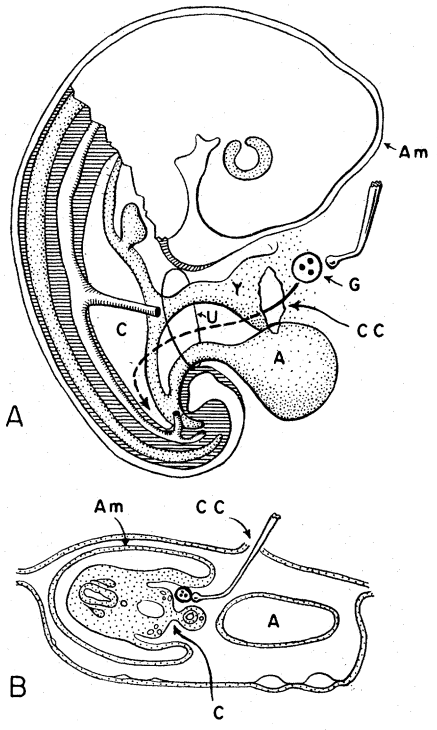

Fig. 1. (*A*) Diagram of 4-day-old chick embryo with right body wall removed; the arrow shows the path along which the graft moves during implantation. (*B*) Diagrammatic cross section through 4-day-old chick embryo showing membranes and the path followed by the graft during the first phase of implantation. Symbols: *A*, allantois; *Am*, Amnion; *C*, coelom; *CC*, cut edge of chorion; *U*, umbilical ring; *G*, carbon-marked graft; *Y*, yolk stalk. (Both diagrams modified after Patten, 1927.)

Within a period of approximately 24 hr, the graft becomes vascularized and firmly attached. At recovery, grafts have been found attached to the mesonephros, intestine, umbilical blood vessels, or body wall. Differentiation of the graft is excellent. Chick limb buds have produced well-defined bone, cartilage, skin, and muscle. One portion of the ventricle of a 3-day-old chick embryo differentiated into a vesicle of pulsating heart muscle. The floor of the pharynx, consisting of pharyngeal entoderm, thyroid vesicle, and mesenchyme, formed normal thyroid tissue, cartilage, bone, and gut epithelium.

Successful takes of both guinea-pig and rat tissues have been reported (*5*). It is to be emphasized, however, that the quality of graft differentiation obtained by means of this method is in no way different from the results afforded by the Hamburger method. This method does have the advantage not only of admitting use of larger pieces of tissue or organs as grafts but also of allowing the operator to utilize older embryos as hosts. The mortality rate from the operation appears to be very low (less than 2 percent). The method is less favorable for very small grafts, which cannot be firmly wedged into place during the implantation procedure and may subsequently be lost because of the movements of the embryo. For this reason, the technique is no substitute for Hamburger's method but is designed to complement it.

The method suggests a number of applications. The possibility of implanting a relatively large piece of tissue, as well as being able to place it with some accuracy next to a developing organ—for example, the heart, kidney, and so forth—may be of value in pathology and cancer research. The effects of hormones, drugs, inhibitors, and other chemicals on embryonic processes might be studied by incorporating such substances in an inert solid carrier and similarly inserting them into the coelom. The method seems simple enough to be useful as a routine laboratory experiment in experimental embryology.

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Structural Modification That Increases Translocatibility of Some Plant-Regulating Carbamates

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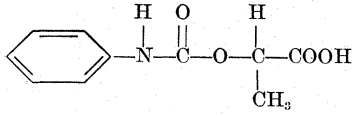
The growth-modifying and herbicidal effects of isopropyl N-phenylcarbamate (IPC) and isopropyl N-3-chlorophenylcarbamate (3-Cl-IPC) have been reported (*1–7*). These compounds are apparently not readily translocated when applied to relatively mature leaves of grasses. Foliar applications have comparatively little effect on plants, whereas soil applications prevent growth of many kinds of germinating seeds, particularly those of grasses. The discovery that alpha-methoxyphenylacetic acid (MOPA) is readily translocated by the roots, stems, and leaves of plants (*8*) led to experiments with two carbamates, lactic acid N-phenylcarbamate (LPC) and lactic acid N-3-chloro-



phenylcarbamate (3-Cl-LPC) (*9*). Structurally, MOPA and these carbamates each have a carbon atom in an alpha position with respect to a carboxyl group and in each this carbon is associated with a hydrogen and a methyl or a methoxyl group.

Approximately 50 μg of the compound being tested was applied in a carrier made of 4 pt lanolin and 1 pt Tween 20 to each first leaf (3 cm long) of barley

plants (Wong var.). In this and succeeding experiments (carried out in a greenhouse) 6 to 12 plants were used for each type of treatment. A thin layer of the paste containing either LPC or 3-Cl-LPC was applied to a 1-cm² area on the upper leaf surface midway between the tip and the base; IPC, 3-Cl-IPC, and the carrier alone were applied separately to other barley plants for comparison. The IPC-, 3-Cl-IPC-, and carrier-treated plants grew vigorously, whereas the LPC- and 3-Cl-LPC-treated ones failed to grow. Six weeks after treatment some leaves of the LPC- and 3-Cl-LPC-treated plants were partially decayed, and finally the plants died.

In a subsequent experiment, 3-Cl-LPC again prevented growth when placed on the first leaf of young barley plants, whereas 3-Cl-IPC failed to do so. In contrast, both compounds prevented growth when applied to the leaf sheath just below the soil level; 3-Cl-LPC was translocated by the leaves to the crown, whereas 3-Cl-IPC was not translocated from a distant portion of the leaf to the crown in sufficient amounts to affect growth measurably.

To determine whether 3-Cl-IPC evaporated (*10*) before absorption could take place, about 50 μg of the compound was applied to the first leaf of each plant. Fresh paste was applied 2 and 5 days later on other areas near the original ones. Growth of the plants was not measurably different from that of comparable plants treated with the carrier alone. When the paste containing 3-Cl-IPC was removed from the leaves 2 days after application and placed on barley seeds, it prevented germination, whereas the carrier alone did not suppress germination.

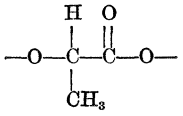
Application of 3-Cl-LPC to an area (0.5 cm²) at the tip of the first leaf of barley plants reduced rate of growth only 22 percent during the following 2 wk, indicating that the compound was less readily translocated from this area than from an area nearer the base of the leaf. The leaf-tip treatment induced growth of lateral buds, and at the end of 2 wk the treated plants had developed more leaves than had the controls. Plants treated with 3-Cl-LPC on the tip of the second or the third leaf responded in a similar way. Roots of six young barley plants were suspended in aerated nutrient solution containing 10 ppm of 3-Cl-LPC, and roots of six others were suspended in aerated nutrient solution as controls. During the following week growth in length of roots and leaves was suppressed 37 percent, and of leaves 47 percent, indicating that the compound was absorbed and translocated.

When 3 or 6 μg of 3-Cl-LPC was applied to the first leaf of young barley plants, growth of subsequent leaves was reduced 36 and 59 percent, respectively, during the 2 wk following treatment. Twelve micrograms reduced their growth 95 percent, and 24 μg completely prevented it.

Leaves of various species of grass translocated 3-Cl-LPC, but the plants varied in their responsiveness to leaf applications (lanolin-Tween 20 paste) of the chemical. Barley, oats, ryegrass, and Alta fescue treated in the first-leaf stage were 70, 53, 34 and 21 percent shorter, respectively, than controls 12 days after treatment. Corn plants did not respond to this type of treatment. However, when the chemical was applied to the soil before germination of corn, or was sprayed on corn plants a few days after germination, it greatly retarded growth. Sprayed on respective groups of young barley plants at 125, 250, 500, or 1000 ppm, the compound completely suppressed growth. At the lowest dosage level, each barley plant received about 10 μg of 3-Cl-LPC.

LPC, 3-Cl-LPC, and the sodium salt of 3-Cl-LPC (*11*) did not evaporate readily from leaf surfaces. In a study of volatility, groups of 12 primary leaves of young bean plants were dipped in aqueous mixtures (500 ppm) of the compounds just named and 3-Cl-IPC. After the leaf surfaces were dry, the leaves were placed in gastight cellophane bags, four leaves per bag, and were supported 10 cm above germinating barley seeds within the bags. Untreated leaves were similarly enclosed for controls. During a period of 6 days at 70° to 90°F, further germination of the seeds was prevented by the vapors of 3-Cl-IPC. Barley in the control bags and in all bags with leaves given other treatments grew vigorously and attained a height of 40 to 50 cm.

The following compounds (*12*) related to LPC were readily translocated when applied to barley leaves: α-carbo (2-chloroethoxy) ethyl N-phenylcarbamate, α-carbocyclohexoxyethyl N-phenylcarbamate, α-carbo-(2,4-dichlorophenoxy) ethoxyethyl N-phenylcarbamate, α-carbobenzoxyethyl N-phenylcarbamate, α-carbododecoxyethyl N-phenylcarbamate, α-carbobutoxyethyl N-phenylcarbamate, α-carbobutoxyethyl N-3-chlorophenylcarbamate, and α-carbobutoxyethyl N-3-cyanophenylcarbamate. Each of these compounds has within its structure the "lactic acid" group



The carbamates (*13*) α-carbobutoxyethyl N-methyl-N-phenylcarbamate, α-carbobutoxyethyl N-3-nitrophenylcarbamate, α-carbobutoxyethyl N-3-methylphenylcarbamate, containing the "lactic acid" group were tested by stem treatment of barley plants, but they induced no visible response. These compounds, too, may be readily translocated, but some means other than growth modification would be required to detect their movement.

The following closely related carbamates (*14*), which do not have the "lactic acid" group within their structure, were not translocated by leaves of barley plants but they greatly affected growth when placed near the crown of the plants: IPC, 3-Cl-IPC, isopropyl N-3-cyanophenylcarbamate, 2-propynyl N-phenylcarbamate, and ethyl N-phenylcarbamate.

Leaves of plants used (with the exception of corn) were able to translocate plant-regulating carbamates

containing the "lactic acid" group but were unable to translocate those that did not contain this group. If incorporated into the structure of some other plant regulators or pesticides, the "lactic acid" group may also enhance their translocatibility.

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30 March 1954.

Enzymatic Oxidation of DPNH by Diketosuccinate and Dihydroxyfumarate

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In the course of an investigation (*1*) of pyridine nucleotide dehydrogenases in higher plants, we have observed that various plant extracts catalyze an oxidation of DPNH (*2*) by diketosuccinate. Preliminary experiments indicate that such an enzyme is widely distributed in the roots, seeds, and leaves of higher plants and in animal tissues as well. The more detailed experiments described here have been done mainly with preparations from pea roots.

Because diketosuccinate is decarboxylated rapidly in aqueous solution (*3*), it was necessary to show that the enzymatic oxidation of DPNH is due to the diketosuccinate itself and not to its decarboxylation product. On standing at room temperature, solutions of diketosuccinic acid lost their ability to oxidize DPNH enzymatically within 20 to 40 min. The rate of loss of enzymatic activity paralleled the rate of decarboxylation. This showed that the enzymatic oxidation is not due to the decarboxylation product.

The enzyme has tentatively been called diketosuccinate reductase. The product of the reduction of diketosuccinate has not yet been unequivocally identified. However, a substance that reduces 2,6-dichlorophenol indophenol (*4*) accumulates when the reduction of diketosuccinate is coupled with the oxidation

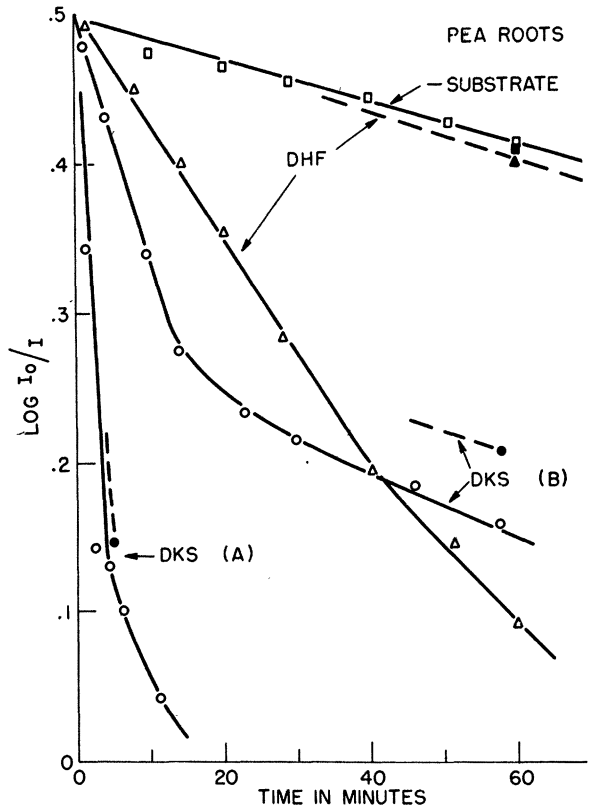

Fig. 1. The oxidation of DPNH by diketosuccinate (DKS) and dihydroxyfumarate (DHF). The enzyme was a dialized extract of an acetone powder prepared from pea roots. Each reaction mixture contained 0.1 ml of enzyme, 0.2 ml of 0.5*M* phosphate buffer of *p*H 6.5, DPNH as shown, and substrate, made up to a total volume of 3.0 ml. The open circles with solid lines indicate aerobic conditions; the solid circles with dashed lines indicate anaerobic conditions (N_2 freed from O_2 with yellow P). The dihydroxyfumarate was prepared according to Hartree (*9*), and the diketosuccinate according to Fenton (*3*). Both substrates were added as solids at the beginning of the experiment to avoid preliminary decomposition.

of ethanol in the presence of diketosuccinate reductase, alcohol dehydrogenase, and DPN^+. This may be regarded as partial evidence that the reduction product is either α-keto-β-hydroxy succinate or an enediol form of this compound. These substances are interconvertible in solution. The reaction may be formulated provisionally according to either Eq. 1 or Eq. 2.

$$COO^- \cdot CO \cdot CO \cdot COO^- + DPNH + H^+ \rightarrow COO^- \cdot CO \cdot CHOH \cdot COO^- + DPN^+ \quad (1)$$

$$COO^- \cdot CO \cdot CO \cdot COO^- + DPNH + H^+ \rightarrow COO^- \cdot COH{:}\,COH \cdot COO^- + DPN^+ \quad (2)$$

Equation 1 would be preferred from analogy with other pyridine nucleotide dehydrogenase reactions.

The enediol of α-keto-β-hydroxy succinic acid is available as a solid; Hartree (*5*) and Gupta (*6*) have shown that this solid is dihydroxyfumaric acid and