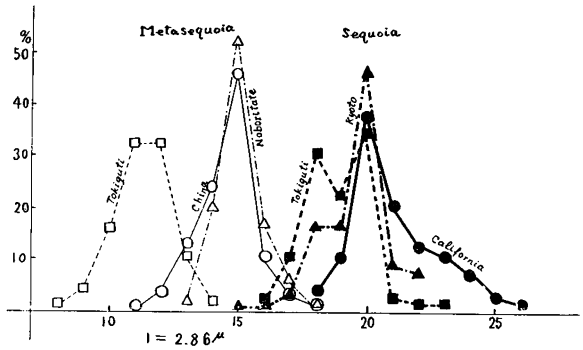

FIG. 1. Distribution of stomatal lengths in living and fossil *Sequoia* and *Metasequoia*.

a circle of 500 μ in diameter was counted in various regions of the leaf, but no significant differences were found between the two genera.

2. *Epidermal cells*: As was observed by Sterling (*5*) in the living *Metasequoia*, and confirmed by the present authors in both living and fossil material, the walls of the epidermal cells in this genus are undulate, a characteristic rarely found in conifers. Since the walls of the epidermal cells are straight in both fossil and living *Sequoia*, this difference is an additional and valuable diagnostic character for separating the two genera. In respect to size, the cells of *Sequoia* are about twice as long as those of *Metasequoia*, although there are no significant differences in width (Table 1).

The close similarity in size between both the guard and epidermal cells of the fossil remains of *Sequoia* and *Metasequoia* with the corresponding cells in living plants of the same genera is strong circumstantial evidence that the fossil *Sequoia* of Japan, like the living *S. sempervirens* of California, had the chromosome number 2n = 66, whereas the fossil *M. japonica* had the chromosome number 2n = 22.

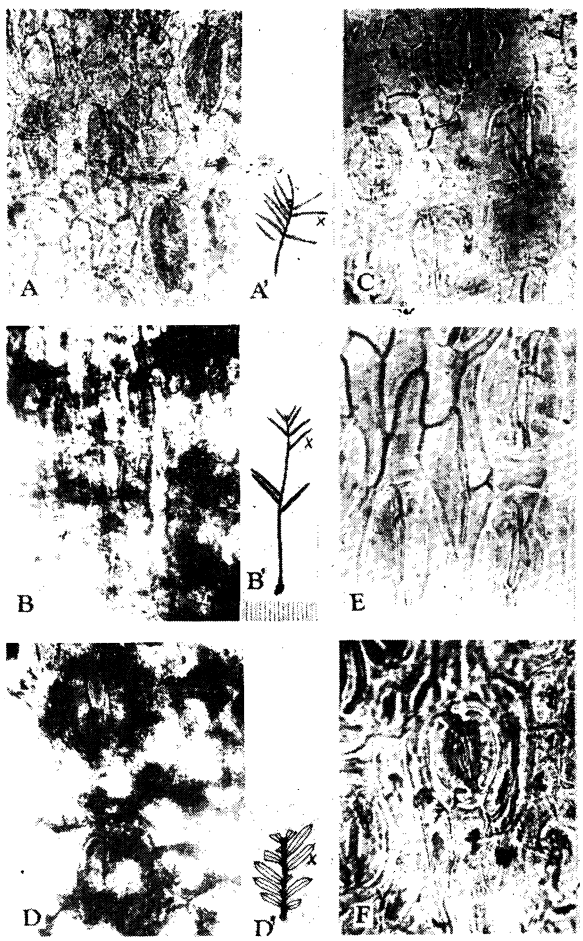

FIG. 2. *A,A'*, *Metasequoia* from Noboritate, Awaji, Pref. Hyogo; *B,B'*, *Metasequoia* from Tokiguti, Pref. Gihu; *C*, epidermis of *Metasequoia* from living species in China; D,D', *Sequoia* from Tokiguti, Pref. Gihu; E,F, epidermis of *Sequoia* from living species; *E*, material from Stebbins, and *F*, from Hirayoshi and Nakamura, *A,B,C,D,E,F*, × 400; *A',B',D'*, × 1; *x* shows leaf measured.

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Destruction of Amino Acids during Filter Paper Chromatography[1,2]

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Berry and Cain (*1*) have stated that one of the most critical operations in the preparation of a filter paper chromatogram involves removal of the solvent. They reported that the intensity of ninhydrin color which resulted from the same amount of an amino acid decreased with an increase of temperature over 80° C or with an increase in the time of heating, and they suggested that the amino acids were oxidized under these conditions. It was concluded that the solvent was best removed by blowing heated air at 85° C over the sheets for 8–10 min. Although many investigators have followed the methods described by Consden, Gordon, and Martin (*2*), which often involve solvent removal in an oven at temperatures up to 110° C, others have allowed the papers to dry at room temperature without giving reasons for doing so; frequently the temperature of drying is not specified in the description of experimental conditions.

Experiments summarized in the present report show that chromatograms wet with phenol should not be heated

[1] This investigation was supported in part by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

[2] After this paper had been submitted, Lowden and Penny (*Nature*, **165**, 846 [1950]), reported that the recovery of certain amino acids was decreased by heat-drying of paper chromatograms.

above room temperature. Almost complete disappearance of certain amino acids resulted when accepted techniques were followed (*3*), depending on the temperature at which phenol was removed from the paper. In one experiment, 4 two-dimensional chromatograms of alanine-2-C^{14} [3] were developed using 80% phenol as the first solvent. The second dimension was run in 63% lutidine. With all other conditions maintained constant, the phenol was allowed to evaporate from the papers at 4 different temperatures: 27°, 60°, 85°, and 105° C. The papers were kept in an oven at the 3 elevated temperatures for about 5 min. Radioautographs of these chromatograms are shown in Fig. 1. The alanine spot from the paper dried

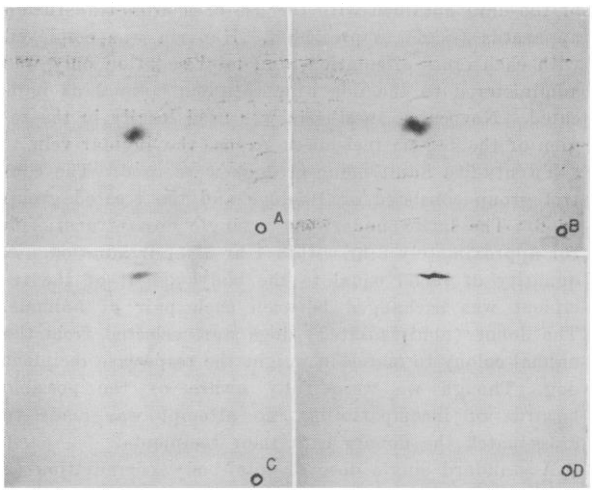

FIG. 1. Radioautographs of chromatograms of alanine-2-C^{14} developed first in phenol (horizontal direction) and then in lutidine (vertical direction). Origon is marked with a circle. After the phenol run, paper *A* was dried in front of a fan at 27° C, papers *B*, *C*, and *D* were dried in ovens at 60°, 85°, and 110° C, respectively.

at room temperatures shows maximum intensity (Fig. 1, *A*), whereas the alanine spot from the paper dried at 85° C is almost undetectable (Fig. 1, *C*). The loss in intensity of the alanine spot was paralleled by the appearance of one or more spots located near the lutidine solvent front. When these unknown spots were eluted and rechromatographed, they moved near the solvent front in both phenol and lutidine. The fact that they appeared directly above the alanine spot in the original chromatograms indicates that they must have been formed after the phenol run. Acid or alkaline hydrolysis of material eluted from the unknown spots led to partial recovery of the alanine which had been altered, suggesting that at least in part these compounds are dehydration products. Glycine-2-C^{14} [3] and tryptophan-β-C^{14} (*4*) were also found by radioautography to be labile to heat in the presence of phenol, with the formation of compounds showing similar high R_f values in phenol and in lutidine.

After the radioautographs had been made, these same 4 chromatograms of alanine were sprayed with ninhydrin. The intensity of the ninhydrin color at the alanine spot

[3] Obtained from Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission.

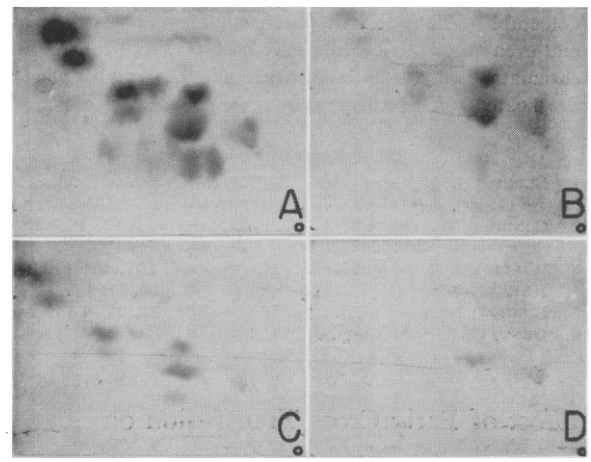

FIG. 2. Chromatograms of a casein hydrolysate developed first in phenol (horizontal direction) and then in lutidine (vertical direction). Origon is marked with a circle. Chromatograms *A* and *B* were made from 400 μg of the hydrolysate; chromatograms *C* and *D* from 100 μg. After the phenol run, papers *A* and *C* were dried in front of a fan at 27° C, and papers *B* and *D* were dried in an oven at 85° C for about 5 min.

paralleled the intensity of the radioactivity spots shown in Fig. 1. However, there was no ninhydrin color near the lutidine front in the region of the compounds derived from the alanine. Furthermore, the intensity of the ninhydrin color reaction with alanine on one-dimensional chromatograms developed with phenol was similarly dependent on the temperature at which they were dried, even though the altered compounds had not been separated from the alanine by a second solvent. This change observed with phenol did not occur when papers wet with lutidine or a butanol-propionic acid mixture (*5*) were heated. The phenol and water were freshly mixed for each run, and the same destruction of amino acids occurred after the phenol (Merck, reagent grade) was distilled from zinc dust.

Apparently all α-amino acids are subject to the reactions involved in this phenomenon, but not to the same extent. This is illustrated in Fig. 2 by chromatograms of a casein hydrolysate. Chromatograms *A* and *B* were made from 400 μg, and *C* and *D* from 100 μg, of the hydrolysate. Papers *A* and *C* were dried at room temperature, whereas the phenol was evaporated from papers *B* and *D* in an oven at 85° C. Most of the spots in these chromatograms may be identified by reference to the map of Dent (*6*). Some of the spots in Fig. 2, *B* are of less intensity than the corresponding spots in Fig. 2, *C*, which suggests that these amino acids had been reduced to less than 25% of their original amounts by the heat-drying of the paper. In contrast, other spots in Fig. 2, *B* are of greater intensity than the corresponding spots in Fig. 2, *C*, indicating that more than 25% of the original amounts of these amino acids survived the heat treatment. Comparison of the chromatograms shown in Fig. 2, *C* and Fig. 2, *D*, in which only 100 μg of the casein hydrolysate was chromatographed, shows that the treatment led to such extensive losses that only 3 ninhydrin spots were

detectable. Thus it is apparent that gross quantitative and even qualitative errors may be introduced into paper chromatography of amino acids if papers wet with phenol are heated during the drying process.

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Effect of Early Cross Transfusion on X-Irradiation Disease[1]

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The literature on the direct and indirect effects of x-irradiation has been reviewed (*1*). The pertinent question that still remains is whether irradiation with roentgen rays results in the formation of specific and/or nonspecific toxic substances which may be transported through the circulatory system and be removed from the irradiated host, thus reducing the process of damage and degeneration on the one hand and/or increasing the process of repair and regeneration on the other. Cross-circulation has been employed to demonstrate the lack of indirect effects *peculiar* to radiation (*1*), as well as to estimate the life span of the neutrophil (*2*) and the thrombocyte (*3*). Studies with parabiotic mice indicate that nonspecific factors are carried from damaged tissue by way of the circulatory system (*4*). The injury incurred by these factors appears to be related to the dosage of radiation and to the volume of tissue irradiated. It has been shown that parabiosis diminishes the deleterious effect of irradiation (*4*).

Arterial cross circulation and parabiotic techniques are formidable surgical procedures of considerable experimental and academic interest but of relatively little practical value. Parabiosis cannot be established in the usual survival period following lethal radiation.

Cognizant of the import of the nonspecific toxicity of massive doses of irradiation and of uncombated infection as causal influences on mortality from irradiation, we have undertaken an examination of possible protective and regulatory factors of cross transfusion in the reduction of toxicity. In this report a summary of some

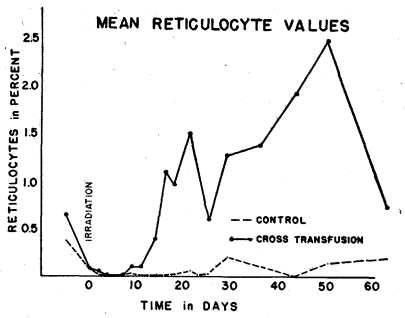

FIG. 1.

of the data obtained with the use of a cross-transfusion apparatus (*5*, *6*) is presented. Heparin was employed with each cross circulation, and mild sedation only was administered to the donor or recipient animal as indicated. Novocaine anesthesia was used locally in the region of the 1–2-cm incision to expose the jugular vein.

Twenty-five adult mongrel dogs were used. The control group consisted of 19 dogs and the treated group of 6. The latter underwent a single cross transfusion for approximately 2 hr within 4 hr after irradiation. A quantity of blood equal to the body weight of the recipient was exchanged between each pair of animals. The donor (nonirradiated) dogs were selected from the animal colony to match in weight the respective recipient dog. Though we were fully aware of the possible hazards of incompatibility, no attempt was made to cross-match the donors with their recipients.

A standard single dose of total-body x-irradiation of 450 r[3,4] (approximately LD 90–100%) was delivered to

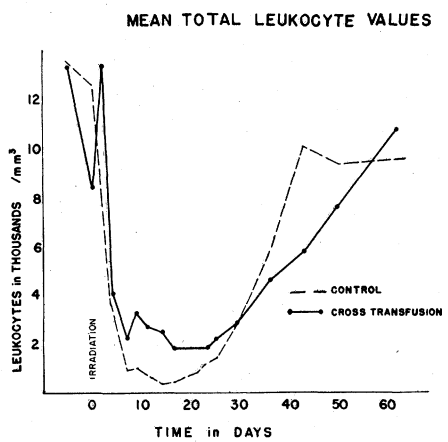

FIG. 2.

each dog used in these tests. Following the irradiation, 16 of the 19 (84%) untreated dogs succumbed in 8–25 days after x-irradiation, whereas only 2 of 6 (33%) cross-transfused dogs died 8 days after irradiation. The 4 surviving transfused dogs presented a clinical picture

[1] Part of this paper is based on work performed under Contract No. W-7401-Eng-49 for the Atomic Energy Project at the University of Rochester, and Contract No. AT(04-1)-290 at the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles 7, Calif.

[2] The authors gratefully acknowledge the counsel of H. Goldblatt. Technical assistance was rendered by N. Papageorges.

[3] This dosage of x-irradiation has been reported to be LD 90–100% in other laboratories (*7*, *8*).

[4] X-irradiation was administered from a Picker x-ray machine of 220 kvp, 15 ma, 45" tsd, and a parabolic copper filter with a half-value layer for copper of 1.0 mm.