Analysis of the serum protein in human beings with diabetes mellitus shows a decrease in albumin and an increase in α_2 globulin. Less consistent changes occur in the β lipoprotein and γ globulin(2). Particular interest surrounds these changes as they may be related to the abnormalities of lipid and cholesterol metabolism found in these patients. Serum protein changes in alloxan-diabetic rats are not entirely comparable to those changes due to diabetes mellitus in human beings(3), but since insulin is necessary for the protein anabolic effect of growth hormone and has been shown to cause an increased incorporation of amino acids into the protein of alloxan-diabetic rat liver(4), the changes in serum protein may result from the lack of an insulin effect on liver protein synthesis.

Studies of liver function after a portacaval shunt in rats reveal changes in plasma proteins that are quite similar to those found in human diabetics and suggest that the biologic effect of insulin on the liver may be a significant factor in these changes.

Six months after end-to-side portacaval shunt, heparinized capillary blood was taken for protein analysis. Six lambda was applied to Schleicher and Schuell 2043A filter paper. After the run, the strips were stained with bromphenol blue and electrophoretic curves for the components determined on a densitometer (Spinco Analytrol).

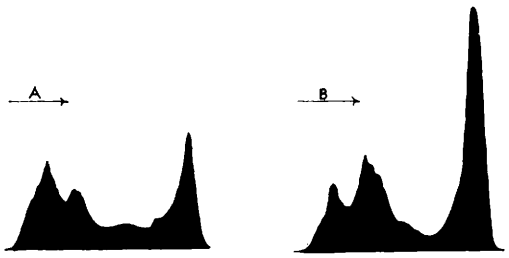

FIG. 1. Typical electrophoretic curves for plasma from (A) rat with portacaval shunt, and (B) normal rat after 1020 min. of electrophoresis at a potential gradient of 2.6 V cm^{-1} at 25°C in diethylbarbiturate buffer ionic strength pH 8.6.

TABLE I. Plasma Protein Fractions in Rats. % of electrophoretic curve area.

		Alb (%)	α_1 (%)	α_2 (%)	β (%)	γ (%)
Normal		42.9	9.2	6.0	28.6	13.4
P-C Shunt	1.	26.5	11.2	14.1	18.8	29.4
	2.	44.6	10.7	7.1	16.1	21.4
	3.	37.4	11.7	9.1	18.7	23.0
	4.	14.5	9.7	7.5	22.0	46.3
	5.	26.7	8.5	8.5	25.6	30.7
	6.	42.3	10.0	9.0	16.7	22.2
	7.	26.2	10.4	10.4	14.5	38.6

The results from a preliminary study (Table I) show an increase in α_2 and γ globulin and a consistent decrease in albumin and β lipoprotein. The electrophoretic curves for a shunted rat (Rat No. 1 of Table I) and for a normal rat are compared in Fig. 1.

The significance of the protein changes and their effect on the pathologic physiology of diabetes millitus is obscure, but the shunted animal provides a biologic system for the study of these changes, where diet and other variables can be controlled.

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Genetic Recombination by Transduction Between Mannitol-Negative Mutants of *Staphylococcus aureus*.* (29302)

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Mannitol-negative *(mtl—)* mutants of *Staphylococcus aureus* strain 233 have been transduced to *mtl+* at relatively high rates

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(1 transductant per 10^4 adsorbed plaque-forming units) using a temperate, serological Group B bacteriophage which was propagated on *mtl*+ cells(1). Genetic recombinants *(mtl+)* could also be obtained when the bacteriophage was propagated on certain of the *mtl*— mutants. This recombination indicated that more than one genetic lesion was present in this group of mutants, and suggested that different biochemical lesions might also be represented.

A study of the wild-type cells indicated that mannitol is oxidized in staphylococci *via* mannitol-1-phosphate to fructose-6-phosphate by an inducible, nicotinamide adenine dinucleotide-linked dehydrogenase(2). This pathway had been suggested earlier in *Escherichia coli*(3) and *Diplococcus pneumoniae*(4) as an alternative to the direct oxidation of mannitol to fructose which is found in *Acetobacter*(5) and *Azotobacter*(6) species.

Although phosphorylation of mannitol could not be demonstrated, a study of the fermentation patterns of the *mtl*— mutants of *S. aureus* and assays of their mannitol-uptake and mannitol-1-phosphate dehydrogenase activities indicated that 15 of the 19 mutants were defective specifically in their utilization of mannitol. The other 4 mutants had a pleiotropic defect(7) in that they were unable to ferment any carbohydrate except glucose. Possible biochemical lesions in the former group include defects in control of mannitol-induced enzyme synthesis, transport of mannitol into the cell, phosphorylation of mannitol, and synthesis of mannitol-1-phosphate dehydrogenase.

This study attempts to correlate the presence or absence of genetic recombination between these *mtl*— mutants of *S. aureus* and their specific enzymatic defects.

Methods. The techniques for demonstrating genetic recombination between *mtl*— mutants of *S. aureus* 233 with phage C have been described(1). Briefly, mannitol-utilizing *(mtl+)* recombinants were isolated from the non-utilizing population on a selective medium, Modified Eosin-Methylene Blue-Mannitol (MEMB-mtl) Agar. Phage stocks, obtained by propagating phage C on the donor mutants, were diluted to 10^9 plaque-forming units/ml and irradiated with ultraviolet light to increase their transducing efficiencies(8). A single drop in each of the 20 phage suspensions (from 1 *mtl*+ and 19 *mtl*— donors) was placed on an MEMB-mtl Agar plate which had been spread previously with the *mtl*— recipient being tested. The plates were incubated in the dark at 37°C for 3 days and examined for the presence of typical, mannitol-fermenting colonies within the areas of phage infection.

Equivocal results were obtained in a few of the crosses due to the relatively high back-mutation rate of the recipient strain or, less frequently, to phage suspensions of low transducing titer. However, in all crosses the density of *mtl*+ colonies within the area of phage infection was compared to that of non-infected areas on the same plate and to the area spotted with phage grown on the recipient strain being tested. Positive and negative results were recorded only when obvious differences existed between the test and control areas. Each cross was repeated 3 times using freshly propagated phage stocks.

Results. Table I summarizes the results of tests for genetic recombination by reciprocal transductions between the 19 *mtl*— mutants of *S. aureus* strain 233. The genetic and/or enzymatic defect of each mutant is also presented as postulated in a previous study(2). Certain mutants (mtl-1, mtl-3, mtl-15 and mtl-19) could be tested only as genetic donors in these crosses since they were not inhibited on the selective medium.

When each mutant is represented as a deletion mutant, 17 of the 19 can be grouped in a linear array as shown in the recombination map proposed in Fig. 1. Genetic recombina-

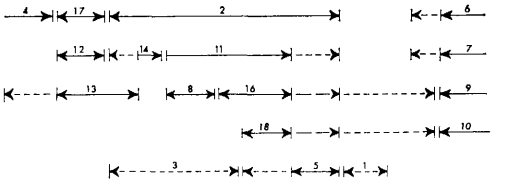

FIG. 1. Recombination map of *mtl*- mutants of *S. aureus* strain 233. Recombination occurs between mutants represented by non-overlapping deletions (solid lines), but does not occur between mutants with overlapping deletions. Recombination is questionable between mutants represented by broken lines.

TABLE I. Genetic Recombination by Transduction Between *mtl*— Mutants of *S. aureus* strain 233.

mtl— recipient	*mtl*— donor																			Postulated genetic and/or enzymatic defect*
	4	17	12	13	14	3	2	11	8	16	18	5	1	6	7	9	10	15	19	*mtl*+
4	0	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	+	+	P or R
17	?	0	0	0	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P or R
12	+	0	0	0	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	P or R
13	?	?	0	0	0	0	0	?	+	?	?	?	+	+	+	+	+	+	+	+	P or R
14	+	+	+	?	0	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	P or R
3†																					D
2	?	?	?	?	0	0	0	0	?	0	0	0	+	+	+	+	+	+	+	+	P or R
11	+	+	+	+	+	0	0	0	0	0	0	?	+	+	+	+	+	+	+	+	P or R
8	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	P or R
16	+	+	+	+	+	0	0	0	?	0	0	?	?	+	+	+	+	+	+	+	P
18	+	+	+	+	+	?	0	0	?	0	0	0	+	+	+	+	+	+	+	+	P or R
5	+	+	+	+	+	+	0	?	?	?	?	0	+	+	+	+	+	+	+	+	P
1†																					D
6	+	+	+	+	+	+	+	+	+	?	?	+	+	0	0	0	0	+	+	+	C
7	+	+	+	+	+	+	+	+	+	?	?	+	+	0	0	0	0	+	+	+	C
9	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	+	+	+	C
10	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	+	+	+	C
15†																					K
19†																					K

+ = genetic recombination occurs; 0 = genetic recombination does not occur within limits of detection; ? = genetic recombination questionable, see text.

* Postulated genetic and/or enzymatic defect:
R = regulatory gene defect
P = defective transport of mannitol into cell (mannitol permease?)
K = defective phosphorylation of mannitol (mannitol kinase?)
D = defective synthesis of mannitol-1-phosphate dehydrogenase
C = pleiotropic defect in carbohydrate fermentations.

† = not suitable as recipients, see text.

tion does not occur within the limits of detection between any of those mutants represented by overlapping deletions (solid lines), but does occur between all mutants with non-overlapping deletions. Recombination is questionable between some of the mutants; these are represented by overlapping broken lines. It should be noted that neither the relative size of a deletion nor the distance between two mutants on the tentative map necessarily represents the true size of the genetic lesion or the distance between mutants along a chromosome.

Three genetic groups are readily apparent in these reciprocal tests. One group of 2 mutants (mtl-15 and mtl-19) behaved as wild-type in crosses with all other *mtl*— mutants. For this reason, these 2 mutants are not included in the recombination map. The previous study suggested that these mutants are defective in their phosphorylation of mannitol.

A second group, composed of mutants mtl-6, mtl-7, mtl-9 and mtl-10, is distinct in that there is no recombination within the group, but recombination does occur between any member of the group and all other mtl-mutants. These 4 mutants have the pleiotropic defect in carbohydrate fermentations mentioned earlier.

The remaining 13 mutants were placed in a third group by these reciprocal genetic crosses. In this group are the mutants with defects in mannitol uptake (mtl-5 and mtl-16), in mannitol-1-phosphate dehydrogenase (mtl-1 and mtl-3), and in permeability to mannitol and/or a regulatory gene. At this time, it is not possible to differentiate by biochemical techniques the mutants with regulatory gene defects from those which are impermeable to the inducer.

Other biochemical characteristics of these *mtl*— mutants have been examined, but no differences are detectable between the mutants and the parent strain in production of coagulase, α-,β-, or δ-hemolysin, or deoxyribonuclease.

Discussion. The proposed recombination map indicates that except for gene controlling phosphorylation, the specific genes controlling mannitol oxidation in staphylococci are linked to one another. It also indicates that no linkages exist between these genes and the locus of the pleiotropic mutation. In these respects, there is correlation between the genetic analysis of these mutants and their biochemical defects.

Subdivision of the *mtl*— mutants may be warranted since mutants mtl-4, mtl-12 and mtl-17 are linked to the other mutants only through the lesion of mutant mtl-13. These mutants could have defects in either a regulatory gene which prevents the synthesis of mannitol-induced enzymes, or in their mannitol permease, *i.e.*, they are cryptic because the inducer cannot penetrate the cell. Mutants mtl-15 and mtl-19 are distinct in that they recombine genetically with all other *mtl*— mutants, indicating a lack of close linkage to the other mutants. These were the only mutants postulated to have defects in their phosphorylation of mannitol.

The true order of the *mtl*— mutants within the group cannot be determined until more refined analyses can be made using closely linked outside markers in 3- and 4-factor genetic crosses. Since none of the mutants were detectably different from the wild-type parent in production of factors associated with virulence in staphylococci (coagulase, α-, β-, or δ-hemolysins, etc.), it seems unlikely that these traits could be used as markers in such an analysis. Streptomycin resistance and xylose fermentation were also considered as possible outside markers because they are linked to the *mtl* loci of *D. pneumoniae* and *E. coli* respectively(9,10); however, xylose is not fermented by staphylococci, and linkage between streptomycin resistance and the *mtl* markers has not been detected in staphylococci(2,11).

Summary. Reciprocal tests for genetic recombination by transduction have divided 19 *mtl*— mutants of *Staphylococcus aureus* strain 233 into 3 genetic groups. One group was composed of 4 mutants having a pleiotropic defect in their fermentation of carbohydrates. One group was composed of 2 mutants postulated to be defective in their phosphorylation of mannitol. The remaining mutants, postulated to have defects at specific

steps in the utilization of mannitol, were linked genetically into the third group.

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Studies on Viral Inhibitors of Biological Origin. I. Inhibition of Viral Replication by Protein-Like Constituents of Bacteria.* (29303)

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Experimental evidence has been presented demonstrating that a factor derived from a Corynebacterium of unidentified species interferes with the intracellular stages of replication of 2 Group A arboviruses, Sindbis and chickungunya(1). This bacterial inhibitory factor (henceforth abbreviated BIF) did not interfere with the cytopathogenicity of 7 unrelated viruses. Further experiments have been performed concerning the nature of the substance and the spectrum of its antiviral activity. In view of the very narrow spectrum of antiviral activity exhibited by the original Corynebacterium, inhibitory substances against other viruses were sought in other bacteria.

Materials and methods. In addition to the original unidentified Corynebacterium, inhibitory factors were obtained from *Corynebacterium diphtheriae* (both a non-toxin producing strain and an intermedius), *Corynebacterium xerosis, Escherichia coli* (B, K12 and 0111) and *Salmonella typhimurium.* In all cases other than the original Corynebacterium, 100 ml aliquots of beef heart infusion medium were used for growing the bacteria. The fastidious growth requirements of the original organism have been described(1). Cultures were incubated at 37°C for 3 days prior to sedimenting the bacteria by centrifugation for 20 minutes at 3000 rpm. Bacteria were then resuspended in 5 ml of bovine amniotic fluid (BAF) tissue culture medium (2) and disrupted by sonication in addition to freezing and thawing as decribed previously. Following disruption, the bacterial suspensions were filtered through a millipore filter with a pore size of 0.45 μ. The resulting filtrates were assayed for inhibitory activity.

Except where otherwise noted all tests were carried out in cultures of primary human amnion cells using 100 $TCID_{50}$ of the virus as test dose. Serial 2-fold dilutions were made of the materials to be tested for inhibitory capacity, and 1 ml aliquots were added to 1-3 cultures immediately before inoculation of virus. The titer of a given inhibitory factor is expressed as the highest dilution which completely suppressed cytopathic effect (CPE) on the day that approximately 50% of the cells in viral control cultures exhibited CPE.

Treatment of inhibitory factors with enzymes (trypsin, DNAase, RNAase) dialysis, and heating were all performed in the manner described for the original BIF(1).

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