The data show that the radioactive iron, which is taken up by pigeon erythrocytes *in vitro,* leaves the cells during reincubation in the various mediums described. This is particularly evident in the iron-free medium. However, the percentage of Fe^{59} present in cytoplasm increased during reincubation, whereas the percentage of Fe^{59} present in the nuclei and stroma declined, primarily because of a considerable drop in the nonhemin iron fraction. This strongly suggests that the nonhemin iron in nuclei and stroma can be utilized for hemoglobin synthesis and that it may be in equilibrium with the iron in the suspending medium. In contrast to these observations, the iron, which is taken up by pigeon red cells *in vivo,* is comparatively stable and undergoes only a very slight decline, which is attributable to the nonhemin iron fraction of nuclei and stroma.

This fact suggests the possibility that the iron taken up by pigeon erythrocytes *in vitro* at the early stage of incubation is attached or combined loosely to the surface of the cells and may be in equilibrium with iron present within them, particularly in the nuclei and stroma.

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* Rockefeller Foundation fellow from the University of Tokyo.

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New Method of Intracoelomic Grafting

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The technique of intracoelomic grafting, originally described by Hamburger (*1, 2*), has found wide application in the field of experimental embryology, and the results obtained through its use have contributed much to our understanding of developmental processes. The technique in its present stage of development (*3*) has, however, certain inherent limitations that restrict its usefulness. One drawback is that the size of the implant is limited both by the small capacity of the coelom of 60- to 70-hr-old chicks and by the necessarily small incision permitted in gaining access to it. For these reasons, successful implantation of relatively large bits of tissue—for example, approximately 0.5 to 1.0 mm^3—is not practicable.

During a recent investigation, it became necessary to make intracoelomic grafts of the thyroid glands of 10- to 11-day-old chick embryos. It was soon discovered that implantation of a half or even a quarter of such a gland was difficult, if not impossible, and that the results to be expected from such a procedure were highly questionable. To circumvent this impasse, a new approach was required. The method that was finally developed appears to have sufficient applicability to be of interest to workers in fields other than embryology.

By 3½ days of development (*4,* Hamburger-Hamilton stage 20), the chick embryo lies entirely on its left side, the allantois is beginning to expand, and the embryonic membranes have grown completely over the embryo and have separated into chorion and amnion. The embryonic coelom and the extraembryonic coelom are still in broad communication at this time, for the umbilical ring has not yet been occluded by the structures that pass through it. Beginning with the time that the embryo first comes to lie completely on its side and ending with the time that the allantois has expanded to such a point that the site of operation is obstructed (approximately Hamburger-Hamilton stage 23), the coelom of the chick is readily accessible for operative manipulations and is in a condition such that tissue placed within its confines readily becomes vascularized.

The operation itself is extremely easy and rapid. The egg is opened in the usual manner, and the embryo is lowered by withdrawing a small amount of albumen from the pointed end of the egg. The opening required in the chorion (Fig. 1 *A* and *B*) may be made either by cutting with iridectomy scissors or by tearing with fine watchmaker's forceps, and should be just large enough to admit the graft; it is essential to distinguish carefully the chorion and amnion and to avoid injury to the latter. The graft is temporarily placed on the chorion next to the incision, and, to facilitate its placement and later recovery, it is marked with a few grains of sterile blood carbon or, alternatively, with a vital dye. Using an L-shaped blunt glass instrument (Fig. 1), the graft is nudged into the incision and gently pushed past the anterior face of the allantois through the umbilical ring. Upon reaching the dorsal surface of the embryonic coelomic cavity (Fig. 1 *B*), the graft may be directed toward the posterior portion of the cavity and pushed into a secure position. It is quite possible to push the graft into the anterior portion of the coelomic cavity at this point. The carbon particles are usually visible through the flank of the embryo and will indicate by their location whether or not the graft has become firmly lodged in the desired location. The volume of tissue grafted is largely dependent upon its shape. A spherical or cubical graft having a volume approaching that of a thin elongated graft is more difficult to implant than the latter, which may be inserted without unduly distorting the embryo. When the graft has been placed in position, the albumen is replaced, the shell window is fitted into place, and both openings are sealed with melted paraffin.

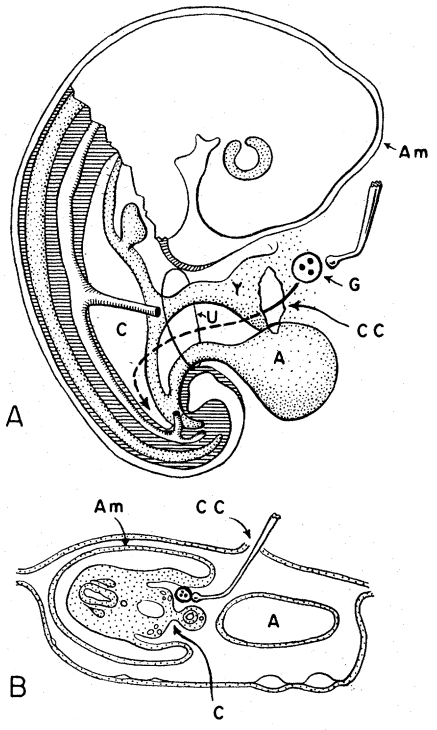

Fig. 1. (*A*) Diagram of 4-day-old chick embryo with right body wall removed; the arrow shows the path along which the graft moves during implantation. (*B*) Diagrammatic cross section through 4-day-old chick embryo showing membranes and the path followed by the graft during the first phase of implantation. 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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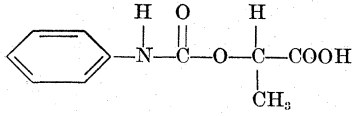
The growth-modifying and herbicidal effects of isopropyl N-phenylcarbamate (IPC) and isopropyl N-3-chlorophenylcarbamate (3-Cl-IPC) have been reported (*1–7*). These compounds are apparently not readily translocated when applied to relatively mature leaves of grasses. Foliar applications have comparatively 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