the A^+ and normal (B^+) enzymes. The enzyme deficiency in A^- red cells is principally caused by an increased rate of degradation rather than by a decreased rate of production of the enzyme.

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6. Normal enzyme (B^+) has been purified and crystallized; its chemical and enzymatical characteristics, and the purification method and method of enzyme assay have been described [A. Yoshida, *J. Biol. Chem.* **241**, 4966 (1966)]. Negro-type variant (A^+) has also been purified and characterized.
7. Antiserum to B^+ was obtained by injecting 0.4 mg of purified homogeneous B^+ glucose-6-phosphate dehydrogenase together with 0.5 ml of sodium alginate adjuvant and 0.5 ml of 0.1*M* $CaCl_2$. This dose was given weekly for 4 weeks.
8. The number of milligrams of enzyme protein absorbed by 1 ml of antiserum is equal to the number of units of enzyme activity neutralized by 1 ml of antiserum divided by the specific activity of enzyme.
9. No quantitative difference was found between the purified B^+, purified A^+, partially purified B^+, and partially purified A^+ by quantitative microcomplement fixation test (unpublished observation by Drs. V. Sarich and A. C. Wilson).
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14 November 1966

Local Recognition of Histocompatibility Differences in Skin Grafts

Abstract. *Studies in rabbits of skin grafts tagged with tritiated thymidine indicate a greater proliferation of endothelial cells and fibroblasts at the site of an allograft than at that of an autograft as early as the first day after grafting. It appears, therefore, that allogeneic differences can be recognized and responded to locally almost at once. Labeled nuclear material is found to be transferred from the epithelial cells of skin grafts to host cells of the adjacent tissues. A mechanism therefore exists which might effect a local transfer of information on histocompatibility differences.*

It is generally accepted that first-set skin allografts are not distinguishable from autografts by morphological criteria for a number of days after grafting, the time depending on the degree of histocompatibility difference. Not until the immune response has progressed far enough to provide an observable difference in the degree of cellular infiltration and vascular development can the allograft be identified. In hundreds of skin cross-grafting experiments, using two outbred strains of rabbits, we have found this time to be hardly ever shorter than 4 days from the time of grafting, rejection occurring between 7 and 10 days. However, when graft sites were examined by microangiography, the impression was gained that the vascular reaction under and about a skin allograft is greater than that at an equivalent autograft in the same animal as early as 1 to 2 days after grafting.

In the present experiments an effort was made to substantiate this difference objectively by determining the uptake of labeled thymidine by the nuclei of endothelial cells and fibroblasts in the graft and graft bed. The demonstration of an almost immediate difference in the response to allograft and autograft would suggest that recognition of "nonself" may be very prompt and may take place locally. Such an early differential response has been reported in the goldfish with respect to scale transplants (*1*), and in the rabbit for hair follicles in transplanted skin (*2*).

Circular full thickness grafts of ear skin (1.7 cm in diameter) were exchanged between pairs of young rabbits (1 kg in weight) of two strains with presumed strong histocompatibility differences (New Zealand Whites and Chinchillas). Each rabbit received a similar autograft on its other ear at the same time. All surgical procedures were carried out aseptically. Grafts were left undressed. At 6 and 12 hours after grafting, and at daily intervals, rabbits were killed 2 hours after they had received an intravenous dose of tritiated thymidine (H3Th) (0.75 μc/kg of body weight, specific activity 3 c/mmole). Autoradiographs, exposed for 2 weeks, were prepared of tissue sections which included graft and graft bed. Cells with tritium-labeled nuclei, excluding cells of obvious epithelial origin, were counted from the epithelium to the cartilage, between grid lines (64 lines per inch) extending perpendicularly to the skin surface along the full length of the graft. Three to eight widely spaced tissue sections were counted in this way for each graft. The mean number of labeled cells per grid space was calculated for each section, and from those, the overall mean and variation for each graft. The allograft/autograft ratio of labeled cells was then determined for each animal.

As seen in Table 1, labeled nuclei were more numerous in the allograft than in the autograft in every animal. Apparently, then, the nonepithelial cells were proliferating at a greater rate in the allografts. Differences did not become statistically significant until 24 hours, but were in the same direction even at 6 and 12 hours. Results are presented for the first 4 days; thereafter standard morphological differences evoked by the immune response became obvious.

Although the time course of the reaction to the allograft might by itself suggest a secondary immune response, it is very unlikely that the recipient in each of the randomly selected animal pairs was presensitized to the donor tissue. Our findings are much more reasonably interpreted as indicating a local transfer of histocompatibility information from the outset of the healing of a tissue transplant. It is of interest that the reaction elicited is a mitogenic one.

"Antigenic recognition" in vitro was inferred by Möller (*3*) from the cytocidal effect of nonsensitized lymphocytes on allogeneic and semi-syngeneic target cells in the presence of phytohemagglutinin. However, in the absence of phytohemagglutinin, nonsensitized lymphocytes have a stimulatory effect on allogeneic target cells in vitro (*4*). We have observed in vivo a mitogenic effect upon the epithelium of skin allografts prior to graft destruction (*5*). The cells displaying labeling during the

Table 1. Labeled cells in allograft and autograft.

Time (hours)	Mean numbers of labeled cells per grid space		Ratio of means: allograft/autograft	*P* value of difference of means
	Allograft	Autograft		
6	0.14	0.10	1.4	
12	.08	.02	4.0	
12	.39	.23	1.7	
12	.50	.28	1.8	
24	2.98	1.81	1.6	
24	5.40	4.10	1.3	
24	7.86	2.19	3.6	<0.01
24	23.48	10.38	2.2	<0.01
24	16.30	11.60	1.4	<0.01
42	29.52	17.07	1.8	<0.01
42	24.32	18.97	1.3	<0.05
42	25.28	21.37	1.2	<0.05
46	20.50	17.57	1.2	<0.05
46	21.95	11.11	1.9	<0.05
46	16.87	11.73	1.4	<0.05
72	4.58	1.64	2.9	<0.05
72	13.19	11.22	1.2	<0.05
72	20.49	18.59	1.1	
96	14.10	8.84	1.6	<0.05
96	24.43	14.23	1.7	<0.05

time discussed in the present experiments are mainly those actively involved in the revascularization of the graft and in healing: capillary endothelium, and fibroblasts (Fig. 1A). These are the cells that effect the functional union of graft and host. They would thus seem to fulfill the precondition of close contact which is said to govern the recognition by cells of surface antigenic configurations without the involvement of the immune reaction (*3*). However, in addition to any surface contact effect that may occur, the following experiments indicate that nuclear components released from graft cells are incorporated into nuclei of neighboring tissue cells of the host.

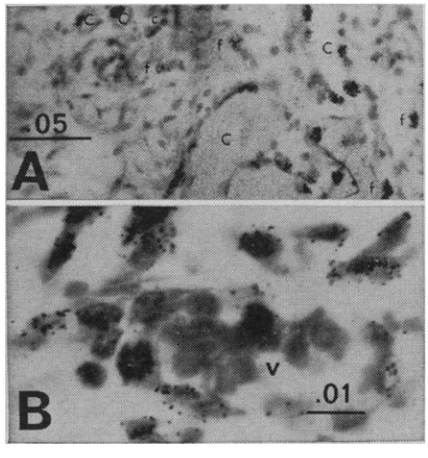

Fig. 1. Nuclear labeling in relation to skin grafts (scales represent fractions of a millimeter). (A) Autoradiograph of a 46-hour allograft, labeled by tritiated thymidine given intravenously to the host. Labeled capillary endothelial cells and fibroblasts are demonstrated at the graft-host interface. The vasculature had been injected with a radiopaque mass (Micropaque) before the ear was fixed, allowing easy recognition of the somewhat distended fine vessels. Abbreviations: c = capillary; f = fibroblast. (B) Autoradiograph of host cells deep in the bed of a 4-day-old allograft labeled prior to transfer. A small vessel (v) containing erythrocytes is lined by labeled endothelial cells, one of which is in mitosis. The remaining labeled cells are fibroblasts. All label in this figure (B) derived from the nuclei of labeled graft cells. (Graft and bed were infiltrated with nonradioactive thymidine; see text.)

Ear skin cells were heavily labeled, either by twice-daily intradermal injections of small doses of tritiated thymidine (5 μc in 0.05 ml of saline) for 4 days, or by slow twice-daily intra-arterial infusion by means of a Harvard pump (30 μc in 1 ml of saline over 30 minutes) for 3 days. Three to five days after the last administration of H3Th, at which time no free interstitial H3Th could be detected in autoradiographs, skin so labeled was transplanted to the ears of 12 rabbits. In two of these rabbits the graft and graft bed were infiltrated with 1 μmole of nonradioactive thymidine twice daily for the life of the graft, starting immediately after grafting. (This dosage schedule almost certainly provided a continuous reservoir of thymidine to cells in the vicinity, since after injections of tritiated thymidine in considerably lower concentrations, autoradiographs showed radioactivity to persist in the interstitium beyond 18 hours.) Recipient animals were killed at intervals starting 2 days after grafting, until the allografts were fully rejected. Autoradiographs of tissue sections of graft and graft bed were prepared and exposed for 8 weeks at 4°C.

At all stages examined we found labeled nuclei within cells unmistakably identified as host cells (*6*). During the period concerned with here—the first 4 days—the local host cells that had taken up label were the endothelial cells and fibroblasts, and some invading lymphoid cells (Fig. 1B). Since the graft contained no free labeled thymidine at the time of grafting, and the transfer of label from graft to host was unimpaired by a high local concentration of unlabeled thymidine, it seems unlikely that the material incorporated into host cells was thymidine itself. If a higher-order compound originating from graft cell DNA, or DNA itself, was exchanged, a potential mechanism of informational exchange on the genomic level would seem to exist, upon which local recognition of allogeneic differences could be based. Some such mechanism for the handing on of information by cells was postulated by Burnet several years ago in connection with the primary hypersensitivity response (*7*). It has recently been shown that lymphocytes become sensitized on passage through an allogeneic organ (*8*), and that specific transplantation immunity may be induced in allogeneic animals by the intradermal injection of cell-free medium of donor cell cultures (spleen) (*9*).

Host-cell labeling was seen several days prior to allograft rejection. Other evidence also suggests that the release of labeled nuclear material was a function of living cells rather than a passive accompaniment of cell death. The labeling itself did not kill the graft cells; the heavily labeled graft cells proliferated profusely until the grafts were rejected (Fig. 2). The epithelium of hair follicles was sometimes seen surrounded by a halo of silver grains, a finding strongly suggesting the release of some labeled compound by these actively dividing cells (Fig. 2a). Cells in culture have been shown to release into the medium a factor that is mitogenic; cell-free media of lymphocyte cultures, for example, will induce allogeneic lymphocytes to transform and divide (*10, 11*). Only living cells produce this mitogenic factor; its elaboration can be suppressed by drugs that inhibit cell synthetic processes and cell division (*11, 12*).

These findings combined can be interpreted as indicating that in the rabbit, in the context of skin-grafting, information on histocompatibility differences is exchanged between host and graft cells directly and virtually im-

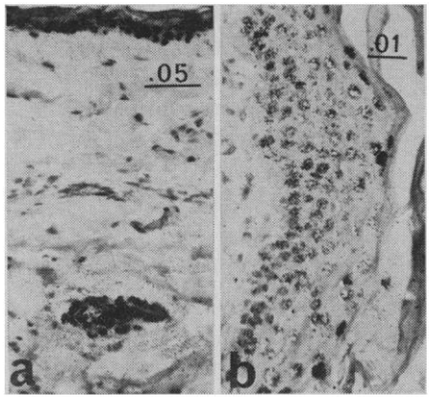

Fig. 2. Autoradiographs of skin labeled before transplantation. (Scales represent fractions of a millimeter.) (a) Skin immediately before grafting; silver grains, considerably in excess of background density, can be seen adjacent to the heavily labeled hair follicle and basal layer of the epidermis, suggesting that some labeled material may have been released by these cells. (b) Epithelium of the same skin 7 days after transplantation as an allograft: the initial heavy labeling of the basal cells has not interfered with the usual extensive proliferation of the epithelial cells.

mediately at the graft site, and that the effect of this exchange is stimulatory to DNA synthesis. Such transfer from an allogeneic tissue to host lymphoid cells may be an initial step in the induction of transplantation immunity.

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6. This transfer is presumably an exchange in both directions. Unlabeled skin grafts, placed on a previously labeled site, acquired a nuclear label. It is more difficult, however, to prove unequivocally that this was a local transfer, since cells of the lymph node draining the graft site were unavoidably labeled also, and label subsequently found in graft cells could have been brought there by circulating lymphoid cells exercising a trophic function (*13*).
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Electrophysiologic Studies during Scanning and Passive Eye Movements in Humans

Abstract. *It has been demonstrated in man that mechanically induced shifts of the retinal image without change in total luminous flux evoke a parieto-occipital electrical response (lambda waves). The technique provides a simple method of quantifying responses in the visual system without complication by voluntary movements and their associated readiness potentials. The observation contradicts the view that lambda waves are directly concerned with mechanisms preventing blur during eye movement.*

Vision remains clear during eye movement, though the mechanism whereby this is achieved is subject to debate. A concomitant of eye movement is the appearance in the parietal region of positive potentials called lambda waves (*1*). It has been postulated (*2*) that these waves are concerned with antiblurring mechanisms during eye movements, since they regularly follow changes in fixation. The observation that passive displacement of the eyeball not only produced blurring of an image but also resulted in lambda waves has some interesting consequences for visual physiology, and was investigated in the following experiment.

Recording electrodes were attached to the parieto-occipital region, and an oculographic pair in a horizontal plane close to one eye. Potentials from the electroencephalographic electrodes were amplified and displayed on an ink-writing oscillograph as well as being summated by a Mnemotron CAT computer. The experimenter's finger, tapping the outer canthus of the subject's eye, produced a transient displacement of the eye; the resulting voltages, after direct-current amplification, were transmitted to the computer. A subject was required to fixate a point in the middle of an illuminated (30 mlam) stimulus card which bore a complex black-and-white picture known from previous work in this laboratory to be effective in producing lambda waves. A tap of this kind caused vertical images to be displaced laterally by about 1° of arc, with blurring; the accompanying electro-ocular transient was sufficient to trigger the computer.

Extensive investigations have been carried out on one subject (D.F.S.) and confirmed on two other subjects; one typical result is illustrated (Fig. 1). Figure 1A shows a clearly defined response with a peak positive latency of 130 to 150 msec resulting from displacement of the left eye. This response is very similar to that shown in Fig. 1C, which was obtained during voluntary monocular scanning of the stimulus card. Related to these averaged observations are insets A_1 and C_1 from the parieto-occipital electroencephalographic tracings. Lambda waves are clearly recognizable in both tracings, though they are larger and more frequent in C_1, where the eye was scanning rather than being moved passively.

Figure 1B is a control recording obtained by tapping the canthus in total darkness. It will be observed that the characteristic deflection of Figs. 1A and 1C did not occur. In addition there are no lambda waves in the electroencephalographic tracing (inset B_1). These results exclude the possibility that the responses shown in Fig. 1A are caused by skin or proprioceptive stimuli associated with passive displacement of the eye and the extraocular musculature.

It therefore appears that when the

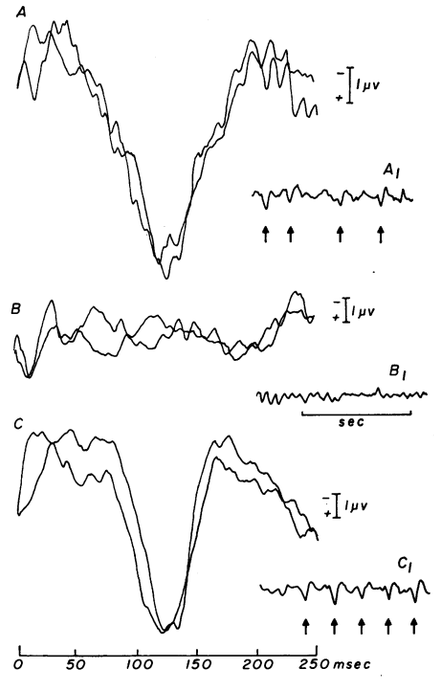

Fig. 1. Summated responses in parieto-occipital region, two sets, of 20, superimposed (subject D.F.S.). (A) Response to tapping of outer canthus of left eye during fixation on complex stimulus card. (B) Response to similar tapping of eye in darkness. (C) Response to scanning of complex stimulus card. Insets A_1, B_1, and C_1 show electroencephalographic tracings, with lambda waves indicated by arrows.