THE PROBLEM OF SPECIFICITY IN GROWTH AND DEVELOPMENT*

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Introduction: Biological Specificity

The frequency with which such terms as specificity, selectivity, conformity, correspondence, etc., appear in biological literature is ample proof that they denote a universal and fundamental trait, running like a common theme through all manifestations of life. Yet, they are used with so many different shades of meaning and degrees of precision that it is impossible to tell whether the various phenomena to which they are applied bear a purely formal resemblance to each other or whether there is essentially a single principle in back of them all. A random list of examples will illustrate the case. We describe as "specific" the absorption by certain compounds of certain wave lengths of light; the relation between enzymes and their substrata; the matching between egg and sperm; the action of a hormone on its end organ; the effect of genes on characters of development; the association between a parasite and its host; the immunological response to a foreign protein; the adequate response of our nervous system to a given stimulus; the acts of recognition and evaluation, which characterize our highest mental functions. What do these various "specificities" have in common? Are they merely superficial parallels, or does one or the other of them perhaps contain the key to the rest so that specificity in all manifestations of life could be resolved to a single operative principle?

It may be too early to attempt an answer to this question, but it does not seem too early to ask it. Therefore, let us take a closer look at relations and activities in growth and development which we commonly describe as "specific," and examine to what extent their specific character might be explicable in terms of better known and better understood specificities at other biological, or preferably simpler physical and chemical, levels. In particular, let us explore the

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pertinence of the model of serological specificities as a model of developmental processes, inasmuch as recent studies in immunochemistry have brought those specificities within our grasp.[16] Perhaps, the study of growth and development could then profit from this faster advance of one of its biological sister lines.

But let us first clarify what biologists mean when they speak of "specificity." In its common connotation, the term refers to that relation between two systems which enables members of one system to exert a *discriminative* effect upon certain members only of the other; it implies *selectivity* of action and reaction even in the absence of separate channels from the acting to the reacting members. A chemical that bathes all tissues, but affects some of them with disproportionately greater potency than others, will be considered as specific for the affected tissues in that sense. By definition, selectivity is the faculty of a process or of a substance to activate, to alter the state of, or to combine with, certain elements in preference to, and to the exclusion of, other elements of the same system. The basic criterion of selectivity, therefore, is the correspondence and mutual fitting between two properties. Primarily, the term specificity applies to this *correspondence,* and to neither of the interacting systems as such. By custom, however, it has acquired a secondary meaning signifying those properties of each system which make selectivity of interaction possible.

Resonance is one simple model of selectivity. Here the specificity is based on time characteristics. The example of fitting keys and locks illustrates specificity of relations based on spatial correspondence. And if we analyze all conceivable types of specificity, it would seem that all can be resolved into characteristic patterns of time or space. Selectivity shows different degrees of sharpness, the intensity of the response falling off more or less steeply from a peak, which marks the point of best correspondence. In these days of radio communication, we need hardly stress the fact that selectivity is a matter of degree. It is important, however, to point out that the degree of selectivity need not be entirely a fixed constitutional property but can often be sharpened as a result of adaptation of the responding system to repeated or lasting exposure to a stimulus of constant configuration.

Perfunctory and incomplete as this definition of specificity is, it will do for the purpose of our further discussion. In the indicated sense, specificity is perhaps the most fundamental attribute of life

processes, from the synthesis of the building stones of protoplasm to the orderly performance of our mind, and its elucidation must remain one of the prime concerns of biology. Let us now turn to our object proper—development.

Developmental Kinetics

Brought to its simplest formula, development consists of three types of events: (1) *Growth*: the reproduction of certain basic compounds by synthesis from simpler elements, duplicating the patterns of existing compounds. (2) *Differentiation*: the gradual elaboration of new chemical systems and compounds not previously present as such, presumably by gradual transformation of the patterns according to which synthesis of the protoplasmic compounds occurs. This transformation of basic protoplasm takes divergent courses in different cell strains, producing lines which become increasingly dissimilar in their biochemical and morphological constitution as development proceeds. (3) *Localization*: the sorting and segregation of biochemically different units into definite locations. The field which has for its object the study of these processes might appropriately be called "developmental kinetics."

As the various biochemically differentiated units aggregate in different predictable locations, associate with their own kind and with certain other units, or relinquish their positions and disperse, all depending on whether and how they fit into each particular site, they furnish us with paradigms of selective behavior, and we may use these phenomena as our point of departure. When we speak of shifts and redistribution of units, we refer to the materials of the undivided egg prior to segmentation, as well as to the movements of individual cells or whole cell layers later in the cellulated germ. The accumulation of certain visibly distinct substances in a given sector of an egg and the shift of germ layers during gastrulation have many basic features in common. Both involve convections by which the units concerned are brought into novel combinations with other units and under new local conditions. Whether such new combinations will be lasting depends on the nature of the components. Since cells are in constant activity and activities of adjacent tissues may differ in kind, only those can remain united whose metabolic activities, chemical requirements, chemical discharges, electrical properties, growth changes, surface properties, etc. are mutually compatible.

As we shall explain below, this compatibility is not merely a matter of communal tolerance, but implies active "affinities" among the partners that are to form durable biological unions. Intracellular streaming, cell locomotion, and the shifting of cell masses may be gross mechanical events, but the forces that tie a part to its final location seem to be subtle and specific. True, in the mature organism the attachment between neighboring tissues is secured by various encasing and cementing structures, such as basement membranes, connective tissue fibers, and the like. However, prior to the development of these accessories, tissues must rely for whatever hold they exert upon each other on forces residing directly in their naked contact surfaces. It is to the exposed cell surfaces then that we must look for the revelation of the factors which make or break specific cellular associations.

The phenomena of developmental kinetics thus present us with the following questions. Why do particles, cells, and cell layers shift during development? Why do they cease to move once they have reached certain localities or have combined with certain other groups coming from other directions? What determines their course and how do they get to their proper destinations?

Embryology furnishes numerous striking examples of shifts of tissues relative to each other, moving either as compact masses or as groups of individual cells. Indeed, the processes molding the early embryo after cleavage are predominantly in the nature of translocations rather than growth. Practically the whole germ is on the move. Later, after the basic form has become fixed, mobility is restricted to certain cell types which move within the now consolidated frame. The neural crest,[8] for instance, spreads into the interstices of the embryonic body, laying down different cell types at different stations: ganglion cells along the vertebral column, sheath cells along the nerve fibers, pigment cells along predetermined lines in the integument and its derivatives, and, at least in Amphibians, cartilage for certain elements of the head skeleton. There is circumstantial evidence that these various cell types are already different in character when they leave their common sites of origin. What, then, guides each to its proper final destination? Nerve fibers offer another example.[38] By outgrowth, which is primarily a matter of the movement of their free tips, motor and sensory fibers span considerable spaces, after which each type forms exclusive connections with the peripheral tissues appropriate to its own kind, motor fibers with

muscle, sensory fibers with skin organs. How do they get where they belong? Germ cells,[45] at least in some forms, have been claimed to originate in embryonic areas far distant from the gonad and to immigrate only secondarily into the latter, presumably conveyed part-way by the blood stream. The oriented migrations of lateral line organs, muscle buds, various ducts, and capillary sprouts offer further examples of the same kind.

One notes a formal resemblance between these developmental processes and the behavior of some parasites, which enter their host at a specified point and end up at a predictable destination. The comparison may be valid even for parasites with well-differentiated nervous systems, reacting to an orderly sequence of sensory cues furnished by landmarks of the host body; for the properties which endow a sensory cell or a nerve cell with discriminatory ability may yet turn out to be of the same nature as those that permit a cell to "sense" its way through the body.

As to the question of how the migratory cells of the embryo come to gather in certain specified locations, it has long been considered good form in biology to answer by circumlocution, stating that the cells get to their proper location by "attraction," "tropisms," a "sense of direction," and so forth. For descriptive classification, a listing of various types of tropisms may be useful, but it serves no analytical purpose. The term "neurotropism," for instance, in use for nearly half a century, has not only explained nothing but actually delayed real insight into the factors orienting the course of a nerve fiber. Even less realistic is the anthropomorphic and animistic terminology, in which cells are described as acting personalities, making decisions, choosing courses, and quite generally "doing" things. The main objection to symbolic expressions of this kind comes from the fact that instead of formulating the problems, they merely label them. We may not be able to dispense with such descriptive terms for some time to come, but we must guard against giving them any explanatory value. We must treat cells as physical systems in space and time, endowed with definable properties which are subject to the limitations of all physical bodies and their laws of behavior. If a cell gets from one place to another, it can do so only in strict compliance with the physical realities prevailing along its course.

Realistically speaking, then, a cell can have no "sense of direction" unless there is a physical directive agent (intensity gradient, oriented guide structure, or the like) operating right at the spot

where the cell is. No agent can affect a cell from a distance other than through the intervening medium, and the physico-chemical constitution and behavior of the latter will determine if and when and from what direction and in what amount an orienting agent will arrive at the cell. What counts, is not the nature, orientation, or concentration of the agent at its source, but only the nature, orientation, or concentration in which it is present in the differential of space immediately adjoining the cell. This commonplace statement is called for by the tendency of some biologists to treat the attractions of cells "toward" distant destinations as if the intervening space were a vacuum, fully transparent, permeable, and non-corporeal.

Just how, in concrete language, does a cell get from its source to its destination? There are three possible answers to this question. They refer to three principles which we shall call (1) *selective conduction,* (2) *selective fixation,* and (3) *selective elimination.* A brief explanation of these follows.

Selective Cell Association

Selective conduction. The locomotor mechanism of tissue cells is still rather poorly understood. Lacking such special locomotor organs as cilia, flagella, and the like, cells move either by rhythmic deformations of their bodies, protrusion of pseudopodia, or a form of gliding. Cell sheets advance actively either by the locomotor activity of the cells along their free edges or by changes in the shape of all their cells, often due to differential contraction or expansion of their surfaces. In general terms, cells move whenever an inner disequilibrium creates pressures and tensions which are unevenly distributed over the surface and yield a resultant in some one direction. If this resultant direction changes at random from instant to instant, the cell will likewise shift at random, in a sort of large-scale Brownian movement, about a stationary center. Any progressive cell advance, on the other hand, implies the presence of constant polarizing forces which make the locomotor pressures and tensions yield resultants in a prevailing direction.*

Accordingly, no cell can make continuous progress in an isotropic field of force. On the other hand, any anisotropy of the surrounding field capable of affecting the pressure-tension configuration of the cell, will thereby have an orienting effect on locomotion. Among

* For recent descriptions of cell locomotion, see Lewis[18] and Holtfreter.[15]

the agents potentially capable of such effects are elastic tensions, pressure, interfacial tension, flow, gravity, electric potentials, electric currents, and radiation, as well as steady gradients in the concentration of any chemicals that affect the physical properties of the cell surface. In some cases, the external polarizing force may provide both the drive and the guide for the movement; in other cases, it determines only the direction of the movement, while the driving power is furnished by the metabolic energy of the cell itself. In either case, the orientation is of external origin. Spindle type cells (fibroblasts, mesenchyme, Schwann cells) and nerve processes, for instance, are oriented by interfacial tension along oriented fibrous structures, which, in turn, are but orderly arrays of groups of linear molecules.[40, 41] The locomotor mechanisms of spindle cells and amœboid cells (e. g., lymphocytes) differ somewhat, but even the latter seem to require contact substrata for continued advance.

"Contact guidance" has been shown to be a necessary condition for directive cell movement. If cells are confronted with guide structures that are all aligned in a common direction, the cells are forced into a single course.[41] In this case, contact guidance is not merely a necessary, but it is a sufficient condition for orientation. Yet, if the medium contains multiple intersecting guide structures, the problem becomes equivocal. Which one among the several available and structurally equivalent pathways is a cell to follow in a given instance? Since they seem to be able to "choose" the right track, we must postulate that the contact substrata have different specific properties that act as cues: one particular type of surface would be uniquely suitable for the application of one particular cell type, and another type of surface for another cell type. I have called such a mechanism, which is based on the specific matching between the cells to be guided and their prospective guide structures, *"selective contact guidance."*[38] Its most plausible explanation would be that temporary linkages are formed between specific molecular groups in the cell surface and complementary groups in the guide structure. The guide structure may be situated in the surface of another cell or in the intercellular matrix.

Selective fixation. This term refers to a mechanism of the following sort. Cells of a given kind spread from their source at random, but certain areas of the body are so constituted as to arrest and hold cells of that particular type when they happen to get there. This local trapping, which might be a matter of mere immobilization

or of true attachment, presupposes again highly discriminative powers on the part of both the cell and its prospective "trap." One could envisage a hypothetical trapping mechanism as consisting of the establishment of firm linkages across the contact surfaces between molecular groups of high affinity.

Selective elimination. This process is the reverse of the one just outlined. As in the former, the cells concerned are at first distributed rather ubiquitously throughout the body, but are then actively destroyed in certain regions while being spared in others. The final distribution would not be a matter of selective affinity between the cell and its permanent site, but rather of some disaffinity between it and all other sites.

It seems that these three principles embrace all the conceivable mechanisms by which localized aggregations of cells originating from distant sources could be effected. In a last analysis, they resolve themselves to a single common principle, namely, the existence of specific contact relationships between the various units of the organism, according to which adjacent units may either be bound to each other, or not bound, or even actively separated. In the case of selective conduction, the ties are those between the cell and its guide structure and are transitory. In selective fixation, they are more durable, anchoring the cell to its surroundings, pending reinforcement or replacement by secondary cementing agents. Being contact relationships, they can easily be conceived of as products of intermolecular forces, and their specificity as the result of steric conformances, that is, fittingly interlocking configurations of the molecular species to either side of the surface of contact.* These relations will have to be viewed as dynamic rather than static, and as statistical rather than rigidly fixed; that is to say, as the bonds in question are presumably incessantly made and broken, the rate and frequency of these events are as instrumental in determining the degree of specificity attained as are the nature and arrangement of the molecular groups involved.

Before going into these matters more fully, however, we wish to strengthen the biological evidence on which this theory of contact specificity rests.

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* We are adopting here essentially Pauling's[24] concept, according to which the strength of intermolecular bonds varies with the degree of correspondence in the shape of the interacting molecules.

Tissue Affinities

In the healing of complex wounds, in which several tissues are involved, it has been repeatedly observed that each tissue component tends to fuse with its own kind. What has not been sufficiently stressed is that after such fusion has occurred, the tissue components automatically cease to expand further. This phenomenon is strictly comparable to the stoppage of the migration of units in the embryo when they meet their own or some properly matching kind. If we try to reduce all observations of this sort to a common denominator, we are led to the following thesis.

Any given cell type remains stationary only as long as certain very specific contact conditions peculiar to its own kind prevail along its exposed surfaces. If these conditions are not fully satisfied, the unit will move and continue to move until it finds itself again either in the original or in an equivalent situation. To use a simile which may prove pertinent, each unit would possess many specifically arranged "valencies," and only if all of them are completely "saturated" by properly matching "valencies" of the surroundings will the unit be immobilized. Any partial unsaturation, on the other hand, would mean instability, and hence, result in mobilization. It is obvious that such "unsaturation" could arise either from without or from within the unit; from without, if the unit is deprived of its matched environment by mechanical lesions or other alterations; from within, if the character or state of the unit itself changes, as happens during ontogenetic differentiation or in pathological states.

Let us now illustrate the operation of this principle on some concrete examples. These can be grouped into three classes, depending on whether we focus on the selective combination of units with their own kind (*"homonomic" affinity*) or with some other matching kind (*"complementary" affinity*), or on the *active detachment* from other units.

The behavior of epithelial sheets may serve as prototype of homonomic affinity. Epithelia with free borders rarely remain quiescent. They expand until edge meets edge and the system becomes closed up in itself. Epithelial coats and linings, therefore, always tend to restore their own continuity and tolerate no gaps. Inflicting a hole deprives the epithelial cells along its border of some of their natural surface contacts, and thus creates the "unsaturated" state which presently sets them in motion. If they remain attached

to the rest of the epithelium, they drag it along. The movement is sometimes directed, at other times rather random. In either case, it ceases only after the gap has been covered and there is no longer any free edge. Observations on explants and transplants indicate that in the presence of several kinds of roaming epithelia, reunion mostly occurs selectively, each fusing preferentially with its own kind and by-passing other kinds. Mixed epithelial mosaics tend to break up into their constituents, each type forming a separate coat or cyst. Exceptions are noted in those cases in which two different epithelia are of the kind that normally have a common border, but whether this is an expression of complementary affinity or of purely mechanical welding remains to be seen.

Further examples of homonomic affinity may be cited from such widely different fields as the development of vascular anastomoses, the growth of Schwann cords in nerve regeneration, the formation of nerve trunks, the healing of transplants, and the reaggregation of dissociated sponges.

Capillary networks arise from the fusion of advancing capillary sprouts. Evidently, the blind processes keep growing until they meet other similar processes, with which they then merge.[4] The fact that they anastomose only with members of their own kind, to the exclusion of all the other cell types they pass on their way, is proof of the distinctive constitution of their surfaces, which alone makes such selective recognition possible.

The transection of a nerve trunk is followed by the emigration and growth of masses of sheath cells (Schwann cells) from both ends. If the gap between the ends is occupied by a diffuse scar, the Schwann cell masses expand in it profusely in all directions and form a tumor-like glioma. If, however, the nerve ends are bridged by a trellis of parallel fibrin fibers, growth is checked as soon as the cell strands advancing from opposite ends have merged into continuous bands.[43] Evidently, the difference in the two cases is this. An irregular scar provides the cells with a diffuse net of pathways, along which the chances for any two cells to meet head-on are relatively low, and consequently, growth continues unchecked; while the presence of oriented guide fibers across the gap necessarily leads the cell cords from opposite ends to advance straight towards each other and eventually to meet, whereupon they come to rest. Since the same Schwann cords are not arrested by their frequent encounters with other cell types, such as endoneurial cells, fibroblasts, and macrophages, it is clear that the effect is highly specific.

Nerve fibers show a marked tendency to associate according to their specific character. Not much is known about this except what can be inferred from the fact that peripheral nerve trunks and central fiber tracts do not contain different kinds of fibers (motor, sensory, sympathetic, etc.) in indiscriminate dispersion, but grouped into relatively homogeneous bundles. In view of the fact that each bundle or tract is made up of fibers of widely different ages, the homogeneity of their content indicates that fibers of a given kind growing out at a later stage have gathered preferentially around older fibers of their own kind. This concept of "selective fasciculation"[38] finds some more direct support in certain experiments with an easily distinguishable nerve cell type (Mauthner's cell): supernumerary Mauthner's fibers developing from grafted brain parts tend to follow the normal Mauthner's fibers of the host if they happen to make contact with them.[23]

These examples find their simplest counterpart in the behavior of sponges dissociated into small fragments. It has long been known that such fragments become highly mobile and upon encounter merge into larger bodies. In this reorganization process, the various cell groups become sorted according to their original characters, partly by selective association during the merger, partly by later segregation and regrouping.[3] Though there is again no evidence of attraction among homologous elements, order is restored by virtue of the fact that those elements that happen to come in contact will join more readily and more firmly if they are of the same kind than if they belong to unrelated kinds.

When there is selective association, temporary or permanent, between units of two different kinds, we may call this "complementary affinity." It implies a sort of "plurivalency" on the part of the units concerned. Examples of this principle are found in many cases of "selective contact guidance." For instance, the lateral line of amphibians develops by the tail-ward migration of a streak of cells along several predetermined routes under the epidermis. It has been shown experimentally that any change in the orientation of those parts of the body through which the lateral line is to travel, causes a corresponding change of the course of the line.[11] We must assume, therefore, that the bed for its growth is marked out by specific characters of the underlying local tissue which the tip of the outgrowing line follows.* The nature of these cues is unknown, but

* Experimental evidence[30] indicates that the relevant conduction pathway is furnished by the mesodermal structures rather than by the epidermis.

it would seem simplest to envisage them again as distinctive chemical characters of the contact surfaces. A purely mechanical concept seems inadequate to explain the facts.

A similar contact affinity must be assumed as guiding the growth of the Wolffian duct to the cloaca. When the posterior trunk of an embryo is experimentally rotated against the anterior trunk, the duct, on reaching the dislocated portion, deviates from its original course and often turns into the rotated position.[14] This indicates that its channel must have been marked out by local characteristics of the surrounding tissues. The fact that the blind end of the duct finally breaks through into the cloaca, in turn, is indicative of some complementary affinity between duct and cloacal wall, while the merging of the segmental mesonephric tubules with the pronephric and later Wolffian duct, is presumably to be classed as "homonomic affinity."

It was mentioned before that sensory and motor nerve fibers tend to group themselves according to type, as older fibers serve as guides to younger ones of the same category. However, even the early pioneering fibers take already divergent courses, depending on whether they are sensory or motor,[31] a fact which can only be explained by some "complementary affinity" between the respective fiber tips and the preneural pathways over which they travel. There seem to be separate pathways in the mesenchyme for motor and for sensory fibers, each of them impregnated with a specific character permitting just the nerve fibers of the corresponding type to follow it. At the end of their pathways, the nerve fibers enter another phase of selective behavior as they connect with their respective end organs. It has been shown, for instance, that sensory fibers which have been diverted into the path of motor fibers and thus forced to terminate on muscle fibers never form transmissive connections with the latter,[10, 42] evidently because of some incompatibility of the respective protoplasms. Thus, sensory fibers and motor fibers are not only constitutionally different themselves, but their differential is matched by corresponding differentials in the embryonic pathway structures and again among the terminal tissues. This mechanism insures not only that nerve fibers are generally guided to their proper terminal recipients, skin and muscles, but actually effect connections in the proper combinations. As will be outlined below, specific interactions with the periphery continue even beyond this stage.

Generalizing these experiences, it would seem reasonable to assume that all associations between tissues of different character,

whether temporary or permanent, are essentially a matter of reciprocal bonds. The development of composite organs by the joining of two or more contributions from separate sources is particularly suggestive; see, for example, the combination of the infundibulum with Rathke's pouch to form the pituitary body, the association between nerve fibers and sheath cells, adrenal medulla and cortex, liver cords and capillaries. These combinations are so unique in each case that it would be difficult to account for them otherwise than by some specific reciprocal bonding between the combining elements. It is only later in development that the mechanical frameworks of connective tissue fibers and tunics come in and consolidate the existing primary unions.

Just as "homonomic" and "complementary affinities" must be postulated to account for the selective association among parts, so some active mechanism is called for to explain the separation among formerly contiguous parts, which is a common embryological phenomenon. In the reassembling of dissociated sponges (see above), one observes not only positive affinity among similar units, but also an active separation among dissimilar ones. Likewise, in embryonic development, certain cell groups regularly leave their former positions, as if forced out.

For instance, the primary mesenchyme of the echinoderm gastrula leaves the entoderm plate for the blastocœle. The neural crest abandons its position along the margins of the neural plate and migrates into the body spaces. The lens and other placodal derivatives become pinched off from the epidermis. The mesenchyme of the limb bud leaves its somatopleural source, the anterior hypophysis separates from the oral ectoderm, and so forth. Again, while some of these effects might be of purely mechanical character, due to differential retraction, dissolution of connections (fibers or membranes) by proteolysis, or extrusion as a result of crowding, there remain many more instances that cannot be adequately explained on such a simple basis, particularly those in which cells leave their former associates individually and sort themselves out from the rest. In these instances, we may conclude that former bonds among cells have been selectively severed. This will happen whenever a cell type, in consequence of its progressive differentiation, has become so modified in its surface composition that it no longer conforms to the surfaces of surrounding cells of other types which have not differentiated in the same direction.

Our discussion up to this point has centered on the demonstration of the fact that living parts engage in, and maintain, their mutual relations primarily by forces resulting from varying degrees of "affinities" between contiguous elements. As indicated in the introduction, specificity is subject to gradations, though, for the time being, the classification rests entirely on crude criteria of biological behavior. Judging from their conduct, most tissues show the greatest affinity to their own kind; that is, "homonomic affinity" dominates over "complementary affinities." Among the latter, there are gradations from those with sharp selectivity ("univalent") among given pairs, which might be termed "conjugated," down to the less restrictive specificities ("plurivalent") permitting multiple combinations to be formed, and in each class there are evidently variations of intensity.* Tissues occurring almost ubiquitously, such as blood vessels and common connective tissue, which prove to be acceptable to a wide variety of tissues other than their own, must be regarded as either endowed with multiple selectivity, their surface being "complementary" to some character shared by all those other tissues, or as altogether non-selective. In view of the notable failure of blood vessels to penetrate into intact cornea, epidermis, and cartilage, one would favor the alternative of multiple selectivity over that of non-selectivity. Following this general scheme of evaluation, it should be possible eventually to develop a systematic list of the various components of the body in which each one would be assigned definite affinities, single or multiple and of different valencies.

The concept here proposed, if valid, immediately raises two further fundamental questions: (1) How do the various "affinities" arise ontogenetically? (2) What is their nature, and can they be expressed in terms of known properties of a simpler order? Let us turn to the first point first.

Ontogeny of Specificity

Biochemical differentials between organs, individuals, races, species, and higher taxonomic categories can be tested by serological reactions in vitro or by the biological reaction to tissue grafts.[21] The

* It is an oversimplification to treat the cell as a unit in matters of affinity, as different faces of the same cell may exhibit different affinities (e.g., the basal, lateral, and apical faces of simple epithelia). The conditions making this situation possible will be explained below.

claim that antigenic specificity increases with the progress of development has not been substantiated by recent immunological studies.[6] On the other hand, there is abundant evidence to show that incompatibility reactions between grafts and foreign hosts increase with age. Combinations that are tolerated when host and graft are young may later be dissolved as a result of progressive biochemical divergence of the components. A similar gradual estrangement has been shown by Holtfreter[13] to occur between different tissues of the same species as a corollary of their biochemical differentiation. The experiments in point consisted of grafting together in arbitrary combinations different portions of amphibian germs.

When fragments of ectoderm and entoderm from blastulae or early gastrulae are combined and explanted in vitro, they merge at first into a common mass, but a few days later begin to separate into their ectodermal and entodermal constituents. Evidently, the divergent differentiation of ectoderm and entoderm gradually produces discrepancies which make impossible further intimate association between them. *Pari passu,* fragments of pure ectoderm and pure entoderm develop increasing resistance to combining with each other, until after four days they can no longer be made to coalesce at all. Only the oral and branchial portions of the entoderm, which in the normal organism remain permanently connected to ectoderm, continue to fuse with it in vitro, too. This, incidentally, gives further support to our view that the various parts of the organism are joined not simply as a result of mechanical accidents, but by virtue of specific fitting linkages between contiguous parts.

In contrast to the sharp antagonism that develops between ectoderm and entoderm, mesoderm shows positive affinities to both of them, and consequently can act as intermediary in cementing ectodermal and entodermal components into common compounds. It is noteworthy that this ambivalence of mesoderm is preceded by a brief phase during which it resists fusion with ectoderm. This coincides with the period in which the execution of gastrulation makes it necessary for the presumptive mesoderm to shift independently. Thus, affinities not only vary with the progress of differentiation, and may even change their sign, but are pre-adapted, stage for stage, to the needs of ontogeny. Indeed, they may turn out to be major means of insuring the proper course of ontogeny.

As differentiation continues within the germ layers, so does the development of further specificities of association. For instance,

isolated entodermal fragments containing material for the formation of liver and gut later segregate neatly into these two components. Similarly, in the ectoderm, the neural portions detach themselves gradually from the epidermal portions; neural crest separates itself from neural tube; eye from brain; and so forth. In all these experiments, the progressive self-sorting of tissues according to their developing idiosyncrasies has occurred under very unnatural conditions, and the methods by which segregation was effected often differed radically from those of normal embryogenesis. The only common denominator was the net result, namely, that they did become separated. Many more similar examples could be cited from Holtfreter's work. They all show clearly the progressive development of "affinities" and "disaffinities" within the differentiating germ.

These results are fully consistent with our concept, that cells, in the course of their ontogenetic biochemical specialization, assume characters which predispose them to make or break connections with other cells; that these properties are instrumental in guiding mobile cells and cell groups and in determining their associations with others or dissociations from others; and that cells will come to rest only after they have become contiguous with others of matching properties, but are mobilized again whenever this correspondence is disturbed by changes from within or without. Cytological differentiation consists of progressive changes in the composition and constitution of the cell. While some of these changes become conspicuous as structural "differentiation products" or as changes in cell behavior, others do not reveal themselves to direct observation. The acquisition of those specific surface configurations on which cellular affinities are based evidently belongs in the latter class. Identical courses of differentiation in a group of cells lead to "homonomic" affinity. Cells whose differentiations take divergent courses, may or may not show affinities to each other, depending on whether or not their differentiations have produced concordant conditions. If surface conditions remain (or become) concordant, then the cell types concerned retain (or acquire) "complementary" affinities. If differentiation results in incongruous surface conditions, the affected cells will fail to combine, or if combined, will become detached.

Surface specificity, thus, is to be viewed not as an independent character, but simply as an outward expression of the specific character acquired by the whole cell during its differentiation. As such,

it must be subject to the same developmental rules and limitations as differentiation in general. This is important in connection with the problem of whether all the specific characters of a given cell are determined by its constitution or whether some may be imparted to the cell by its environment. We know from experimental evidence that most of the characters which a cell differentiates are released from an inherent response repertory, with which each cell is genetically endowed,[36] but we also know from the process of antibody formation to introduced foreign antigens that cells can be made to acquire new specific properties not originally contained in their native endowment.

As for embryological differentiation, we know of at least one example in which specific characters are not evoked, but actually impressed by one cell type upon another. This occurs in the "specification" of neurons by their terminal organs. An extensive series of experiments has revealed that each muscle represents a constitutional entity, possessing specific properties that distinguish it from any other (non-homologous) muscle.[35] By virtue of this specificity, which is presumably biochemical, each muscle secondarily modifies its young motor neurons in such a manner as to confer upon them its own precise specificity.* Each muscle thereby "tunes" itself in on the action systems operating in the nerve centers. Sensory end organs exert a similar "specifying" effect on the afferent nerve fibers.[29, 39] The responses are so strictly selective for a given individual muscle or sensory ending that we must assume there to be in operation as many discrete specificities as there are individual muscle specimens and distinct sensory organs. It has been previously suggested[35] that this "specification" process may proceed farther into the centers, the ultimate neurons passing their acquired specificity on to the penultimate ones, and so forth, but the extent of this chain process is still wholly conjectural. It has also been concluded from the slowness with which the "specification" process spreads, that it may be in the nature of an antigenic reaction, the individually distinguishing protein group of each terminal organ impressing its configuration on receptive protein groups in the nerve fiber, where it would be passed on in similar manner down the line.[37] Moreover, one could speculate that specific conformances of this nature along synaptic interfaces

* This specification of neurons by their muscles has been called "modulation"[35, 37] but we refrain from using the term here to avoid confusion with another type of "modulation" which will be discussed below.

may determine the course of impulse transmission, so that the specificities of developmental and mental processes may yet rest on common grounds. At present, the experimental evidence is confined to the described phenomenon of peripheral specification. Whatever its nature, it is clear that it involves the imposition of specific characters from one protoplasmic system upon another, even though only in addition to, and on top of, other specific characters already previously acquired by ordinary divergent differentiation. Whether this "infective" type of specification is peculiar to the nervous system remains to be seen.

Molecular Ecology

To speak symbolically of "affinities," is merely to outline the problem, not to attack it. It remains to resolve the described biological phenomena into known phenomena of physical and chemical order. How such resolution could be envisaged will be indicated in the following. It will be essentially an elaboration of an earlier similar attempt to interpret cellular affinities in terms of molecular structure and organization.[38]

By way of preparation, it seems appropriate to transcribe the symbolic concepts of "cell" and "protoplasm" into terms of molecular phenomena. This transcription has a purely pragmatic purpose, namely, to create a more workable model of the cell. Its utility will soon become evident. It has led me to introduce a concept of the cell which can best be characterized as "Molecular Ecology." That is, a cell is to be viewed as an organized mixed population of molecules and molecular groups of the following properties and behavior.

(1) Each population is made up of molecular species of very different composition, sizes, densities, rank, and stability, from trivial inorganic compounds to the huge and highly organized protein systems. Some segments of these populations occur in relatively constant "symbiontic" groupings, often of a limited size range; these form the various particulates of the cell content.

(2) It is one of the fundamental characteristics of cellular organization that the various species constituting the population are not self-sufficient, but depend in various degrees upon other members of the population as well as upon the physical conditions prevailing in the space they occupy. Survival and orderly function of the total

population are predicated on the presence of all essential members in definite concentrations, combinations, and distributions.

(3) In view of this intricate interdependence, given molecular species can exist and given interactions between species can occur only within a certain limited range of conditions specific for each kind. We might call these conditions the "existential and operational prerequisites" for each molecular species or group. The probability of members of a given species to persist, hence to be found, in any but the appropriate setting, would be extremely low.

(4) If the specific existential and operational prerequisites for the various molecular species and groups differ at different sites of the cell, different species will automatically become segregated into their appropriate ecological environments. As a result, even a wholly indiscriminate mixture can become sorted out into a definite space pattern. Certain species will assemble in relatively stable combinations, like biotic groups, while others, mutually incompatible, will separate.*

(5) While the conditions and forces which determine the molecular regrouping are of the most diverse sorts—electric charges, surface tensions, coacervation, solubility, chemical affinities, adsorption, enzyme-substrate relations, mobility, elasticity, etc.—their resultant in each case is of such character as to insure relative stability of composition, density, and localization of the given group of species. As they combine, larger units of supramolecular, submicroscopic, and finally, of microscopic order arise, each durable or "viable" only in a particular typical constellation of conditions.

(6) Organization in space of the content of the cell, and of any of its constituent particulate elements as well, therefore, presupposes a primordial system of spatially organized "conditions" to set the frame for the later differential settlement of different members of the dispersed molecular populations. Such conditions can presumably only exist in systems with stability like solids. Systems answering this demand are presented by all surfaces and interfaces in the cell, which include the interfaces between one cell and another,

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* We are omitting here from consideration the fact that many large organic molecules, such as the native proteins, seem to undergo constant metabolic renovation, exchanging constituents with their environment, but preserving their identity.[26] In terms of our analogy, this is the counterpart of the turnover of cells within the individual members of an animal population.

between cell and medium, nucleus and cytoplasm, nucleolus and nuclear sap, chromosomes and nuclear matrix, chromatic and achromatic substance, as well as between all other formed cell components and the interstitial fluid.

(7) A given surface area of given constitution will therefore favor the adsorption of a given assortment of molecular species, which will thus concentrate in that area and thereby crowd out other species not equally fit to occupy that particular zone. In this manner, the various surfaces will gradually become settled by mosaics of "frontier populations" recruited from the subjacent territories.*

(8) Owing to their frontier position, these surface populations acquire a unique rôle in determining the subsequent course of events in the interior. Without necessarily being morphologically distinct, they assume the functional properties of membranes. That is, they control the selective transfer of substances and energy between the molecular realms they divide.

(9) Polar molecules (e. g., the biologically prominent lipoproteins), in becoming fixed to an interface, are forced into a definite orientation relative to that interface, and hence, relative to one another.[17, 27] This orderly array makes it possible for the resulting polarized layer to serve now, in its turn, as a new surface along which further molecular layers from the interior can become fixed, with the selection depending on the physical and chemical properties of the free ends of the righted molecules of the first layer. Thus, a stacking up process is initiated through which organization can be gradually extended into the interior, creating an increasing diversity of conditions as it proceeds.

(10) If the conditions along an interface change in such a manner that the new conditions are no longer compatible with the continued existence of the old frontier population, the latter will be crowded out by a new assortment of species better fitted to the new situation. As this new frontier population settles in the controlling master position, it sets a new master pattern for the events in the interior, causing the further fate of the cell to take a radically different turn. Different contact surfaces can thus entail qualitative

* Again for the sake of simplicity, we are ignoring here the fact that specific local conditions favor not only the adsorption of certain existing molecular species, but synthesis of new species as well. This point will be more fully discussed in a later section.

changes in the cell by bringing different segments of the molecular population into the controlling surface positions.

The concept formulated in these ten points takes into account the growing realization that the structural and working order of the cell is based not on the presence of a fixed mechanical framework pervading it—abundantly disproved by the facts—but on a regular distribution in space of the various intracellular processes: a dynamic rather than static skeleton, maintained by metabolic energy and determined in its characteristics by some definite geometrical order in the field of its operation. This order we conclude to be an order of "conditions," going back in last analysis to the typical organization of surfaces—"organization" in this sense referring to the particular non-random distribution of physical and chemical properties (see later). Pending evidence to the contrary, it is also possible to view the organization of genes as residing in their surface properties. In other words, the organization pattern of many, and perhaps all, living systems can be derived from a two-dimensional ground plan to which the third dimension is secondarily added by the selective stacking-up of various polar compounds in consecutive layers.

This concept likewise makes allowance for the statistical variability of many cellular and developmental events. Only the frame in which these events occur is relatively invariant. The highest degree of invariance has been assigned to the gene with a molecular population of remarkable constancy in composition and arrangement; mutations being explicable either by the loss of a given member species of the population or by its mere expulsion from a controlling surface position. However, the microdeterminism of chromosome structure is not matched by an equally rigid determinism of cellular characters. Only "norms" of development and statistical probabilities of the occurrence of given characters, rather than stereotyped constancies of details, are predictably determined. This is precisely what the "molecular ecology" concept of the cell implies in its emphasis on local "conditions," meaning simply *probabilities* that certain processes rather than others will occur with varying degrees of definiteness.

Contact Relations in Molecular Terms

Let us now consider, in terms of this concept, what will happen when two such systems, e. g., two cells, to be named A and B, come

together in a common boundary. The surface of each will become a conditioning factor for the other. If the surface constitution of A satisfies the existential prerequisites of the adjacent frontier population of B, the latter will remain unaltered. However, if the A-surface introduces conditions incompatible with the B-surface population, the latter, if sufficiently mobile, may retreat from the surface to be replaced from the interior by another group of species better adapted to the new contact area.

It must be remembered here that stationary frontier populations tend to become rapidly congealed by the recruitment of additional layers, very probably accompanied by the cross-linking of fibrous molecules into fabrics, which with the incorporation of water compose the gel crust described for so many cell forms. Molecular mobility in these gel layers is greatly reduced. Whether and how fast a cell will respond to a new surface by regrouping will, therefore, depend on the condition of its crust. A cell in motion (locomotion or mitosis) or immediately after settling will be more responsive than will one that has been stationary for some time. On the other hand, there are indications that the very presence of an incompatible surface condition may lead to a solvatization and mobilization of the crust, thus restoring the freedom of movement necessary for molecular regrouping.

Now, just what are the surface conditions to which the molecular populations will react? As stated above, they are of complex character, and in part merely a combination of electric charges and surface tensions of the sort that is exemplified by the adsorption and concentration of detergents along inorganic interfaces. However, forces of this description seem too general to account for the high degree of specificity in intercellular relations illustrated in the earlier part of this article. Unless we want to invoke entirely unknown principles, no other explanation of such specificity seems at hand than one based on a concept of interlocking molecular configurations. This concept, traceable to Ehrlich, and culminating in the recent work of Pauling, maintains that the specificity of intermolecular relations is based on "steric conformance," i. e., corresponding or complementary spatial configurations between molecules, or certain exposed atomic groups of them, enabling them to conjugate in key-lock fashion. The theory is that such structural fitting allows the fitting particles to come within range of strong binding forces. A second and equally important point of the theory is the thesis that master molecules of

specific configuration may serve as templates or models which would force other surrounding molecules to assume a complementary configuration, in mould-cast fashion.

Suggestive evidence for this concept can be derived from studies on antigen-antibody systems, enzyme-substrate systems, hormone-effector cell relations, and drug action; in other words, from a sufficient variety of biological phenomena to suggest fundamental validity. On the strength of this evidence, an extension of the concept to problems of growth, differentiation, and tissue behavior becomes a legitimate task.

Figure 1 is the slightly modified reproduction of a diagram used previously to explain selective adhesion and non-adhesion among cells in terms of molecular configurations along the contact surfaces. The key molecules, which numerically perhaps constitute only a small fraction of the surface population, are symbolized as bars with characteristically shaped ends.

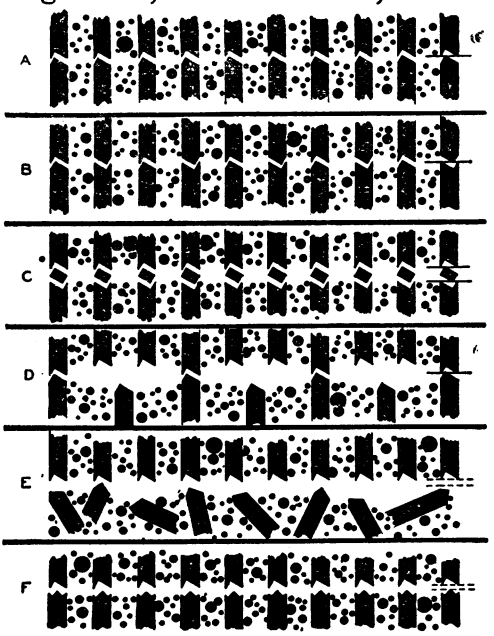

FIG. 1. Diagram of hypothetical molecular configurations in adjacent surfaces of two cells or cell and medium (modified from Weiss, 1941). For further explanation, see text.

The assumption is that two complementarily shaped molecules meeting in proper orientation will become linked by intermolecular forces, which thus become forces of attachment between the two contiguous systems. Properties of the sort required in our model are commonly associated with proteins, or combinations of proteins with lipids and other substances.

Let us now consider the implications of our hypothesis in greater detail. Each horizontal row, from A to F, symbolizes one hypothetical molecular state along an interface either between two cells or between a cell and its medium. In diagram A, for instance, the upper row (notched ends) represents the critical surface molecules of one cell, the lower row their complementary counterparts in the

other cell; the white zigzagging band between them is the cell boundary. In this instance, the linkage occurs between members of two different populations, illustrating "complementary affinity" as outlined in an earlier section.

For simplicity, the molecules are shown evenly spaced. This could be true only if the cell surface were a highly organized lattice with the key units disposed in regular two-dimensional periodicity, somewhat along the ideas of Wrinch.[46] Otherwise, we would have to give the diagram a statistical interpretation, in which the even spacing would merely indicate the average density of the surface population. Probability of encounter between complementary units, rather than absolute spatial congruity between the two complementary populations, would be the determining factor. This will be further explained in diagram D.

Diagram B illustrates the linking between two identical systems —"homonomic affinity" in our terminology—on the assumption that they both contain units of complementary configuration and that both types have identical chances to settle in the surface. The question as to why these molecules should combine with their counterparts across the boundary, rather than with their neighbors, could be answered by reference to the orienting effect of the surface, which would greatly enhance the chances of the former over the latter. As we shall mention below, there is some evidence for the coexistence of complementary proteins in cells, but in the few instances where their position could be ascertained, they were found to be in different locations, one near the surface and the other in the interior. If this condition were to hold generally, it would, of course, invalidate scheme B.

An alternative explanation of "homonomic affinity" is illustrated in scheme C. Here an intermediary substance of complementary configuration is assumed to act as the link between identically shaped molecules. According to Schmitt,[25] histones might play such a cementing rôle in the formation of epithelia. The cementing substances could still be products of the very cells they unite. That is, the cells would again be endowed with complementary units, but the one type of smaller size would be exuded to act as intercellular cement, while the other and larger one would be retained. Evidently, this concept would be wholly compatible with the observed separation of complementary species into surface and subsurface sites, respectively.

Scheme D is intended to show how affinity can vary in strength. The key molecules are assumed to be present in the two surface populations in different concentrations (in the given instance, in a ratio of $3^2 : 2^2$, i. e., 9 : 4). This means fewer points of coincidence between complementary units, if one thinks in terms of regular surface fabrics; or a lowered probability of encounter, in a statistical concept. In other words, the "bonding" between the two surfaces is weaker than in scheme A, where concentrations are equal.

Scheme E illustrates the effect of disorientation. It is evident that even in a completely random array some complementary molecules would happen to come to lie in interlocking positions. However, the incidence of this occurrence would be very low as compared with the orderly array produced by adsorption to a surface, which turns the key molecules with their receptive groups all in the same direction. Surface orientation thus becomes an indispensable prerequisite for interlocking on an effective scale. This being the case, disorganization of the surface as a result of some change in the condition of the cell would automatically lead to detachment from its neighbors. In none of these speculations does the surface population have to be visualized as static, so long as its *average* composition remains unaltered.

In scheme F, detachment (or non-attachment) is due to lack of correspondence of shape between the key members of the two frontier populations. The molecules facing each other do not interlock. This condition will often arise secondarily as a result of cellular differentiation, which implies profound transformations in the character of the molecular populations. Differentiation gives rise to new species of molecules and presumably also modifies many of the existing ones. These changes will necessarily be reflected in the composition and distribution of the surface populations. Whether or not two systems, which differentiate in contiguity, remain attached or become separated, will, therefore, depend on whether their respective frontier populations undergo parallel or divergent changes. In the latter case, incongruities as depicted in diagram F, will arise. This explains in principle the observations of Holtfreter and others, quoted earlier, that tissues which at one time are closely combined may later separate, as differentiation progresses.

In addition to the permanent incompatibilities resulting from divergent differentiation, the scheme provides an explanation for temporary and reversible separations such as sometimes occur

between cells of the same character in response to certain stimuli. If the surface is altered in such a way that it ceases to offer optimal existential conditions to the former frontier population, other molecular species will emerge from the interior and occupy the modified surface. The new settlers may be wholly unrelated to the populations across the border (as in diagram F), and consequently, the old links will be severed and will not be reformed until the old surface condition has been restored. In the meantime, the new frontier population, in its master position, may initiate rather profound and conspicuous changes in the appearance and behavior of the particular cell, but these changes, as one can readily see, are primarily changes in the distribution of existing compounds, and not in basic composition. They represent the transient processes which I have designated as "*modulations*,"[36] in contradistinction to the progressive and irreversible transformations of character and composition to which the term "differentiations" had better be reserved.

It would exceed the scope of this paper to illustrate in greater detail how a wealth of familiar phenomena of development and pathology can be interpreted in terms of this concept.* For the time being, we merely want to point out that it can satisfactorily account for all the phenomena of selective affinity and disaffinity dealt with in the earlier sections. The question, therefore, is no longer whether the concept of "steric conformances" as mechanisms of intercellular relations is fruitful as a working hypothesis—for this seems to have been answered in the affirmative—but whether it can be verified. We have borrowed the concept of "complementariness" of configuration from immunology, where antigen-antibody linkage has been tentatively explained on the basis of steric conformance. We are now to explore how pertinent this model is.

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* It is evident, for instance, why different faces of the same cell can behave differently, and even show different affinities, as mentioned before: Surface areas exposed to different conditions (either neighboring cells or ambient medium) will become occupied by different segments of the molecular population. The marked polar architecture of epithelial cells is a notable example. In one given type, for instance, compounds mediating attachment to the basement membrane will be drawn toward the base, compounds, say with proteolytic potency, toward the apical face, and compounds for "homonomic" adhesion with like cells, to the sides. It can easily be understood that changes in the physico-chemical conditions along either the basal or apical surfaces could destroy this polar organization of the cell and turn it into a "pathological" course.

Immunological Models

In terms of "molecular ecology," the cell can not be considered as an antigenic unit. It contains numerous and diverse molecular species, which if specific steric properties are a prerequisite of antigenic action, represent a wide variety of antigenic agents. To a certain extent, it has been possible to demonstrate this fact by fractionating the cell content and testing the antigenicity of the various fractions separately. Head and tail fractions of spermatozoa, which can be conveniently separated, provoke each a corresponding antibody.[12] Considering the great limitations of techniques of fractionation, it is reasonable to assume that any given cell harbors an infinitely greater variety of specifically configurated proteins than we can reveal by present immunological techniques.

Next to the existence of such specifically shaped units, our concept postulates that those occupying surface positions can act as links between the cell and its surrounding structures. The clumping of scattered cells and blood corpuscles by agglutinins or precipitins is evidence that comparatively large bodies can become affixed to one another by intermediary molecules of fitting complementary configuration. Obviously, there is thus no fundamental difficulty in envisaging bonds between cells in general as effected by a similar principle. The factual basis for such a view, however, is still extremely meager. There is perhaps only a single well-attested case known that could be quoted as supporting evidence. This is the combination of egg and sperm in fertilization, which could be classed under our general heading of "complementary affinity," as it involves the permanent selective union between two cell types of different origin and character.*

The first one to call attention to the similarity between fertilization and immunological phenomena was F. R. Lillie[19] in his classical experiments on "fertilizin." This work has later been carried on by Tyler,[32] and according to his version, provides definite evidence for

* The fact that egg and spermatozoon fuse after combining while somatic cells in "complementary" combinations usually retain their identity, is of minor importance. There are somatic cells which behave similar to germ cells; for instance, those that merge into syncytia, or those that penetrate into neighboring cells (e.g., the nurse cells of some oocytes; the melanoblasts that feed pigment into epidermis cells of feathers). Evidently, it requires special mechanisms to cause two cells to coalesce after they have become joined.

the contention that egg and sperm hook on to each other, as it were, by the interlocking of surface substances of complementary configuration, acting precisely like antigen-antibody systems. "Fertilizin" of the egg surface combines with "antifertilizin" of the spermatozoon, and the experimental evidence available strongly supports the view that either of these substances closely resembles the true antibodies to the other, as obtained by immunological procedures. Moreover, the interior of the egg contains a substance which behaves just as "antifertilizin" does and must, therefore, be regarded as complementary to the surface "fertilizin." At least for the egg, the simultaneous presence of complementary substances in the same cell, but at different sites, has thus been made highly probable, and Tyler has correctly appraised the possible general significance of this fact. It is immaterial for the general concept whether the key compounds are still incorporated in the cell surfaces proper, when they act, or are segregated in a distinct outer coat, as is the case with fertilizin.

The egg-sperm relation thus illustrates precisely the type of relation our concept has postulated as controlling compatibility and incompatibility or affinity and disaffinity between tissues in general, and since the former relation has proved to be reducible to simple terms of immunochemistry, particularly interlocking molecular configurations, there is good reason to suspect that the latter relation may yield to the same interpretation. Although the proof remains to be produced, the expectation is logically well founded, and attempts at verification hold some promise of success. Should they prove unsuccessful the problem of "specificity" of tissue relations would revert to the descriptive stage, in which it is now, and a fresh solution would have to be attempted in some other, as yet unforeseeable, direction.

Differentiation in Molecular Terms

It is of interest now to examine how the basic problems of ontogeny present themselves in the light of the concepts developed in this paper. While the bulk of this task must be reserved for a future occasion, a few pertinent comments and experimental data that fit into the present context may be advanced here.

Ontogenic differentiation is characterized by the fact that cells of demonstrably equivalent constitution turn into strains which become increasingly diverse.[86] There is overwhelming evidence that in this

process, content and character of the cell undergo irreversible transformations.* At the same time, it has become clear that a cell at any given stage of differentiation can assume a variety of morphological and physiological expressions, which are commutable; these fluctuating states have been designated as "modulations,"[36] in contradistinction to the unidirectional differentiations proper. Translated into terms of molecular ecology, "modulation" would consist of the mere regrouping of the existing molecular key species without basic change in their character, while "differentiation" would involve a change in the composition of the population, with the appearance of new key species and the loss of old ones.

Some examples of "modulation" in mature cells are: the cyclic changes which hormone-responsive cells undergo in accordance with cycles in the hormone concentration in the blood[22]; the reversible morphological changes in lateral line sense organs which accompany the transition of certain newts from aquatic to terrestrial, and back to aquatic life[7]; the conversion of fixed histiocytes to macrophages and the resettlement of the latter; the temporary conversion of osteoblasts to fibroblasts[2]; and many similar cases. In all of these instances, the change in the cell occurs in response to a definite change of the cellular milieu. According to our concept, this would happen whenever that part of the medium which is in immediate contact with the cell surface becomes so altered in its composition or physical properties that it no longer satisfies the needs of the old frontier population of the cell, and the latter retreats and gives way to some other species better adapted to the new conditions. These changes initiated from the surface can then produce a thorough reshuffling of the cell content, in the course of which formerly inactive species may assume prominent functions, and formerly active ones go into eclipse, with the result that the whole cell changes conspicuously in appearance and behavior.

* How far this process goes in any given cell is an empirical question, which must be explored separately for each particular cell type of each particular group of animals; moreover, it cannot be properly answered unless cytoplasm, cytoplasmic products, nucleus, and chromosomes are considered separately. The criterion of differentiation is the irrevocable restriction of potency. The test of this is not whether or not a given cell can lose some of its specialized aspects, but whether in doing so, it regains the capacity to redifferentiate in other, new directions. In all higher forms, perhaps all cœlomates, irreversible differentiation of various degrees is the rule in somatic cells. In cœlenterates, on the contrary, true differentiations seem to be rather the exception.

Differentiation does not appear to differ at first from modulation, and only by the criterion of reversibility can we distinguish them. This suggests the possibility of deriving one from the other. Each embryonic cell possesses an inherent endowment of molecular key species, whose properties, specific requirements, and possibilities of interacting narrow the capacity for future differentiation to a limited number of possible courses. This is the material basis of the embryological term "potency." The actual realization of differentiation requires (a) some factor that initiates one particular among the several potentially feasible courses, e. g., makes the pluripotent cell turn into a neuroblast or spongioblast or myoblast or chondroblast or melanoblast, etc.; and (b) the proper physical and chemical setting for the realization of the selected course, e. g., conditions that permit neuroblasts to transform into nerve cells, myoblasts to produce contractile fibrils, melanoblasts to produce pigment, etc. Now, factor (a) could be assumed to consist of some condition that attracts specifically one particular segment of the mixed molecular population to the surface and fixes it there in a master position. Once settled, these oriented surface molecules would specifically control intake and output between the cell and its environment, and would act as a ground plan for the progressive segregation of further species from the original intracellular pool. They not only would furnish a structural frame to which other species of molecules could be built on in a series of steps leading to a fabric of increasing complexity, but they could also catalyze specific reactions among the other species and thus take the lead in changing the chemical composition of the basic constituents of the population. Evidently, once this change in composition has been effected, reversible modulation has turned into irreversible differentiation. What has started as a mere redistribution and relocation of the cell content has ended in a change in character. Tentatively, all differentiations may therefore be considered to have started as modulations—a view fully in accord with the results of Experimental Embryology which have proved the reversible character of the early steps of differentiation.

Divergent differentiation among initially equivalent cells is to be explained by the fact that these cells were exposed to critically different surface conditions. As outlined earlier, the surfaces of such cells will then become occupied by wholly different segments of the molecular population, and in consequence, subsequent chemical events will take quite disparate courses. Experimental Embryology teaches

that the "position" of a cell determines its fate in differentiation. Since, physically speaking, "position" can only mean exposure to certain physical and chemical conditions prevailing at that particular site, our concept proves again in harmony with experience. Some sample applications may be briefly outlined.

Following the classical studies of Spemann, "inductive" effects exerted by one embryonic tissue on an adjacent one have been extensively explored. To quote the most common examples, we refer to the hetero-induction of neural formations in ectoderm by underlying mesoderm,[28] the induction of a lens in epidermis by the underlying eye cup,[28] the induction of feathers in the epidermis by an underlying dermal papilla.[20] It is doubtful that all influences described as "inductions" operate through a common mechanism. Yet, if we confine ourselves, for the present, to "hetero-inductions" of the type just mentioned, the following points may be considered as fairly well established. (1) The "inductive" effect is in the nature of an evocation,[33] rather than an imposition; that is, it merely calls forth a response for which the affected cell has had a latent endowment, but does not impart upon the cell entirely new properties.[36] (2) The "inductive" effect can, in favorable cases, be shown to be reciprocal; that is, both adjacent tissues are subject to each other's influence, although usually one partner is in a less responsive condition. (3) The effect is transmitted by contact. The original supposition that it is mediated by a single chemical entity has not been confirmed.[34] (4) The "inductive" exposure need only last for a relatively brief period (minimum: a few hours), after which the affected tissue continues the induced course on its own.

To these points, we may add a brief reference to a highly suggestive, though cursory, observation. In some cases, the first sign of an "inductive" influence is a marked re-orientation of the cells of the "induced" layer relative to the "inducing" substratum in such a manner that the cell axes of the former become aligned with those of the latter. Part of this phenomenon could be ascribed to tensile stresses between the two intimately adhering layers. On the other hand, it is quite possible that we are faced with the results of a potent alignment effect on polar molecular groups in the "induced" cells; that is, with a direct index of an orderly molecular regrouping, set in motion through contact with the "inductive" substratum. A closer study of the fine-structural reorganization that accompanies "induc-

tion" phenomena is urgently needed. It may produce a direct test of the speculative interpretation tentatively set forth in the following.

We will assume that the capacities for the various courses of differentiation potentially open to a given cell ("differentiation potencies") are based on the presence in that cell of groups of molecular key species which can set up master patterns, each a different one, to which the rest of the cell content will then conform. Ectoderm cells of an early amphibian gastrula, for instance, have actually been found capable of giving rise, under normal or experimental conditions, to epidermal cells, pigment cells, nerve cells, gland cells, muscle cells, notochord cells, pronephric cells, etc.[28] We assign to them, accordingly, a corresponding variety of master compounds of specific configuration. These key molecules need not be present as such from the beginning, but may themselves have their ontogenic history (see above in the section of ontogeny of affinities). None of these key species can gain dominating influence on the fate of the cell so long as they lie all intermingled in the interior. But as soon as one of them succeeds in occupying the surface to the exclusion of the others, it gains a dominant position from which to influence the further course of events in the cell in accordance with our earlier statements.

Thus, we submit that when an ectodermal cell turns into a neural cell, the decisive initiating step is the selective condensation along its surface of the key molecules for neural development which up to then had been mixed with the other species in the interior. Conversely, if the surface of the same cell were to become settled by the key molecules for lens development, this would set the cell on its course toward becoming a lens cell. Embryonic determination of cell fate would thus consist essentially of the accumulation in the cell surface of selected species of master compounds. Any factor that makes such surface segregation possible, thereby becomes a "determining" factor, and in line with the general theme of this article, we might again turn to the principle of steric molecular interlocking across cell boundaries as a possible mechanism.

Let us suppose that the surface of the "inducing" substratum is saturated with a certain species A of polarized molecules of such configuration as to match precisely one single component α of the molecular populations α, β, γ, δ, etc., of the overlying ectoderm cells. Due to their complementary shapes, these two types, A and α, would form strong unions (see Fig. 1A). Thus, given a certain degree of

mobility of the cell content, all the α units will gradually be trapped along the surface exposed to A, just as a film of antigen traps antibody molecules. Faced with a different substratum, containing key molecules B complementary to β, the same ectoderm cell would have become covered with a β layer, furnished again from its own stock, and thus become turned into a wholly different course of differentiation.

Progressive determination would occur through a succession of such steps. A given contact situation would bring a certain key species to the surface. Its residence there would affect the chemical processes in the interior, entailing presumably further regrouping along internal interfaces, setting off a chain of effects which will reach the nucleus and chromosomes, whose reactions, in turn, will rebound on the chemical composition of the cytoplasmic population. As a result, new compounds will arise, and when the cell is later faced with a new contact substratum, this may attract some of these new species, initiating the next phase of differentiation, and so forth. At any one stage, the cell will thus have only a limited assortment of specific key species, and its reaction to "inductive" surface contact will therefore vary with time. This is the molecular version of what is usually referred to as the development of responsiveness, or "competence,"[33] in embryonic cells.

A further possibility to be kept in mind is that some "induction" effects may involve actual changes in the morphology of the molecules exposed to the "inducing" surface. It is conceivable that specifically shaped molecules of the "inducing" substratum would impose conforming shape on the adjoining molecular layer of the "induced" cell. That such an impression of specific properties from one molecule to another is feasible is demonstrated by the mechanism of antibody formation, and at least suggested by the fact reported above, that muscles and sense organs impart highly specific characters upon the nerve cells with which they are connected. But we have no way of predicting how common this type of "infective induction" may be in development in general.

Any discussion of "inductions" must take into consideration the many instances in which effects normally exerted by adjacent cells can be experimentally duplicated by rather trivial inanimate agents. Such agents are frequently referred to as "dead organizers." The name is highly objectionable, but the facts as such are on firm ground.[34] There are several ways of reconciling them with our con-

cept. Either the agent in question acts in "skeleton key" fashion, i. e., has key properties in common with the natural conditions; or it provides merely physical conditions for the attraction into the cell surface of a single segment of the molecular population, not necessarily a selected one, thus setting the cell on some accidental course of differentiation; or it may cause the responding cells to release certain specific compounds which, after adsorption to the outside, would then operate somewhat in the fashion of the intermediate layer represented in Fig. 1C. There may be other possibilities, but in view of the lack of pertinent information it would seem futile to enlarge upon this matter at the present time.

The hypothesis of induction here advanced explains satisfactorily (1) the specificity of inductions, depending on the contact substratum; (2) the "evocative" character of "induction" in the sense that it can only operate through the instrumentalities preformed in the responding cell; (3) the "exclusiveness"[36] of cellular differentiation (i. e., the fact that once a cell has entered a given course of specialization, other courses are automatically suppressed), as saturation of the surface with one selected molecular species automatically precludes others from assuming the same vantage position; (4) the potentially reciprocal character of "induction," inasmuch as a surface with a settled α-population could attract A units from within a less consolidated cell containing A, B, C, D, etc., just as the A-surface in the above example attracts α units; (5) the transmission of "induction" effects by contact only; (6) the initial reversibility ("modulation" phase; see above) of the "inductive" effect; (7) the irreversibility of the effect after the critical surface condition has prevailed for a critical length of time; this is the period required for a given surface population to establish permanent chemical changes in the cell.

The hypothesis presupposes a high degree of macromolecular mobility in the intracellular matrix. We know that during mitosis there is free streaming of cytoplasm, so that at this stage at least the cell content would parade, as it were, past the surface and enable the latter to recruit selected key species. However, a great many potent "induction" effects (e. g., neural plate, lens) seem to be quite independent of cell division and to proceed during stages in which most of the responding cells are mitotically inactive. It is for these cases that one will have to ascertain whether or not intracellular liquidity

is high enough to permit the comprehensive macromolecular reshuffling postulated by our theory.

Growth

The foregoing discussion has been focussed on cellular *differentiation*, that is, changes in the complexion of the molecular populations, but has ignored *growth*, that is, the increase in their size. Yet, growth confronts us with the same fundamental problem of specificity: Why and how do the various molecular populations that eventually distinguish one cell type from another continue to reproduce more and more of their own kind?

Simple as it is to deal with growth in formal terms, in which the cell is treated as a unit, the problem assumes forbidding complexity when viewed on the molecular level. Of the molecular species entering the cell and available for its growth, some retain their identity (water, electrolytes, etc.) while others are combined into new compounds. Of the synthetic products, some are rather ubiquitous and trivial, others highly specific for a given species or organ or cell type. It is the latter which present the crux of the problem of growth, as they can evidently not be synthesized from simpler ingredients except with some of their own kind present as models. Current studies and speculations on protein synthesis, virus reproduction, gene multiplication, cell morphology, and enzymology are aiming at some tangible scheme that could explain "self-reproduction" of these highly specific key systems of the growing cell. It is noteworthy that in these speculations, the concept of "templates" or molecular master patterns assumes increasing prominence. The cue is taken from immune reactions, in which cells turn out large amounts of antibody in the presence of an antigen template. Guided by this analogy, I started in 1938 a series of experiments to test whether specific antigenic systems of different organs exercise specific catalytic functions in the growth of the respective organs. The work* was done with the collaboration of Dr. Dan H. Campbell, who carried out the immunological part, and Dr. Sewall Wright, who did the statistical calculations. Other commitments and war research prevented the completion of these studies, and only a preliminary abstract has been published thus far.[44] Being pertinent to our present discussion, the results may be briefly reported here.

* Aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

It is common knowledge that organ-specific antibodies exert deleterious effects on homologous organs of the *mature* organism. Our experiments were designed to explore possible specific actions of such antibodies on the growth of *embryonic* organs. The procedure was as follows.

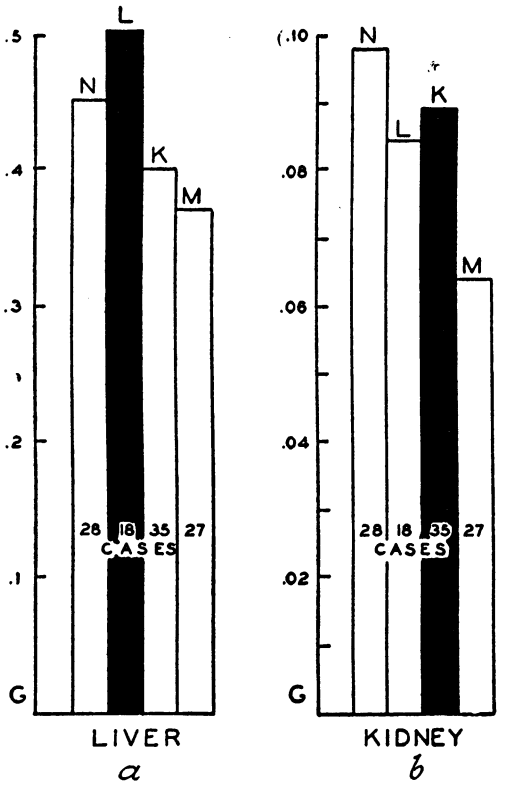

FIG. 2. Weights of livers (a) and kidneys (b) of chick embryos on the 20th day of incubation, which had been injected during early development with liver- (L), kidney- (K), and muscle- (M) antisera, and of normal controls (N).

Autolyzed suspensions of three organs of adult chickens, namely, liver, kidney, and pectoral muscle, were injected into three groups of guinea-pigs over a period of 47 days. Ten days after the last injection, blood serum was recovered from the guinea-pigs, supposedly containing, among others, specific antibodies against the injected organ substances. We shall call these antisera "L," "K," and "M," indicating the liver-, kidney-, and muscle-injected series respectively. These antisera were then injected into chick eggs of ages ranging from sixty hours to eight days of incubation. Each egg received only a single injection of 0.4 cc. of one of the antisera, deposited near the embryo. Treated embryos and normal controls were fixed at various ages, but only those of the oldest group, sacrificed on the twentieth day of incubation, have thus far been studied. This group is composed of 28 normal controls, 35 K-embryos, 18 L-embryos, and 27 M-embryos. Their livers and kidneys were weighed by a standard procedure. The M-series serves as test of the general effects of antisera injection, while the K- and L-series were intended to reveal any specific effects

on homologous organs. Owing to the great variability of organ weights in both normal and treated embryos, the data had to be evaluated statistically.

Figure 2 shows the average weights of livers and kidneys in controls (N) and treated embryos. Total weights of injected embryos averaged considerably below those of the controls, a fact which is evidenced in the smaller livers in the K- and M-series and the reduced kidneys in the L- and M-series. In contrast to this general growth depression, the one organ type in each series for which the injection had been specific shows evidence of positive growth stimulation. In the case of liver, this has led to an absolute increase of ten per cent above normal (Fig. 2a), while in the kidney (Fig. 2b) the specific stimulative effect has not been large enough to offset the unspecific depressive effect of the treatment. The organ-specific antisera thus seem to have specifically promoted the growth of the homologous embryonic organs. This is clearly illustrated by the fact that the livers of L-embryos are larger than those of K-embryos, whereas the kidneys are larger in K-embryos than in L-embryos. By statistical calculations, one can determine the average liver weight for a given mean weight of kidney, and the average kidney weight for a given mean weight of liver (regression coefficients). These values, graphed in Fig. 3, reveal the following facts. Kidneys of all embryos injected with non-homologous (L, M)

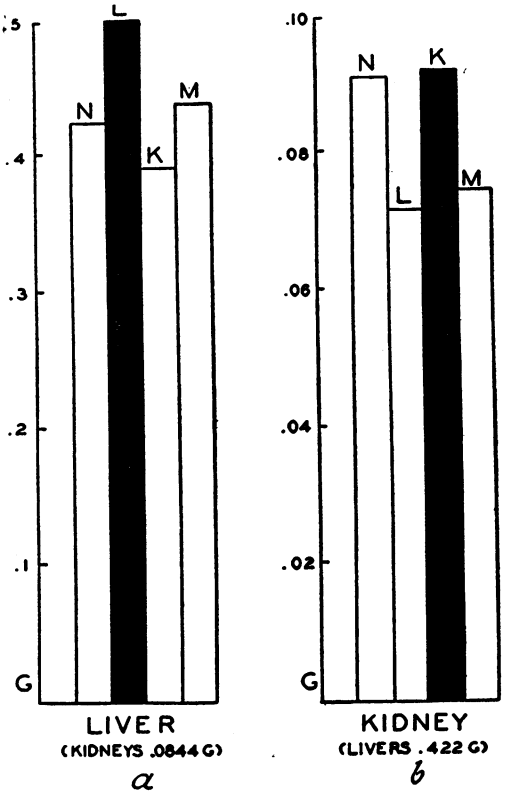

FIG. 3. Weights of liver (a) and kidney (b) of the same group of embryos as in Fig. 2, adjusted by regression coefficients for normal variability.

sera (Fig. 3b) are much more markedly reduced in size as compared to normals (N) than are the livers of embryos which had received non-homologous (K, M) injections (Fig. 3a). On the other hand, the livers of the L-embryos (Fig. 3a) and the kidneys of the K-embryos (Fig. 3b) are substantially increased relative to the reciprocal combinations. The adjusted liver weights are 29 per cent larger in the L-embryos than in the K-embryos, while almost the reverse holds for the kidney weights, those in the K-series being 28 per cent higher than the ones in the L-series. The statistical significance of the observed differences between the L- and K-series is .00001 for liver and .001 for kidney; that is to say, the probabilities of obtaining the results by mere chance are 1 in 100,000 in the former, and 1 in 1,000 in the latter, which makes the results appear as of high statistical significance. Two in 100 is conventionally considered the safety limit.

One may conclude, therefore, that some distinctive biochemical principles of chicken liver and kidney extracts call forth corresponding organ-specific products in the guinea-pig, which, in turn, when transmitted through serum, affect the growth of the homologous structures in the chick embryo. The fact that the specific effect consisted of stimulation rather than depression is at first surprising. Apparently, the manner in which antibodies affect the physiology of mature cells differs from their mode of action in growth. Only the general growth depression observed in the experiments corresponds to the conventional type of antibody action. The specific growth promotion of the homologous organs, on the other hand, is in an altogether different category. If corroborated, it would prove that antibodies to a given organ protein can act as catalysts in the synthesis of more of that particular protein. This would lead us directly to a "template" concept of growth, with molecules of complementary configuration acting reciprocally as moulds for each other's synthesis. In that case, the experimental transfer with double reversal, from chicken to guinea-pig to chick, would actually have been but a complicated version of what happens within the organism itself without transfer. Each differentiating cell would contain complementary key compounds, each of which would act as mould for the other. However, their respective syntheses would have to be assumed to occur at different rates, so that one would always prevail numerically.

Growth rates, according to this concept, would be governed by the concentrations in which the two complementary systems would be present and by the extent to which they would become conjugated

and thereby inactivated as specific catalysts. Evidently, if some of the specifically configurated portions of these compounds are liberated from their source cells, their concentration in the medium could exert a growth-controlling influence on distant homologous cells. It is not inconceivable that many examples of "compensatory hypertrophy," which cannot be explained by the "functional overloading" of the residual tissue, go to the credit of such systemic balances.*

In order to test this principle more directly, I had a series of experiments carried out with the technical assistance of one of my students, Hsi Wang.† Essentially, it was a repetition of the preceding series, but leaving out the guinea-pig as an intermediary. Fragments about 0.5 mm.[8] in size were taken from livers of 6-day chick embryos and implanted in the area vasculosa of 4-day hosts. Substances from the grafted material could thus be carried by the blood stream into the host embryos. The latter were allowed to develop further for periods ranging from two to nine days after the operation (six to thirteen days of total age), at which time the weights of the whole embryos as well as of their livers were determined. The results, based on 137 cases with liver implants and 107 controls, were very striking.

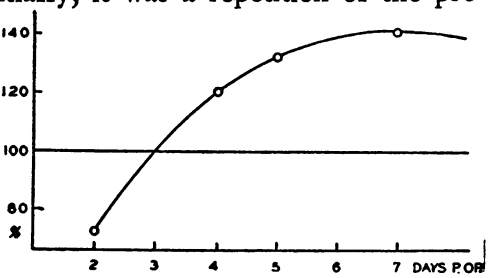

FIG. 4. Effect of implantation of liver fragments to extra-embryonic area upon weight of liver of host embryo. Ordinate: ratio of liver indices for hosts over controls (liver index $(\frac{W_l}{W_t - W_l})$ being the ratio of liver weight (W_l) over total body weight (W_t) minus liver weight). Abscissa: time in days after implantation.

Average body weight (minus liver) of the experimental lot was 10 per cent below that of the controls, indicating a slight general depression of growth in consequence of the treatment. The livers of the host embryos, however, were greatly enlarged. In extreme cases, they were twice to three times as large as the largest of the controls. The time course of this effect is illustrated in Fig. 4. Liver weights were expressed in percentage of body weight, and the averaged ratios of these values for the hosts over the controls were plotted against time, counted from the implantation of the grafts.

* It will be of interest to explore the relation between this concept and the theory of "antihormones."[5]

† Only a brief preliminary abstract of these experiments has been published.[44]

The graph reveals that after an initial depression during the first two days, the growth of the host livers greatly overshoots that of the controls, the excess mounting steadily up to the seventh day, when it reaches an average of over 40 per cent. After that, the magnitude of the effect seemed to decline.

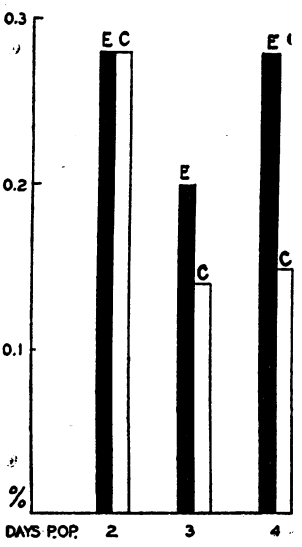

FIG. 5. Mitotic indices (percentage of mitoses in total nuclear count) in host livers of the experimental series of Fig. 4. E, experimental embryos; C, control embryos.

The observed enlargement of the liver is reflected in both the size and mitotic rate of the constituent cells. Cell size, determined for 30,000 sample cells, was larger in the host livers by 9 per cent after two days, 10 per cent after three days, and 20 per cent after four days. The mitotic index, determined for a total of 815,000 cells was the same for experimental host and control livers on the second day, but on the third and fourth days the former exceeded the latter by 43 and 87 per cent respectively (Fig. 5). Evidently, cellular hypertrophy antedates the increase in mitotic activity.

The mechanism of this effect is a matter of conjecture. Either specifically shaped parts of liver cell proteins can act directly as nuclei for further synthesis when entering the appropriate environment, which is another liver cell, or they act merely as models for moulds which then would turn out more of the original product. The latter alternative would bring the experiments with direct liver implantation and those using liver antisera to a common denominator. However, our information is far too sketchy for detailed speculations.

Moreover, the specificity of the effect is not absolutely sharp. Not only is the growth of other organs aside from liver affected by liver implants, but liver enlargement can likewise be provoked by implants other than liver, although the homologous effect is always strongest. In most embryos with enlarged livers, for instance, the kidneys were also somewhat enlarged; this could perhaps be regarded as a secondary functional effect. On the other hand, increased liver growth was observed not only in embryos with grafts of liver, but also, in descending order of intensity, with grafts of blood clots, skin, mesonephros, and perhaps muscle, none of which,

however, approached liver tissue in effectiveness. Sham grafts of paraffin were wholly ineffective.

In conclusion, these experiments contain strong indications that substances released from the grafts and carried by the blood stream into the embryo, exert a catalyzing effect on the growth of the homologous tissue. But the admixture of the less localized effects just mentioned leaves the interpretation somewhat in doubt. The main purpose of presenting the results here has been to call attention to a promising line of work which is badly in need of systematic and intensified pursuit.

Pending verification by such future work, the whole concept of growth advanced in these pages remains hypothetical. It has points in common with the scheme of gene reproduction suggested by Sterling Emerson,[9] which likewise resorts to immuno-chemical analogies. In its emphasis on the existence of molecules of complementary configuration, our concept has points of contact with Tyler's thesis of "auto-antibodies,"[32] which has not, however, been explicitly applied to growth. Yet, for the time being, there seems to be nothing more to these convergences than a common conviction that the phenomena of biological specificity have a common stereochemical foundation: reproduction to be based on the ability of a compound to serve as a model for the synthesis of more of its kind; adaptation, on the ability of a compound to impose a conforming configuration upon other compounds; and selectivity, on the interlocking of matching compounds.

This past discussion leans heavily on current concepts of immunochemistry, particularly those developed by Pauling.[24] It may be premature to tie the phenomena with which we have been dealing too closely to the antigen-antibody model. Rather than trying to force all biological specificity into the immunological compartment, we might have to consider the latter as merely a special case of a more universal biological principle, namely, *molecular key-lock configuration as a mechanism of selectivity*, whether involving enzymes, genes, growth, differentiation, drug action, immunity, sensory response, or nervous co-ordination.

Conclusion

The purpose of this article has been to point out how some problems of specificity in development can be resolved into terms of molecular theory. Some of the premises and conclusions are fairly

well substantiated, others are in need of experimental validation. From a pragmatic standpoint it is immaterial how many of the more detailed suggestions that were tentatively advanced will actually be borne out by future work, so long as the main line of thinking followed in our discussion proves of value and stimulates such future work.

There can be little doubt that many of our statements will have to be revised, as more facts become known. What we have called "contact" relations, for instance, might very well turn out to be "proximity" relations, still operating at close range, but exceeding the effective limits of the more common intermolecular forces. Or in stressing selective surface adsorption as an ordering principle in the rallying and sorting of molecular species, we may have unduly neglected some faculty of self-sorting of mixed molecular populations (as in the formation of tactoids[1]). The possibility of specific interactions through effects of radiations has not even been mentioned. Also, some phenomena bearing signs suggestive of specificity may yet find a more simple mechanical, electrical, or colloid-physical explanation.

Yet, these and similar reservations notwithstanding, our discussion will have served to illustrate at least the feasibility of breaking the rather abstract notions of specificity in development down into concrete and verifiable issues. We thus prepare what has been a domain of purely formal description for precise analytical investigation at the molecular level. In preparing the transition to this level, the biologist is prone to overstep his competence. Thus, in stealing a leaf from the chemist, I have risked the charge of trespassing on foreign and unfamiliar ground. I have done this in the conviction that, in science, a step forward, even in the wrong direction, is better than stagnation.

References

1 Bernal, J. D.: Structural units in cellular physiology in "The Cell and Protoplasm." Publ. Am. Asso. Adv. Sci. No. 14, 1940, 199.

2 Bloom, W., M. Bloom, and F. C. McLean: Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeons. Anat. Rec., 1941, *81*, 443.

3 Bronsted, H. V.: Entwicklungsphysiologische Studien über *Spongilla lacustris* (L.). Acta Zool., 1936, *17*, 75.

4 Clark, E. R., and E. L. Clark: Microscopic observations on the growth of blood capillaries in the living mammal. Am. J. Anat., 1939, *64*, 251.

5 Collip, J. B., H. Selye, and D. L. Thomson: Anti-hormones. Biol. Rev., 1940, *15*, 1.
6 Cooper, R. S.: Adult antigens (or specific combining groups) in the egg, embryo and larva of the frog. J. Exper. Zool., 1946, *101*, 143.
7 Dawson, A. B.: Changes in the lateral-line organs during the life of the newt, *Triturus viridescens*. A consideration of the endocrine factors involved in the maintenance of differentiation. J. Exper. Zool., 1936, *74*, 221.
8 DuShane, G. P.: The embryology of vertebrate pigment cells. Part I. Amphibia. Quart. Rev. Biol., 1943, *18*, 109.
9 Emerson, S.: Genetics as a tool for studying gene structure. Ann. Missouri Bot. Gar., 1945, *32*, 243.
10 Gutmann, E.: Reinnervation of muscle by sensory nerve fibers. J. Anat., 1945, *79*, 1.
11 Harrison, R. G.: Experimentelle Untersuchungen über die Entwicklung der Sinnesorgane der Seitenlinie bei den Amphibien. Arch. f. mikr. Anat. u. Ent., 1904, *63*, 35.
12 Henle, W., G. Henle, and L. A. Chambers: Studies on the antigenic structure of some mammalian spermatozoa. J. Exper. Med., 1938, *68*, 335.
13 Holtfreter, J.: Gewebeaffinität, ein Mittel der embryonalen Formbildung. Arch. exper. Zellforsch., 1939, *23*, 169.
14 Holtfreter, J.: Experimental studies on the development of the pronephros. Rev. Can. de Biol., 1944, *3*, 220.
15 Holtfreter, J.: Structure, motility and locomotion in isolated embryonic amphibian cells. J. Morphol., 1946, *79*, 27.
16 Landsteiner, K.: *The Specificity of Serological Reactions*. 2nd ed., 310 pp., Harvard Univ. Press, 1946.
17 Langmuir, I.: Molecular layers. Pilgrim Trust Lecture. Proc. Roy. Soc. London, Ser. A, 1939, *170*, 1.
18 Lewis, W. H.: The rôle of a superficial plasmagel layer in changes of form, locomotion and division of cells in tissue cultures. Arch. exper. Zellforsch., 1939, *23*, 1.
19 Lillie, F. R.: *Problems of Fertilization*. 278 pp., Univ. of Chicago Press, 1919.
20 Lillie, F. R., and H. Wang: Physiology of development of the feather. VII. An experimental study of induction. Physiol. Zool., 1944, *17*, 1.
21 Loeb, L.: *The Biological Basis of Individuality*. 711 pp., C. C Thomas, 1945.
22 Moore, C. R., and D. Price: Gonad hormone functions, and reciprocal influence between gonads and hypophysis with its bearing on the problem of sex hormone antagonism. Am. J. Anat., 1932, *50*, 13.
23 Oppenheimer, J. M.: The anatomical relationships of abnormally located Mauthner's cells in Fundulus embryos. J. Comp. Neurol., 1941, *74*, 131.
24 Pauling, L.: Molecular structure and intermolecular forces. [In Landsteiner's *Specificity of Serological Reactions*, pp. 275-93, Harvard Univ. Press, 1946.]

25 Schmitt, F. O.: Some protein patterns in cells. Third Growth Symposium. Growth, 1941, *5*, (Suppl.), 1.
26 Schoenheimer, R.: *The Dynamic State of Body Constituents.* x + 78 pp., Harvard Univ. Press, 1942.
27 Schulman, J. H.: Monolayer technique. In Bourne's *Cytology and Cell Physiology*, Oxford, 1942.
28 Spemann, H.: *Embryonic Development and Induction.* 401 pp., New Haven, Yale Univ. Press, 1938.
29 Sperry, R. W.: Visuomotor coordination in the newt (*Triturus viridescens*) after regeneration of the optic nerve. J. Comp. Neurol., 1943, *79*, 33.
30 Stone, L. S.: Primitive lines in Amblystoma and their relation to the migratory lateral-line primordia. J. Comp. Neurol., 1928, *45*, 169.
31 Taylor, A. C.: Selectivity of nerve fibers from the dorsal and ventral roots in the development of the frog limb. J. Exper. Zool., 1944, *96*, 159.
32 Tyler, A.: Specific interacting substances of eggs and sperm. Western J. Surg., Obst., & Gynec., 1942, *50*, 126.
33 Waddington, C. H.: *Organisers and Genes.* 160 pp., Cambridge, England, The University Press, 1940.
34 Weiss, P.: The so-called organizer and the problem of organization in amphibian development. Physiol. Rev., 1935, *15*, 639.
35 Weiss, P.: Selectivity controlling the central-peripheral relations in the nervous system. Biol. Rev., 1936, *11*, 494.
36 Weiss, P.: *Principles of Development.* 601 pp., Henry Holt & Co., New York, 1939.
37 Weiss, P.: Self-differentiation of the basic patterns of coordination. Comp. Psychol. Monogr., 1941, *17*, 1.
38 Weiss, P.: Nerve patterns: The mechanics of nerve growth. Third Growth Symposium. Growth, 1941, *5* (Suppl.), 163.
39 Weiss, P.: Lid-closure reflex from eyes transplanted to atypical locations in *Triturus torosus*: Evidence of a peripheral origin of sensory specificity. J. Comp. Neurol., 1942, *77*, 131.
40 Weiss, P.: The technology of nerve regeneration: A review. Sutureless tubulation and related methods of nerve repair. J. Neurosurg., 1944, *1*, 400.
41 Weiss, P.: Experiments on cell and axon orientation in vitro: the role of colloidal exudates in tissue organization. J. Exper. Zool., 1945, *100*, 353.
42 Weiss, P., and M. V. Edds Jr.: Sensory-motor nerve crosses in the rat. J. Neurophysiol., 1945, *8*, 173.
43 Weiss, P., and A. C. Taylor: Histomechanical analysis of nerve reunion in the rat after tubular splicing. Arch. Surg., 1943, *47*, 419.
44 Weiss, P., and H. Wang: Growth response of the liver of embryonic chick hosts to the incorporation in the area vasculosa of liver and other organ fragments. Anat. Rec., 1941, *79* (Suppl.), 62.
45 Willier, B. H.: Experimentally produced sterile gonads and the problem of the origin of germ cells in the chick embryo. Anat. Rec., 1937, *70*, 89.
46 Wrinch, D.: The native protein theory of the structure of cytoplasm. Cold Spring Harbor Symp., Quant. Biol., 1941, *9*, 218.