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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5. This interesting circumstance and related experiments will be discussed more fully in a subsequent publication.
6. This investigation was supported by grants from the National Vitamin Foundation, Inc., New York, the United Fund of Allegheny County, Pa., and the Division of Research Grants (No. RG 4624), National Institutes of Health, U.S. Public Health Service.

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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.

ratio of 1:2 made up at *p*H 6, stored for several hours, and then adjusted to 5.5 just before it was used had a small but measurable suppressing effect (about equal to the 1:½ mixture at *p*H 7).

Obviously both the molarity of the ascorbic acid and the *p*H of the mixture are important factors. The latter is borne out by the fact that treatment of root tips with mixtures of colchicine and ascorbic acid at *p*H 5.5 produces an enhancement of the colchicine effect, the degree of which is dependent on the molarity of the ascorbic acid. Interestingly enough, pretreatment of root tips with ascorbic acid at two times the molarity of colchicine had virtually the same effect as combining the two compounds at *p*H 5.5 before use. While the latter might be considered to be an in vitro effect, the former must be an in vivo one.

Obviously, before a compound is designated as an enhancer or antagonist, the conditions of treatment must be stated. L-Ascorbic acid falls into both categories, depending, in part at least, on *p*H. However, alkaline *p*H's which produce rapid hydrolysis of the lactone ring (*8*) render the ascorbic acid ineffective. The effect of L-ascorbic acid on the colchicine reaction is determined by three factors (i) the *p*H, (ii) the relative molarity, and (iii) the integrity of the ascorbic acid molecule. Exactly how these factors are related mathematically remains to be determined.

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Reaction of Epinephrine with Ethylenediamine

The condensation of epinephrine with ethylenediamine (EDA) to form a fluorescent product was first reported by Natelson, Lugovoy, and Pincus (*1*). Weil-Malherbe and Bone (*2*) adapted this reaction to the fluorimetric quantitation of epinephrine and norepinephrine in blood.

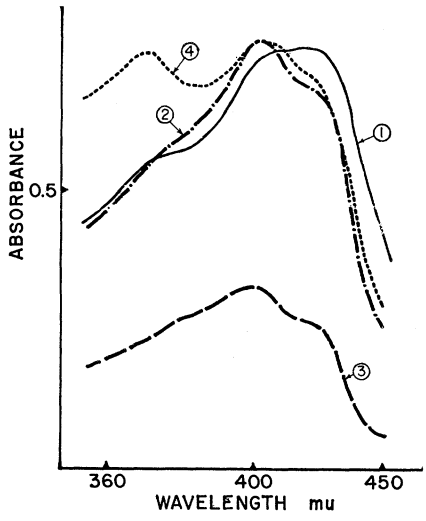

Fig. 1. Absorption spectra of fluorescent derivatives in isobutanol. Curve 1 is the product from 0.8 μM epinephrine, 1×10^{-2} mole EDA, 1×10^{-3} mole EDA dihydrochloride; curve 2 is the product from 0.8 μM epinephrine, 1×10^{-3} mole EDA, 1×10^{-4} mole EDA dihydrochloride; curve 3 is the product from 0.8 μM epinephrine, 1×10^{-4} mole EDA, 1×10^{-5} mole EDA dihydrochloride; curve 4 is the product from 0.8 μM epinephrine, 1×10^{-2} mole EDA, 1×10^{-3} mole EDA dihydrochloride, heated at 70°. Spectra were obtained using a Beckman DK-2 recording spectrophotometer.

While considerable attention has been given to improving the method of Weil-Malherbe and Bone, little work has been reported on the mechanism of this reaction and the nature of the products formed. Burn and Field (*3*) reported the formation of two fluorescent derivatives from norepinephrine by heating at 50° to 70°C with 5 percent EDA for 1.5 to 2 hours at *p*H 11. They obtained two fluorescent derivatives from epinephrine under similar conditions.

A study of the reaction of various catechols with EDA is underway in our laboratory. As part of this study, the reaction of epinephrine with EDA was carried out under various conditions of temperature, *p*H, and EDA concentration, and the products were examined by spectrophotometric and chromatographic techniques. The absorption spectrum of the products obtained under the conditions established by Weil-Malherbe and Bone (*2*) is represented by curve 1 of Fig. 1. The peaks at 400 and 415 mμ and the shoulder at 370 mμ each represent a different fluorescent derivative as shown by chromatographic separation on filter paper (*4*) and by spectrophotometric measurement of the eluted products. The compound with $\lambda_{max.}$ 370 mμ had an R_f value of 0.22 and fluoresced blue-white; that with $\lambda_{max.}$ 400 mμ, R_f 0.35, fluoresced blue-green; and the derivative with $\lambda_{max.}$ 415 mμ, R_f 0.49, fluoresced yellow.

Changes in EDA concentration affected the relative proportion of the fluorescent derivatives formed in this reaction. A stepwise reduction of the EDA concentration resulted in a gradual increase in the ratio of the absorbance at 400 mμ to that at 415 mμ. Curve 2 of Fig. 1 represents the effect of a tenfold decrease in EDA concentration. A 100-fold decrease in EDA concentration resulted in lesser amounts of all three derivatives (curve 3, Fig. 1). The highest EDA concentration (curve 1, Fig. 1) gave a *p*H of 10.6. All other concentrations were adjusted to this *p*H with saturated trisodium phosphate solution.

The effect of increased temperature is illustrated by curve 4 in Fig. 1. At 70°C a significantly greater amount of derivative with $\lambda_{max.}$ 370 mμ was formed. Heating for longer than 20 minutes at 50°, 70°, or 100°C decreased the yield of all three derivatives.

Maximum amounts of the blue-green and yellow fluorescent compounds were formed at *p*H 10.6, lower yields being obtained at *p*H 9.5 or 11.0.

Weil-Malherbe and Bone furnished evidence that adrenochrome is an intermediate in this reaction (*2*). This fact has been verified in the present study. Adrenochrome and epinephrine yielded identical products with EDA as demonstrated by absorption spectra and paper chromatography.

Bubbling oxygen through the reaction mixtures increased only slightly the yields of fluorescent material. In fact, substitution of adrenochrome for epinephrine, in which case oxygen is not required (*2*), does not increase the yields of fluorescent compounds. This is contrary to the results of Burn and Field (*3*) who reported that the oxygen dissolved in the solution was not sufficient for maximum yields of fluorescent material.

Norepinephrine, in contrast to epinephrine, formed only two fluorescent derivatives under the same conditions; one having R_f 0.33 fluoresced blue-green, the other, R_f 0.49, fluoresced yellow (*4*). The spectrum of this fluorescent material was similar to that reported by Burn and Field (*3*), which showed a single peak at 420 mμ in isobutanol. The difference in the reaction of epinephrine and norepinephrine with EDA cannot be explained at this time (*5*).

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