Reports

Effect of a Pyridoxine Deficiency on Skin Grafts in the Rat

It has been amply demonstrated that the antibody response to a variety of antigens is markedly inhibited in a number of vitamin-deficiency states (*1*). The effect of a pyridoxine deficiency has been particularly well documented. In addition to induction by the usual dietary procedures, a pronounced deficiency of this vitamin can be rapidly induced by the administration of the antagonist, desoxypyridoxine. The latter experimental approach has been used to advantage in investigations on the role of pyridoxine in antibody production (*2*). It is generally accepted that the rejection of skin grafts from genetically dissimilar members within a given species (homografts) results from an immune response of the recipient to the antigens of the donor skin (*3*). The possibility was considered that this acquired immune response would be diminished in pyridoxine-deficient rats, with a resultant decrease in the magnitude of the rejection process. Thus, the study described in this report was undertaken to investigate the effects of a pyridoxine deficiency, induced in the rat by dietary means or by the administration of desoxypyridoxine, upon the rejection phenomenon of skin homografts.

Male, weanling rats of the Wistar and Long-Evans strains were purchased from commercial sources and were housed in individual cages. They were fed ad libitum a purified diet in which the B vitamins were furnished as a daily pill (*4*). Desoxypyridoxine, dissolved in isotonic sodium chloride, was administered daily by intraperitoneal injection. Pieces of skin of full thickness, measuring 2 by 2 cm, were excised from either the middorsum or the anterior abdominal wall of donor animals. All underlying fat and subcutaneous tissue were removed, and the skins were immediately applied to the host area to be grafted. The latter was prepared by removing a similar sized square of skin of full thickness from the middorsum, together with underlying fat and connective tissue, including the panniculus carnosus. Grafts were applied in reversed fashion so that hair growth was in the opposite direction to that of the host. No dressings were applied. Progress of the graft was followed closely by gross observation. Rejection was determined by ulceration, contraction, and final appearance of a fibrous scar. Histological examination supplemented the gross observations in doubtful cases. The absolute criterion of a successful graft ("take") included the absence of any of the above processes and the ultimate appearance of new hair growth in a direction opposite to that of the host. The success or failure of a graft could usually be assessed 3 weeks after the grafting procedure.

In the first series, comprising five separate experiments with a total of 400 rats, middorsal skin grafts were exchanged between (i) control, (ii) pyridoxine-deficient, and (iii) control and pyridoxine-deficient non-litter-mates of the Wistar strain after the animals had been maintained for 4 weeks on the experimental diets. Pyridoxine deficiency was produced by the omission of pyridoxine from the daily pill. Control rats received an identical diet supplemented daily with 50 μg of pyridoxine. Unless it is noted otherwise, the dietary regimen was not altered during the course of the experiment. Autografts of similar type were performed in both control and pyridoxine-deficient rats. The experimental period in this series varied from 3 to 12 weeks after the grafting procedure. During this time, many of the pyridoxine-deficient rats succumbed, while others, along with controls, were sacrificed. The results of these experiments are shown in Table 1. Grafts designated as "takes" at 3 weeks following grafting did not regress during the remainder of the experimental period. In three experiments involving 90 pyridoxine-deficient recipients (excluding autografts), 52 were alive 6 weeks after grafting. At this time, 80 percent of the grafts on these animals were considered "takes." In a separate experiment, seven pyridoxine-deficient recipients with successful grafts from pyridoxine-deficient donors were transferred to the control diet 4 to 8 weeks following grafting. All of these grafts were in excellent condition 24 weeks following their transplantation.

Table 1. Skin grafts in pyridoxine-deficient and control rats.

Donor	Recipient		Total No. of rats	Percentage of "takes"		
	Strain	Type		3–12 wk after operation	3 wk after operation	10 wk after operation
Series 1 (dietary-induced deficiency)						
Control		Control	78	15		
Deficient		Control	67	28		
Control		Deficient	60	62		
Deficient		Deficient	62	90		
Autografts						
Deficient			64	86		
Control			69	45		
Series 2 (desoxypyridoxine-induced deficiency)						
	Long-Evans	Control	71		9	1
	Long-Evans	Deficient	50		92	60*
	Long-Evans	Control†	16		0	0
	Wistar	Control	70		4	1
	Wistar	Deficient	43		63	2
	Wistar	Control†	16		0	0
Autografts‡						
	Long-Evans	Control	24		100	
	Long-Evans	Deficient	14		86	
	Wistar	Control	23		100	
	Wistar	Deficient	9		100	

* Seventeen animals in this group have been observed for 18 to 21 weeks. Of these, five still have successful grafts.

† This group was treated exactly like the corresponding deficient group except that 1 mg of pyridoxine was given daily by intraperitoneal injection during the period of desoxypyridoxine treatment. The Long-Evans rats were fed the control diet immediately following cessation of desoxypyridoxine administration. No symptoms of pyridoxine deficiency were noted in these animals.

‡ Thirty of these autografts have been under observation for 10 to 18 weeks following grafting. All are in excellent condition. Autografts successful at 3 weeks following operation have never been noted to regress.

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The number of "takes" observed in control recipients indicates a certain genetic similarity between members of the Wistar strain employed (Table 1). However, it is apparent that resistance to skin grafts is markedly diminished in pyridoxine-deficient recipients. The use of donor skin from pyridoxine-deficient animals results in a greater percentage of successful grafts than does that of donor skin from controls in corresponding groups of recipients (*5*).

In the second series, consisting of three separate experiments with a total of 336 rats, skin grafts were exchanged between members of the Wistar and Long-Evans strains after the animals had been maintained for 3 weeks on the control diet furnishing 10 μg of pyridoxine daily. The rats grew well on this regimen and did not manifest any of the symptoms characteristic of a pyridoxine deficiency. Donor skin was taken from the anterior abdominal wall and grafted to the back. Autografts were performed on both Wistar and Long-Evans rats. Immediately following the grafting procedures, each strain was divided into two groups. One continued to receive the control diet for 5 to 6 weeks. Thereafter, survivors in this group were fed a commercial stock ration (Purina chow). Another group received the pyridoxine-free diet and was treated with desoxypyridoxine. Animals of the Long-Evans strain were given daily injections of 250 or 500 μg of desoxypyridoxine for 10 days and were continued on the pyridoxine-deficient diet for an additional 5 to 8 days. The Wistar rats were given similar daily injections of desoxypyridoxine during this period of 15 to 18 days. Typical symptoms of pyridoxine deficiency were evidenced in all desoxypyridoxine-treated rats. These pyridoxine-deficient animals were then fed the control diet for 3 to 5 weeks and the Purina chow ration for the remainder of the experiment. Results obtained with both levels of desoxypyridoxine were similar and are summarized together in Table 1.

As in series 1, the skin grafts in pyridoxine-deficient recipients of series 2 were far more successful than those in control recipients. In contrast to series 1, many successful grafts in series 2 were subsequently rejected. This was particularly true for the pyridoxine-deficient Wistar rats, in whom the incidence of rejection following an initial "take" at 3 weeks was exceedingly high. It should be noted that, at 3 weeks, the grafts of the pyridoxine-deficient Long-Evans rats were superior to those of the corresponding Wistar group. In further comparison with series 1, the absence of any mortality, the lesser incidence of "takes" in control homografts, and the higher incidence of "takes" in control autografts in series 2 are noteworthy. This last observation illustrates the superiority of full-thickness abdominal skin over full-thickness dorsal skin as donor material.

In summary, the survival time of skin homografts is increased markedly in pyridoxine-deficient rats. Some grafts are still in excellent condition 5 to 6 months following grafting. It is possible that this effect may be related to an inhibition of the immune response to the antigens of the donor skin in this deficiency state. The specificity of this effect and its mechanism are under study (*6*).

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References and Notes

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3. *Ann. N.Y. Acad. Sci.* 59, 277 (1955); 64, 735 (1957).
4. A. E. Axelrod *et al.*, *Proc. Soc. Exptl. Biol. Med.* 66, 137 (1947).
5. This interesting circumstance and related experiments will be discussed more fully in a subsequent publication.
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Influence of L-Ascorbic Acid on the Colchicine Reaction

Because of its relative specificity of cytological effect, the colchicine reaction should provide valuable information regarding the mechanism of mitosis (*1*). That the reaction has not done so to date is apparently due to the lack of knowledge concerning its mode of action in the cell (*2, 3*). One obvious approach to this problem is the careful study of substances which either suppress or enhance the reaction. Many such substances are known, but the majority are either moderately good antimitotic agents in themselves or must be used at very high dose levels (*2*).

Ascorbic acid has been reported to inhibit partially the effect of colchicine on tissue cultures of rabbit fibroblasts (*4*). Since L-ascorbic acid is a normal metabolite, reinvestigation of the reported antagonism of this substance on the colchicine reaction appeared to be worth while (*5*). To do this, use was made of the Bowen-Wilson *Pisum* test (*6*). This test consists essentially of treating standard size (2.5 to 3.5 cm) primary roots of young pea seedlings under standard conditions and examining their meristems for quantitative cytological changes in terms of frequency of diagnostic chromosome configurations relative to dose-time changes. Colchicine activity was measured by means of the colchicine index which was devised and studied for validity in our laboratory (*7*). This index is based on assigning values to specific chromosome configurations according to the severity of the colchicine effect represented by such configurations. Previous studies have shown that such an index changes smoothly with time and that the rate of change is dependent on dose. A given index at a given time represents a specific colchicine potency. Modification of the colchicine reaction may therefore be measured by differences between control and treatment indices at given times.

Table 1 summarizes our findings. Essentially, it was noted that treatment of colchicine for several hours with ascorbic acid at *p*H 7, followed by adjustment of the *p*H of the mixture back to 5.5 immediately prior to use, resulted in a lower index than that obtained in the colchicine control. While ascorbic acid has only a slight but measurable effect when it is used at one-half the molarity of the colchicine, it does have a much greater effect when its molarity is equal to that of colchicine (1.25×10^{-4}). At twice the molarity of colchicine, ascorbic acid produced no significant change in effect over that produced by the equimolar concentration. A mixture of colchicine and ascorbic acid in a molarity

Table 1. Effects of L-ascorbic acid on the colchicine reaction.

Test No.	Colchicine mol. ($\times 10^{-4}$)	Ascorbic acid. mol. ($\times 10^{-4}$)	*p*H		Colchicine equivalent (%)	Enhancement in time
			Initial	Treatment		
1	1.25		5.5	5.5	100	0
2	1.25	0.63	7.0	5.5	94	−30
3	1.25	1.25	7.0	5.5	84	−69
4	1.25	2.5	7.0	5.5	88	−54
5	1.25	2.5	6.0	5.5	94	−32
6	1.25	0.63	5.5	5.5	100	+ 2
7	1.25	1.25	5.5	5.5	101.5	+22
8	1.25	2.5	5.5	5.5	102	+36
9*	1.25	2.5	5.5	5.5	102	+30

* Roots were pretreated with ascorbic acid for 1 hour and then treated with colchicine.