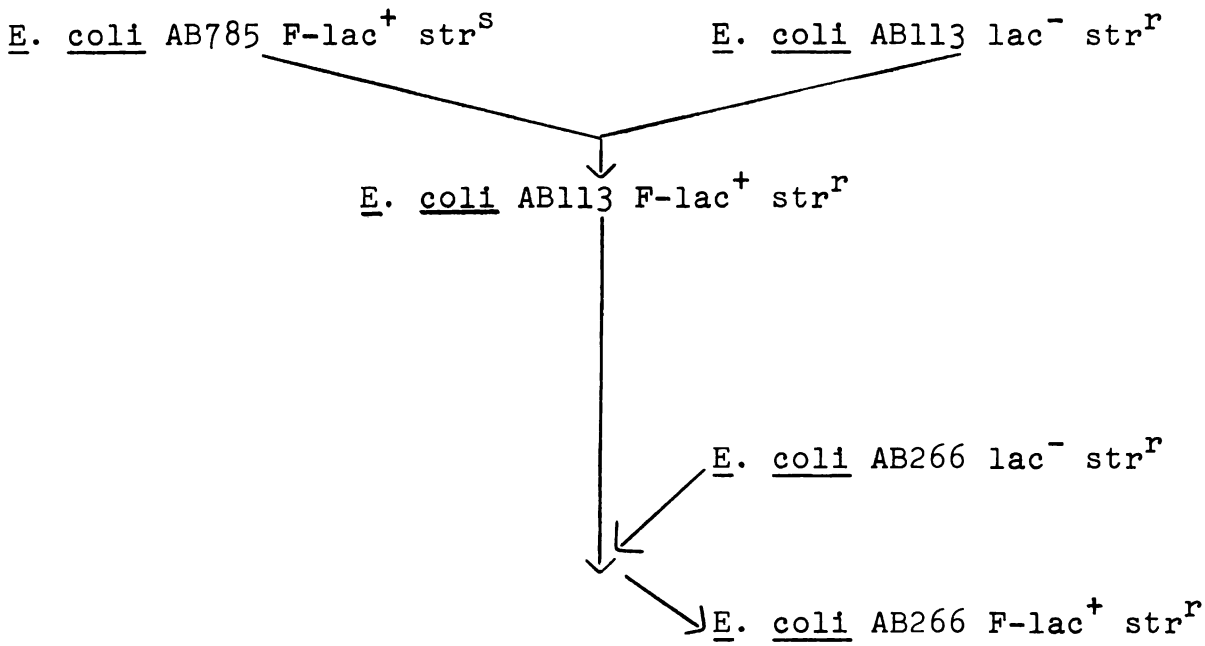

Figure 1.--Summary of the transfer of F-lac$^+$ in the control crosses of Series I.

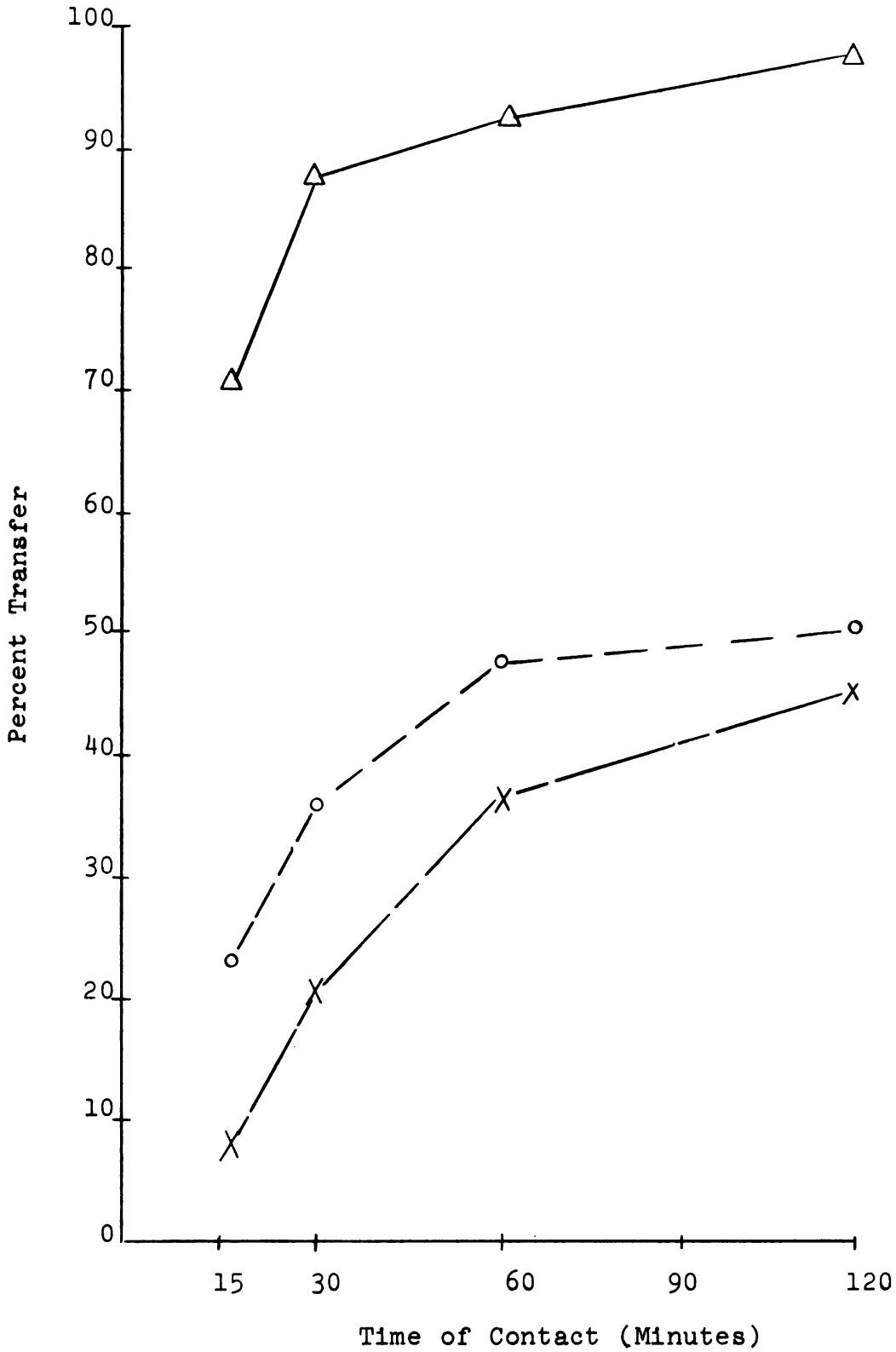

Figure 2.--Frequency of transfer of F-lac$^+$ from E. coli AB785 F-lac$^+$ to E. coli AB113 lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

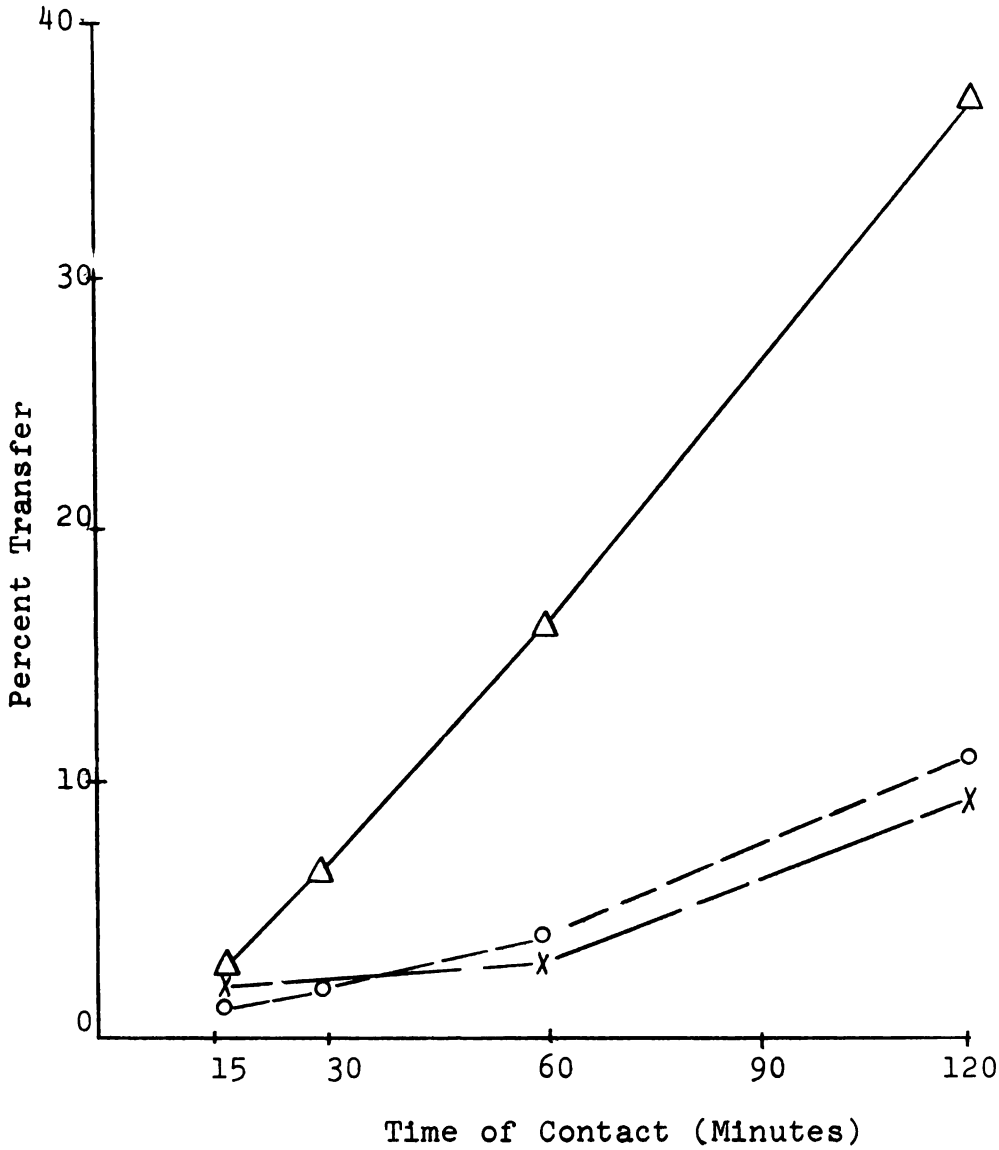

Figure 3.--Frequency of transfer of F-lac$^+$ from E. coli AB113 F-lac$^+$ to E. coli AB266 lac$^-$ via milli-pore (Δ), centrifuge (X), and flask (o) methods.

TABLE 4.--Frequency of transfer of F-lac$^+$ with restricting and nonrestricting recipients.*

Cross	Method	After 60 Minutes		
		Recipient	Recombinant	Frequency
785 x 113	Millipore	2.3×10^8	2.1×10^8	9.1×10^{-1}
	Centrifuge	1.3×10^8	4.7×10^7	3.6×10^{-1}
	Flask	1.9×10^8	8.9×10^7	4.7×10^{-1}
113 x 266	Millipore	2.0×10^8	3.4×10^7	1.7×10^{-1}
	Centrifuge	8.9×10^7	2.0×10^6	2.2×10^{-2}
	Flask	2.7×10^8	8.0×10^6	3.0×10^{-2}

*The donors were mixed with the recipients in a ratio of approximately 10 to 1 to give a total cell concentration of about 10^9 per ml. Variations in the ratio from 5-15 to 1 did not markedly affect the frequency of transfer.

Series II

In the second series of crosses E. coli AB785 F-lac$^+$ was mated with E. coli BB lac$^-$. The initial mating indicated the presence of restriction when compared with the control matings of Series I. However, passage of the F-lac$^+$ then proceeded quite readily from E. coli BB F-lac$^+$ to E. coli BB lac$^-$ as would be assumed by host modification. Subsequent passage into E. coli AB113 lac$^-$ also gave results that indicated little, if any, restriction. Again in these crosses, the millipore method produced better results than either the filter or centrifuge method (Figures 5, 6, and 7).

During the production of streptomycin resistant mutants, two types of E. coli BB lac$^-$ str^r mutants were detected. One mutant, designated as "high" received donor material with a frequency five times greater than another mutant labelled as "low" when employing the centrifuge method. All the experiments reported were performed with the "high" mutant.

A contradiction to the above occurred when E. coli BB F-lac$^+$ was mated with S. pullorum 35 lac$^-$ (Figure 8). An increase of transfer to 21% at 120 minutes occurred via the centrifuge method; however, as noted by the graph, a quick rise and then a sharp decline was observed when using the millipore method.

Table V lists the frequency of transfer and Figure 4 summarizes the above crosses.

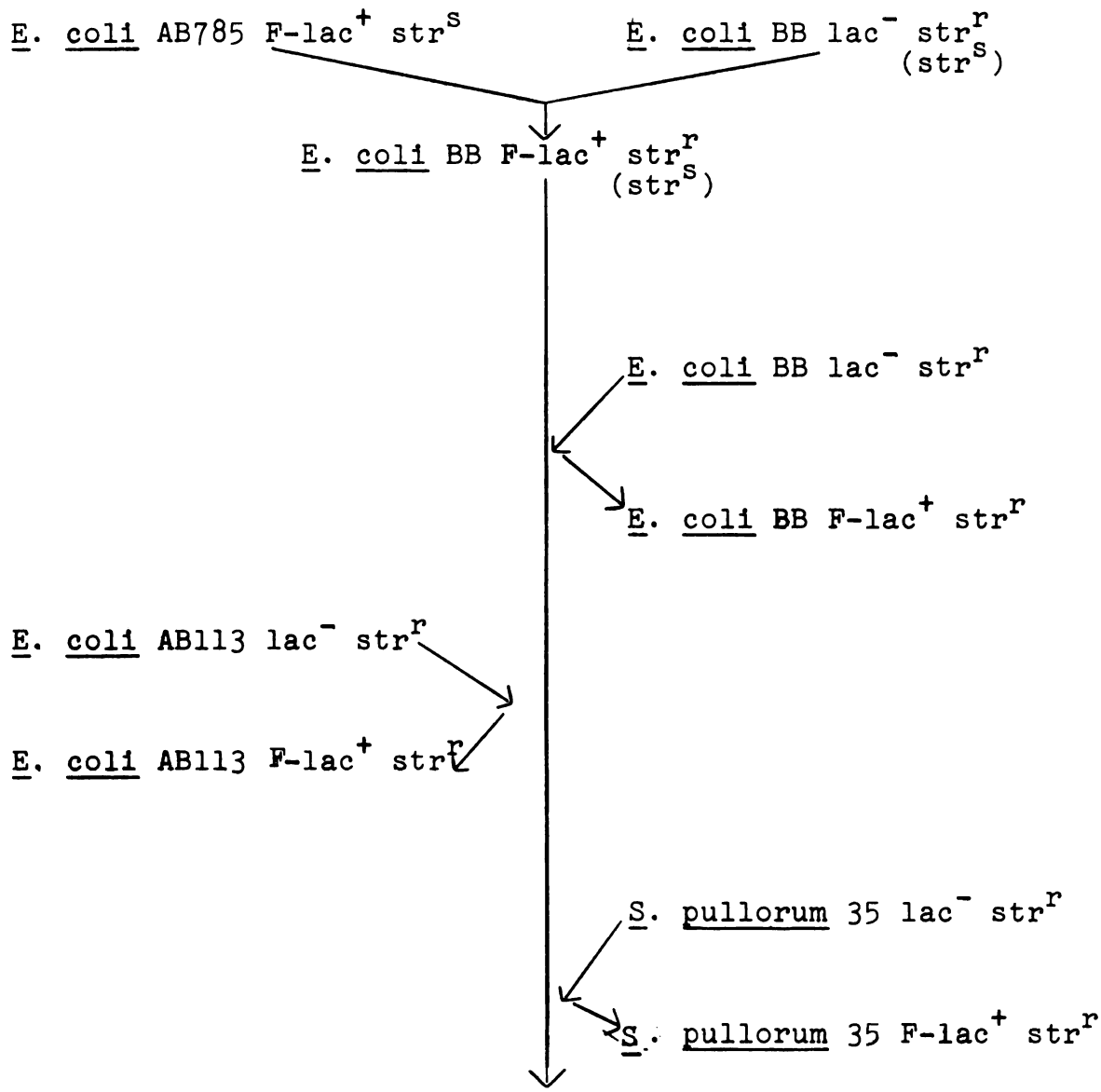

Figure 4.--Summary of the transfer of F-lac$^+$ in Series II.

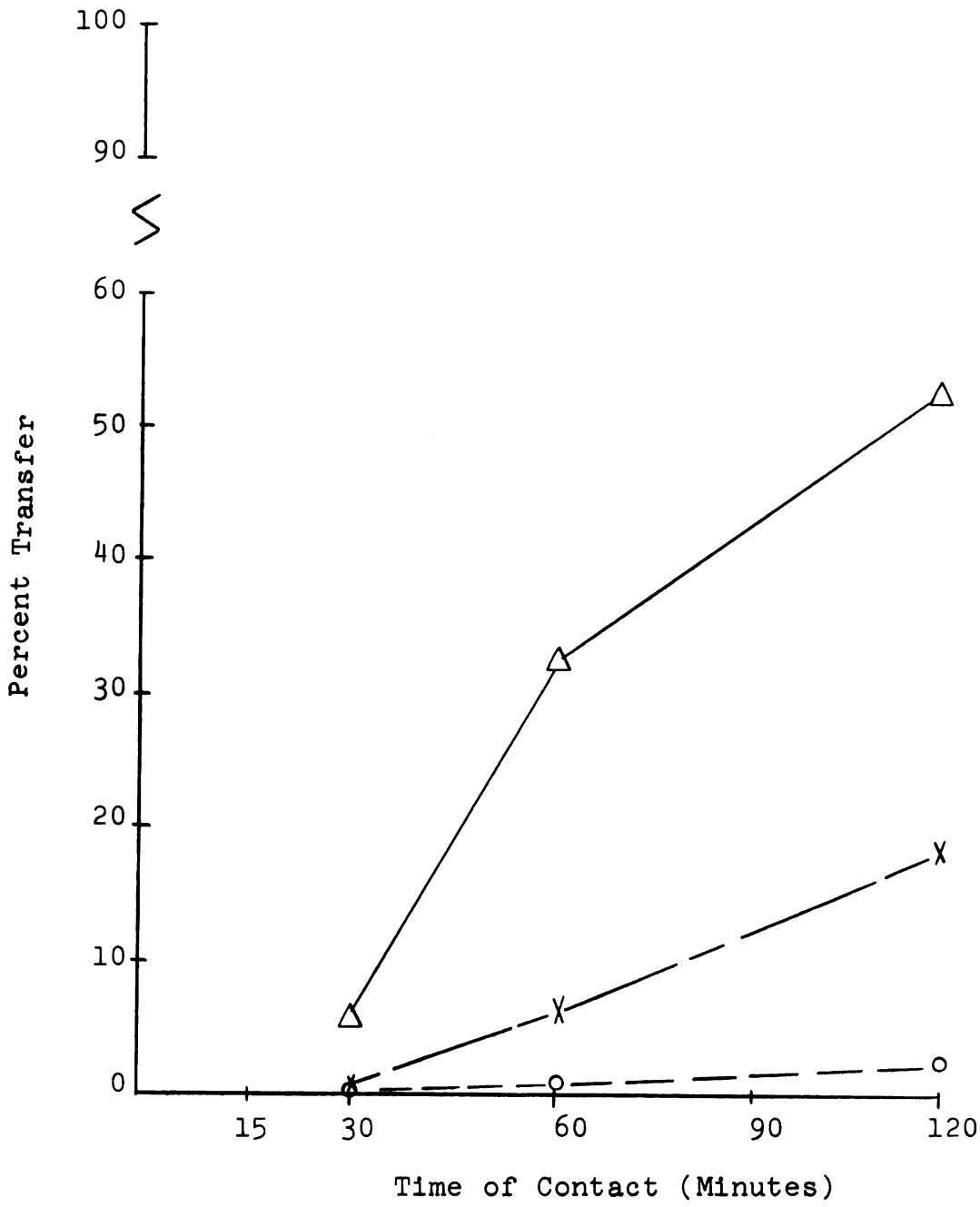

Figure 5.--Frequency of transfer of F-lac$^+$ from E. coli AB785 F-lac$^+$ to E. coli BB lac- via millipore (Δ), centrifuge (X), and flask (o) methods.

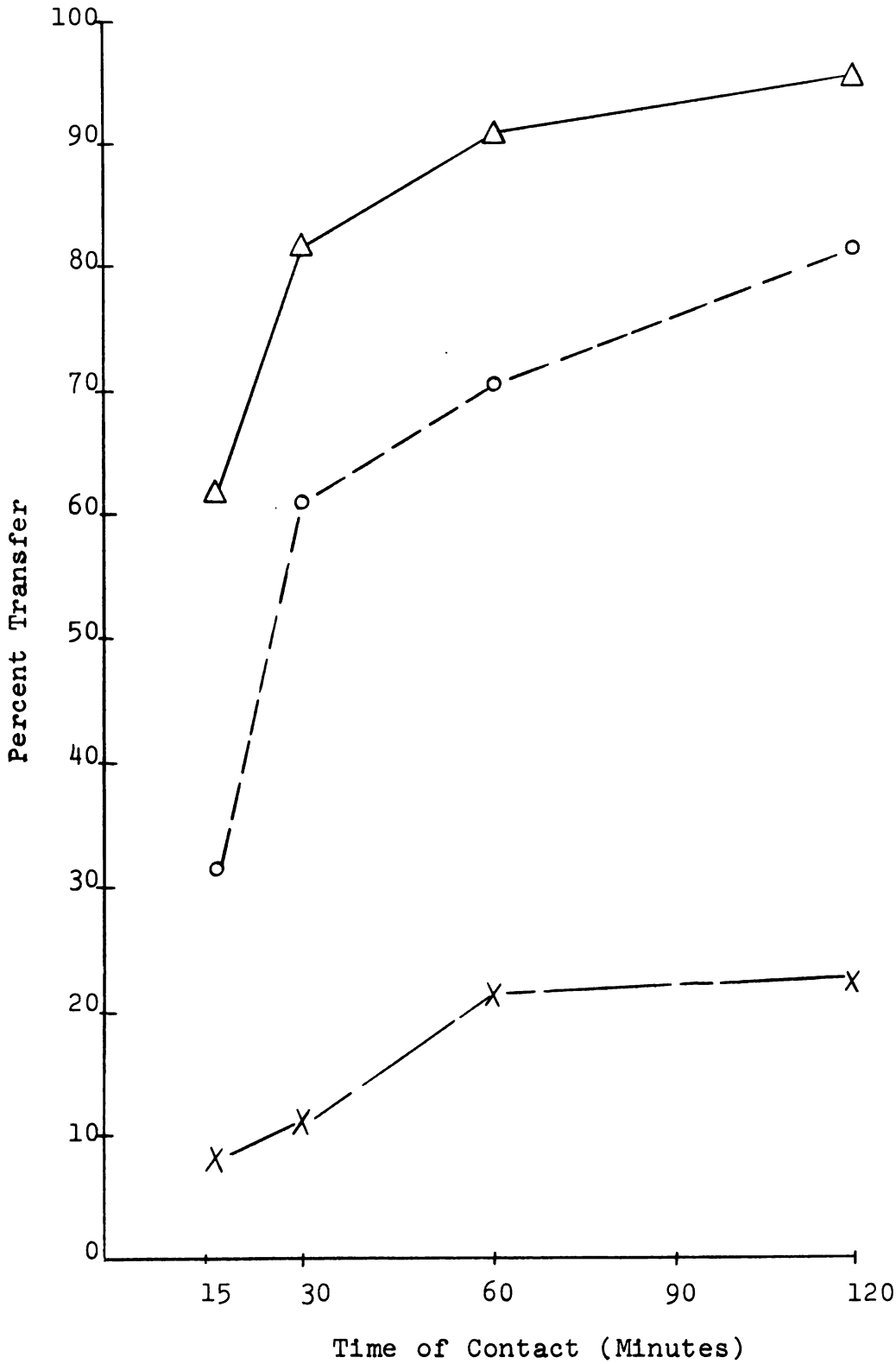

Figure 6.--Frequency of transfer of F-lac$^+$ from E. coli BB F-lac$^+$ to E. coli BB lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

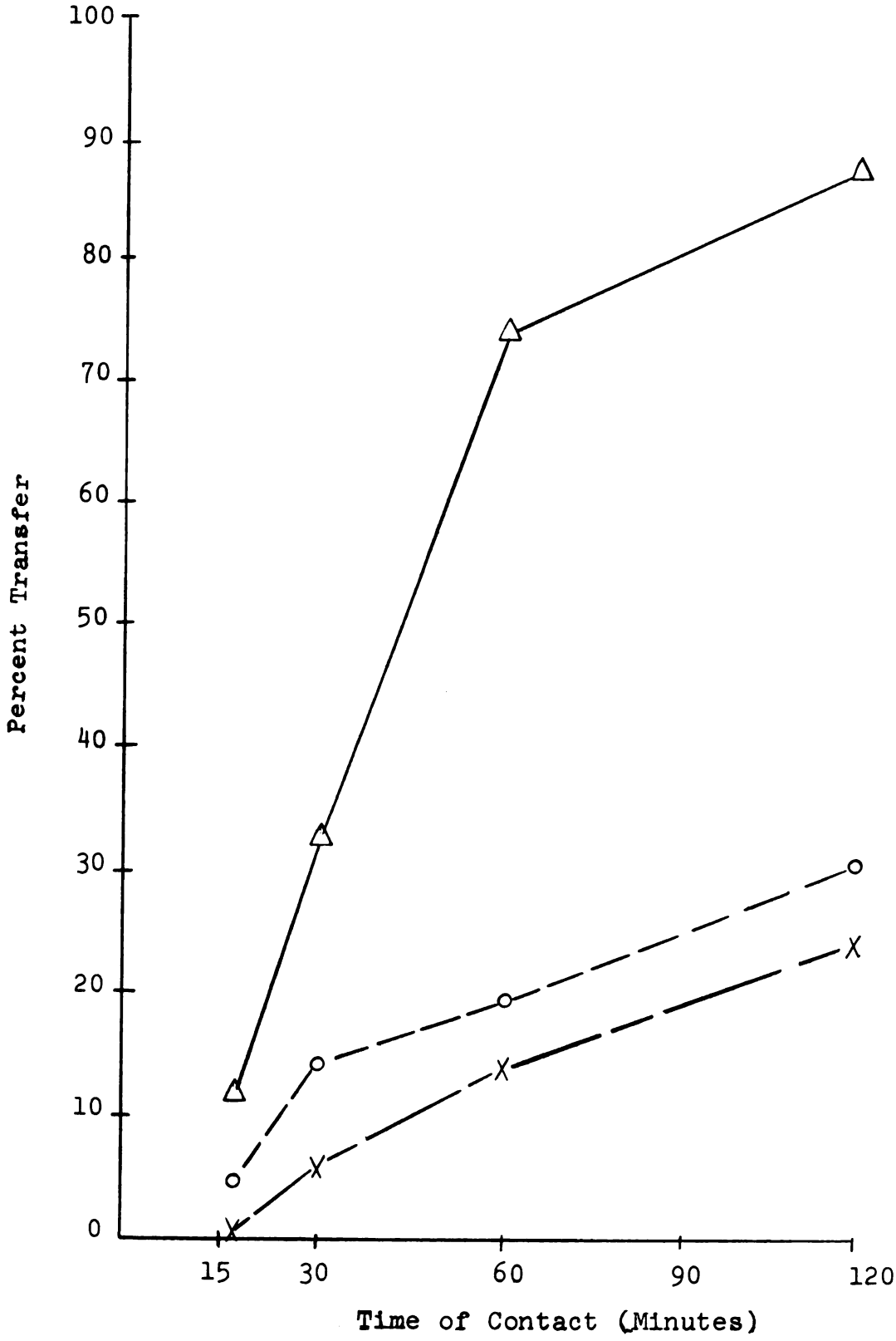

Figure 7.--Frequency of transfer of F-lac$^+$ from E. coli BB F-lac$^+$ to E. coli AB113 lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

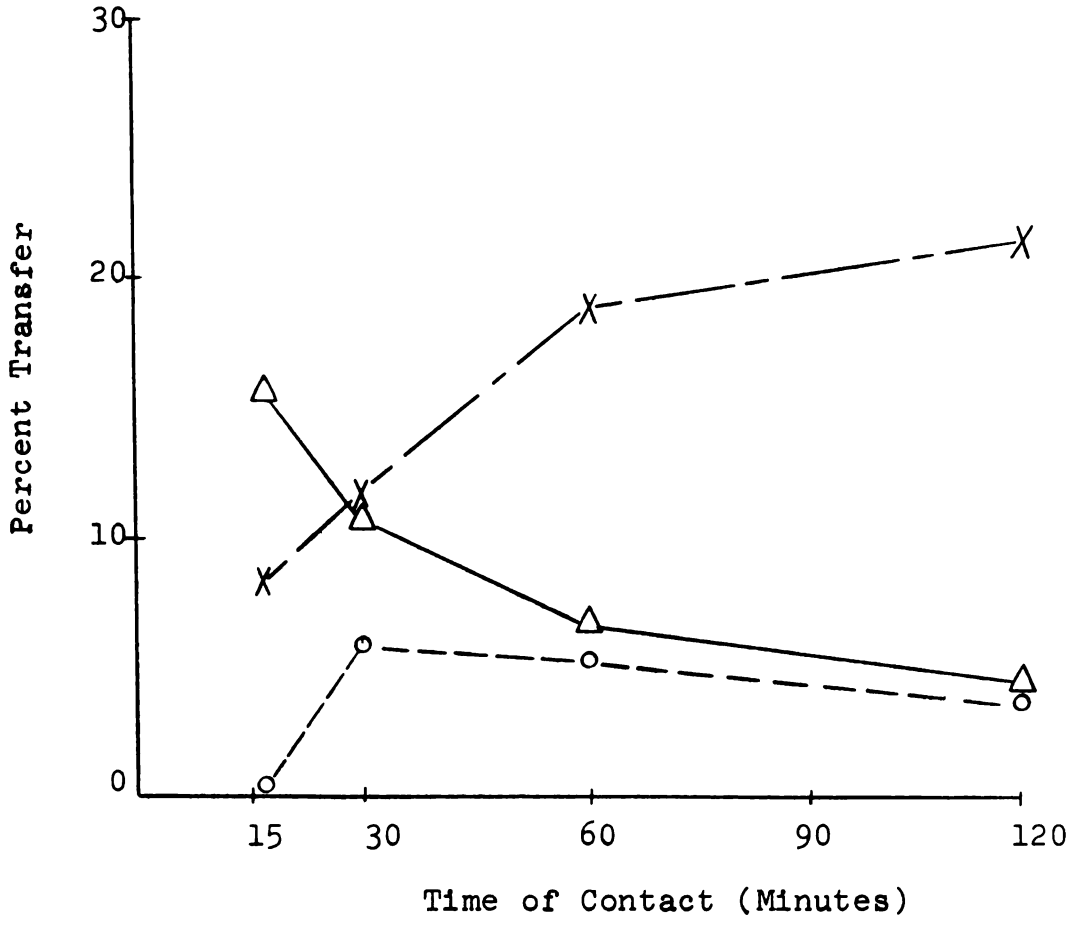

Figure 8.--Frequency of transfer of F-lac$^+$ from E. coli BB F-lac$^+$ to S. pullorum 35 lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

TABLE 5.--Frequency of transfer of F-lac$^+$ with restricting and nonrestricting recipients.*

Cross	Method	After 60 Minutes		
		Recipient	Recombinant	Frequency
785 x BB	Millipore	3.4×10^8	1.1×10^8	3.2×10^{-1}
	Centrifuge	1.5×10^8	1.0×10^7	6.7×10^{-2}
	Flask	4.7×10^8	5.6×10^6	1.2×10^{-2}
BB x BB	Millipore	2.2×10^8	2.0×10^8	9.1×10^{-1}
	Centrifuge	1.5×10^8	3.0×10^7	2.0×10^{-1}
	Flask	1.5×10^8	1.1×10^8	7.3×10^{-1}
BB x 113	Millipore	1.9×10^8	1.5×10^8	7.9×10^{-1}
	Centrifuge	1.3×10^8	1.8×10^7	1.4×10^{-1}
	Flask	1.1×10^8	2.1×10^7	1.9×10^{-1}
BB x SP	Millipore	2.8×10^8	2.0×10^7	7.1×10^{-2}
	Centrifuge	1.4×10^8	2.5×10^7	1.8×10^{-1}
	Flask	1.4×10^8	6.3×10^6	4.5×10^{-2}

*The donors were mixed with the recipients in a ratio of approximately 10 to 1 to give a total cell concentration of about 10^9 per ml. Variations in the ratio from 5-15 to 1 did not markedly affect the frequency of transfer.

Series III

In the third series of matings *E. coli* AB785 F-lac$^+$ was crossed with *S. pullorum* 35 lac$^-$. Subsequent crosses were then made with *S. pullorum* 35 F-lac$^+$. The millipore, centrifuge, and flask methods gave fairly equivalent results throughout this series of matings. The frequency of transfer with *E. coli* AB785 F-lac$^+$ was lower than that observed for any of the other initial crosses. The most significant difference, however, occurred when *S. pullorum* 35 F-lac$^+$ was mated with either *E. coli* BB lac$^-$ or *E. coli* AB113 lac$^-$. Very little, if any, transfer was observed; in some experiments none was observed. When *S. pullorum* 35 F-lac$^+$ was crossed with *S. pullorum* 35 lac$^-$, the frequency of transfer increased to an observable rate, but still did not nearly approach the 100% level that would be expected with a homologous system.

Table VI and Figures 10 and 11 give the percent transfer during the two hour interval, while Table VII lists the frequency of transfer at 60 minutes. Figure 9 gives a summary of the crosses.

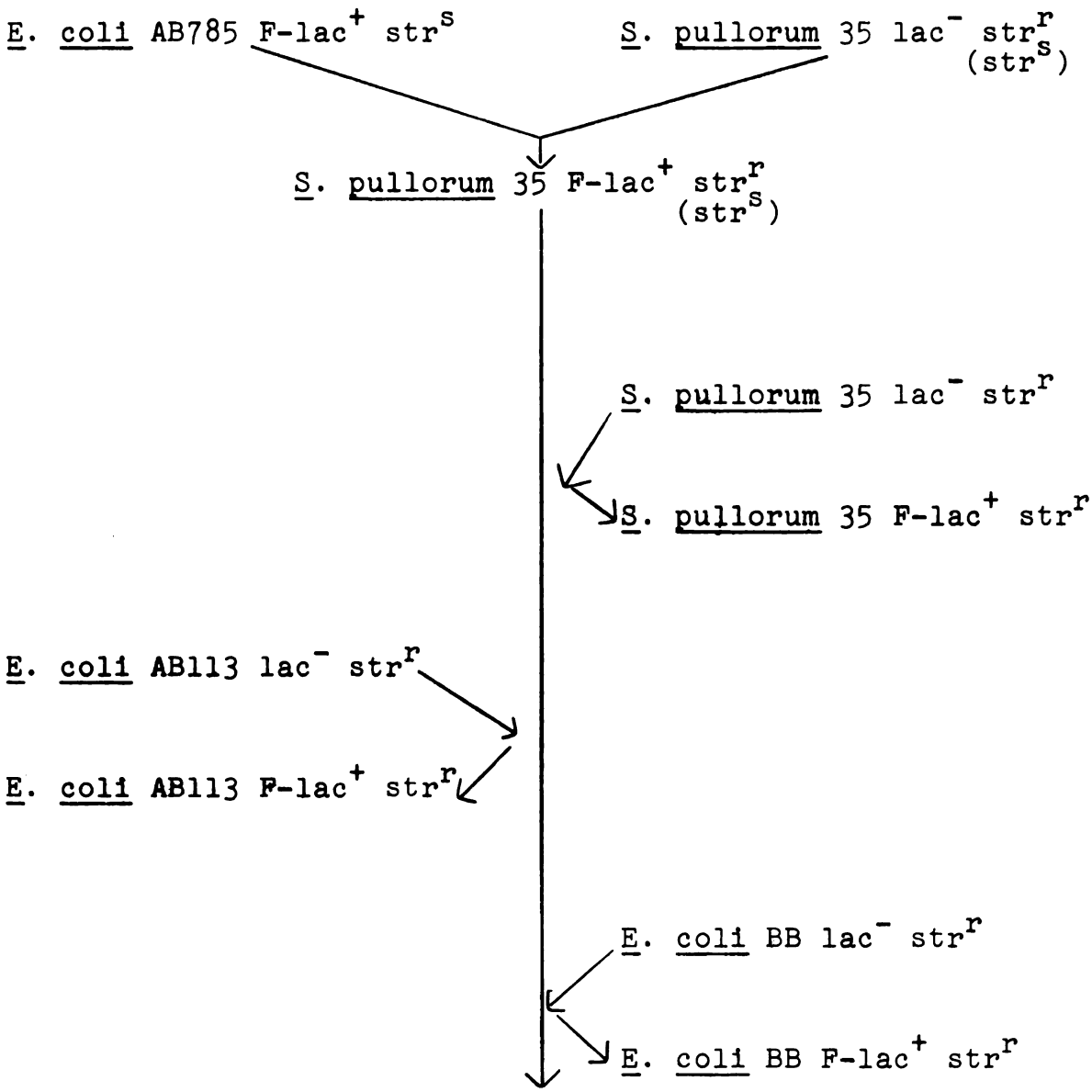

Figure 9.--Summary of transfer of F-lac$^+$ in Series III.

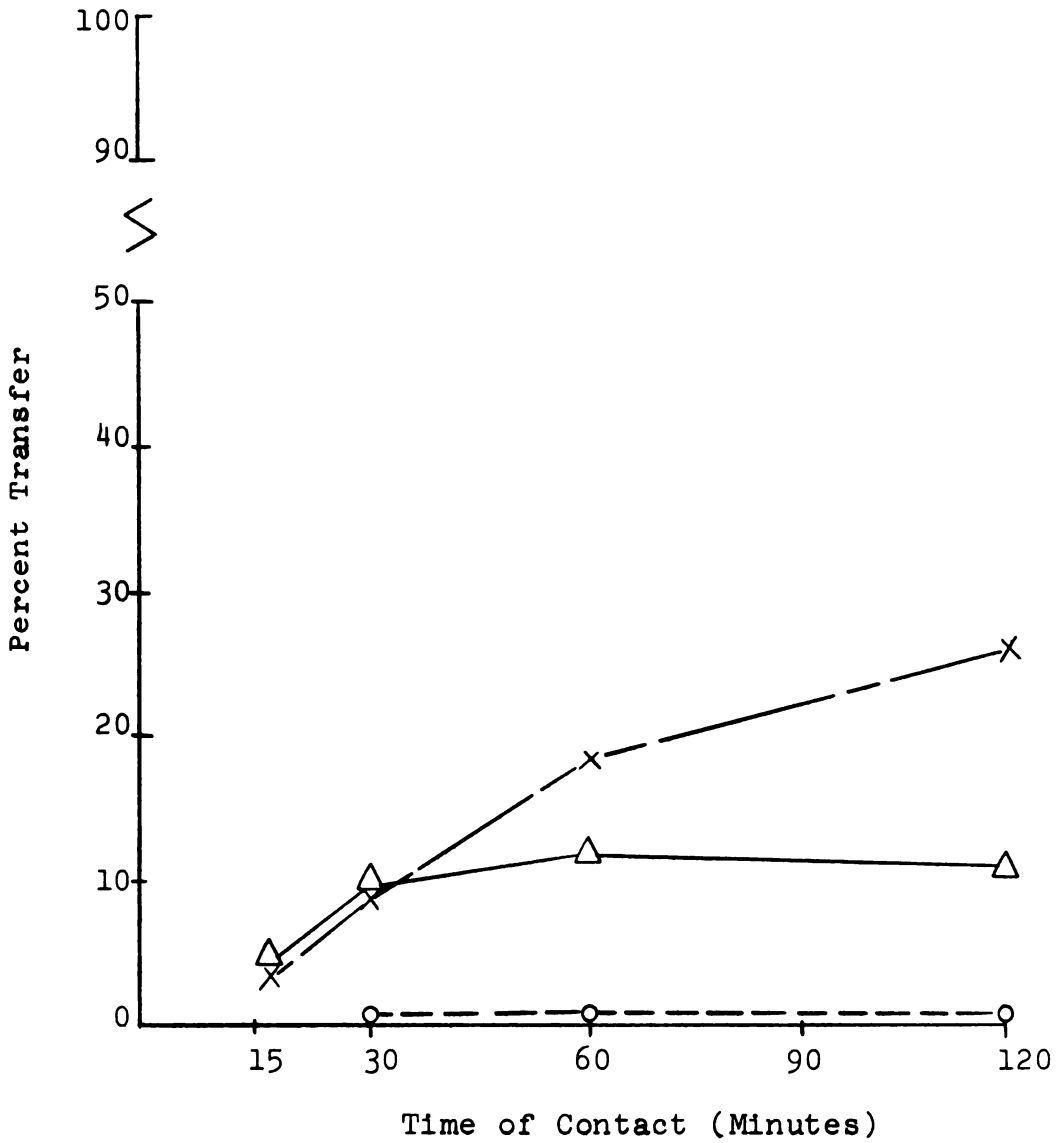

Figure 10.--Frequency of transfer of F-lac$^+$ from E. coli AB785 F-lac$^+$ to S. pullorum 35 lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

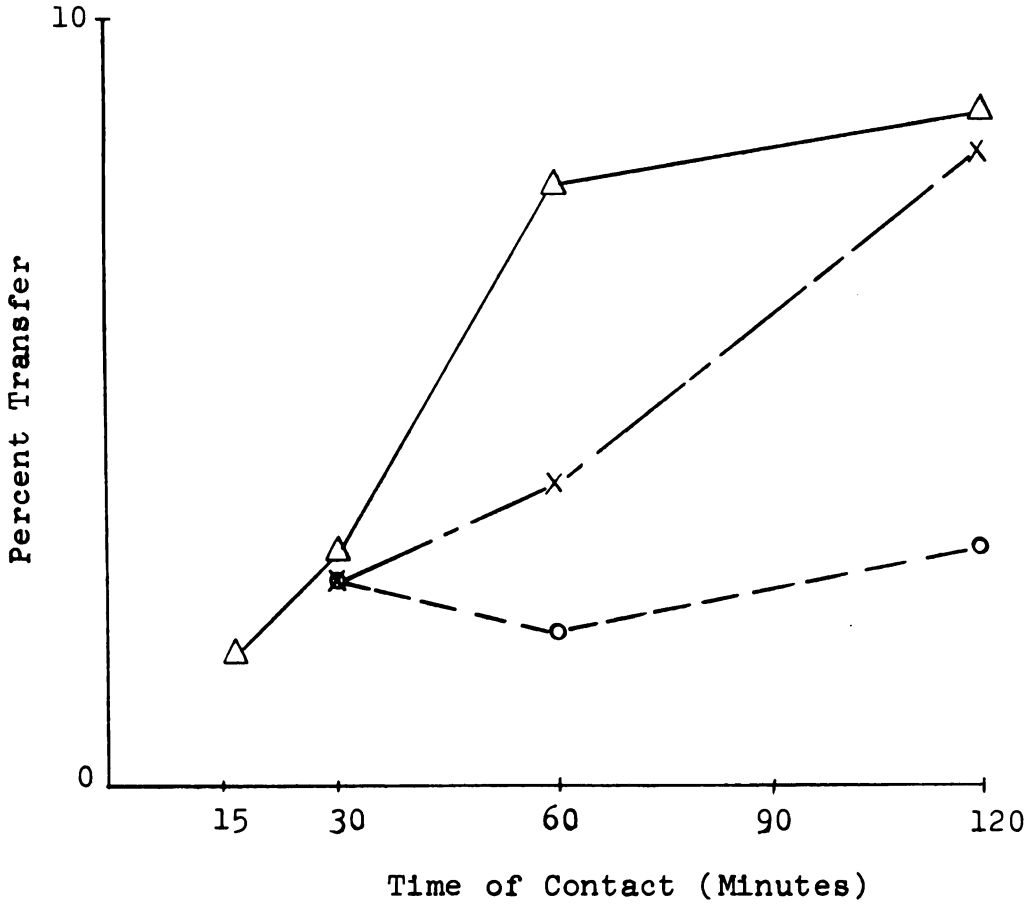

Figure 11.--Frequency of transfer of F-lac$^+$ from S. pullorum 35 F-lac$^+$ to S. pullorum 35 lac$^-$ via millipore (Δ), centrifuge (X), and flask (o) methods.

TABLE 6.--Frequency of transfer of F-lac$^+$ from S. pullorum 35 F-lac$^+$ to E. coli AB113 lac$^-$ and E. coli BB lac$^-$.

Cross	Minutes	Method		
		Millipore	Centrifuge	Flask
SP x 113	15	----	----	----
	30	----	----	----
	60	.12%	.17%	.38%
	120	.17%	1.3%	.40%
SP x BB	15	----	----	----
	30	----	----	----
	60	----	----	----
	120	.05%	.10%	.05%

TABLE 7.--Frequency of transfer of F-lac$^+$ with restricting and nonrestricting recipients.*

Cross	Method	After 60 Minutes		
		Recipient	Recombinant	Frequency
785 x SP	Millipore	2.1×10^8	2.5×10^7	1.2×10^{-1}
	Centrifuge	8.7×10^7	1.6×10^7	1.8×10^{-1}
	Flask	1.2×10^8	1.7×10^6	1.4×10^{-2}
SP x SP	Millipore	7.8×10^7	6.0×10^6	7.7×10^{-2}
	Centrifuge	4.3×10^7	1.7×10^6	4.0×10^{-2}
	Flask	5.2×10^7	1.0×10^6	1.9×10^{-2}
SP x 113	Millipore	5.4×10^8	6.6×10^5	1.2×10^{-3}
	Centrifuge	1.9×10^8	3.3×10^5	1.7×10^{-3}
	Flask	3.4×10^8	1.4×10^6	4.1×10^{-3}
SP x BB	Millipore	7.5×10^8	3.3×10^5	4.4×10^{-4}
	†Centrifuge	3.4×10^8	3.3×10^5	9.7×10^{-4}
	†Flask	6.8×10^8	3.3×10^5	4.9×10^{-4}

*The donors were mixed with the recipients in a ratio of approximately 10 to 1 to give a total cell concentration of about 10^9 per ml. Variations in the ratio from 5-15 to 1 did not markedly affect the frequency of transfer.

†After 120 minutes.

DISSCUSSION

Methods of Conjugation

Three series of crosses were initiated to determine the frequency of transfer by the millipore, centrifuge, and flask methods. In all crosses but one (*E. coli* AB785 F-lac$^+$ X *S. pullorum* 35 lac$^-$), the millipore method was found to be the most efficient means of transferring the F-lac$^+$ from donor to recipient strains. In many of the crosses, the difference in rate of transfer between this method and the other two was great enough to preclude any error that might have been incurred in technique.

An explanation for the millipore method giving the best results might be related to the following. The bacteria are impinged upon a solid surface in proximity to one another and thus the rapid separation encountered in broth is eliminated (Matney and Achenbach, 1962). However, they are still in proximity with the atmosphere and can continue receiving a constant supply of nutrients--both factors which appear to be necessary for conjugating cells (Fisher, 1957). The above results were inferred from platings in which the number of bacteria had increased, sometimes to such an extent that the usual dilution was not sufficient for the reading of results.

The centrifuge method produced results that were usually comparable to those obtained by the flask method (exceptions: Figures 6, 8, and 10). In these crosses the bacteria did not readily multiply while in the pellet; this suggests that optimal conditions for growth were not present.

The exception noted in Figure 6 occurred when E. coli BB F-lac$^+$ was crossed with E. coli BB lac$^-$. A possible explanation might be gleaned from the clumping that occurred when the cells were suspended. Perhaps the clumping between these two strains of E. coli BB had the same physical effect as that encountered with the millipore method. The two other exceptions will be discussed later.

Utilizing the flask method, a rapid multiplication of the bacteria occurred with time. However, as noted by a few of the graphs, the percent transferred did not rise considerably with this method when compared with the centrifuge method.

Whenever an E. coli K-12 or BB was mated with a S. pullorum, the results tended to improve if the centrifuge method were employed. This suggests that the packing of cells gradually permits a greater percentage of transfer than could otherwise be obtained by the millipore method. Perhaps either the proximity or the configuration of the cells plays a role.

Therefore, by experimenting with all three methods, it appears that many of the results in the literature could

be improved upon by using that method that most readily facilitates the transfer of the genetic material whether it be of a Hfr, F', or F^+ variety.

Host-controlled Modification

The presence of host-controlled modification was found in S. pullorum 35. The F-lac^+ was transferred from E. coli AB785 F-lac^+ to S. pullorum 35 and then passed to another S. pullorum 35 at a reasonable, but low rate. Restriction probably plays a role in both the initial and homologous cross; nonhomology should be nonexistent since integration does not necessarily occur (Arber and Morse, 1965).

Even though restriction appears to be present in both the initial and homologous cross, it may be coupled with effective contact. The decrease in the efficiency of transfer between an E. coli-S.pullorum or S. pullorum-S. pullorum cross when compared to an E. coli-E. coli cross suggests that a missing surface component may contribute to this decrease. Mutants have been isolated which have an increased ability to act as recipients; neither technique nor condition has been discovered which impairs the ability of the cell to act as recipient (Gross, 1964).

The possibility exists that the F-lac^+ might carry the host specificity from its previous host. However, by repeated isolations, any host specificity should have been lost during replication. When the S. pullorum was used as the donor to infect E. coli AB113 or BB, the frequency of

transfer dropped significantly which would imply that the F-lac$^+$ in the S. pullorum had been modified.

Another interesting result was observed in the matings of the control crosses. Although no restriction would be expected in a homologous cross between various strains of E. coli K-12, some was noted. This restriction also has been noted by Mäkelä et al.(1962) and Arber and Morse (1965).

The crosses of Series II also yielded some noteworthy information. Whereas some restriction was noted in the initial cross (E. coli AB785 F-lac$^+$ X E. coli BB lac$^-$), none was noted when E. coli BB F-lac$^+$ was crossed with a homologous recipient or with E. coli AB113 lac$^-$. Therefore, it appears that both recipients are capable of receiving a modified F-lac$^+$ equally well and that the mechanism involved in host-controlled modification may not be as selective as might be hypothesized or that E. coli K-12 and BB are more homologous than assumed. Perhaps the base sequence is modified by methylation (Arber, 1965; Klein and Sauerbier, 1965) so that it is recognized by both the nucleases present in E. coli K-12 and BB as "self," rather than "nonself."

When E. coli BB F-lac$^+$ from Series II was crossed with S. pullorum 35, a gradual rise in percent transfer occurred. However, when the same cross was performed via the millipore method, a rapid increase, followed by a gradual decline

was observed. Arber and Morse (1965) observed similar results when they crossed F^+ K-12 gal^- X F^- K-12(P1) gal^+ and F^+ K-12 gal^- X F^- B gal^+. They suggested that the lethality was only present in those cell pairs that persisted after the end of donation of the transferred DNA molecule. Clowes (1963) and Gross (1963) both observed that lethality occurred at a high ratio of Hfr to F^- cells and surmised that these effects were due to damage to F^- cells. Gross also found that not all of his Hfr strains produced this effect. Thus, perhaps in this cross, the conditions imposed by the millipore filter, i.e., the immediate proximity of the bacteria to one another, also cause this lethality.

SUMMARY

Of three methods utilized in the transfer of the intermediate sex factor, F-lac$^+$, the millipore method was found to be generally superior to either the centrifuge or flask method. The transfer of F-lac$^+$ also was affected by the strains involved.

E. coli AB785 was used as the universal donor of F-lac$^+$. F-lac$^+$ was transferred readily to both an *E. coli* K-12 and BB, but with a lower frequency to a *S. pullorum*. Subsequent transfer with *E. coli* F-lac$^+$ to both *E. coli* BB lac$^-$ and AB113 lac$^-$ readily occurred, but was method-dependent when *S. pullorum* 35 was used as the recipient.

When *S. pullorum* 35 was used as the donor, restriction was readily observed if *E. coli* AB113 or BB were the recipient. When *S. pullorum* 35 was the recipient, some restriction was noted.

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