RECONSTITUTION OF COMPLETE ORGANS FROM SINGLE-CELL SUSPENSIONS OF CHICK EMBRYOS IN ADVANCED STAGES OF DIFFERENTIATION

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Embryonic development proceeds in long sequences of interactions among cells and cell groups. Some of these interactions determine essential characteristics of tissues and organs, whereas others are merely of a permissive nature. For a given portion of an egg or embryo, the relevant interactions may (*a*) occur almost entirely within its own confines, or (*b*) be initiated, expedited or specifically supplemented by interactions with other portions. Principle (*a*), referred to under various names ("autonomization,"[1] "emancipation,"[2] "segregation,"[3] "self-organization,"[4, 5]) has been stressed by Weiss as the most basic, yet also most obscure, property of a developing organism.[2] Principle (*b*), commonly referred to as "induction" [5, 6] and implying all sorts of adjuvant and permissive steps along the course of (*a*), however, has commanded an ever greater share of attention, work, and emphasis in recent years, to the point of creating the illusion, especially among those not fully familiar with the intricacies of embryology, that the principle of "induction" holds the major, if not the exclusive, key to the understanding of development. It is with a view of restoring a more balanced perspective that we present the results reported in this paper, even though they are still incomplete. They are so unexpectedly demonstrative in proving the scope and power of principle (*a*), i.e., of self-organization without instructive outside intervention, that it seems warranted to communicate them at this time. They demonstrate the fact that cells which have already constituted a functional organ can, after complete isolation, dispersal, and random recombination, reconstitute that same type of organ once again, and can do so in an indifferent environment from which they could have received no cues as to how to do it.

The study of the behavior of metazoan cells liberated from their organized tissue associations in the body, begun in sponges and hydroids,[7, 8] has recently been extended with signal success to higher forms. The new trend had two points of origin. In 1950 and '52, Weiss and Andres[9, 10] used single cell suspensions, obtained by dissociating embryonic tissues, to study the fate of such cells when disseminated in older embryos by the vascular route. Also in 1952, Moscona and Moscona[11] used cells from dissociated embryonic tissues to test their ability to reaggregate and to proceed with typical histogenesis in tissue culture.

The methodology of tissue dissociation has since confirmed its value for the study of development in further work by Moscona, Grobstein, Zwilling, Wolff, and others. The foci of interest and emphasis in these studies varied, centering on the following issues (giving only sample citations): (1) Effects of the isolating procedure—mechanical; enzymatic (trypsin); chelation (versene)—(2) Mode of reunion (clustering) of dispersed cells—(3) Type-specific self-sorting of mixed cell populations[12]—(4) Mechanism of self-sorting[13]—(5) Interactions between separated and then recombined components (e.g., epithelium and mesenchyme of composite tissues[14, 15])—(6) Retention versus loss of properties by cells in isolation[16]—(7) Residual alterations imposed upon cells while in the isolated stage[17]—(8) Problems of critical mass minima[18, 19]—(9) Capacity for continued *histogenesis* of isolated cells—and (10) Capacity for progressive *morphogenesis* of reaggregated cell groups.

For our present purpose, the last two points are the key items. The categorical distinction between histogenesis and morphogenesis is based on the greater simplicity and uniformity of the results in the former case, as contrasted with the complex nature, coupled with a high degree of over-all regularity and spatial order, in the latter. For instance, callus formation in a fractured bone is histogenesis, whereas the regeneration of a complex, bone-containing limb from a blastema in amphibians rates as morphogenesis. Plain keratinization of skin is histogenesis, but the formation of a feather is morphogenesis. Yet, this distinction is not to imply that morphogenesis might not eventually be resolved into sequences of ordered interactions according to specific space-time patterns of simpler histogenetic components.[20]

As Moscona[21] was the first to show, cells destined to give rise to cartilage or kidney, when reassembled after isolation and reared in tissue culture, continue in their erstwhile courses of histogenesis, producing cartilage and nephric tubules, respectively. Moreover, if provided with the proper stroma, a glandular blastema may give rise not just to a random coil of tubules, but to a system of branched ducts of such regularity of dimensions, arrangement and mutual spacing[22] that this can definitely be classified as an elementary process or morphogenesis.

However, the powers for organ resyntheses of a more complex order have received scant attention. Andres[23] observed that random scrambled embryonic cell suspensions incorporated in the yolk sac of the chick embryo can develop into organized, very complex bodies, containing many different and well-differentiated organ parts (brain, ganglia, skeleton with joints and muscles, skin with feathers, etc.), and that, furthermore, these parts tend to appear in certain constant constellations relative to one another. Although this was presumably the first clear indication of the far-reaching faculty for morphogenetic self-organization within heaps of random-scrambled embryonic cells, the instructiveness of these experiments suffered somewhat from the fact that they were mostly done with cells from very young stages, (1–4 days of incubation age), when cellular potencies are still rather wide. Subsequently, however, Weiss and James[17] found that the general principle held for later stages as well, as they could show that a random scramble of skin cells from the 8-day chick embryo, when the general cutaneous specialization is already fixed and actual feather germs are present, could still, after recompacting and cultivation on a blood plasma clot *in vitro*, give rise to feathers, the formation of which presupposes a highly coordinate group performance of the

participating cells.   But after having given evidence of their competence for specific self-organization, these miniature feather buds ceased to develop further, obviously for lack of vascularity in their pulp.

It was a natural step from these experiences to try to get the cell masses to develop beyond the stages they had managed to reach in tissue culture, by letting them acquire blood supply, yet still in a sufficiently neutral environment.   In line with standard practice, we chose for this purpose the chorio-allantoic membrane of chick embryo.

The cells to be tested were taken from chick embryos between 8 and 14 days of age.   In the present article, we shall confine our discussion to the results with kidney, liver and skin.   Mesonephros and liver at this age have reached an advanced stage of differentiation and functional activity.   Although they are yet to undergo much further growth, their extant parts exhibit already the essential cytological and histological features by which one ordinarily identifies the particular mature organ.   The metanephros is still largely in a blastematous state.   The skin is still primitive, but already contains young feather germs.

Organs of one type freshly excised from several embryos were pooled, minced, and incubated for one-half hour in a mixture of trypsin and pancreatin in Ca-Mg-free Earle's solution.   The resulting cell suspension was washed, in some cases passed through a nylon strainer to remove any residual clumps, concentrated by mild centrifugation, and moderate amounts of the mushy aggregate were then deposited by pipette on the chorio-allantoic membranes of 8-day embryos.

The grafts were left to develop for an additional 9 days, giving them a total age of from 17 to 23 days.   They were then located, excised, fixed in Bouin's, and sectioned.   Results of the microscopic examination of some of the farthest developed sample specimens are summarized in the following:

The most striking features were (1) the completeness and normal texture of the whole assortment of histological components typically to be found in the respective developed organs; (2) the typical architectural pattern and distribution of these components within the reconstituted organs; (3) the polarization of this architecture along an axis transversely from the outer to the inner side of the chorio-allantoic membrane; (4) the graft-specific configurations assumed by connective tissues and blood vessels within the grafts; (5) the manifestations of functional activity; and (6) the lack of residual signs of large-scale cell disintegration, such as pycnotic cells or necrotic patches, within solid blocks of healthy tissue.

The detailed observations were as follows:

Kidney.—The case of metanephros illustrated in Figure 1 shows a bilaterally symmetrical organ between an outer capsule and an inner pelvis-like cavity, with typical secretory and collecting tubules, the latter radiating as a rete toward the lumen, into which some of them can be seen to open.   Glomeruli are found in the outer portion, where blood vessels abound, but in this particular specimen they are relatively scarce.   By contrast, the case of Figures 2 and 3 shows a more flattened kidney, richly stocked with Malpighian corpuscles, each with a blood-filled glomerulus, evidently formed by invading host capillaries, enveloped by the membranous funnel of a tubule.   The straight portions of the tubules follow again a general transverse direction, merge in their course and, some at least, break through the allantoic wall.   The cytological distinction between the different segments of
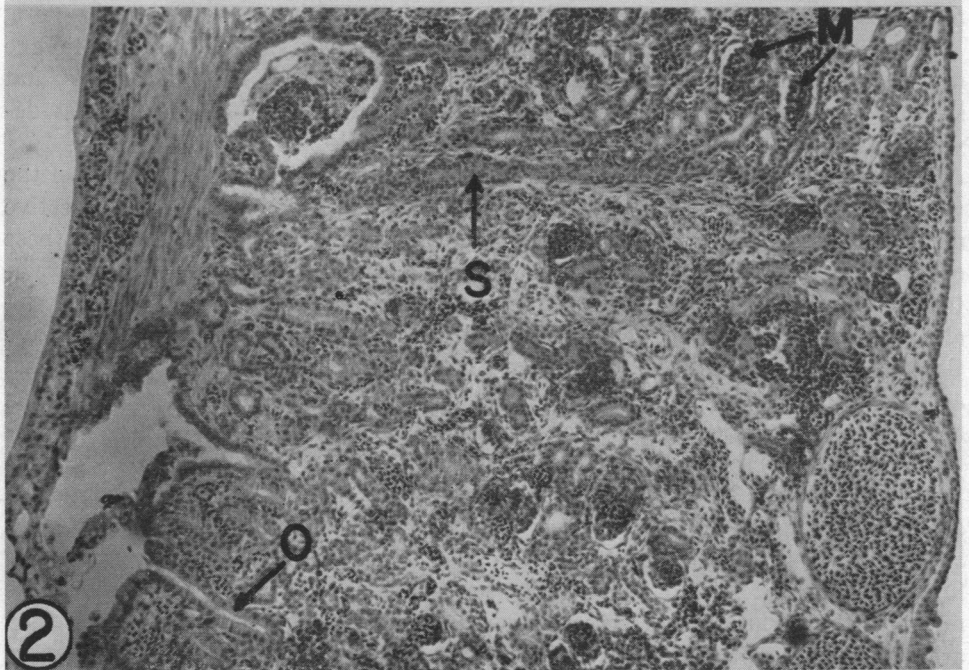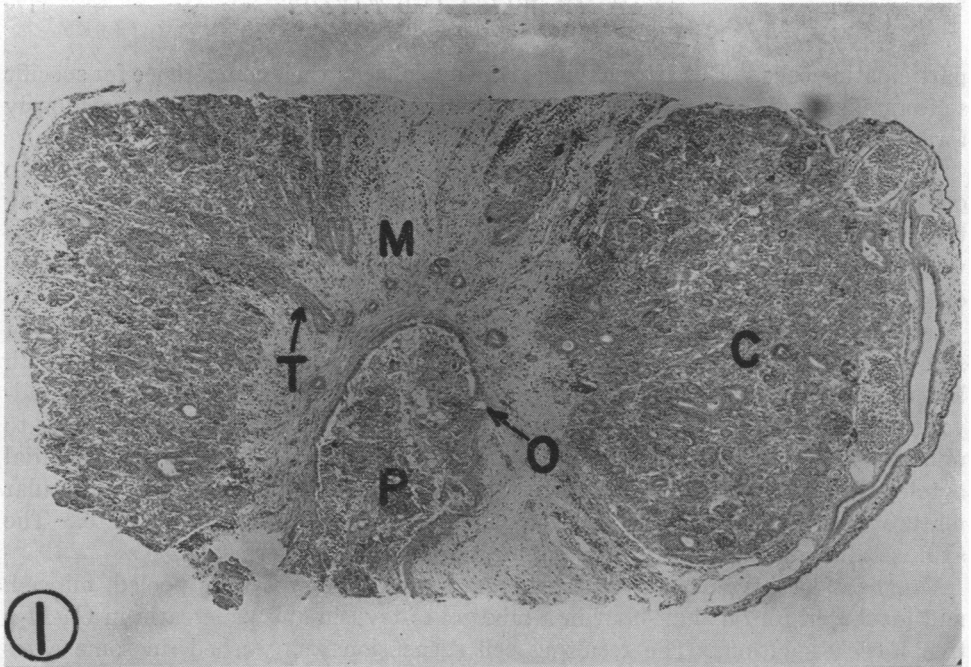
Fig. 1.—Section through a graft of scrambled metanephric cells 9 days after being placed on a chorio-allantoic membrane. Reorganization has resulted in a symmetrical organ with cortex (C), medulla (M), and pelvis-like cavity (P). Radial collecting tubules (T) are seen, some opening into the pelvis (O). × 38.

Fig. 2.—Section through another graft of kidney cells. M - Malpighian Corpuscle. S - Straight portion of collecting tubule. O-Tubule opening into allantoic cavity. E - Nest of eosinophil leucocytes. × 103.
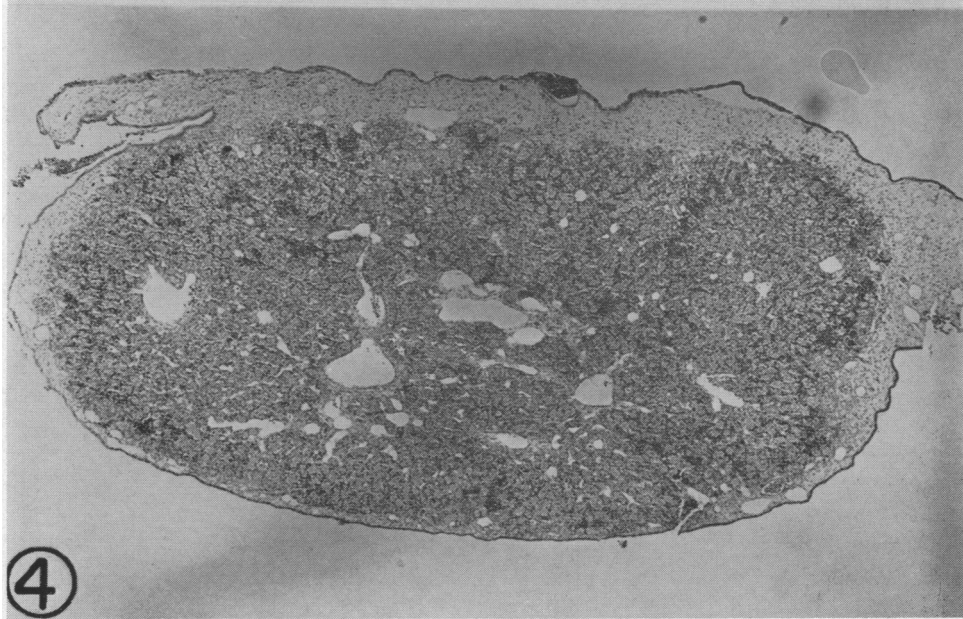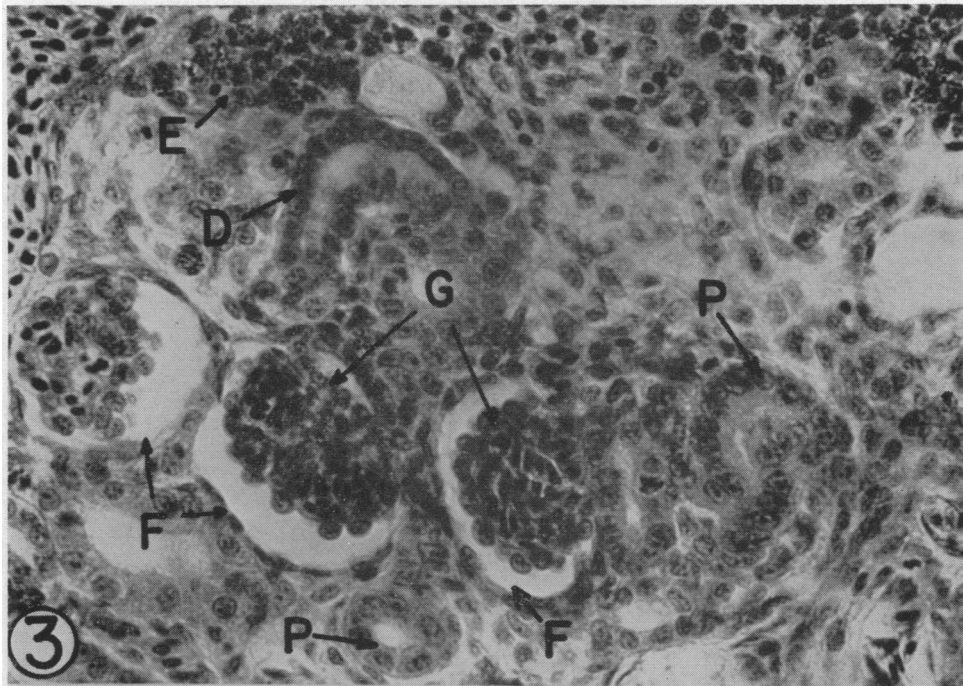
FIG. 3.—Several Malpighian Corpuscles from the same graft as that shown in Fig. 2.   G - Glomerulus.   F - Funnel of collecting tubule.   P - Large cells of proximal convoluted tubule. D- Small cells of distal convoluted tubule.   E - Nest of esosinophil leucocytes.   × 450.

FIG. 4.—Section through a graft of scrambled liver cells showing reorganization attained by the ninth day after being placed on a chorio-allantoic membrane.   × 38.
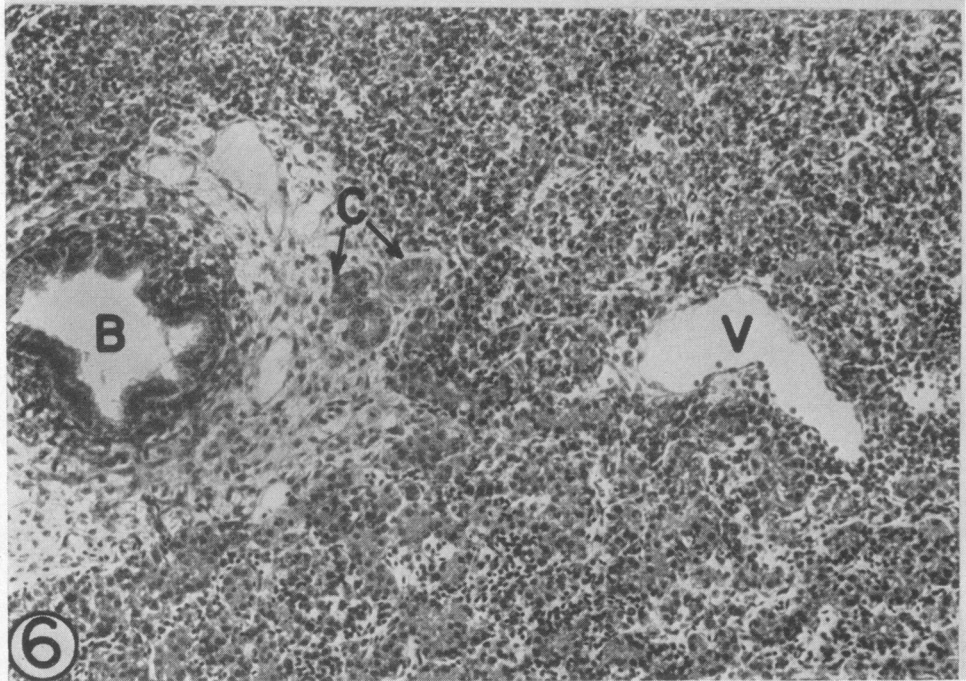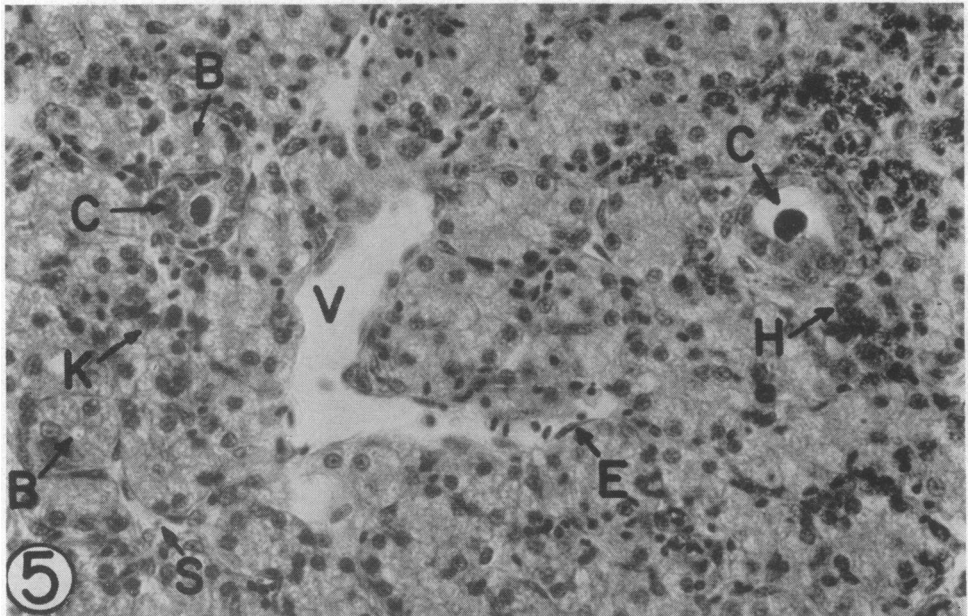
FIG. 5.—Higher magnification of the same chorioallantoic graft of liver cells as that shown in Fig. 4. B - Bile capillary in a cord of parenchymal cells. S - Sinusoid. C - Canaliculus containing bile concrement. V - Venous sinus. E - Endothelial cells. H - Hematopoietic island. K - Kupffer cell.

FIG. 6.—Another graft of liver cells. B - Bile duct. C - Bile canaliculi. V - Venous sinus.

tubules (large cuboid cells lined by a brush border or smaller cells with smooth contours) are quite well marked. Mitotic activity is prominent. Interspersed with the formed structures are nests of eosinophil leucocytes, which undoubtedly represent hematopoietic sites, although their origin, whether from graft or host cells, remains undecided. One is tempted to identify the sequestered coagulum in the pelvic enlargement in Figure 1 as a secretory product of the graft, but since it contains remnants of cells and no uric acid tests were made, it may be simply a sloughed-off part of tissue. On the other hand, the turgor of the tubules is strongly indicative of their having been in functional operation. More conclusive signs of functional sufficiency have been found in the liver grafts to be described next.

Liver.—Figure 4 shows a compact oval-shaped organ within a wide connective tissue cortex rich in blood vessels. The parenchyma consists of typical ramified liver trabeculae, each cell column holding a bile capillary in its center and bordering on sinusoids at the periphery. In higher magnification (Fig. 5) are seen bile capillaries which feed into canaliculi, whose content of green bile concrements gives evidence of active secretion. In Figure 6, a well developed duct with large ciliated cells, which proximally connects with canaliculi and distally discharges into the allantoic cavity, presumably represents a bile duct; it has diverticula of the sort described for certain species. Rather centrally located in the mass of liver parenchyma, there are wide venous sinuses, the vascular connections of which have not yet been traced. Within the parenchyma lie numerous hematopoietic islands with various types of blood cells in formation, and interspersed among the liver cords are large macrophages (Kupffer cells) with greenish tinted cytoplasm, suggesting the presence of hemosiderin from digested erythrocytes. Mitotic activity is present throughout the parenchyma.

Skin.—As in the prior experiments of Weiss and James,[17] feathers have formed, but by virtue of their vascularization they have developed far beyond the stage of simple buds. The reaggregated epidermis sheets have closed into vesicles into which the sprouting feathers protruded, crowding each other as they grew. Each was set in a separate regular follicle over a typical feather papilla and consisted of cornified barbs symmetrically flanking a typical shaft. In the most advanced cases, these feathers measured a few millimeters in length.

The described specimens were the best thus far obtained in these preliminary and not numerous experiments, in which, moreover, a variety of procedures had been tried. Some major variables were the tissue type and species of origin of the inoculum; the degree of compacting (cell density) and the size of the clumps grafted to the membrane; and the manner in which the cells were deposited. In some of these combinations, no traces of the grafts were later found, in others, especially heteroplastic grafts (e.g., rabbit to chick), heavy inflammatory reactions and graft necrosis were observed, and in still others, only small scattered islands were recovered, which had undergone histogenesis, but no morphogenetic organization. In view of the variability of conditions and of the small number of trials in each set, the erratic nature of the results is not surprising. At any rate, while the failures remain to be accounted for, the positive cases here described are incontrovertible proof that, given the proper conditions, cells that had already been parts of a functional organ can, after dispersion and random recombination, re-engage in a collective reconstitution of that particular organ without outside in-

structions or specific "inductive" help. To grasp the cogency of this point, it is essential to bear in mind that whatever the blood supply and the chorio-allantoic environment may have contributed to letting the observed formations accomplish their advanced development, they certainly could have added nothing to make the liver cells reconstitute a typical liver; the kidney cells, a typical kidney; and the skin cells, feathers; for blood supply and site were identically the same for all of them.

In other words, the cells of the various tested organs, at the time of their isolation, must have already contained some specific properties, or what in modern lingo would probably be called "information," distinctive of the kind of organ of which they had formed part and must have been capable of translating that "information" into a repeat performance. Now, obviously this does no more than rephrase the problem. The only certain fact for the present is that the cells collectively *can* do it. Just how, will have to occupy research workers for quite some time to come.

The answer will not be simple. Off hand, at least the following phenomena will have to be taken into account as possibly contributory to the effect. (1) Reassortment of cells that were already terminally specified according to kind, like to like, (e.g., secretory tubule cells joining other secretory tubule cells). (2) Reassociation of such cells with complementary cell kinds (e.g., epithelial cells combining with their corresponding stroma cells). (3) Recruitment and assimilatory induction of younger blastema cells to join such more advanced groupings as listed under (1) and (2). (4) Migration or other displacement of specific cell groups into locations within the whole unit where conditions are uniquely favorable for their kind. (5) Internal inductive actions emanating within the cell collective from a cell group of type α and acting on an adjacent one of type β, calling forth in the latter a conforming response of differentiation (e.g., the process by which the blind end of a kidney tubule causes a nearby capillary loop of a chorionic vessel to produce a glomerulus). (6) Reciprocal inductive actions of β on α (e.g., perhaps the formation of a Malpighian unit by the nephron in response to the glomerulus). (7) Selectively enhanced-proliferation of tissue components in their appropriate relative positions, representing "ecologically favorable sites" in the metabolic economy of the growing organ; as well as, conversely, selective destruction at unfavorable sites. (8) Mutual regulation of growth among different components of an organ so as to harmonize their growth rates in the joint production of composites.

Some of these points are well attested, others are quite hypothetical. At any rate, in view of our limited data, it would be quite impossible to assess their respective shares in the reported results. The only firm conclusions that can be drawn at the moment are: (1) Since the grafted cells, whose morphological arrangement had been completely disrupted, accomplished on a neutral test site a second organogenesis strictly corresponding to the organ from which they had been isolated, they must have achieved their transformation from the random scrambled into the morphologically fully organized state wholly by "self-organization," that is, by virtue of properties residing *within* the isolated cell population, unaided by specific inductive instructions from *without*. (2) Since an arbitrary sample of a mixture of cells from an organ could reconstitute a miniature replica of the respective organ of rather harmonious proportions, with a rather full complement of the typical

cell types in rather normal mutual distributions, one must (*a*) take seriously the "field properties of such cellular collectives (Weiss '39,[2] more specifically, Weiss '53,[24]), and (*b*) make more systematic efforts to fill "field" terms (or even more urgently, "information" terminology), wherever possible, with concrete data along such lines as indicated by the eight points just listed.

For the rest, the best we can hope for is that the confrontation with phenomena as inexplicable, though analyzable, as the ones dealt with in this paper, will deflect at least some attention and investigative effort back towards the fundamental problems of self-organization from such one-sided, illogical and illusory pursuits as the quest for "the" inductive agent that "causes" embryonic differentiations.

Summary.—Single-cell suspensions prepared from kidney, liver, or skin of 8-to-14-day chick embryos, scrambled, recompacted, transplanted to the chorio-allantoic membrane of 8-day embryos, and examined 9 days later, proved to have been able to give rise to remarkably complete and morphologically well organized organs of the respective kinds, with the various tissue components in their normal mutual relations and functional activity. The results re-emphasize internal "self-organization" as one of the most basic problems in the study of development, in contradistinction to contemporary preoccupation with external "inductions."

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