The Effect of Bacteria on the Take of Split-Thickness Skin Grafts in Rabbits

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The influence of different species of bacteria on the take of split-thickness skin grafts on granulating wounds in man has not been well documented except in the case of Group A beta hemolytic streptococci. Reports by Jackson and co-workers,[6] and by our group[7], clearly indicate that Group A beta hemolytic streptococci cause graft failure. A similar effect of the other micro-organisms that frequently colonize granulating surfaces has not been convincingly demonstrated. Some evidence for a deleterious effect of *Pseudomonas aeruginosa* and of other gram-negative bacilli was presented by Jackson and co-workers.[5] The belief that *Ps. aeruginosa* is deleterious to grafts is shared by Brown,[3] while Ackman[1] and Gerrie[4] consider the gram-negative micro-organisms to be harmless saprophytes. A specific effect of the coagulase positive *Staphylococcus aureus* on graft takes has never been established, possibly because this micro-organism is almost always present on granulating wounds. Similarly, in the case of alpha and gamma hemolytic streptococci and of Group C and D streptococci, a deleterious effect could not be demonstrated either by Jackson and co-workers,[6] or by us.[7]

Since granulating wounds are almost always colonized by a mixed flora of gram-positive cocci and gram-negative bacilli together with gram-positive sporulating and nonsporulating rods, there is a possibility that bacterial synergism or antagonism have an important effect on the take of grafts. There is considerable evidence in the literature that gram-negative bacilli, particularly pseudomonas species, are antagonistic to the gram-positive cocci both *in vitro* and *in vivo*. This literature was extensively reviewed by Waksman[9] in 1941.

In a large series of wounds recently studied by our group, a correlation between the bacteriologic flora of the wounds and the graft takes was performed in an effort to define the pathogenicity of the micro-organisms when they were present on the wounds singly or in various combinations. The pathogenicity of Group A beta hemolytic streptococci could be established,[7] but a specific deleterious effect exerted by other micro-organisms and combinations of micro-organisms escaped detection. Since our ability to control the bacterial flora on granulating wounds is limited, chance burns do not appear to afford proper conditions for studying specific bacterial combinations with regard to their effect on graft takes. Therefore, an animal experiment was designed to elucidate some important aspects.

The purpose of the study was (1) to define, quantitatively, the effects of Group A beta hemolytic streptococci on the graft takes in freshly excised wounds in rabbits; (2) to define the same effects of *Ps. aeruginosa* and coagulase positive *Staph. aureus;* and (3) to define the effects of the interac-

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tion, synergistically or antagonistically, between *Ps. aeruginosa* and Group A beta hemolytic streptocci on one hand and *Ps. aeruginosa* and coagulase positive *Staph. aureus* on the other hand.

TABLE I. *Experimental Groups.*

Group	Micro-organisms	Amount of Broth
	Streptococcus.....	0.25 cc.*
A		
	Pseudomonas.....	0.25 cc.
	Staphylococcus.....	0.25 cc.
B		
	Pseudomonas.....	0.25 cc.
C	Pseudomonas.....	0.5 cc.
D	Streptococcus.....	0.25 cc.
E	Staphylococcus.....	0.5 cc.
F	————	——

* In all cases the broth volume was diluted to 1 cc. with normal saline before it was injected under the graft.

EXPERIMENTAL PROCEDURES

Adult white rabbits were used. The flank of the animal was shaved, and the rabbit was anesthetized with intramuscularly injected paraldehyde. The shaved area was prepared three times with 7 per cent tincture of iodine. When dry, the iodine was washed off with 70 per cent alcohol. The field was draped with sterile towels, and an area 7 x 4 cm. was outlined with methylene blue by use of a transparent grid. First a split-thickness skin graft, 0.020 inches thick, was obtained from the outlined area with the Brown electric dermatome. Then the complete thickness of the skin was excised down to the areolar tissue covering the external oblique muscles. Following this, the previously obtained graft was sutured into the defect by use of a continuous silk suture, and a thick occlusive dressing was applied over fine mesh petrolatum gauze. The dressing was kept in place with adhesive tape that encircled the entire length of the trunk. A strict aseptic technic was used during the operation. The take of the graft was determined on the fourth postoperative day with the aid of the transparent grid, and was expressed as a percentage of the area grafted. Take of an area was recorded if the graft adhered to its base, was of a healthy, pink appearance, and if there was gross evidence of a healing process as judged by bleeding when the graft was dislodged.

TABLE II. *The Take of Grafts and Mean Percentages in the Experimental Groups.*

Micro-organisms	Streptococci	Staphylococci	No Cocci	Totals and Mean %
	A	B	C	
	0	10	31	
	5	0	22	
Pseudomonas	0	0	92	
	0	44	56	
	0	0	10	
Totals.....	5	54	211	270
Mean %....	1	11	42	18
	D	E	F	
	6	63	80	
	15	90	89	
No pseudomonas	15	95	100	
	25	35	100	
	95	95	85	
Totals.....	156	378	454	988
Mean %....	31	76	91	66
Over-all Total..	161	432	665	1258
Over-all Mean %	16	44	67	42

Thirty adult white rabbits of both sexes were divided into six groups of five animals each by use of a table of random numbers. Immediately after the graft had been sutured in place, a known quantity of trypticase soy broth culture, diluted to 1 milliliter with normal saline, was injected under the graft. The broth culture had previously been incubated for 18 hours at 35° C. One group of rabbits served as sterile controls. All strains of bacteria had been recently isolated from granulating areas on burned patients. Five strains of each species were used. The different groups and the volumes of broth cultures used to inoculate the rabbits appear in Table I. Each streptococcus strain was used once in Group A and once in Group D. Each staphylococcus strain was used once in Group B and once in Group E, and each pseudomonas strain was used once in Group A, once in Group B, and once in Group C. The streptococci were all of the Group A beta hemolytic variety, the pseudo-

TABLE III. *Analysis of Variance.*

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F	P
Columns (Table II)	12,725	2	6,362	10.6*	P<0.001
Rows (Table II)	17,184	1	17,184	28.7*	P<0.001
Interaction	1,498	2	749	1.3†	not signif.
Duplicates	14,073	24	586	———	———
Interaction and duplicates	15,571	26	599	———	———
TOTALS.....	45,480	29	———	———	———

* Interaction and duplicate variation used as the denominator.

† Duplicate variation used as the denominator.

Sample calculation: Column variation of Table II.

$$\text{Sums of squares} = \frac{1}{10}(161^2 + 432^2 + 665^2) - \frac{(1258)^2}{30} = 12{,}725$$

Interaction sums of squares =

$$\frac{(156-5)^2}{10} + \frac{(378-54)^2}{10} + \frac{(454-211)^2}{10} - \frac{(156+378+454-5-54-211)^2}{30} = 1{,}498$$

monas strains were all *Ps. aeruginosa,* and the staphylococci were all coagulase positive *Staph. aureus.* The number of bacteria per milliliter of broth culture was determined for pseudomonas, staphylococci and streptococci, and was found to be approximately 530 x 10^6, 665 x 10^6, and 104 x 10^5 respectively. These determinations were made by counting colonies on pour plates inoculated from saline serial dilutions of the thoroughly agitated broth culture after incubation of the pour plates for 72 hours at 35° C.

Trypticase soy agar was used as pour plate medium. In the case of the streptococci, a special, enriched trypticase soy agar medium was used. Microscopic examinations of the broth cultures and the saline dilutions showed that the agitation was sufficient to break up cluster and chain formations.

The inoculum was chosen so that approximately the same number of micro-organisms was injected under each graft in Groups A, B, C, and E. However, when only streptococci were inoculated (Group D), the inoculum had to be reduced to 0.25 ml., since rabbits infected with 0.5 ml. of the streptococcal broth invariably died.

At the inspection on the fourth postoperative day, cultures were taken from the wounds to see if the strains originally inoculated could be re-isolated. This was always possible. Controls (Group F) that had graft sites contaminated with bacteria were discarded if the number of micro-organisms recovered, as judged by the growth on blood agar plates, was more than 10 to 15 colonies per plate. This elimination of contaminated controls was justified, since the purpose was to determine the effects of micro-organisms on the take of grafts. Therefore, virtually sterile controls had to be obtained for comparison.

It was decided, for the purpose of statistical evaluation of this study, that a P value of 0.01 or less would be judged "significant," a value of 0.01 to 0.05 "questionably significant," and a value of more than 0.05 "not significant."

RESULTS

The results of all grafting procedures appear in Table II. The mean per cent of take in the controls was 91 per cent, and in the infected animals 32 per cent. This difference is statistically significant ($t_{28} = 3.6$; $0.001 < P < 0.01$). The results were then subjected to a complete analysis of variance. This method of analysis is described by Mather,[8] p. 61-85, who should be consulted for details. The results of the analysis, by means of the "F"-test, appear in Table III. It can be seen that there was a statistically significant variation

TABLE IV. *Analyses of Data on Takes of Grafts (see Tables II and III).*

Effects	Method of Calculating Variations	Sums of Squares	Denominator	F	P
Effect of strep.	(A+D) − (C+F)	12,700	599*	21.2	P<0.001
Effect of staph.	(B+E) − (C+F)	2,714	599*	4.5	P<0.05
Effect of pseudo.	(A+B+C) − (D+E+F)	17,184	599*	28.7	P<0.001
Interaction strep.-pseudo.	(A+F) − (D+C)	423	586†	— —	not signif.
Interaction staph.-pseudo.	(B+F) − (E+C)	328	586†	— —	not signif.
Effect of strep. *vs.* effect of staph.	(A+D) − (B+E)	3,672	599*	6.1	P<0.05
Effect of pseudo. *vs.* effect of staph.	C − E	2,789	599*	4.7	P<0.05
Effect of strep. *vs.* effect of pseudo.	D − C	303	599*	— —	not signif.

* Interaction plus duplicate variation.
† Duplicate variation.
All of the comparisons in this table are based on one degree of freedom
Sample calculations:

$$\text{Effect of Strep.: } \frac{1}{10}(161^2 + 665^2) - \frac{(161+665)^2}{20} = 12{,}700$$

$$\text{Effect of Pseudo.: } \frac{1}{15}(270^2 + 988^2) - \frac{(1258)^2}{30} = 17{,}184$$

$$\text{Interaction Strep.-pseudo.: } \frac{1}{10}(459^2 + 367^2) - \frac{(459+367)^2}{20} = 423$$

between the "columns" in Table II as represented by Groups A + D, B + E, and C + F, or expressed differently, between the grafts infected with streptococci, staphylococci and those not infected with any cocci (P<0.001). There also was a statistically significant variation between the "rows" as represented by Groups A + B + C and D + E + F, or expressed differently, between grafts infected with pseudomonas and grafts not so infected (P<0.001).

The method for determination of the effects of the streptococci, staphylococci and pseudomonas, as well as the synergistic or antagonistic interaction between the streptococci and pseudomonas and between the staphylococci and pseudomonas appear in Table IV, together with the "F"-values and probabilities. These comparisons were envisioned when the study was planned; they were not made because of effects suggested by the results. In Table IV, only the pertinent comparisons are shown, and a complete partitioning of the degrees of freedom is not given.

Both the streptococci (P<0.001) and the pseudomonas (P<0.001) exerted a significantly deleterious effect on the take of the grafts. Such an influence seemed also to be exerted by the staphylococci (P<0.05). There was no significant synergism or antagonism between the streptococci and the pseudomonas, nor between the staphylococci and the pseudomonas, though a tendency in the direction of synergism was noted in both combinations. Because of this lack of significant interaction (synergism or antagonism), the best estimate of error is the mean square based on the pooled sums of squares and degrees of freedom of interaction variation and duplicate variation, as Mather has pointed out.[8]

An effort was made to determine the relative importance of the species of bacteria on the take of the grafts (Table IV). It can be seen that the effect of the streptococci was questionably larger than that of the

staphylococci ($P<0.05$) but not significantly different from the effect of the pseudomonas. The effect of the pseudomonas was also questionably larger than that of the staphylococci ($P<0.05$).

DISCUSSION

The results of this study indicate that Group A beta hemolytic streptococci, coagulase positive *Staph. aureus,* and *Ps. aeruginosa* cause graft failure in freshly excised wounds in adult white rabbits. The study also seems to indicate that the streptococci and the pseudomonas are more deleterious than the staphylococci. An interaction, synergistic or antagonistic, between various bacteria used in combination, could not be proved.

The use of approximately the same number of organisms per inoculum was originally planned in order to eliminate the size of the inoculum as a factor influencing the results. This was possible except for the procedures where the streptococci were used alone (Group D). If 0.5 milliliter of streptococcal broth was used, the rabbits invariably died, and it was necessary to decrease the inoculum to 0.25 milliliter of broth. This might be responsible for the finding that the streptococci influenced the graft take approximately to the same extent as the pseudomonas. If it had been possible to use the large inoculum, the streptococci might have produced a still more deleterious effect on the take of the grafts.

It must be pointed out that the properties of a certain strain of a species producing graft failure in man may not be the ones that are responsible, or mainly responsible for graft failure in rabbits. For instance, it is known that fibrinolysin, produced by Group A beta hemolytic streptococci, produces lysis of human fibrin but not of rabbit fibrin.[2] Therefore, it is not possible to apply our results directly to the problem of graft failure caused by bacteria in man.

SUMMARY

1. Group A beta hemolytic streptococci and *Ps. aeruginosa* deleteriously affect the take of skin grafts in white rabbits.

2. *Staph. aureus,* coagulase positive, seemed to produce a less deleterious influence.

3. Interaction (synergism or antagonism) was not demonstrated between the streptococci and the pseudomonas or between the staphylococci and the pseudomonas.

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