the estradiol exerted its maximal protection when coupled with castration of the male and the consequent removal of the male hormone. Estradiol in the body of the male appears to overcome the deleterious effect of testosterone to a large extent. The intact males which received estradiol showed survival of only 5% less than the castrates plus estradiol. This implies the greater potency of the female as compared with the male hormone, at least in the concentrations available.

Graham and Graham(3) suggested that the resistance to radiation is not related to a simple sex hormone activity. They feel that some extra-gonadal system may be stimulated and protection results. Patt *et al.*(2) suggest that the protective action of estrogens is mediated through the adrenals whereas Ellinger(5) expands this hypothesis stating that "the effect of estrogens may well be mediated by the spleen which is recognized to be under the influence of the adrenal cortex and the functional status of which appears as of great importance for the radiosensitivity in total body irradiation." Bethard, Simmons, and Jacobson(4) report that the survival of mice given estrogens, followed by irradiation with spleen shielding, was greater than when either method was used alone.

Summary and Conclusions. The differential in survival of male and female mice, when exposed to whole body x-irradiation, seems to be due in part to the gonadal hormone present. Intact males, with the normal quota of testosterone, have less survival value than do castrate males. Further, castrate males injected with the female hormone, estradiol benzoate, exhibit maximum survival, approaching that of the intact female.

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Development of Heterotypic Combinations of Dissociated Embryonic Chick Cells.* (22495)

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Some time ago, attempts were initiated to study the behavior *in vitro* of dissociated embryonic chick cells(1-3). Suspensions of viable cells were obtained by disintegrating organ rudiments of the early chick embryo into their constituent cells, using a procedure based on treatment with trypsin. It was found that, under suitable conditions of cultivation, the discrete cells re-aggregated into clusters, re-established a tissue-like association, and subsequently resumed their characteristic histotypical development. Thus, aggregates of limb-bud mesoblastic cells gave rise to cartilage and muscle, and aggregates of mesonephric cells to characteristic nephric tubules and vesicles. These observations were in line with the results of investigations by Weiss and coworkers(4-6) on the fate of embryonic cells in suspension introduced into embryos or grown *in vitro*. The combined evidence strongly indicated that, under the conditions explored, the dissociated cells retained their ability to restore the special histogenetic pattern of their erstwhile associa-

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tion. In most of these experiments, both the identity and proportions of cells in the suspension were the same as in the original tissue. They provided, therefore, little information as to how qualitative and quantitative variations in the original composition of the cell population might affect the outcome. The pertinence of these questions was stressed by diverse observations on chick cell aggregates(2, 5-7), suggesting that cells of different kinds, intermingled in the same suspension culture, tended to become grouped according to kind. Phenomena like these could evidently not be studied in suspensions containing essentially one major cell type only ("isotypic" suspension); their elucidation required a system consisting of 2 or more different cell types ("heterotypic" suspension) clearly distinguishable by their appearance and histogenic properties. The cellular combinations arising in such heterotypic mixtures proved suitable for the study of cellular properties and activities, operative not only in the formation and the development of aggregates, but also in more general histogenetic processes. Of the numerous factors that might affect the final outcome of cell aggregation, the following groups of variables should be considered: 1. The origins and types of cells; their age; proportions of the component types in a given mixture. 2. The physical, chemical and immunological properties of the substrate and the nutrient(8). 3. Cellular interactions; rates of mitotic activity; metabolic activities of the cells; production of extracellular materials or structures. These factors, and others, may affect each or all 3 major steps in the development of aggregates, which are: a) the formation of the primary cluster, *i.e.*, the aggregation of cells by random encounter, directional migration, surface interaction, contraction of extracellular matter; b) organization within the aggregate, either by preferential proliferation and development of some foci with the suppression of others; or by regrouping of the cells within the initial cluster; in either case, the resulting pattern might or might not be identical with the original one; c) the development of the aggregate as an "organoid" system with the possible occurrence of field phenomena, metabolic or diffusion gradients, tissue interactions, etc.

The present report summarizes some observations on a relatively simple heterotypic system, consisting of suspensions of combined mesonephric and limb-bud cells of the chick embryo. Future reports will deal with more complex homologous and heterologous combinations.

Material and methods. Chick embryos from stage 16 (Hamburger) to 5 days' incubation were used. The isolation of tissue blastemas and preparation of cell suspensions and cultures were done as previously described(1), with the following modifications. Final dissociation of the tissue, following treatment with trypsin, was done after rinsing and transfer of the still compact fragments into the culture medium. Stickiness, although rarely a problem with this material, was avoided by keeping the rinsing solutions at pH 7.2-7.6(9). Different concentrations and proportions of cells in the iso- or heterotypic suspensions were obtained by dissociating varying numbers of rudiments, or fractions of a rudiment, in identical quantities of medium. Aliquots of suspension in a mixture of embryo extract (10-12-day embryos), chicken serum and Earle's saline in equal amounts, were placed in hollow ground slides, sealed and incubated. Aggregates that formed during the following 48 hours were then transferred for further cultivation by Fell's watch-glass procedure. The results to be reported are based on a total of 280 cultures, studied in the living and preserved state. The following terms will be used: (1) *isotypic* and (2) *heterotypic,* to designate suspensions consisting of (1) predominantly one type of cell and (2) two or more cell types; (3) *isochronic* for cells obtained from embryos of equal age; (4) *heterochronic* for cells from embryos of different ages.

Aggregates of lateral mesoderm cells from embryos of stage 16. The lateral mesoderm from embryos of ca. 54 hours' incubation representing prospective limb-bud and nephrogenous tissues, was dissected and dissociated either in a single piece or following separation into the respective organ-forming regions. At

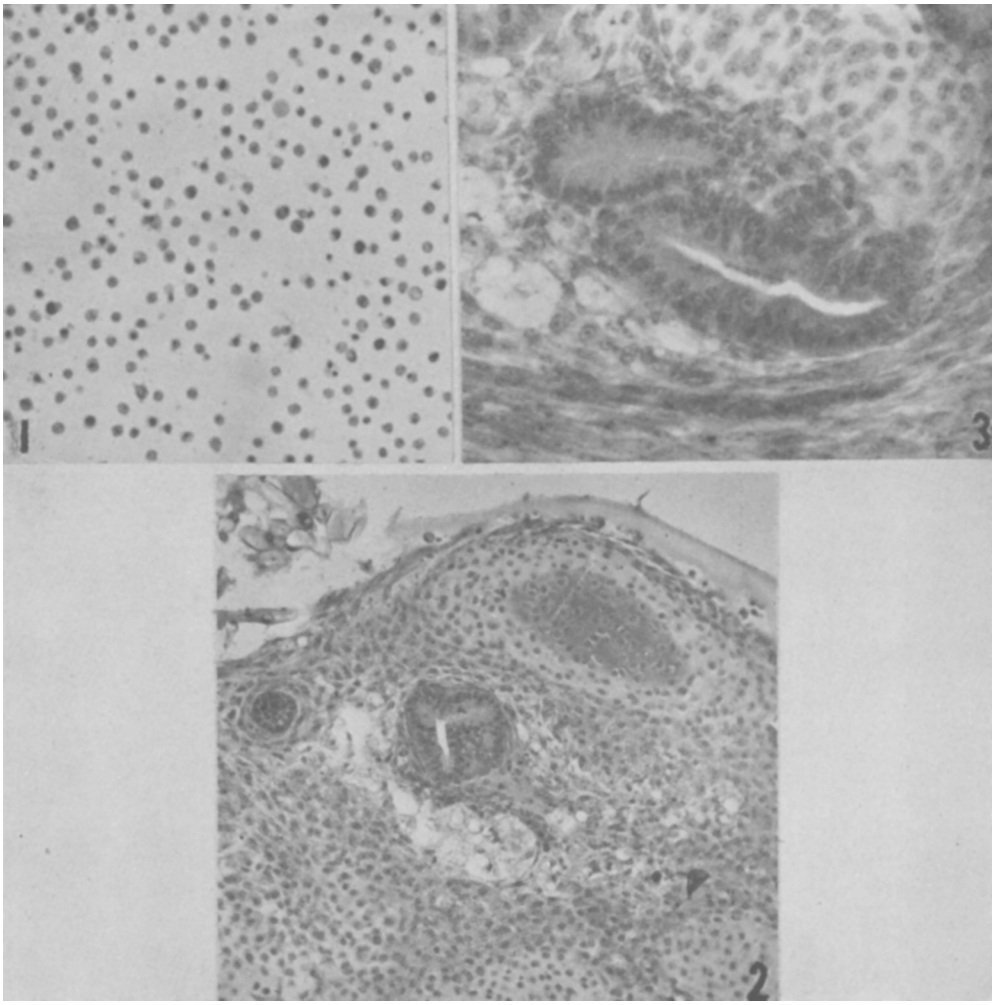

FIG. 1. Cell suspension of lateral mesoderm from embryos of stage 16. ×160. Fixed in formalin-acetic vapour; hematoxylin.

FIG. 2. Section of an aggregate of cells as in Fig. 1, cultured for 6 days on a plasma clot and showing cartilage, nephric tissue, epidermal structures and keratin, fat-cells and macrophages. × 160. Zenker's fix.; hematox.-Biebrich's.

FIG. 3. Same as Fig. 2, showing cartilage, nephric tubules and myoblasts. ×280.

this stage, these regions show no clear structural indication of their subsequent development. The resulting aggregates were grown for 6 to 8 days, and, when examined histologically, were found to have developed in accordance with their normal fate in the embryo: The prospective limb-bud cells had formed cartilage, while nephrogenous cells developed tubules and vesicles. In aggregates formed in the composite suspensions of the whole mesodermal ridges, cartilage and nephric tissue developed side by side (Fig. 1-3). In some cases, myoblasts in the early stages of cytodifferentiation were present. Epidermal cells that had been included in the suspensions gave rise to keratinized epidermal pearls and layers. It was thus established that, even at this early stage of development, dissociated and intermingled cells were able to reconstitute distinct and characteristic tissue fabrics and that their subsequent differentiation followed the type-specific course evidently initiated in them prior to dissociation.

Aggregates of isochronic limb-bud and mes-

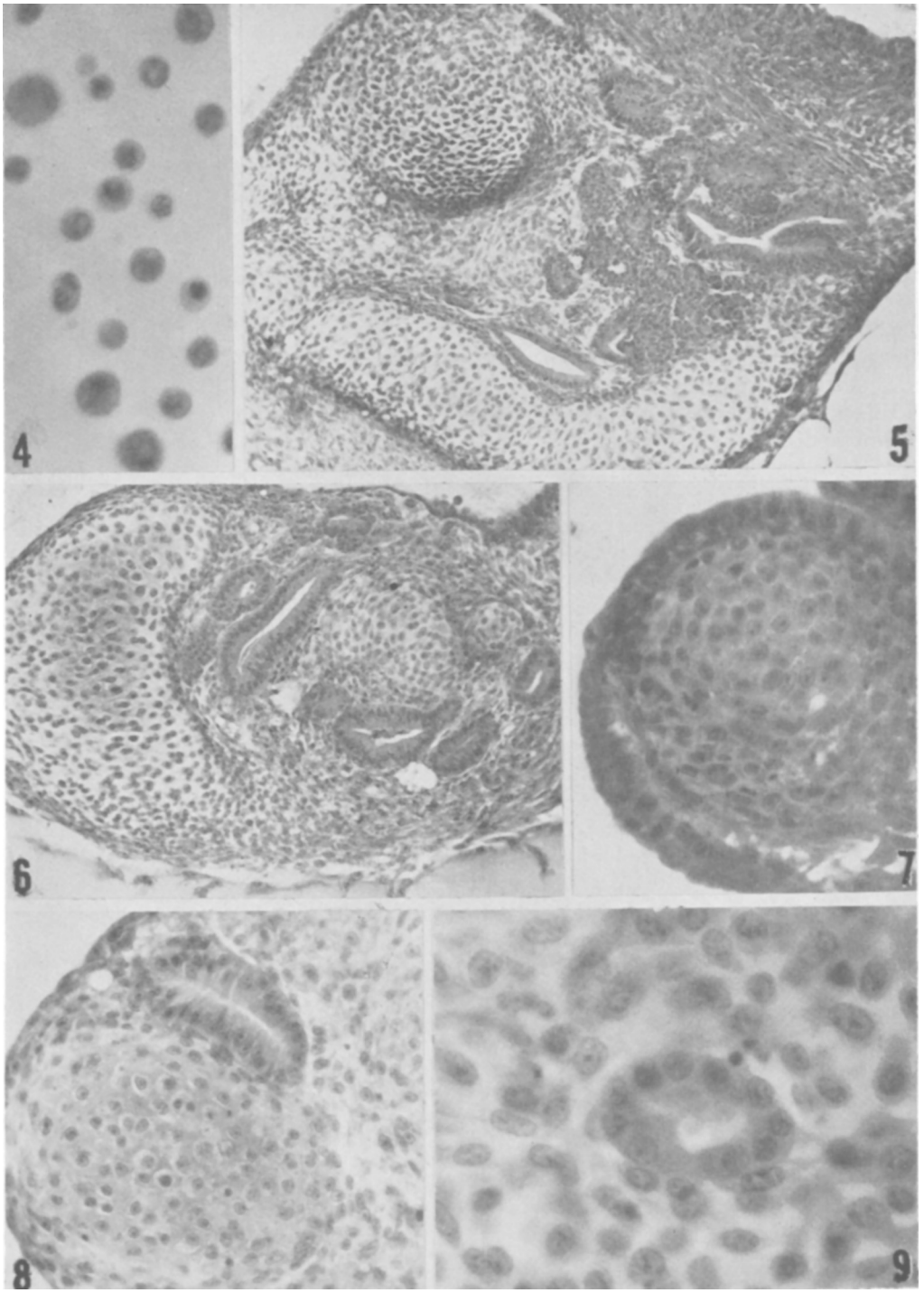

FIG. 4. Suspension of mixed mesonephric and limb-bud cells from a 4-day embryo. ×480.

FIG. 5. Section of an aggregate of cells as in Fig. 4, following 7 days in culture; cartilage, nephric tissue, myoblasts and fibrocytic stroma. ×120.

FIG. 6. Section of an aggregate of 3½-day mesonephric and 5-day chondrogenic cells in equal proportions, cultured for 7 days. ×160.

FIG. 7-9. Parts of section through aggregates of mesonephric and chondrogenic cells to show close association between the two resulting tissues. ×260, 220, 460.

onephric cells from older embryos. This series consisted of aggregates formed in suspensions of combined mesonephric and chondrogenic cells (in some cases whole limb-buds were used) of identical ages, from 3, 4, and 5-day embryos. Both types of tissues then reappeared in these heterotypic aggregates, following 4 to 7 days' cultivation, in their characteristic differentiated form (Fig. 4.5). The progress of differentiation varied according to the types and ages of the precursors. Chondrogenesis at a given total age (age at explantation plus length of cultivation) compared favorably with that in embryos of comparable age. By contrast, the differentiation of nephric tissue lagged behind its progress in the embryo. In aggregates formed in suspensions including myogenic cells, the identified myoblasts were never as numerous as could have been expected from their original proportion. They rarely developed beyond the trapezoid or early spindle-shaped stage (2). This confirmed prior observations *in vitro* that, in the presence of chondrogenic cells, myoblasts did not readily attain advanced differentiation, unless present in proportions exceeding those in the normal limb-bud(3). Yet it was shown(2) that the cause of this impairment did not reside within the explanted myoblasts as such and that given the right conditions they would readily differentiate into muscle cells(10-13). In contrast with other tissues, epidermal cell-groupings, whenever present in the aggregates, showed a precocious formation of keratin as compared with the skin of embryos of equivalent total age. This acceleration has been noted by others(14,5).

Aggregates of heterotypic cells in varying ratios. Considerable evidence exists to suggest that there is a limiting mass minimum for tissue explants, below which continued morphogenesis and development will not occur(15,16). The following observations indicate that in heterotypic systems, histogenesis may be affected, above the level of the limiting mass minimum, by the ratios of cells of different types present in the mixtures. Results obtained by varying the proportions of mesonephric and chondrogenic cells from 4-day embryos illustrate this point. To aliquots of a suspension of chondrogenic cells, mesonephric cells were added in concentrations decreasing serially by a factor of 2. The controls were run as a parallel series of cultures. Examination of the resulting aggregates, following 6 days' cultivation, showed clearly that the lower the proportion of mesonephric cells in the initial suspension, the lower was the frequency of appearance of nephric structures and the smaller was their average size. Ultimately, a certain liminal proportion was reached, below which no recognizable nephric structures were found, although scattered epithelioid cells and patches were in evidence. In the absence of epidermal or other epithelial admixtures, these cells were evidently nephroblasts that had not reconstituted a tissue fabric. By contrast, an equal amount of mesonephric cells cultured separately in the same quantity of medium reaggregated into characteristic tubules. It was not, therefore, the low absolute number of mesonephric cells that interfered with the expression of their typical histogenetic activity, but rather their association with an apparently excessive amount of cells with a basically different histogenetic pattern. Comparable experiments were made with a graded series of decreasing proportions of chondrogenic cells, combined with constant amounts of mesonephric cells. Here again, the size and the number of cartilage foci decreased in the lower concentrations of chondrogenic cells, and below a certain liminal concentration no cartilage appeared in the cultures during 6 days' cultivation. Such cultures showed normal nephric tissue and fibroblastic cells. And yet, a similar quantity of chondrogenic cells in an isotypic suspension reaggregated and did develop into cartilage. In fact, it was noted by Fell(17), as well as in this study, that even quite minute, isolated fragments of chondrogenic tissue, consisting of 30 to 40 cells, if grown as such under suitable conditions, differentiated readily into cartilage.

This evidence seems to suggest that the manifestation of histogenetic activity in a heterotypic suspension might be profoundly affected by the relative proportions of the cells in the original mixture. The mere presence of cells of a certain type in a heterotypic sys-

tem does not, therefore, insure their expression as a typical tissue. Unless present above a liminal proportionate concentration, they do not give rise to a coherent tissue fabric. It may be assumed that these liminal values vary for different cell types as well as for each type at different stages of development and in different heterotypic combinations.

Heterotypic-heterochronic aggregates. Such aggregates were obtained by intermingling mesonephric and chondrogenic (or limb-bud) cells from 3- and 5-day embryos. Whenever the cell ratios were above the respective liminal values for histogenetic activity, cartilage, nephric tissue and epidermis (if such was originally included) appeared regularly in the cultures, following 4 to 6 days' cultivation (Fig. 7). Histogenesis and the ultimate appearance of the tissues were normal. Taking into account the expected differences in rates of development of tissues in the heterochronic combinations, no essential difference was noted, within the tested range, between the development of the isochronic and the heterochronic aggregates. The different types of cells, originally incorporated in the suspensions, appeared in contiguous or scattered groupings, each manifesting the typical structural properties of the tissue of origin.

It should be mentioned at this point that Trinkaus and Groves(7) reported an inhibition of cartilage formation in aggregates of mixed 3-day mesonephric and 5-day limb-bud cells, as well as in aggregates of limb-bud cells only. In view of the theoretical considerations raised by the authors, it should be stressed that in our experiments with the same type of material we have found no evidence for this effect.

Regional groupings of cells in heterotypic aggregates. As already pointed out, the reaggregated cells, in the fully developed heterotypic aggregates, appeared in histotypically distinct, major groupings. These easily identifiable clusters of cartilage, nephric or epidermal structures were interconnected by a stroma of fibrocytes, mainly of connective tissue and myoblastic origin, sometimes including fat-cells, macrophages and possibly also cells of chondrogenic and mesonephric origin. In their chaotic architecture, the aggregates strikingly resembled teratomatous structures (6). On closer scrutiny, small groups of cells were found scattered in various locations in close association with cells of other types. Small groups of nephric or epidermal cells were frequently observed either tucked in between masses of cartilage (Fig. 8) or right inside the chondrified tissue (Fig. 9). On some occasions, a layer of nephric cells was found to have enveloped a chondrified core (Fig. 7). In spite of their close association, it was possible to identify the different components with a fair measure of certainty. It seemed, therefore, that the cells had not only preserved their original identity throughout the phase of dissociation and aggregation, but had also re-established specific and selective contiguity, as evidenced by their assortment into type-specific groupings(18,19). Considering the complete initial intermingling of the cells in suspension, their ultimate arrangement suggests the following possibilities to account for the final structure of the aggregate: (a) that it is determined during the initial phase of aggregation by differences in the rates of movement of different cell types, by contact interactions, etc.; (b) that it is due to reorganization within the primary clusters through active cell movements and selective cell groupings; (c) that it is attributable to differential survival and proliferation of the various components; (d) that it is affected by all of these factors. The evidence presented here does not allow a critical evaluation of these possibilities.

Summary. 1. The behavior *in vitro* of dissociated and reaggregated mesonephric and limb-bud cells of the early chick embryo was studied in heterotypic, isochronic and heterochronic combinations. 2. Under the conditions examined, all cell types incorporated in the cell suspensions reappeared eventually in the aggregates to form tissue-like groupings that developed in accordance with their tissues of origin. 3. Manifestation of the characteristic histogenetic activity by cells of a given type in a heterotypic combination decreased in proportion to their share in the total population. If present below a certain liminal proportion, the cells did not reconstitute their typical tissue fabric, although equal

amounts, when cultured separately, would differentiate histotypically.

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Effect of Varying Dosages of Potassium Iodide in Experimental Atherosclerosis. (22496)

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Several investigators have reported that potassium iodide(1,2), organic iodides(3) or desiccated thyroid(3) inhibited formation of atheromatous plaques in cholesterol-fed rabbits. While KI and organic iodides had a tendency to cause hypercholesterolemia, thyroid powder lowered serum cholesterol somewhat. Similar effects of these compounds on serum cholesterol have also been observed in chickens(4), although no anti-atherogenic influence was found. Recently, administration of KI to rats for very short periods was shown to cause a 2-fold increase in serum cholesterol (5).

The present study was designed to examine effects of various dosages of KI as well as of 2 thyroid-active substances (diiodotyrosine and thyroxine) on development of atheromata and on serum cholesterol and b-lipoproteins of rabbits fed cholesterol. The anti-atherogenic effects of large dosages of KI are confirmed.

Methods. Male rabbits of the Dutch belted strain were maintained on a diet of rabbit chow (Wayne Rabbit Ration, Allied Mills, Chicago, Ill.) which had been thoroughly mixed with a suspension of cholesterol in corn-oil so that every 100 g of food contained 2% cholesterol and 6% corn-oil. The basic diet was augmented with amounts of KI or of diiodotyrosine as noted in Tables I and II by spraying the appropriate solution on the pellets while they were tumbled in a concrete mixer. This was done before adding the cholesterol-oil suspension. The thyroxine was administered by the intraperitoneal injection of 0.05 mg, 3 times weekly. After 8 weeks the animals were sacrificed and aortas examined visually for atheromata and graded on a 0-4 scale. Averages for each group are given in Table I. In Exp. 1 and 2 the entire aorta was graded, in Exp. 3 the arch and thoracic portions were graded separately. Sera were assayed for cholesterol(6) and lipoproteins(7) and livers were excised, weighed wet and analyzed for cholesterol content. Average values of these are stated in Tables I and II.