close sequence of events and complete degeneration was attained about 10 days after inoculation. At the peak of the changes effected, the contrast between healthy and infected cultures was very striking. As is well known, eventually healthy cultures will also undergo degeneration and necrosis and the gross appearance of these changes may closely resemble the changes called cytopathogenic when referring to infected cultures. However, this normally happens several weeks after injury and destruction of cells in virus infected cultures takes place.

Specificity of virus action was observed in several experiments, where specific antiserum from patients known to have a high antibody titer to vaccinia, was allowed to act on the virus and apparently had a decelerating and, in some cases, completely inhibitory action. This aspect of the study, and the question of survival and quantitation of the virus in supernatant fluids, is being studied at present.

Conclusions. The cytopathogenic effect of vaccinia virus in roller tubes and Maximow double cover slip tissue cultures was established in its principal aspect of degenerative and pathological changes in cell morphology. Specificity of this effect was corroborated as far as possible at the present with coordinated serological tests and animal experiments. Further studies and analysis of visual changes by phase microscopy and photomicrography are planned.

1. Robbins, F. C., Enders, J. F., and Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.

2. Cameron, Gladys, *Tissue Culture Technique,* New York, Academic Press Inc., 1950, p. 92.

3. Vissher, M. B., Editor-in-chief: *Methods in Medical Research,* Chicago, The Yearbook Publishers, Inc., Section 4: Tissue Culture Methods, 1951.

4. Robbins, F. C., Enders, J. F., Weller, T. H., and Florentino, G. L., *Am. J. Hyg.,* 1951, v54, 286.

5. Hanks, J. H., and Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.

6. Gey, G. O., and Bang, F. B., *Viruses and Cells,* New York Acad. Sc., 1951, v14, 15.

7. Kempe, C. Henry, *Yale J. Biol. and Med.,* 1952, v24, 328.

8. Feller, A. E., Enders, J. F., and Weller, T. H., The Prolonged Co-existence of Vaccinia Virus in High Titre and Living Cells in Roller Tube Cultures of Chick Embryonic Tissues, 72:367-388, Oct. 1940.

9. Bang, Frederik, *Bull. Johns Hopkins Hosp.,* 1950, v87, 511.

10. Bang, F. B., Levy, E., and Gey, G. O., *J. Immunol.,* 1951, v66, 329.

Received December 8, 1952. P.S.E.B.M., 1953, v82.

Eosinophile in Human Skin Homografting.* (20167)

BLAIR O. ROGERS,† JOHN MARQUIS CONVERSE, A. CECIL TAYLOR, AND ROSS M. CAMPBELL. (Introduced by J. H. Mulholland.)

From the Department of Surgery, New York University College of Medicine

The revival of interest in skin homografting is evidenced by the increase in the number of papers which have appeared in the medical literature within the past several years. Many of these reports explain the sloughing of most skin homografts on the basis of an *acquired immunity reaction.* In reviewing the literature(1-4) it becomes apparent that skin homograftings have been permanently successful only between identical twins(5), or between members of the same family(6,7). That the compatibility of blood types between donor and recipient remains an important factor necessary for permanent survival of homografts is indicated by other recent reports (4,8,9). Extensive skin homograft research in animals has been described(10-15). Brown and McDowell(16) found "many eosinophiles" in biopsies taken of the sloughing homograft in split-thickness homografting. Split-thickness homografting in a child re-

* This investigation was made possible by grants from the Atomic Energy Commission and the Milbank Memorial Fund. Presented at the Tissue Transplantation Conference, Arden House, Harriman, N. Y., Oct. 7 and 8, 1952.

† Milbank Fellow in Plastic Surgery (1951-1952). (At present, Department of Surgery, Presbyterian Hospital and College of Physicians and Surgeons, Columbia University, New York).

ceiving ACTH therapy was reported by McNichol(17); the eosinophile count began to rise prior to and during the breakdown of the homograft despite the depression of the total eosinophile count by ACTH therapy early in the immediate post-operative course. Animal experiments have demonstrated similar findings. Rise in the eosinophile count at the time of homograft sloughing in rabbits, despite depression of the eosinophile by ACTH and desensitization procedures has been described (18).

Our interest in the eosinophile response was stimulated by observations of a split-thickness homograft from a white donor placed in a forehead defect of a Negro. Microscopic examination of biopsies taken at the time of the sloughing of this *first-set* homograft disclosed eosinophilic engorgement under the homograft dermis. The eosinophilia, in this case, differed from the classical reports(19) of an increased number of lymphocytes in the region of a sloughing skin homograft. A rise in *circulatory* eosinophiles was also noted in our patient at the time of a *second-set* homografting with skin taken from the same donor. On the basis of these observations, a study was made of the circulating eosinophiles and of tissue eosinophiles in ten cases of full-thickness skin homografting.

Experimental homografts in man. Full-thickness homografts of identical size were placed in similar locations (anterior thigh) in surgically created defects and in two cases, full-thickness autografts of identical dimensions were employed on the opposite thigh. All autografts took initially and survived as permanent grafts. The subcutaneous fat was trimmed away from the under surface of the dermis in both homografts and autografts, and the grafts were secured in position by similar types of dressings. *Full-thickness* homografts were used as a matter of expediency. It was simpler to remove a uniform-sized, round piece of full-thickness skin and to fill the resultant defect with a full-thickness homograft of equal size. The uniform size employed served to minimize variations in the eosinophile response which might be attributed to the dosage-phenomenon(11).

Circulatory eosinophilia. Daily white cell counts and differential counts were made in each homografted patient, the total number of circulating eosinophiles being determined by multiplying the white count by the number of eosinophiles in the differential count; this eosinophile count was found comparable to that obtained in total eosinophile counts determined by the Dunger chamber technic. The white counts, differential counts and total eosinophile counts in these patients were taken at various times of the day; in the majority of instances in the mid-morning, about 2 hours after breakfast. The effect of meals on the eosinophile count can be disregarded (20). The normal eosinophile count in ungrafted, control patients is 80-500 cells per cubic mm. The highest eosinophile counts obtained in each of 10 homograftings (4 of these were second-set homograftings), were 4,301; 4,080; 3,047; 1,374; 1,138; 1,033; 975; 800; 540; and 484 cells per cubic mm. The highest eosinophile counts obtained in each of 4 additional autograftings which served as controls were 960; 868; 639; and 552 cells per cubic mm. Penicillin was employed in all of our cases. In 6 of the 10 homograftings, the counts were more than twice the normal and in two were 8 times normal; one was 6 times normal and another 3 times normal; in none of the autograftings did the count exceed twice the normal range. In each case of homografting, however, the rise in the number of circulating eosinophiles exceeded twice the normal range (Fig. 1). Following disappearance of the rejected homografts, the eosinophile counts fell to pre-operative levels. A single first-set homograft and a single second-set homograft from the same donor may provoke a highly specific and similar response from the host. This is suggested by the graphs of the eosinophile levels of the first- and second-set homografts (Fig. 1 and 2).

In one patient, can it be considered mere coincidence in both sets of homografts (Fig. 2) that the eosinophilia charted for each set so closely paralleled each other, or that the return of the eosinophile count to normal occurred on the very same day (34th post-operative day) in both sets, and further, that a secondary eosinophilia with a similar but less

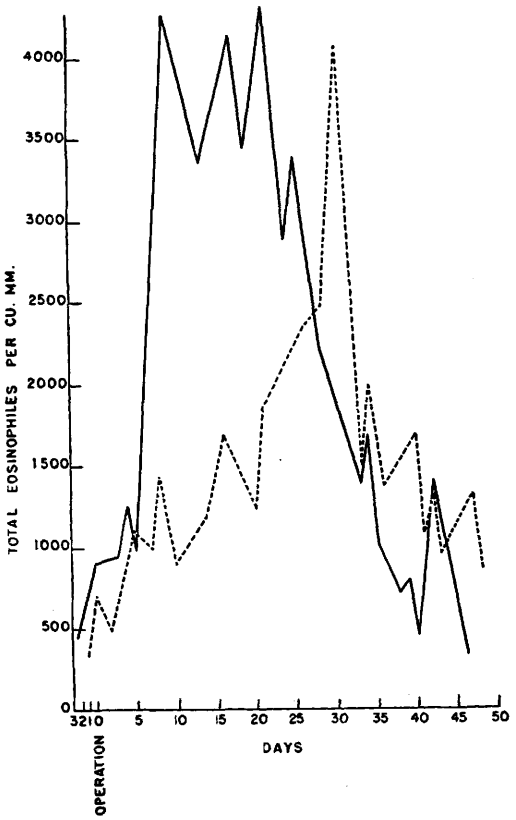

FIG. 1. Case A—The eosinophilia represented by black line for the first-set homografting, and by a broken line for the second-set. Note that the intensity of eosinophilia for both sets exceeds 4000 cells per mm³. These lines fall back to normal levels only when the last vestiges of the homograft dermal pads have disappeared.

intense peak of activity followed the primary eosinophilia? Is a significant factor of homografting, as yet unexplained, involved in this occurrence?

No similar increase in eosinophiles could be demonstrated in the 4 autografttings, nor was eosinophilia demonstrable in an additional case in which a refrigerated *split-thickness* Negro homograft failed to take initially on its white recipient. The graft was found to have sloughed at the first change of dressing on the 5th post-operative day. The failure to "take" in this case was attributed by us to the fact that the method of refrigeration employed had affected the graft and that a devitalized graft had been applied. If a devitalized graft fails to elicit an eosinophile reaction, is the eosinophilia then a response only to "living" homograft cells? Swanson *et al.* (20) found that diurnal variation of the eosinophile count resulted in a greater *eosinopenia* in the morning hours. The findings of morning *eosinophilia* in the homografted patients of this study, therefore, seem to be more striking when compared to the eosinopenia in control patients; the eosinophilia is also in marked contrast to the eosinopenia noted in the post-operative courses of patients who have undergone major surgery (21-23).

Tissue eosinophilia. Circulatory eosinophilia in skin homograftings was accompanied by an increase in *tissue* eosinophiles in the region of the sloughing homograft; the eosinophile count in the tissues was determined by the following method: Freshly removed biopsy specimens consisting of equal halves of homograft and of marginal skin of the host were immediately immersed in Bouin's fixative, infiltrated and embedded in paraffin, sectioned at 8 micra and stained with hematoxylin and eosin. Magnification with an oil immersion objective (x95) and a No. 10 ocular lens was used to count the eosinophiles in one field (x950), the slide was moved one field diameter, and the number of eosinophiles again recorded; fifty adjacent fields were thus counted in two separate parts of each biopsy section. One of the areas included the junction of the host and graft tissue; the other consisted of the graft tissue. Counts were made on 4 alternate sections from each biopsy specimen; the total number of eosinophiles in the 400 oil immersion fields was recorded. The tissue eosinophilia was found comparable in magnitude to the intensity of the circulatory eosinophilia. This would seem to support the impression that the eosinophile response is directed against the presence of a homograft.

Interpretation of findings. a) The greatest intensity of eosinophilia occurred when the epithelium of the homograft had sloughed and only the dermis remained intact. b) The eosinophilia subsided and the count returned to normal levels as the last vestiges of the homograft's dermal pad sloughed away. c) The number of eosinophiles in a differential count increased at the expense of the polymorphonuclear cells, thus establishing an in-

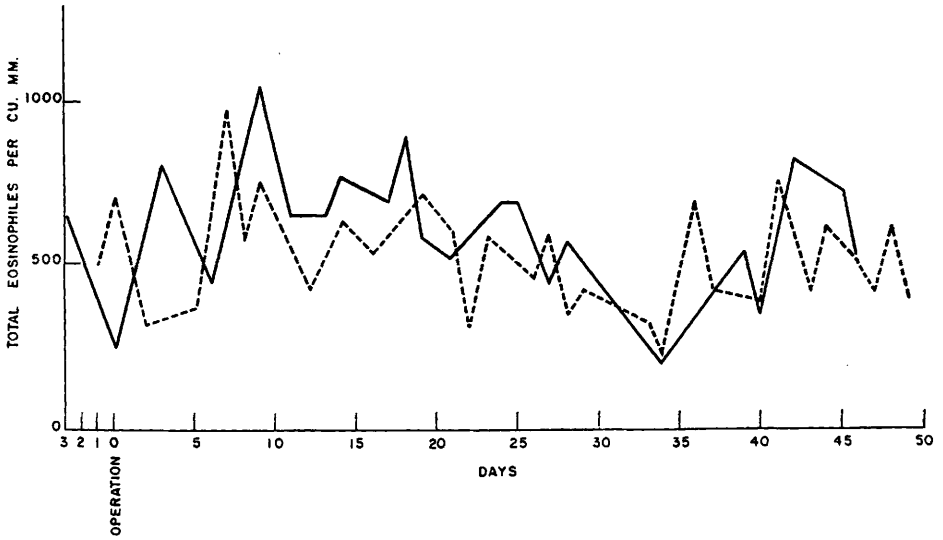

FIG. 2. Case B—Close parallelism between eosinophilia charted for first-set homografting (black line) and that charted for second-set homografting (broken line). In both cases the lines dropped to an almost identical number of eosinophiles per mm³ on the very day (34th post-operative day) when the last vestiges of the homograft dermal pads have disappeared.

verse relationship between the two types of cells in their responses to homografting.

Summary and conclusions. 1. In full-thickness homografts in man, an increase of eosinophiles occurred in the circulating blood stream and in tissues at the site of the graft. 2. Tissue eosinophilia is comparable in magnitude to the intensity of the circulatory eosinophilia. 3. Eosinophilia seems to be most intense when the epithelium of the homograft has sloughed and only the dermis remains intact. 4. Eosinophilia subsides and the count returns to normal levels as the last vestiges of the homograft's dermal pad sloughs away. 5. The number of eosinophiles in a differential count increases at the expense of the polymorphonuclear cells, thus establishing an inverse relationship between the two types of cells in their responses to homografting.

We wish to thank Dr. John H. Mulholland for his stimulating criticism and suggestions in the course of this investigation, Dr. Harry H. Shapiro for his editorial assistance, and Miss Frieda Steinschneider for her technical assistance.

1. Rogers, B. O., *Plast. and Reconstr. Surg.*, 1950, v5, 269.

2. ———, *Plast. and Reconstr. Surg.*, 1951, v7, 169.

3. Dempster, W. J., *Brit. M. J.*, 1951, vII, 1041.

4. Woodruff, M. F. A., *Ann. Roy. Coll. Surgeons, England*, 1952, v11, 173.

5. Converse, J. M., and Duchet, G., *Plast. and Reconstr. Surg.*, 1947, v2, 342.

6. Kearns, J. E., and Reid, S. E., *Plast. and Reconstr. Surg.*, 1949, v4, 502.

7. Wolf, F., *Med. Klin.*, 1946, v41, 350.

8. Spaeth, E. B., and Cappriotti, O., *Plast. and Reconstr. Surg.*, 1948, v3, 707.

9. Caby, F., *Plast. and Reconstr. Surg.*, 1952, v10, 14.

10. Medawar, P. B., *J. Anat.*, 1944, v78, 176.

11. ———, *J. Anat.*, 1945, v79, 157.

12. ———, *Brit. J. Exp. Path.*, 1946, v27, 9.

13. ———, *Brit. J. Exp. Path.*, 1946, v27, 15.

14. ———, *Brit. J. Exp. Path.*, 1948, v29, 58.

15. ———, *Quart. J. Micro. Sciences*, 1948, v89, 239.

16. Brown, J. B., and McDowell, F., *Ann. Surg.*, 1942, v115, 1166.

17. McNichol, J. W., *Plast. and Reconstr. Surg.*, 1952, v9, 437.

18. Allen, H. L., Williams, R. D., Lovingood, C. G., and Ellison, E. H., *Ann. Surg.*, 1952, v135, 329.

19. Loeb, L., *Physiol. Review*, 1930, v10, 547.

20. Swanson, J. N., Bauer, W., and Ropes, M., *Lancet*, 1952, vI, 129.

21. Laragh, J. H., and Almy, T. P., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 499.

22. Roche, M., Thorn, G. W., and Hills, A. G., *New England J. Med.*, 1950, v242, 307.

23. Coppinger, W. R., and Goldner, M. G., *Surgery*, 1950, v28, 75.

Received February 4, 1953. P.S.E.B.M., 1953, v82.