CONTROL SYSTEMS LABORATORY

ESSAYS ON BIOLOGICAL
UNITIZATION

Report Number R-52

Summer 1953

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Project 8-103A, D/A Project 3-99-10-101

UNIVERSITY OF ILLINOIS · URBANA · ILLINOIS

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FOREWORD

One of the original aims of the Bio-Systems Division is the study of the organization of living things. This organization is characterized by a hierarchy of multiple levels of integration, populated by more or less well-defined units. Hence, unitization appears to be a key phenomenon in the making of a complex organization.

The present report represents an intermediate stage in one particular approach to the study of unitization. The papers are largely based on a Symposium on Organ Integration which H. B. Chase arranged for the Bio-Systems Division in the Summer of 1952. They were revised and amplified during 1953. It is hoped that they will serve both as source material for further generalizations, and as a stimulus for parallel investigations.

Henry Quastler

January 1954

INTRODUCTION: UNITIZATION IN BIOLOGICAL SYSTEMS

Henry Quastler

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Every complex natural system, be it a community, an organism, a cell, or of any other kind, contains some well-defined unitized sub-systems. If the system is sufficiently complex, the sub-systems turn out to be composed of sub-sub-systems, which are themselves composite . . ., and so on, and so on. In most natural systems, the units, sub-units, sub-sub-units, etc., form a hierarchy of levels of organization with similar complexity. For instance, in the mammalian organism, one usually counts (from the individual downward): organ systems -- organs -- tissues -- cells -- organelles -- macromolecules -- molecules: and on into the world of the particles of physics. The hierarchy is only moderately regular. Ideally, a sub-system should be unitized anatomically, developmentally, and functionally, and represent unequivocally one of the levels named. Actually, there are many instances of unitization in one respect but not in the two others, and there are some sub-systems which could be assigned to more than one level.

The occurrence of unit sub-systems within systems is so common that one might take them for granted; it is so easy to produce any number of rationales for their exist-

ence that one might think it trivial. But, if one considers any one particular unit and asks why it is just what it is and does just what it does; why its components are integrated, why it contains just so many parts and not more or less -- then one usually does not find a satisfactory answer.

The definition of a sub-system is not always unequivocal. Indeed, the boundaries of all natural units are hazy. A system, says Webster, is "an organized integrated whole made up of diverse but interrelated and interdependent parts." The terms referring to interaction between parts -- "organized, integrated, interrelated, interdependent" -- specify one feature which makes a system out of a set. Of course, the distinction is not absolute. One may form a set consisting of a person's left eye, the most recently formed cell in his right adrenal cortex, and his duodenal loop. It is difficult but not impossible to conceive this set as a unit sub-system, with its three components interacting strongly in some event of critical importance. In fact, there is no set of parts in the universe which could not be called a system if one wants to be facetious enough to accept any kind of interaction. For a useful concept of systems, one must admit only <u>significant</u> interactions, where the decision of what constitutes a significant interaction depends, in last analysis, on the interest of the particular investigator at the particular occassion. The restriction to significant interaction may be arbitrary, but it is nec-

essary in order to approach the "wholeness" part of the definition without the certainty of winding up with the whole universe. The wholeness criterion is satisfied by the inclusion of every component which contributes significantly to the interplay of parts by which a system is integrated; where "significance" refers to the particular occassion.

At the present state of the art, a scientist would be quite justified in restricting his endeavors to the clean concept of a set and dismissing the concept of a unified system as a mere figment of illusion or a mere descriptive convenience. However, he would be equally justified in considering the unification concept as the very basis of the understanding of all of nature. Our own crystal ball asserts that the analysis of units within systems will be the key for the analysis of very complex systems.

One of the earliest tasks in the study of units within systems is to assemble a number of descriptions with emphasis on systems features. The first steps are the identification of a unit and its components. This has been done, for a number of systems, in the essays by Chase and Montagna, Edds, Fenton, and Weisz which are here assembled; also in the article by Bragdon, Nalbandov and Osborne in "Information Theory in Biology." In every case, the description is given by experts in the field; the amount of detail included is just what was judged necessary to just-

ify the identification of the unit and its components.*

Even the limited material collected to date tempts one to make one generalization; it concerns the span of diversity in unified systems. This is the topic of the following sections.

I. The Span of Diversity

In the social sciences, one frequently encounters the phenomenon of the limited span. For instance, the basic unit of many military organizations comprises some 6-10 men; research teams seem to stabilize at sizes of from 5-15; the number of subordinates directly reporting to an administrator, his "span of control," is recommended to be restricted to about 6 (or some number between 3 and 11). It is easy to see why some optimum size should exist; a typical argument -- not the only one -- is following: if the group is very large, then the difficulties of control and communication within the group are forbidding; if the individual groups are small, then the number of such units within the embedding organization increases and, with it, the problems of control and communication between the groups.

The concept of a "limited span" may have applications in biology too. I believe Paul F. Fenton was the first to observe that a count of the major factors involved in the

* It is hoped that the material presented will serve as a nucleus for similar studies; indeed, all recipients of this preliminary edition are invited to consider the possibility of adding a contribution of their own (it takes about 2-4 weeks of work).

control of any single organ or function yields a number not far from 8. It is true that the "magic number" in sociology applies to the number of components in a system; in biology, to the number of different kinds of components. Still, the reasons why there should be magic numbers could be largely the same, (although we have not thought yet of any reason why it should be approximately the same magic number). Consider, for instance, the control system described by Paul B. Weisz in this volume. In the organism which he investigated there occur five categories of granules which form a hierarchy; each granule is capable of transformation into the next higher category. The pattern is maintained by the following control mechanism: (1) each granule produces a substance which, through a feedback loop, acts to preserve its own kind; (2) each granule produces a substance which prevents the formation of its own kind from granules of the lower categories. All substances concerned seem to be of similar activities, and are transported by diffusion and turbulence throughout the whole organism; thus, any molecule, whenever produced, may impinge upon any granule. Under these circumstances, an orderly functioning of the whole control system is possible only if every substance involved has sufficient specific features to make its actions distinct from that of every other substance in the system. The requirements for specificity of elaboration at the origin, and for specificity of response at the target, increase with the number of distinct categories

(but not with the number of granules, because what counts is communication between classes of granules, not between individual granules). In the language of communication engineering, one would say: the greater the number of categories, the longer the address which must be emitted by each sender and deciphered by each receiver. Each new signal introduced is an additional burden on the whole unit system, because it must be distinguished from every other signal in the system.* We thus arrive at the following proposition:

> The span of diversity will be limited by difficulties of internal control and communication, unless the trend to specialization is checked earlier by some other mechanism.

The communication problem is basically the same when control messages are carried in nerves instead of by hormones. To establish a proper nervous connection, specific control must be exercised either during embryonic development, or during the functional stabilization of connections (see Edd's paper, this volume). The mechanism of specifying the proper connections is different, but the amount of control needed is the same as in the case of chemical communication. Nervous and humoral communication systems differ in many ways; each has definite advantages and weaknesses; but both are subject to the limitation of span by communication difficulties.

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* Of course, each signal must also be distinguished from signals related to other sub-systems in the same embedding organization. Such distinctions could be made through some feature which distinguishes all signals relating to one particular sub-system from all other signals, but it is also possible that one signal may have functions (possibly different functions) in more than one sub-system. As long as we restrict our consideration to single units, we can be sure that all signals must be distinguished from each other.

II Sub-Systems Unitization and Information Processing

We have used considerations of communication problems to deduce a plausible argument for the restricted diversity within a unit; a similar consideration can be used to deal with the very existence of unitized sub-systems.

Unitization plays a major role in control and communication within a complicated system. One may think of an organism as a communications network, an abstraction which I believe, represents a large share of an organism's activity; each unitized system represents a node, which may or may not be anatomically confined to a coherent volume. The inputs to the node are all events within or around the organism (including the unit under consideration) which activate the function of this particular unit; the outputs, the effect upon other parts of the organism or the surrounding.

It is a matter-of-fact that in every biological unit the outputs are much less varied than the inputs. We will mention a few examples. The process of collateral nerve regeneration is performed by a system consisting of normal axons, degenerated axons, and muscle fibers; the inputs are a variety of peripheral and central stimuli, metabolic events, specific substances like neurocletin; the output is simply the percentage of functional connections established. The blood sugar level is controlled by a widely dispersed system; its inputs are most varied; they include the activities of various parts of the body, excitement, absorption of food from the intestine, etc. The outputs are much less varied; in fact, most inputs are absorbed by the system and produce

no output: the blood sugar level remains constant. Consider a ganglion cell: the whole staggering complexity of events within and in the neighborhood of it, generates a tri-valued output -- resting, inhibited, or firing; in some cases the output can be a little varied by frequency modulation. Much of normal and pathological biology is dominated by the limitations of the reactive repertoire; many biological units follow the all-or-none law, responding with only binary outputs to a vast range of inputs.

In the language of communication engineering, unitized sub-systems are transducers with strong filter properties; incoming information is partly absorbed, details are fused together, subtle shadings are converted into simple alternatives. Such filtering causes a net loss of information; the important point is that it may be a very necessary evil.

Consider the following model: Given a multi-nodal network, with numerous connections internally and with the surrounding. The network is supposed to act as a system. That means, it must be able to process information coherently. Thus, whatever goes on anywhere in the network should, at least potentially, be made use of everywhere. The nodes are necessarily limited in their capacity of processing information. Now suppose that the total flow of information is much beyond the capacity of any single node. Thus, the amount of information which should be available at each node (under the postulate of coherence) is much larger than what it can handle. How can this communication dilemma be solved? Only by the use

of powerful filters, which take out of the total flow of information that small portion which is presumably of interest to the whole network. The model here described strikes one as a reasonably realistic representation of important aspects of living organisms; accordingly, one is tempted to suggest that the filter effect may be a major factor in the establishment and perpetuation of sub-system unitization.

III Span of Diversity and Information Functions

One can talk about "difficulties in communication" and "requirements for specificity" without attempting to introduce numerical values. It is usually correct to say that the difficulties in a 6-men-communication consist of all the difficulties of a 5-men-communication, and then some; similarly, that the specificity requirements for discrimination between 6 signals are all those which hold for discrimination between 5 signals, plus some additional ones. The concept of a limiting amount can be used without the introduction of a scale. But, if one wishes to understand not only the existence of "magic numbers" but also their actual values, then measurements have to be introduced.

Our consideration of diversity, with its associated difficulties in communication and requirements for specificity, has been restricted to components of united systems. Accordingly, we need a measure of diversity which refers only to diversity between components of a given system; that is, a measure restricted to those features which differentiate components of a given system from each other, without regard

for differences from anything else. We propose to use the following measure:

> The measure of diversity, for the components of a given system, is the average number of statements needed to distinguish any one component from all other components of the system. In order to make the definition unequivocal, it is also stipulated that all statements be binary (i.e., of a yes-no nature), and that they are constructed and arranged in such a way that their average number is minimized.

The difficulties of communication and the requirements for specificity depend on the diversity. Hence, their measures must be functions of the measure of diversity. It will be most convenient to simply equate all three measures. The measure here defined is also identical with the Quantity of Information of Shannon and Wiener. The unit of this function is called an information unit, or bit (contracted from "binary digit").

There is no need, at this point, for a general description of how to compute measures of diversity; we only need the results for two particular cases. Both concern a system with N equiprobable* components. For such a system we need (i) a measure of diversity, and (ii) a measure of the amount of information needed to establish the whole set of signals.

The measure of diversity has been equated to the number

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* The more general case where classes of components have no equal probabilities will not be discussed here.

of binary statements needed to distinguish one component from all others. Now, with one binary statement one can differentiate between two components or whole groups of components; a second statement can subdivide each group into two sub-groups, for a total of four. In general, ν statements will distinguish 2^{ν} classes. Therefore, N components can be distinguished by ν statements if $2^{\nu} \geqq N$ or $\nu \geqq \log_2 N$. One will choose the smallest ν which satisfies the inequality. For our purpose, it will not be necessary to be restricted to an integral number of statements; a "fractional statement" is quite admissible. Therefore, we can define:

For a system with N equiprobable components:

Measure of diversity $\equiv \log_2 N$

In the system considered, the amount of specificity required to elaborate or receive a single signal is $\log_2 N$; this applies to a single node. However, if one were to design the whole set of signals to be used, one would need $\log_2 N$ bits for each of the N components, or a total of $N \log_2 N$ bits. We use, again, the particular assumption that all possibilities are equiprobable (because the general case of unequal probabilities requires a much more elaborate discussion, and we feel that for most biological systems the equiprobability assumption is not a bad approximation). Then, we obtain:

Measure of specificity required to define all communications within systems $= N \log_2 N$

The measure of diversity, (and of specificity, information, etc.) is a measure of the difficulty of establishing

communication. It can also be interpreted as a measure of the effort which would be spent in establishing communication by an ideal operator, given optimum conditions of maximum efficiency in (generalized) coding and error-free operation. What is really "spent" in terms of material and structure will depend on the specifying mechanisms used. Thus, if we ascribe, say, to an enzyme a specificity of 9 bits, we definitely do not want to say that it is equipped with 9 binary switching elements; what is meant is that there is an indication of a selective mechanism at work, with results which could be achieved by 9 binary decisions between equiprobable alternatives, under ideal conditions. Usefulness of the Shannon-Wiener information function in the analysis of living systems hinges on the question whether there is a simple relation between the functions calculated for ideal conditions, and the effort actually spent. There is evidence that simple relations exist for certain classes of mental functions; in the field of general biology, we have not yet progressed to critical experimentation. All that has been established, thus far, are some estimates. These are given in the following table.

ESTIMATES OF BIOLOGICAL SPECIFICITY

Unit	Unitizing process (feature)	Basis for Estimating Specificity	Estimate (bits)	Source
Structural Elements:				
Protein Molecule	Addition of one residue	Bits per residue, if protein considered a linear array of amino acids and decision in adding each residue refers only to selection of appropriate amino acid	4	Branson [1]
Functional Elements:				
Enzyme - Substrate pair	Single interaction	Bits of specificity needed for selective reaction of enzyme with proper substrate, if both are embedded in a cellular systems containing many enzyme-substrate pairs	7-9	Quastler [1]
Antigen - Antibody	Single reaction	Upper limit of antigenic specificity knowing range and number of antigenic determinants (the latter inferred from size of antigenic spot and structure of protein molecule).	9	"
Gene - Trait	Single allelic determination	Lower limit of genic specificity needed to account for observed allelic variation in B gene in cattle (under certain assumptions about mechanism of diversity of traits).	9	"

Organs and Functional Units:

System controlling blood sugar level	Functional integration	A-Specificity required to distinguish between 6 hormones plus 2 vegetative nervous stimuli (humoral mediation). B-Specificity required to set up system of signals	3 24	Bragdon et al [1]
Skin	Organ integration	A-Specificity required to distinguish between signals B-Specificity required to set up system of signals	3 21	Chase and Montagna [2]
Collateral nerve regeneration (general)	Functional integration	A-as above B- " "	2.3 11.5	Edds [2]
Specific nerve connection	Functional association	Specificity required to establish or re-establish proper central connection	7-9	Edds [2]
Parietal cell	Control of secretion	A-Specificity required for 3-6 signals B-Specificity required for set of signals	2.1 10	Fenton [2]
Stomach (mammalian)	Organ integration	A-as above (about 10 signals) B- " "	3.3 10	"
Gastro-intestional tract	Organ system integration	A-as above (about 18 signals) B- " "	4.2 76	"
Morphological differentiation a protozoan	Organism integration	A-as above (19 signals) B- " "	4.2 80	Weisz [2]

Unit	Unitizing process (feature)	Basis for Estimating specificity	Estimate (bits)	Source
Whole Individuals:				
Smallest viable set of enzymes	Self-reproduction	Number and specificity of a set of enzymes which can synthesize replications of themselves	10^4	Quastler[4]
Smallest living unit	Formation of a spore	Specificity of a small bacterial spore (in terms of macromolecules)	10^4	"
Bacterial cell, Wt. 10^{-12} gm.	Growth of a cell	Upper bound, from entropy change in converting food into cell constituents	10^{13}	Linschitz[1]
"	"	Same, but allowance made for dissipative and maintenance activities	10^{12}	"
Bacterial cell, Wt. 10^{-13} gm.	Construction of a cell	Structural specificity of non-aqueous portion of cell, at 0^0 K	2×10^{10}	Marowitz[3]
Bacterial cell, Wt. 4×10^{-13} gm.	Growth of a cell (E. coli)	Entropy change in conversion of food into all body (allowing for dissipative and maintenance activities)	10^{11}	"
Man	Construction of an individual	Structural specificity of whole body, in terms of atoms	2×10^{28}	Dancoff et al[1]
"	"	Same, in terms of molecules	5×10^{25}	"
"	Genic individuality	Structural specificity of chromosomes	10^{11}	"
"	"	Specificity of genome from (estimated) allelic variability	10^5	"

Relevant Psychological Data:

Information per single act of perception (maximum)[7]	Single-pulse display (letters)	Amount of information displayed which subject can transmit	17	5)
Information per act per degree of freedom[8]	Single-pulse display	Amount of transmissible information assimilated from single-pulse display, where stimulus variation of single degree of freedom	2.5 to 6.0	5),6)

Footnotes:

1) Essays on the Use of Information Theory in Biology. University of Illinois Press, Urbana, Illinois, 1953.
2) This volume.
3) Harold J. Morowitz, "The Information Content of Living Cells," To be published in Bull. Math. Biophysics.
4) Transactions, 9th Conference on Cybernetics, Josiah Macy, Jr., Foundation. New York 1253.
5) Staff of Bio-Systems Division, Control Systems Laboratory, "Human Performance in Information Transmission II." Single-pulse Display, C.S.L. Report.
6) Garner, W. R. and Hake, H. W., "The Amount of Information in Absolute Judgement," Psych. Rev. 56, 446-459 (1951), and personal communication.
Ward, W. D., Personal Communication.
Klemmer, E. T., and Frick, F. C., "The Assimilation of Information from Dot and Matrix Patterns." J. Exp. Psychol. 45, 15-19 (1953).
7) Intuitively, this estimate is related to estimates "N" for "Organs and Functional Units."
8) Intuitively, this estimate is related to "Functional Elements" and to estimates "A" for "Organs and Functional Units."

CONTROL SYSTEMS IN THE INTEGRATED SKIN

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The skin is an integrated system in both space and time. The hair follicle and associated sebaceous gland, the pilo-sebaceous unit, is an integrated unit within this system. We do not yet know the full extent of the role of the external sheath of the follicles but are certain that it is extremely important and not particularly in its function as a sheath. There is a tremendous difference between conditions existing when there are active hair-producing follicles and conditions existing when there are inactive, or so-called resting, follicles. This difference which was first observed in 1946 at the University of Illinois (H.B.C.) with relation to greying of hair following x-radiation has been our modus operandi ever since. In mice and rats there are normal waves of hair growth and succeeding inactivity. These can be ascertained and areas chosen for treatment and study. Better yet, it is possible by plucking the club-hairs of the resting or telogen stage, to initiate growth in prescribed areas only, which will be out of step with subsequent waves. This can be done with mice, rats, rabbits and hamsters. Plucking of feathers in birds, as the pigeon, also initiates a new growth. By plucking in the small mouse, for example, it is feasible to obtain 7 areas in different stages of activity on the same animal. In the guinea pig and man, hair growth is mosaic;

that is, individual hairs seem to have their own rhythms and a region does not all come into and out of activity at the same time. Plucking in these cases is effective on resting follicles, we believe, but since they are interspersed with follicles already active at the time, no well-defined area is obtained where all the follicles are in precisely the same stage. Likewise, no appreciable area has all its follicles in a normal resting stage at the same time.

The distinction between active and inactive has been extremely useful but it has seemed advisable to know the whole sequence of events in a hair-follicle cycle. Consequently, 6 substages of the growing stage, or anagen, have been worked out in some detail. All the stages and substages will be described with relation to the layers of the skin and the pilosebaceous units. We shall describe some morphological, cytological, and cytochemical details associated with these stages. Also with respect to stages, we shall describe some of the changes which occur in hereditary hairlessness in mice, and changes which occur following x-irradiation and methylcholanthrene treatment.

There is a rather definite thickness of each skin layer with each state of hair-growing activity. The thickening of the epidermis (2-3 times) during the early anagen is the result of a burst in mitotic activity. As the hair shaft is produced, however, the epidermis returns to "normal" or less in thickness. The adipose layer increases 2-3 times its "resting" thickness and the corium, or dermis, by 50%. This is an enlargement of cells, not mitosis, at least in the adi-

pose layer. At birth the skin is much as it is at the 3-day stage, substage II, of the regular cycle. For some follicles the stage is about anagen III, 5-day type, for others it is earlier, and in other cases the follicles have not yet formed. New follicles on the body are laid down beginning at 3-4 days before birth and ending usually at about 3-4 days after birth.

Before considering the growth cycle of a follicle, we shall consider the formation of a new follicle. A solid bud of cells forms from the basal layer of the epidermis and grows downward into the corium as a solid peg. Next a dimple forms at the end, and as this enlarges with continued growth downward of the peg around this, some dermal cells become included in this cavity. By this time there is then a bulb enclosing a dermal papilla and a connective tissue sheath begins to surround the whole structure. This is anagen II is type and all subsequent development is identical with that found in follicles which have been resting and now start the new cycle. Dendritic pigment cells are included in the peg and some begin to form melanin at about this time as they do for all later hair generations. At about 3 days after birth, practically all the follicles which are going to form have started. At this time the slope of hairs is determined, apparently by a relative shift of layers of the skin. The follicles or pegs, have been perpendicular to the surface; now they approach a 30° angle. Keratinization occurs as a core in the upper end of the peg and in the epidermis above, or in line with it. The result is a future hair canal with keratin-

ized wall extending into the dermis. This is continuous with the corneum. At the lower edge of the keratization, the cells of the follicle, continuous with the basal layer of the epidermis, form the sebaceous gland on the oblique angle side. The internal sheath has been formed from the bulb below and the shaft is being produced.

In a cycle, not the first, we can start with the resting stage, telogen, in which we find a club, a germ, and a dermal papilla. Around the club itself there is a "capsule" of epithelial cells. Below that is the germ (a plate of 20 or so cells in small follicles) which is continuous with the external sheath. There is already present a sebaceous gland and a pilosebaceous, or hair, canal. The first burst of mitotic activity is in the germ, also in the external sheath. This is anagen I. For anagen II there is a downgrowth of the germ plate around the ball of papilla cells. A thin dome of keratinization appears above the germ plate and is the beginning of the internal sheath. The lengthening of the whole follicle due to excessive mitoses in the external sheath commences in this substage. For anagen III the follicle lengthens rapidly and the adipose layer thickens. The internal sheath becomes pointed and extends distally making a tube within the external sheath. There is now a bulb of matrix cells around the inverted pear-shape papilla. Dendritic pigment cells in this bulb start developing melanin granules. Cells in the external sheath now cease dividing and accumulate glycogen. The dermal papilla now becomes metachromatic, indicating presumably the presence of certain polysaccharides. By anagen IV,

6 days after plucking, the follicle is completely elongated, the hair shaft is forming and the tip is at the base of the sebaceous gland. The papilla cavity is narrow. Pigment granules are now found in the matrix cells of the upper third of the bulb. At anagen V the tip of the hair is at the surface of the skin, the papilla cavity is further compressed. The corium and adipose are at their full extension and the epidermis has returned to its "normal" level or even less. Anagen VI is the stage of hair proliferation at the rate of nearly 1 mm. per day. Clearly then, the first half of anagen is "embryonic," and the last half is "fetal," the phase of rapid growth. At the end of this last substage, glycogen in the external sheath and metachromasia in the papilla disappear.

The catagen stage is the transitional stage between anagen and telogen. In the short period of about 2 days the follicle changes from a long, highly active hair-producing structure to a relatively short, resting follicle. Cell division ceases in the bulb, a germ is set aside, the papilla becomes a "ball," and a club at the end of the hair is formed. The base of the resting follicle with its ball of papilla cells comes to lie well up in the corium. Shortly before this, the pigment cells cease producing pigment and the medullary cells of the hair shaft are no longer formed. As the matrix cells stop dividing, no new cells are fed into the internal sheath and hair shaft. The last cells to be keratinized but not compacted into the hair cortex constitute the hollow, brush-like club. The surrounding capsule consists of cells

little if any keratinized, but attached by fibrils to the club. These cells come up from the bulb. The cells of the "basal layer" of the upper bulb, and continuous with the external sheath probably constitute the germ. Cells of the lower bulb which do not make up the germ and are not incorporated in the capsule come to lie between these 2 structures and largely degenerate. As the bulb "disgorges" and the external sheath-germinal layer rounds up, the papilla is left as a ball of cells below the germ. The great shortening of the whole follicle seems to occur by degeneration of some external sheath cells and by a decrease in volume of all the other external sheath cells. Around the follicle, including the papilla, is a connective tissue sheath which apparently "takes up the slack" as these changes take place. The adipose layer and dermis return quickly to the thickness characteristic of the resting stage.

Several aspects of our work can now be discussed with respect to these stages of hair growth. The extent of epidermal damage from methylcholanthrene and x-irradiation is far less when follicles are in anagen rather than telogen. Repair is also rapid. Sebaceous glands are eliminated regardless of the stage within 4 days after a single treatment with 0.6% methylcholanthrene-in-benzene. New glands are formed from the external sheath at once, however, if the follicles are in anagen. X-irradiation destroys dendritic pigment cells, being especially efficient on the few dormant cells of telogen. On the other hand, moderate doses of x-radiation cause epilation only when follicles are active, possibly

by the effect on the --SH groups in keratinization. Methylcholanthrene, however, does not disturb a growing hair but does allow club hairs to fall out. In starvation (caloric restriction to 1/3 normal) new waves of growth are prevented and even plucking will not initiate growth after a while. If hairs are growing, however, when the restriction becomes critical for initiation, these hairs will complete their growth. The adipose layer becomes very thin in restricted animals. The external sheath, with its store of glycogen in the active phase and its great potentiality for forming sebaceous lipids, hair keratinization, and even epidermis, apparently plays an all-important role in skin regeneration, morphology, and function.

In hereditary hairlessness in mice, the first pelage appears normal but then falls out at the end of growth without a normal club being formed. No new pelage forms, except for an occasional scanty second growth. The skin becomes progressively abnormal, with hypertrophied cells in the epidermis, and sebaceous and keratinized cysts in the dermis. Except for the presence of cysts, the skin resembles that following treatment with methylcholanthrene, even to the excessive pigment cells in the epidermis. The cysts develop by sebaceous transformation and later keratinization, particularly from the epithelial (external sheath) tabs left below the original sebaceous gland.

That the failure of new hair growth here is associated with drastic skin disturbances is obvious. That the "germinal" external sheath cells still have lipid and keratin po-

tentialities is also obvious. A mechanism which allows normal cycles in the pilosebaceous units and the skin is lacking. The nature of the initial error associated with the hr hr genotype is as yet unknown. Certain features, however, are apparent. Anagen is normal, but catagen, the transitional stage to telogen, is not. Instead of a keratinized club forming below the internal sheath, a few unkeratinized bulb cells at the end of the shaft pass up into the internal sheath canal. This knot of cells later becomes keratinized, but is not a club and does not prevent the hair from falling out. The rounding up of the catagen appears at first normal with the ball of papilla cells at the end. There is no capsule, however, since there is no club. The bulb cells in the stalk between the internal sheath and the papilla undergo degeneration but not in the normal regular fashion. Certain areas degenerate more than others. The whole strand is not shortened but becomes thinner and more coiled. Soon this strand breaks, leaving a distal tab and a proximal portion deep in the adipose which consists of papilla and epithelial germ. Where 2 breaks occur there may be a middle piece of epithelial cells left stranded in the adipose layer. The germ and papilla portion may produce later an abortive hair but without the normal continuity of the follicle, it grows poorly and does not approach the surface. Epithelial tabs and isolates can and do produce sebaceous and keratinized cysts. The nature of the failure to maintain the continuity of the follicle is not yet clear. The connective tissue sheath may fail to contract and to hold the lower follicle together at catagen. There may be a failure of intercellular bridges

and general cohesiveness of cells. Of possible bearing on this problem, is the condition following irradiation with x-rays (1000 r). Here the active follicle loses its actively growing hair by a failure of continuous keratinization, the already keratinized portion falls out, having no anchor such as a club. The catagen-like strand below becomes beaded in appearance as in the hairless condition. The principle difference is that eventually a new hair does grow, the strand not being broken. Even at this dose, hair growth may be sparse (some follicles fail) and with higher doses, permanent epilation is possible. Conceivably, breaks may occur, but that point has not yet been investigated for the high doses. With doses around 400 r, there is the epilation of growing hairs, but all follicles are capable of producing hairs again after a short delay. Unique in the x-rayed active follicles is the huge accumulation of pigment in the dendritic cells. It would seem that melanogenesis continues but no hair cells are available for taking up these melanin granules. A similar situation does not occur in the hairless mouse because the normal catagen stage, when no further pigment is being formed anyhow, is reached before keratinization ceases.

The relation of all these observations and others to the clinical problems of baldness, skin disorders, wound healing, etc. is not yet known. Certain academic speculations, however, are possible. In any case, it appears that continuity of the follicle, i.e. continuity of the external sheath including the germ with its adjacent dermal papilla, is essential for hair growth cycles and for the consequent normal skin.

In the accompanying diagram (Fig. 1) involving the epidermal (ectodermal) structure of the skin, a system of controls is suggested. An outside stimulus of unknown or "spontaneous" origin occurs to initiate mitotic activity, as in the normal waves of growth. This outside stimulus can also be evoked by plucking of club, or "resting," hairs. Im any case this stimulus, associated with increased vascularity of the area, continues for about 17 days in the mouse and acts on the germ plate, the resting external sheath cells, the peripheral cells of the sebaceous gland, and the basal layer of the epidermis. The stimulus may pass up from the germ plate through the external sheath to the peripheral sebaceous cells and the basal layer of the epidermis, but the responses are so nearly simultaneous that it would appear that there is a general stimulus with the germ plate and external sheath responding slightly earlier (12-24 hours) than the other "germinal epithelia." In the case of the basal layer of the epidermis there is a negative feedback. The thickness of the corneum has long been suspected of determining the mitotic level of activity in the basal layer and this hypothesis has recently been demonstrated in human skin by Hermann Pinkus (personal communication). With the outside stimulus the mitotic rate rises to about 4%, then drops to less than 1%, then returns to about 1% which is the normal maintenance level. Such an oscillation is the result of a feedback system which has been suddenly "over-stimulated." The situation for the peripheral sebaceous cells and sebum is similar. In the case of the external sheath there is possibly a negative feedback.

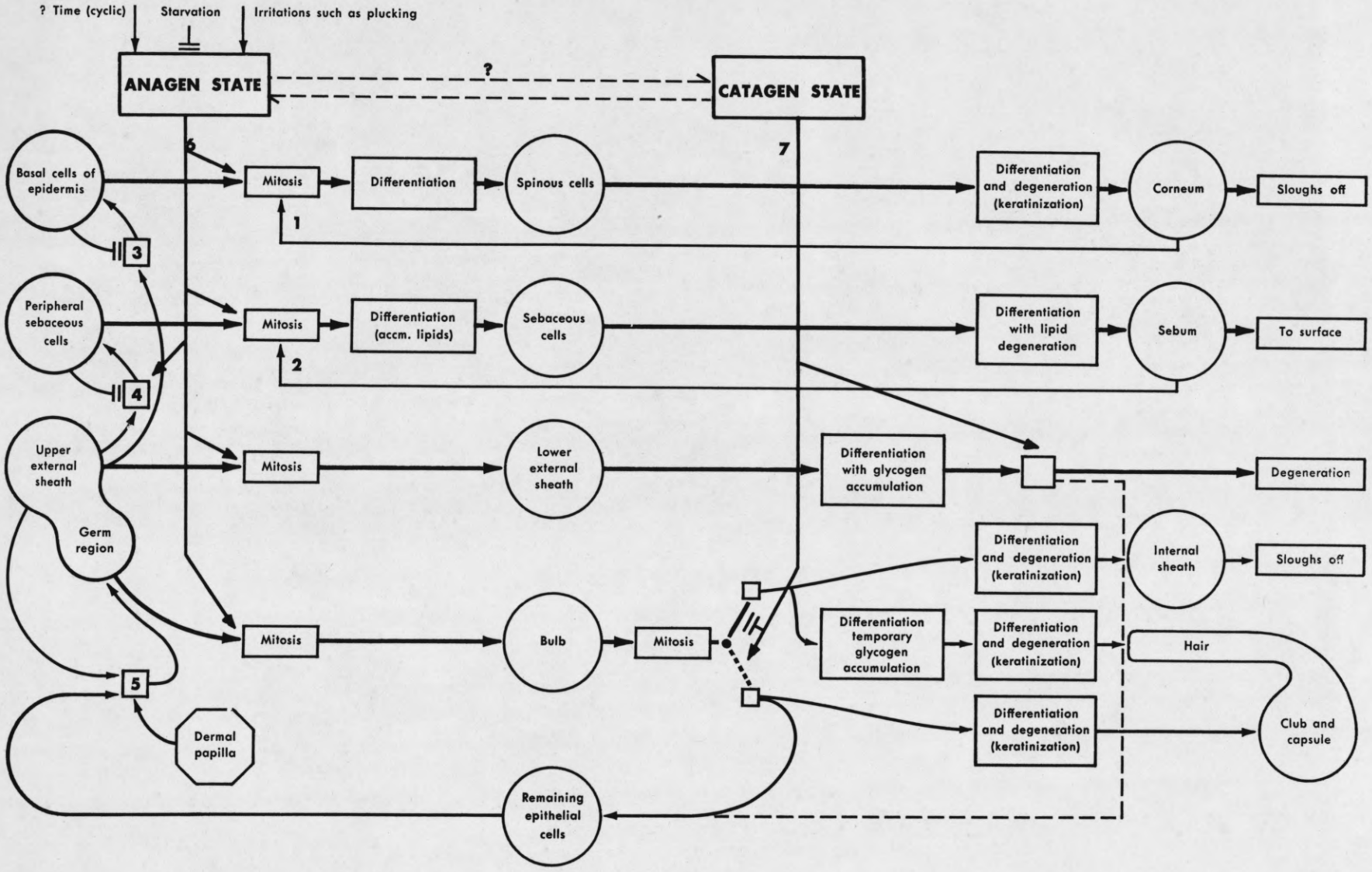

Fig. Control system for growth in skin

The hair and internal sheath-producing system apparently has no negative feedback and will grow as long as the outside stimulus continues. Angora hair in many mammals and human scalp hair, as examples, grow for long periods before the resting stage occurs.

Such a system as described here is in accord with the normal skin dynamics and with the phenomena associated with the hair growth cycle. In terms of control systems there is a non-specific stimulus which affects all "germinal epithelia" of the skin, but most of this epithelium has self-regulatory mechanisms except the germ-bulb. The increased thickness of the corium and the adipose layer also follow this outside stimulus, not by cell division but undoubtedly associated with the coincident increased vascularity. It should be pointed out that the hypertrophied epidermis of older hairless mice and of methylcholanthrene-treated telogen skin suggests that an active bulb may have some effect on the skin either by inhibiting growth in the other germinal epithelia or by "draining" the stimulus into itself.

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CONTROL OF NERVE REGENERATION

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Two early steps in the quantitative analysis of biological control systems are: (i) the identification and counting of control elements, and (ii) an estimate of the amount of control exercised (1). In this reports, an account is given of such an analysis of nerve regeneration. Particular attention is paid to the collateral regeneration of intact nerve fibers in partly denervated mammalian muscles. The degree of specificity exhibited by regenerating fibers as they reconnect with their end organs is also discussed.

I. Repair of Completely Divided Nerves - Terminal Regeneration

The regeneration of an interrupted nerve is described first to provide a factual background. The regenerative process depends on the fact that a nerve is a cable of nerve fibers. Each fiber is a cytoplasmic extension of a cell body which generally lies in or near the central nervous system. The fiber extends through the nerve and ends finally in a specially structured portion of a muscle fiber, gland or sense organ.

Following severance of the nerve, degenerative changes occur in the distal portions of the individual nerve fibers which are cut off from their cell bodies. Different parts of the fiber react differently. The axon and its myelin

sheath fragment, and the resulting debris is cleared away by phagocytic cells. The rest of the fiber, and especially its associated sheath cells, undergoes changes which convert it into an elongated cord of fibrous and cellular elements. Several days, at least, are required for completion of these events which in many respects are more constructive than destructive.

Meanwhile the central ends of the cut fibers undergo terminal regeneration. That is, they send out delicate, almost submicroscopic processes from their amputated tips. These filaments of naked cytoplasm enter the distal part of the nerve trunk where they then preferentially follow the cords of converted sheath cells. In this way, the regenerating tips are eventually led back to the periphery. There, they reestablish connection with appropriate end organs. In muscles, this results almost at once in the onset of functional recovery and the arrest of atrophic changes which always follow denervation.

Subsequent phases of regeneration depend on the fact of reconnection and fail in its absence. That is, renewed interplay between nerve and muscle does not await the completion of regeneration. The final events involve particularly: 1) the synthesis of new cytoplasm in the cell body and the transport of that cytoplasm out along the fiber, thus permitting the latter to "grow;" and 2) the development of a new myelin sheath and the realignment of the sheath cells.

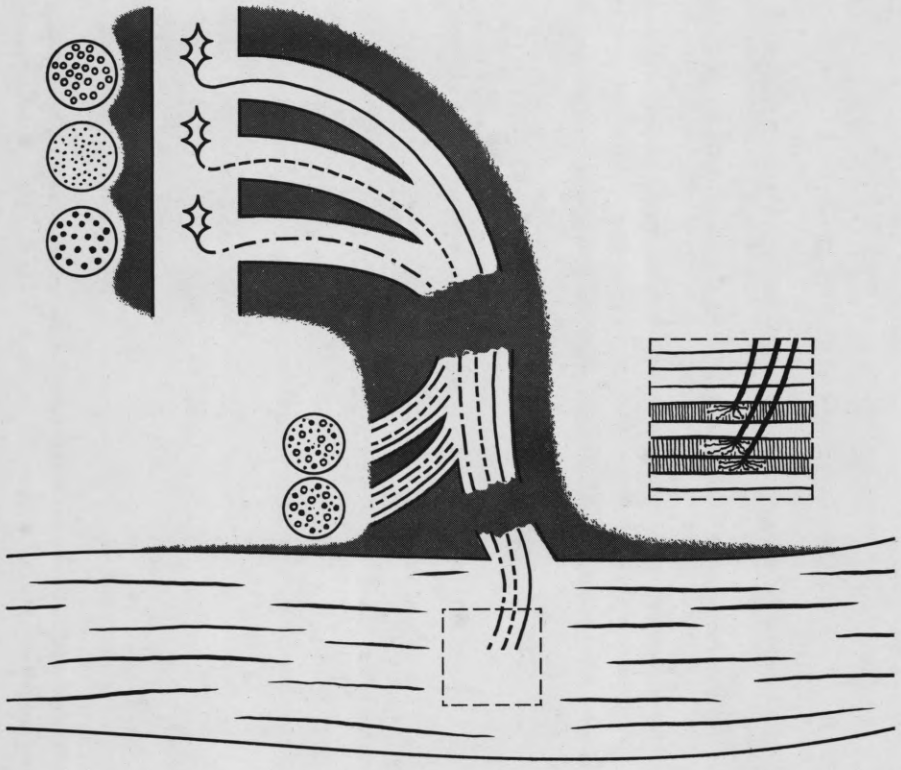

Fig. / Intermingling of fibers from different spinal segments in peripheral nerve.
Insert: neuro-muscular end plates

II. Partial Denervation - Collateral Regeneration

The picture changes when, instead of complete interruption, only some of the fibers in a nerve are severed. If the cut fibers are free to regenerate, then subsequent events are identical to those just described. However, there are several situations where such freedom does not exist. For example, in poliomyelitis, some of the fibers may be wiped out because their cell bodies are killed by the virus. Adjacent fibers may be quite undamaged. Here terminal regeneration is impossible.

It is also feasible to produce partial denervation experimentally. In most limb nerves, the constituent fibers arise from cell bodies which lie in two or three adjacent segments of the spinal cord (Fig. 1). In their initial course, close to the cord, the fibers from each segmental level run in separate bundles. Only more distally do they join into common nerve trunks which distribute them to the limb. One of these separate bundles can be cut and blocked mechanically so that it will not regenerate. The result will be a partial denervation of the entire system distal to that point. The fine anatomy of nerves is such that, out in the muscles, the divided and degenerating fibers will be freely intermingled with the unaffected, intact fibers (cf. Fig. 1).

Under these circumstances, the residual nerve fibers exhibit a new type of regenerative behavior, beginning about 4 days after the operation and continuing for several weeks

(2,3). Somewhere alone the last few millimeters and especially the last 100 or 200 μ of their intramuscular course, the azons develop localized weak points in their surface membrane. Each such area temporarily loses its semi-rigid, gel-like consistency. Cytoplasm flows out laterally and establishes a collateral branch or sprout. Being surrounded by numerous cords of sheath cells (from degenerated fibers), the new sprout readily makes contact with or reneurotizes a "guide-rail" leading to an empty ending or a denervated muscle cell. It then rapidly traverses the short distance to the latter and penetrates it.

The reestablished connection becomes functional within a few days at most, as judged both by the recovery of muscle strength and the arrest of muscle atrophy (4,5,6). After these events, the rest of the reparative process is devoted to enlarging the new collateral branches and ensheathing them with myelin. As in terminal regeneration, the latter changes occur only if the sprout is actually innervating a muscle cell.

The collateral regeneration which follows partial denervation is remarkably adaptive. The original degree of denervation inevitably varies in different parts of a muscle. Some regions are almost wholly deprived of nerve supply, others suffer relatively light losses. Often, within a few mm^3 of muscle, the entire range of possibilities is represented. Yet in each local area, roughly appropriate numbers of new sprouts are finally established - almost

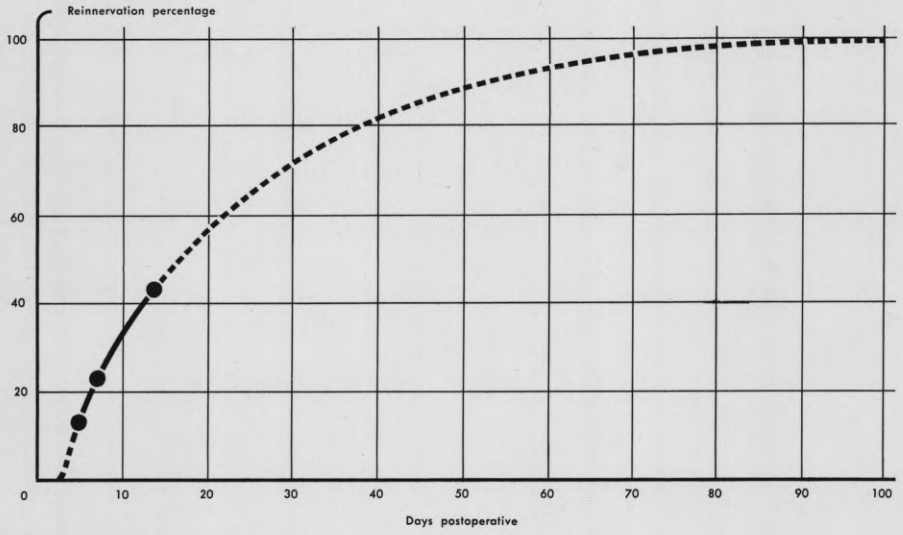

Fig. 2 Rate of reinnervation

never too many and only occasionally too few. That is, the regenerating collaterals tend to saturate the available end plates (2).

The percentage of sheath cell cords which have become reinnervated (reinnervation percentage: R.P.) increases with the duration of the postoperative interval. R.P.'s have only been computed for postoperative periods of 4, 7 and 14 days (7) but extrapolation combined with data on the recovery of muscle strength (6) and with increases in the number of muscle fibers supplied by individual nerve cells (2) gives rough values for the remainder of the course. The curve in Fig. 2 gives a crude estimate of the increase in R.P. with time; it shows that while nearly half of the empty cords are reinnervated after 2 weeks, values approaching 100% are not reached for many weeks. Further, the curve portrays an "average" situation only. The rate of R.P. increase is roughly inversely proportional to the amount of denervation originally effected. In severely affected regions, full reinnervation usually does not occur.

III. Causal Analysis

Our concept of the controls operating during collateral regeneration is presented symbolically in Fig. 3. The following account considers the more critical evidence and the reasoning on which the diagram is based.

The collateral sprout arises in response to some influence originating outside the parent axon. Whether this influence is an active stimulation or a release of some

inhibition of a normally-occurring sprouting potential is uncertain. But both on the grounds of available evidence and on the grounds of seeking the simplest possible hypothesis to explain the known facts, an active stimulation seems more likely.

The stimulating agent is probably a chemical substance ("neurocletin") (3,8). The evidence for this is entirely circumstantial: ether extracts (especially the fatty-acid containing fractions) of myelinated nerve tissue (either intact or degenerating) initiate collateral sprouting when injected into normal muscles; extracts of other tissues are ineffective (3). There is, however, no crucial evidence against the possibility that local changes in such factors as pH or salt balance may constitute the effective stimulus (cf. 9, p. 636).

Neurocletin (or some locally altered chemical state) is a product of the activity of sheath cells of degenerating fibers. This conclusion can be reached by a process of elimination. Denervated muscle tissue itself is not a source of any effective agent (10). Although myelin contains neurocletin and presumably can release it during degeneration (3), sheath cells probably represent the original source. Moreover, collateral formation can occur long after all traces of myelin debris have been resorbed (11). Sheath cells show marked changes in behavior during this period. They divide rapidly and undergo cytochemically detectable alterations (12,13); some transform into phagocytic cells (14);

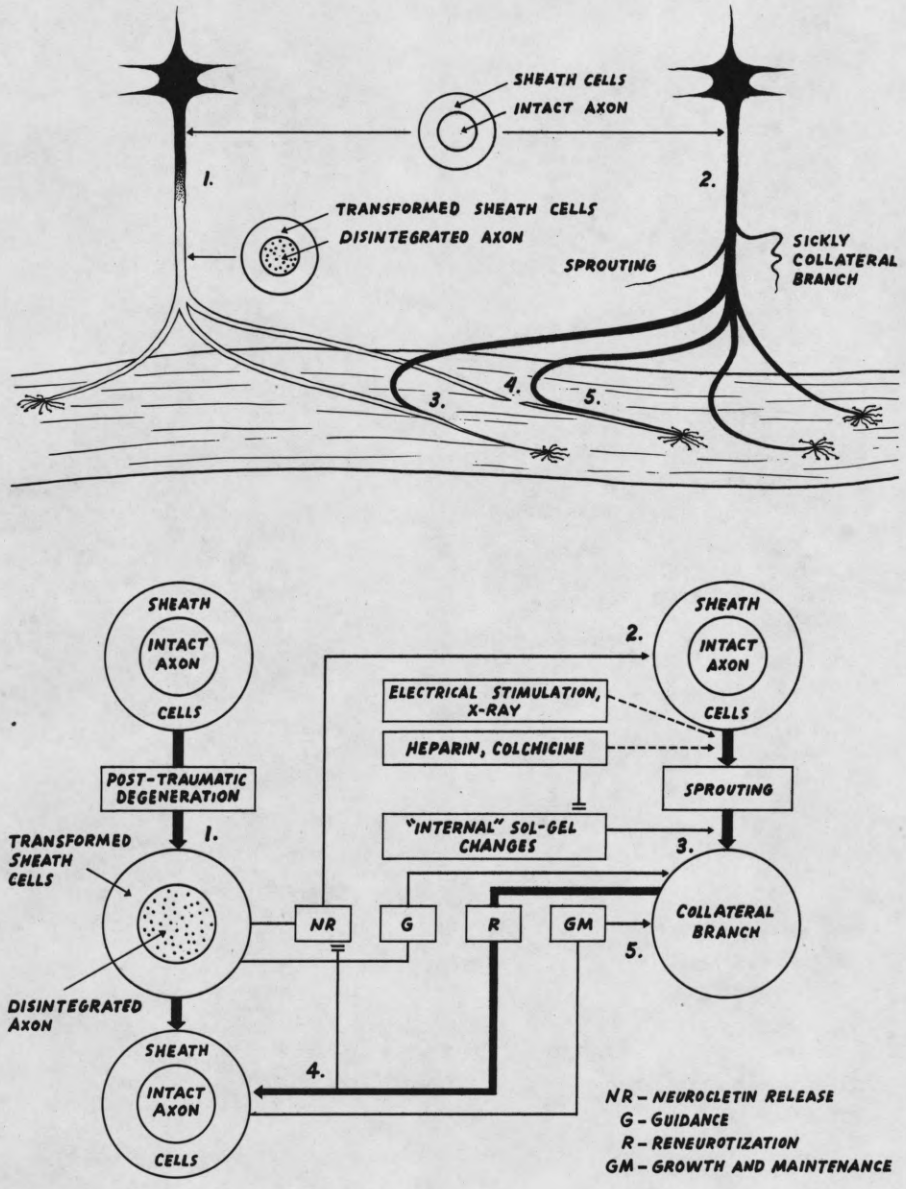

Fig. 3 Control system for collateral nerve regeneration

others become realigned into the cords mentioned above. The suggestion that they also release neurocletin therefore seems a reasonable inference.

Neurocletin, once released, is readily diffusible and is not quickly destroyed; it is effective at a range of several millimeters. Evidence for this assertion is suggestive but not very satisfying. Regenerative activity proceeds more rapidly in partially denervated muscles which lie next to completely denervated muscles (10,15); localized intramuscular injections of neurocletin-containing extracts lead to collateral formation outside the immediate zone of injection (3). Therefore, gradients or differential patterns of neurocletin distribution cannot be invoked to explain variations in the number of collaterals which develop in various local regions.

The primary effect of neurocletin on intact nerve fibers is to produce localized liquefication of the gelated cortical axoplasm (axogel), thus creating a breach through which internal axoplasm (axosol) can move. Virtually all recent workers, beginning especially with Weiss (16), have visualized the initiation of fiber branching in these terms. A fatty acid (cis-vaccenic), possibly related to neurocletin (8), hemolyzes erythrocytes and also inhibits bacterial respiration (17,18). It is therefore not unreasonable to assign a comparable role to neurocletin which could exert its effect by

modifying the trans-membrane potential and hence the permeability of the axon membrane (cf. 19).

The subsequent outgrowth of the collateral is independent of neurocletin and depends on properties of the parent axoplasm. As axosol flows out, its cortical layer gels, establishing a cylindrical contour for the sprout. The advancing tip either remains liquefied or becomes a readily reversible sol-gel system. These statements synthesize views on cell locomotion in general - a process which is still poorly understood (9). No commitment need be made as to the nature of the factors which produce locomotion of the collateral but presumably both intra- and extra-axonal forces are involved (16). The inhibitory effect on sprouting of such mucopolysaccharides as heparin and colchicine (7) is probably exerted during the establishment of the sprout since both these agents prevent cytoplasmic gelation and can hardly be assumed to interfere with initial sprout emergence (11). There is some evidence (7) that, under as yet undefined conditions, colchicine may synergize neurocletin and permit multiple break throughs at a single level of the parent axon. X-irradiation, as well as electrical stimulation of the axon, may have similar synergizing effects (7,20).

The outgrowing collateral has a selective, although not an exclusive, affinity for cords of sheath cells. This affinity is both a matter of observation (2,3) and an inference from studies on terminal nerve regeneration where the advance of the axonal tip is facilitated by contact with

sheath cells. After making contact with one of these cords, which lie a few micra at most from the point of sprout emergence, the collateral is guided directly to an "empty" end plate. Probably few outgrowing sprouts fail to make connection with sheath cells and of these, the great majority are resorbed.

The release of neurocletin by sheath cells is halted when they are reneurotized by collaterals. As successively larger numbers of cords are reneurotized, the concentration of neurocletin around the intact axons diminishes. Finally, at some critical value, sprouting ceases. This is speculation but it is apparently the simplest hypothesis to explain the termination of collateral formation (11). Direct observation shows that reneurotized sheath cells alter their behavior in that they begin to envelop the new axon branch and cooperate in forming a myelin sheath. Further, the sheath becomes refractory within a few days to innervation by another axon branch (20). This implies other changes in sheath cell activity, one of which may be the diversion of metabolism away from neurocletin synthesis or release.

The continued existence of any given collateral depends critically on the establishment of contact with a cord. More collaterals are produced than there are cords available; the supernumerary branches are resorbed. Observation indicates that connected collaterals are stable and not subject to resorption (2). In some unknown manner the cells with which the collateral comes in contact influence both its survival and its growth in size. In this

respect, the collateral behaves in the same manner as a terminally regenerating axon which also depends on its peripheral connection for some "trophic" influence (21). In the latter case, the effect is probably exerted indirectly through modification of the metabolic activity of the cell body from which the axon derives all of the axoplasm necessary for growth and maintenance (22). During collateral regeneration, the effect may not extend as far back. But a signal must extend at least as far as the origin of the collateral if appropriate amounts of axoplasm are to be diverted from the parent fiber. Unconnected collaterals are very rare. When they do occur, they fail to grow in caliber and extend for long distances (up to 200μ) in the endomysium (2,20).

The assertion that supernumerary branches are produced and then resorbed is an inference in this context. But observations on neurons in tissue culture and on nerve fibers of the living tadpole tail provide indirect supporting evidence (23,24). The proposal would provide a satisfactory explanation for the occurrence of appropriate numbers of collaterals in the finished product.

IV. Number of Control Points

In the system just described, there are five obvious control points. The following phenomena result from activities occurring at those points (cf. Fig. 3):

1) transformation of sheath cells following axon damage;

2) gel-sol changes in the membrane of intact axons under in-

fluence of "neurocletin;" 3) guidance of new collaterals by sheath cell cords; 4) inhibition of neurocletin release from sheath cells following reneurotization; 5) maintenance and "growth" of new collaterals under influence of sheath cells and/or end plates. The remaining events do not depend on controls unique to this system but result from properties which are characteristic of the component cells.

V. Specificity in Regeneration

In the preceding section we have discussed processes which lead to the connection of a nerve fiber with an end organ; we now turn to mechanisms which control the connection of specific fibers with specific end organs. The problems considered deal essentially with such questions as: What degree of specificity is exhibited by regenerating nerve fibers as they reestablish their terminal connections? Does a particular motor axon have an affinity for a particular type of muscle fiber and for no other? Is a sensory fiber similarly restricted? Can axons connect with "foreign" end organs in a manner which though structurally atypical, still permits function (in the sense of conducting appropriate impulses to or from those end organs)? Does the reestablishment of central synaptic relations follow the same or different rules?

A. Specificity During Reconnection

The degree of specific interaction exhibited during the reconnection of nerve fibers varies markedly in different animals and in different nerves of the same animal (Fig. 4).

1. Peripheral Reconnection

In general, peripheral nerve fibers are relatively non-selective both during development (Fig. 4A) and during regeneration (Fig. 4B). A motor axon detects the difference between a motor and a sensory end organ and will not connect with the latter either structurally or functionally. The same is true both for sensory and for autonomic fibers (21). In the latter case, adrenergic fibers will not even form connections with cholinergic endings (25). However, any motor fiber will form a structurally and functionally typical association with any muscle fiber. Similarly, sensory axons can connect with and carry impulses from any peripheral sensory station. In either case, the periphery exerts a trophic influence which is not distinguishable from the normal (21).

In view of the disorderliness of at least part of the pathway followed by regenerating fibers, the orderly linkages previously existing between peripheral tissues and the nerve centers are inevitably disarranged. Unless some kind of central reorganization occurs, varying degrees of malfunction will result (mislocalization of sensory stimuli, uncoordinated muscle contraction, etc.). In amphibian larvae, such central reorganization apparently does occur - either by a reshuffing of synaptic association (Sperry, 26) or according to a physiological resonance principle (Weiss, 27) - and normal functional patterns are reestablished. Metamorphosed amphibia have mostly lost this ability and for some muscles (e.g., the extrinsic muscles of the eye), the capacity for reorganization

disappears in early larval stages. In mammals, the plasticity is already gone at birth; presumably, it does exist in embryonic stages (26).

Although these findings, taken by themselves, imply relatively low-grade specificity for peripheral nerve regeneration in the adult organism, some more refined processes of specification clearly must intervene during development. Otherwise, we cannot account for the origin of the orderly pattern of interconnections. Evidence that such specification does occur comes from studies on the re-establishment of synaptic relations during regeneration.

2. Synaptic Reconnection

The classic studies of Langley (25) demonstrated that during regeneration of preganglionic autonomic fibers into the superior cervical ganglia, a marked tendency exists to restore original linkages among fibers of at least 6 different functional types and the neurons of the ganglion. More recently, Sperry (26) has shown that an even higher degree of specificity obtains during the regeneration of the amphibian optic nerve into the visual centers of the midbrains (Fig. 4C). The optic fibers systematically reconnect in (or near) their original terminus so that each retinal locus again possesses, at least roughly, the same midbrain end stations. These relations are set up in a predefined manner, regardless of the adaptiveness of the functional result. For example, section of the nerve followed by rotation of the eye through 180° leads after recovery to permanently reversed visuomotor reactions - permanent unless

the eye is rotated back to its original position.

The extact degree of specificity which must be postulated to account for these results is uncertain. The difficulty lies in part in determining the precision of visual localization in Amphibia, and in estimating the degree of precision recovered after regeneration. The projection of retinal loci on the optic tectum is less ordered than in man, but just how much less is not accurately known (28). The behavioral tests used by Sperry (e.g., the ability to capture a moving lure) only permit a coarse estimate of the range of the specificity involved. At a maximum, it would be the equivalent of between 13 and 15 bits since there are some 10 to 30,000 fibers in the optic nerves of the salamanders and frogs studied (29). If, however, a given regenerating axon had merely to select any one out of a clustered group of, say, about 100 almost functionally equivalent midbrain neurons, this would reduce its task to the equivalent of some 7 to 9 bits. If most of the fibers from its 99 adjacent ganglion cells terminated in a statistically orderly geometrical pattern in the same cluster of 2nd order neurons, then the ability to distinguish among a maximum of 100 to 300 retinal loci would be recovered. This number seems barely sufficient to account for Sperry's observations. Assuming a retina with a surface area equal to one-half that of a sphere 5 mm. in diameter, 300 equidistant loci would lie roughly 360μ apart. A lure the size of a fly held 15 cm. from the eye would produce a retinal image with linear diminsions of some 200μ. With only 300 loci, the visual image would be very coarse

gained but a moving lure might be localized with sufficient precision to permit an accurate strike. Actually, the loci are probably not equidistant; in the area centralis where objects are normally perceived with greatest acuity, distinquishable loci, both before and after regeneration, are doubtless much more densely concentrated. But this does not alter the essential character of the problem and we can only conclude that a closer estimate will depend on a more refined quantitative analysis of such things as behavioral responses, retinal organization and projection on the midbrain and motor nuclei.

Sperry has also studied the regeneration of sensoricentral connections in the vestibular, cutaneous and proprioceptive systems (26). In each case, behavioral patterns after regeneration is complete, again indicate an orderly, systematic reestablishment of connections leading, at least approximately, to a recovery of the original synaptic pattern. Presumably, therefore, sensory neurons in general are specified and are thereby able to discriminate rather precisely between various central neurons. There are at present no quantitative data on which to base an estimate of the degree of specificity involved. As a guess, it is probably less than that of the optic system.

The matching specificity of the 2nd order central neurons which this conclusion requires is more than an inference; it can be demonstrated experimentally (26). Section of the brain stem between the mid and hind-brain severs (among numerous others) the tectobulbar and tectospinal

tracts which are composed of 2nd order fibers conducting visual impulses into the motor centers of the medulla and cord. Despite conditions favoring extreme intermixing of fiber types during regeneration, synaptic reconnections are nevertheless made in an orderly fashion (cf. Fig. 4D). The recovered visu-motor responses are normal unless, for example, the eye has meanwhile been rotated. Then, the responses are reversed.

B. Conclusions

On the basis both of the evidence considered here and of other results which will not be presented in detail, Sperry has constructed the following working hypothesis. During development (Fig. 4A) various peripheral structures (retina, skin, joints, muscles, etc.) "spontaneously" acquire a highly refined, field-like specification. Outgrowing peripheral nerve fibers reach roughly appropriate end stations due to such relatively unspecific factors as short distances and mechanical guidance. The fibers are then specified by the tissues which they innervate. Second, third, etc. order central neurons develop matching specificities by unknown mechanisms, but independently of the periphery. Synaptic relations between central and peripheral neurons are then established in accordance with their respective "chemo-affinities." These synaptic relations remain plastic and subject to readjustment (e.g., after experimental misconnection of center and peripheral organ) for periods of time which vary in different regions of the body and in dif-

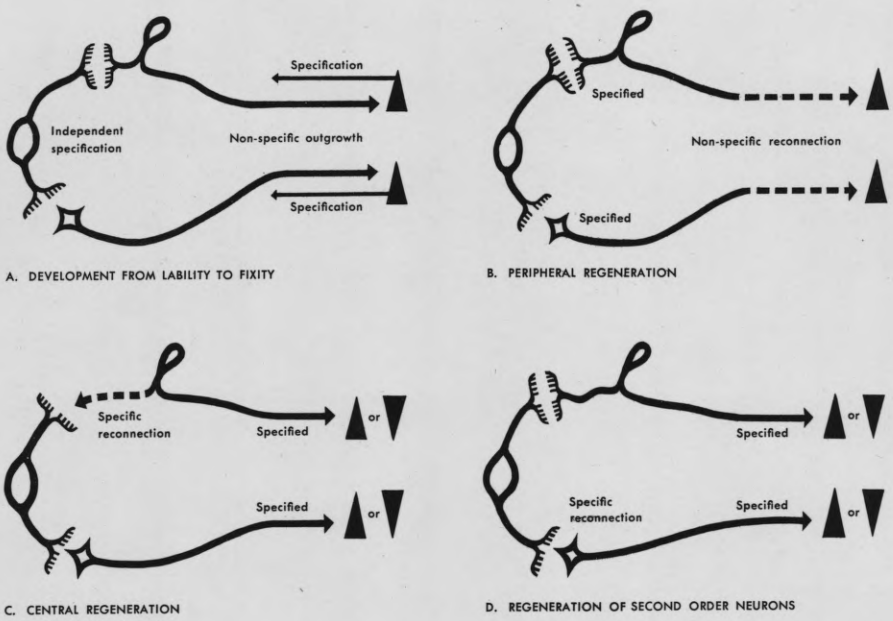

Fig. 4 Specificity of nervous connections

ferent species. Eventually, the specification becomes fixed. Thereafter, neurons retain their local sign properties despite the fact that, following their severance and regeneration, severely maladaptive function may result (Figs. 4B, C and D).

Despite the relatively large amount of evidence which underlies this hypothesis, it is evident that the origin and the number of controls of neural organization can only be considered in vague generalities. Considerable and detailed specificity seems to be an essential inference. In the best known case - the visual system - the arguments presented above suggest that the specificity involved may be about 7-9 bits. This implies that the controls are not so complex as to make further analysis hopeless.

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INTEGRATION OF GASTROINTESTINAL FUNCTIONS

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This essay is an attempt to view the alimentary tract as a control system - its components and its channels of communication. It is therefore by no means a complete account of the physiology of digestion. Only a few particularly pertinent and well-studied aspects are discussed. Furthermore, no attempt is made to present the several sides of existing scientific controversies. For the sake of simplicity some very arbitrary choices have been made. If some of these should later be revealed to be erroneous, the details, but not the general principles, of the story must be changed.

The primary function of the alimentary tract is the transfer of nutrients from the lumen of the tract into the circulatory system. For most of the chemical constituents of the diet this process of absorption must be preceded by a certain degree of physical and chemical preparation. The motor activity of the digestive tract, achieved normally through the integrated contractions of circular and longitudinal smooth muscle fibers, serves to propel the ingested food through the canal. During its passage the food is exposed to a variety of secretions of which some contain hydrolytic enzymes. The chemical action of these enzymes together with the solvent action of water is largely ade-

quate to prepare ingested foods for absorption. In general terms, motility and secretion are under dual control - nervous and hormonal. Response to nervous stimulation is swift and incisive, while hormonal control is slow to manifect itself. While it has been suggested that absorption is likewise under nervous and hormonal control, the evidence is not convincing, and one must look elsewhere for an explanation for the control which the organism is known to exert over the absorption process.

Nervous control of gastrointestinal functions is effected through the agency of several intrinsic plexuses - interlocking nerve fibers embedded in the tissues of the stomach and the intestines. These nerve nets are linked to the central nervous system (spinal cord and brain) by two sets of fibers, parts of the parasympathetic and the sympathetic nervous systems. These extrinsic fibers exert a modifying influence on the alimentary tract. However, the intrinsic plexuses are capable of regulating much of the gastrointestinal activity even when the extrinsic supply is interrupted.

While nerve fibers are in intimate contact with the site of stimulation and the responding structures, hormones are liberated by suitably stimulated cells and enter the blood stream, which eventually carries them to a responsive tissue. For this reason response to nerve impulses may occur in a fraction of a second, while it may take several minutes to convey a message via the endocrine system. For further details see Fenton (1948).

The evolution of multicellular organisms from unicellular forms tempts one to speculate about the evolutionary history of biological control systems in terms of the complexity of control at different levels of organization - cell, tissue, organ and organ system. Superficially the gastrointestinal tract would seem to lend itself to such considerations since appreciable information is available about the controls at several levels of activity: the parietal cell and its special task, the secretion of HCl; the stomach and its chief functions of gastric juice production and gastric emptying; the entire gastrointestinal tract and its tasks of movement, secretion and absorption.

Secretion of HCl by the Parietal Cell

In the normal intact animal the control of acid secretion begins in the mouth. Contact of food with the lining tissue initiates the cephalic phase of gastric secretion. Under suitable conditions the sight or the thought of food is sufficient to initiate secretion. This aspect of secretory control vanishes when the vagi are severed. Although this fact led originally to the conclusion that the control mechanism is purely a nervous one, Uvnäs (1942) and others have obtained evidence suggesting that nerve impulses via the vagi stimulate the pyloric region of the stomach to liberate a humoral agent which finally stimulates the parietal cells to secrete acid.

Contact of certain poorly characterized chemical substances (secretagogues) with the gastric mucosa or distension of the stomach wall initiates the gastric phase of

secretion. Both types of stimuli act upon sense organs in the gastric mucosa and may be rendered ineffective by the application of cocaine or the injection of atropine. Further transmission of the signal occurs via two paths: (1) the liberation of the hormone gastrin which presumably acts as a direct stimulus to the parietal cells, and (2) the nervous pathways via the central nervous system and efferent fibers of the vagus. Impulses coming over the vagi under these conditions may act by liberating histamine which then incites the parietal cells to secrete (Grossman and Robertson, 1948). The integrity of the vagal innervation is important in maintaining the sensitivity of the parietal cells to histamine.

Essentially the same stimuli which initiate the gastric phase of secretion also set in motion the evacuation process and thus commence the intestinal phase of gastric secretion. Although the evidence is not clear-cut, it suggests that both humoral and nervous pathways (via the vagi) are involved.

Three mechanisms exist which can effectively diminish the production of acid. The first is a short loop consisting of the parietal cell, its product - HCl, and the inhibition of the parietal cell by its product. The second loop involves the combined action of HCl and pepsin on the gastric contents, hydrolyzing and liquefying, thus reducing the intensity of the stimulus. The third mechanism involves the initiation of gastric motility and gastric evacuation - again serving to reduce the intragastric stimulus.

In addition a special mechanism exists. When fats or hypertonic solutions are brought in contact with the intes-

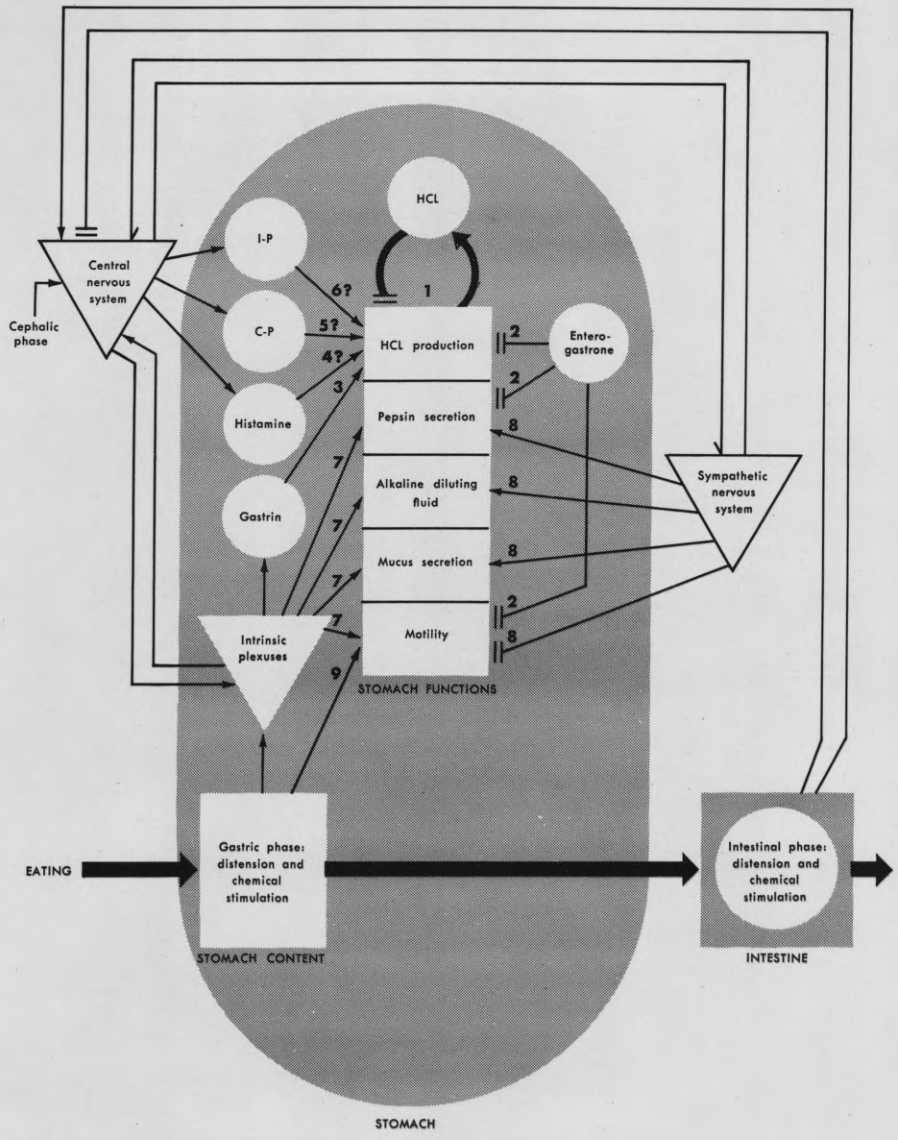

Fig. 2 Control system — stomach

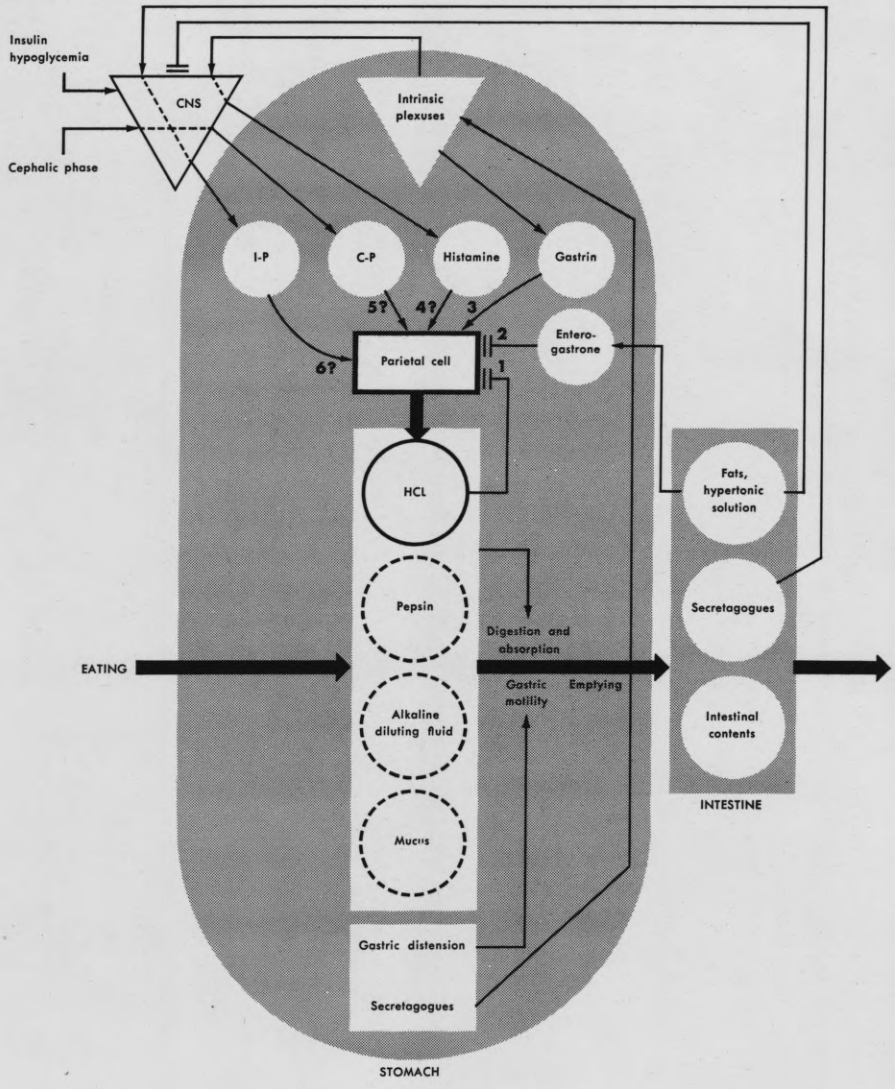

Fig. 1 Control system for parietal cells of stomach

tinal mucosa, acid secretion is inhibited. Nervous and hormonal (enterogastrone) factors mediate this effect.

Two uncertainties combine to make difficult a count of the number of primary control points in acid secretion (Fig. 1). First it is possible that gastrin and the hormones mediating the cephalic and intestinal phases are identical. Second it is conceivable that any or all of the three stimulating hormones act by liberating histamine; the latter could possibly be the final common path in the cephalic, gastric and intestinal phases of secretion. Thus the least number of primary control points is 3; the largest 6. On the basis of present evidence the controls which finally act upon the parietal cell are exclusively chemical.

Functions of the Stomach

In addition to the acid secretion already discussed, the stomach produces the enzyme pepsin (as well as rennain and possibly one or two others). It also produces a slightly alkaline diluting fluid and mucus. Furthermore, the stomach exhibits motility which, in integrated form, is manifest as gastric evacuation. The effective stimuli are distension and a group of chemical substances (Fig. 2).

The controls involved in these complex secretory and motor processes overlap in several important respects, i.e., stimuli which initiate gastric motility also elicit under certain conditions the production of mucus; enterogastrone which inhibits acid secretion also inhibits pepsin output and reduces gastric motility. Thus, in addition to the

controls indicated for the acid producing mechanism, only a relatively few new controls must be added to regulate all secretory and motor activities of the stomach.

Pepsin output appears to be almost entirely under nervous control involving the vagi acting through the intrinsic plexuses. Sympathetic stimulation has some small effect on pepsin secretion. The flow of the alkaline diluting fluid and mucus is stimulated by the vagi and to a lesser degree the sympathetics.

Motility may be initiated directly by distension of the smooth muscle fibers or through reflexes involving the vagi and the intrinsic plexuses. It may be inhibited by impulses via the sympathetic fibers or through the chemical action of enterogastrone.

Thus the control of acid production by the parietal cell has been shown to involve 3-6 control points. The addition of 3 control points and 4 new components permits the stomach to carry out its functions.

Control at the Organ System Level

It is possible to think of the gastrointestinal tract as being composed basically of a multitude of similar functional units having the ability to contract, conduct, secrete and absorb. Obviously the unit must be composed of several cell types: smooth muscle fibers to contract and to conduct impulses; secretory cells to produce alkaline diluting fluid and mucus; nerve fibers of the intrinsic plexuses to conduct and coordinate. These units must be thought of as

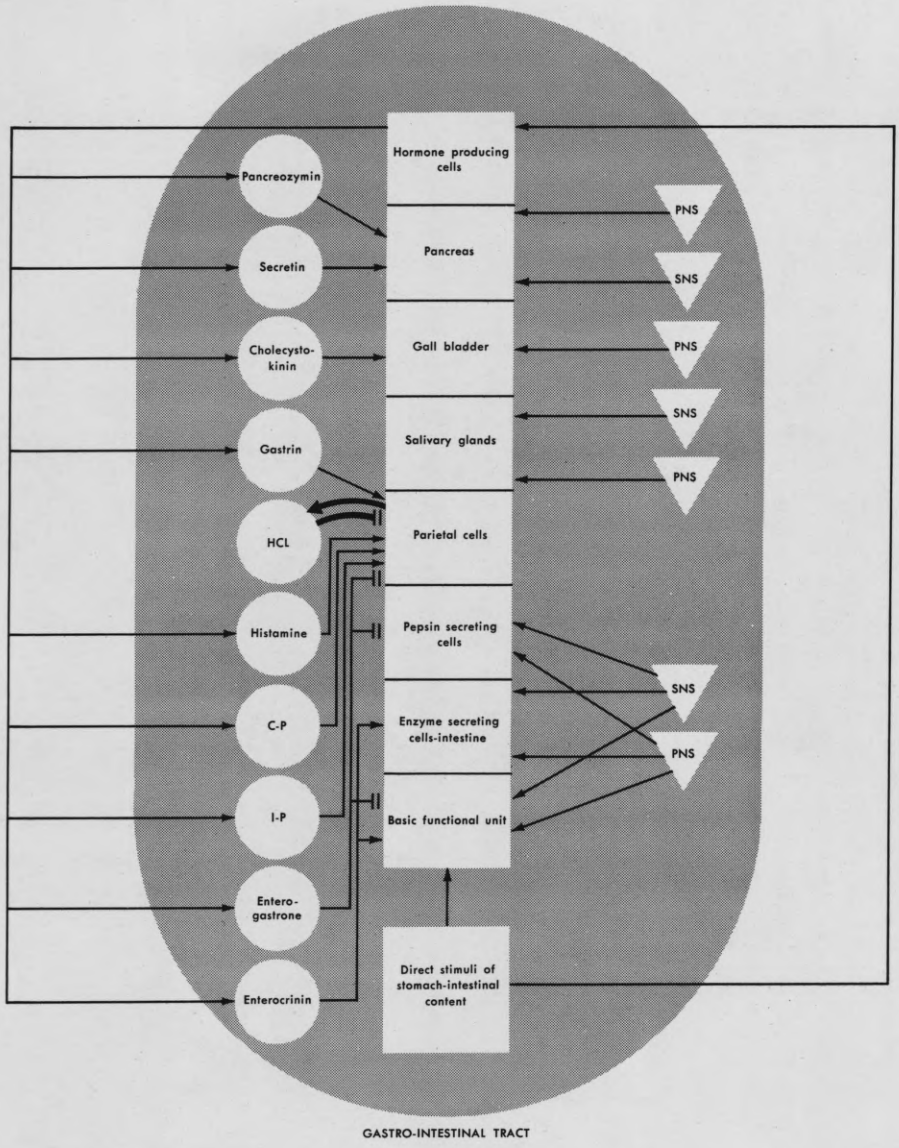

Fig. 3 Control system — gastro-intestinal tract

differing very slightly from one another in a quantitative rather than qualitative sense. They are so arranged that the most reactive units are placed orally with less and less reactive ones following aborally. No specific size needs to be assigned to the unit.

These basic units are controlled by direct stimuli (distension and chemical agents) and by impulses carried over parasympathetic and sympathetic fibers. Chemical inhibition presents a perplexing problem. Enterogastrone has been shown to inhibit gastric motility. It does not seem to have been tested for its effect on the intestinal musculature. It is therefore debatable whether this hormone does have a generalized effect on alimentary smooth muscle or whether it is a specific device for the control of gastric activity.

In addition to the basic units certain special components are built into the digestive system: salivary glands, parietal cells, pancreas, gall bladder, enzyme producing cells of stomach and small intestine, and hormone producing cells (Fig. 3). The total number of control points for the entire organ system must then be the sum of controls for the basic and special units. Corrected for situations in which two or more components are influenced by identical control points, the total count for the entire gastrointestinal tract is 18. Of this number 5 are concerned solely with the secretion of the parietal cells.

Absorption

The driving force in the passage of a nutrient from the gastrointestinal lumen into the circulatory systems is the concentration gradient between the two sides of the absorbing membrane. In some instances this is considerably reenforced by specific cellular processes (i.e., phosphorylation of some carbohydrates) which endow the absorbing membrane with "selective" permeability. It is difficult to count control points since none of the factors influencing absorption rates exert a large enough effect to warrent their being placed in a unique category. It is equally difficult to arrive at an accurate count of the total number of forces which exert some small effect on the absorption rate. A minimum value might be about 24. This is in sharp contrast to closely controlled processes such as HCl secretion where a very few control points modulate activity between zero and some unknown maximum in order to meet fairly specific requirements. Absorption is in a sense a clean-up job whose actual rate is not of great significance to the organism. While the process can occur even with a dead membrane, it is somewhat refined by cellular participation.

Components and Controls

Looking upon the gastrointestinal tract from the standpoint of control systems, one is immediately impressed by the multiplicity of components carrying out identical functions. Amylase is produced by the salivary glands, the pancreas and the mucosa of the small intestine, each enzyme differing

somewhat from the other in structure but not in function. Three protein-splitting enzymes are produced, two or possibly three lipases. In most of the cases cited the mechanism controlling secretion differs. It is possible to visualize a digestive system in which a single cell type, distributed over the length of the tract, produced all the needed enzymes. The feasibility of such a design is suggested by the simultaneous secretion of three or possibly more enzymes by the acinar cells of the pancreas. Thus, the question becomes "What benefit, if any, is derived from the seemingly happazard duplication of components and controls?" Ome thought suggests itself immediately: no disease seems capable of abolishing all digestive activity of the alimentary tract. This may well be because the diversity of components and controls makes it unlikely that all secretory structures be simultaneously affected.

A comparison of components and controls must take into account the complexity of the task. In the case of the digestive tract the task is threefold: hydrolytic cleavage of food constituents, motility and absorption. Conceivably all this could be accomplished with fewer than the eight components listed earlier.

The fact that eight components are regulated by 18 control points is in harmony with the impression that the control of gastrointestinal activity is not very precise. As far as adequate digestive function is concerned, it matters little whether secretory or motor activity deviates from the

mean by 20% or so. This is reflected in the high degree of variability encountered under experimental conditions.

The type of control most frequently found to operate in the digestive system is of some interest because of its simplicity. Almost without exception the response to a given stimulus reduced directly or indirectly the intensity of the stimulus.

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CONTROL IN PROTOZOAN MORPHOGENESIS

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That orderly, reproducible developmental sequences in living organisms require operational control has long been suspected. Experimental demonstration of such control, and elucidation of its nature, has become increasingly possible during the last few decades. This report describes a specific case of this kind, i.e., morphogenesis in the heterotrich protozoan Stentor coerulsus (cf. Weisz 1951).

I. The Morphogenetic Repertoire of Stentor

Stentor may be affected by three categories of situations which elicit a morphogenetic response:

1. A severely disadvantageous environment bringing about loss of body parts. The response to this is regeneration: loss parts are redifferentiated from the unaffected remainder, in correct size, location, and function.
2. A moderately disadvantageous environment through which optimal internal operating conditions are disturbed. Crowding, starvation, chemical irritation, mild injury, and a variety of other stimuli are in this category. The response to this is reorganization: existing differentiated structures are resorbed, and a new set is developed in replacement. The animal acts as if a

freshly formed set of structures could circumvent the imperfections of the old, whether these imperfections are explicit (through injury), or implicity (through blocking of function).

3. A favorable environment which allows undisturbed growth and the execution of all life functions. The response to this is reproduction by fission: a second whole set of structures is newly differentiated and the two morphologies then cleave.

It is now established that the developmental aspects of these three processes are identical, both in pattern and in mechanism. In other words, Stentor is capable of only a single, unique morphogenetic response, which can be elicited by a large variety of different stimuli, and which may be paralleled by a variety of other events, depending on the nature of the stimulus.

In the execution of this response, two groups of structures are involved principally. One is the infraciliature, the second is the nuclear apparatus.

The infraciliature (Fig. 1) is a cortical system composed of longitudinal kineties. Each kinety is made up of a fine fibril, the kinetodesma, and of a row of kinetosomes running parallel to the kinetodesma. Kinetosomes are the basal gransules of the body cilia. These granules are now known to be the critical controllers and maintainers of the diagnostic morphology in ciliate protozoa. Certain kinetosomes are particularly and specifically involved in morphogenesis. In

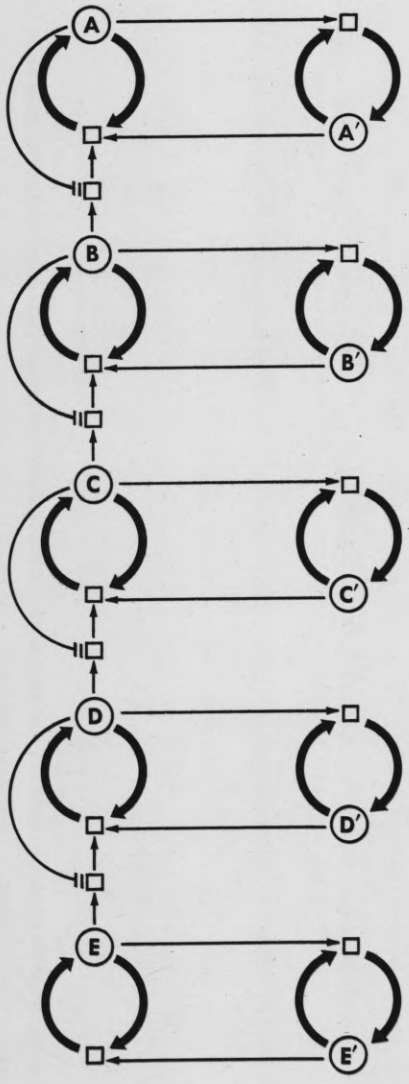
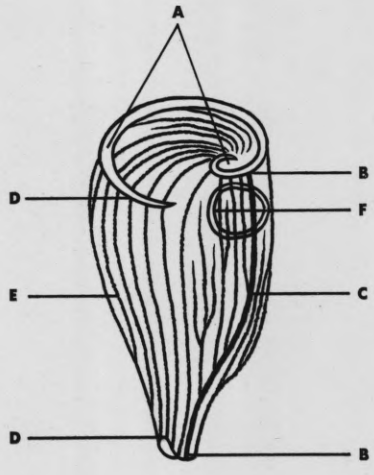


Fig. 2-Control system for morphological maintenance in Stentor



A. gullet and peristome kinetosomes
B. terminal kinetosomes of Kinety 1
C. non-terminal kinetosomes of Kinety 1
D. terminal kinetosomes of other kineties
E. non-terminal kinetosomes of other kineties
F. contractile vacuole

Fig. 1 Morphology of Stentor

Stentor, these kinetosomes are located in kinety I (Fig. 1).

The following generalizations may be made. Kinetosomes are self-reproducing, and cannot arise de novo. Their functioning depends on nuclear influences (v.i). When a kinetosome divides and gives rise to a new one, the latter is at first undifferentiated. Soon, however, it differentiates in one out of five ways, depending on the micro-environment in which it finds itself. It thus adds to one of the five populations of kinetosomes which can be distinguished in Stentor. These populations may be described as follows:

Group A. Kinetosomes of the gullet and peristome, and of the holdfast

" B. Terminal kinetosomes of kinety I

" C. Non-terminal kinetosomes of kinety I

" D. Terminal kinetosomes of all kineties other than I

" E. Non-terminal kinetosomes of all kineties other than I.

Under normal circumstances, a new kinetosome formed in the C group, for example, differentiates like all C's and adds to this population, i.e., kinety I grows in length. Similarly for the other groups. But a new kinetosome formed in one group is potentially able to assume the status characteristic of any of the groups above it in the classification. Thus, a new D kinetosome may potentially differentiate like a C kinetosome, or first like a C, then like a B kinetosome; or first like a C, then like a B, then like an A kinetosome. That this is so is shown in morphogenetic studies. When all

A kinetosomes are excised (as before regeneration), or when the functioning of A kinetosomes is interfered with (as before reorganization), then kinetosomes newly produced by B may differentiate into new A's (as well as into more B's). Evidently, the A's normally inhibit the B's from producing more A's. It can be shown also that B's similarly inhibit C's, C's inhibit D's, and D's inhibit E's. In general, as one population is removed, the next lower is permitted to fill the empty niche.

It will be recognized that the series A to E describes a hierarchy or dominance through which Stentor not only retains its normal morphology, but also restores this morphology should it be partially destroyed by environmental vicissitudes.

As noted above, kinetosomal functioning depends on nuclear influences. In ciliates generally, the micronucleus primarily subserves germinal and the macronucleus a somatic function. In Stentor, the micronucleus may be excised without interference with morphogenesis (although, of course, complete fissional cleavage requires micronuclear mitosis). It is the beaded, elongate macronucleus which maintains kinetosomal functioning. Experiments suggest that for every kinetosomal population there exists a specifically correlated macronuclear agent. Thus, maintenance of A would require a (presumably chemical) signal a' from nuclear agent A' (which is not necessarily a gene but might be a genic derivative). B', similarly, would send a signal b' to which B responds; etc. If A is excised, new kinetosomes produced by B can

differentiate into new A by virtue of the fact that a' signals are no longer used by A, hence can be used by B-derivatives. In the normal organism, A and B-derivatives compete for a', with A having the advantage.

A converse maintaining influence from kinetosome to nucleus also can be demonstrated experimentally. Thus, continued operation of A' depends on a (chemical) signal a from A. Analogously for B', C', etc.

With these data, a diagram as in Fig. 2 can be constructed. It depicts control sequences in the normal, undisturbed organism, and it indicates the consequences when either the kinetosomal or the nuclear apparatus ceases to be optimally functional. To arrive at a measure of the number of messages involved in the above control scheme:

1) Each kinetosomal population sends a message with address "nucleus" and the further specification "act on A'" or "act on B'", etc., i.e., differentiated from four others in this class of messages. This amounts to five distinct signals.
2) Each nuclear agent sends a message with general address "kinetosome," specifying differentiation into one of the five classes of kinetosomes. This amounts to five distinct signals.
3) Kinetosomal population A sends a message "inhibit B," with specifications distinguishing this message from three others produced by B, C, D (reading "inhibit C" "inhibit D," "inhibit E," respectively). This amounts to four distinct signals.

Thus, it takes 14 distinct signals to control and maintain the morphogenetic repertoire of Stentor. These signals are given off into a freely flowing cytoplasm, that is, they are broadcast. This implies that both address and order must be incorporated into the signal itself. Consequently, each signal must have enough specificity to provide distinction from 13 competing ones.

II. The Pattern of Morphogenesis

How does morphogenesis take place, and what kind of control is involved here?

Experiments have shown that the necessary and sufficient stimulus for any of the forms of morphogenesis in Stentor consists of some efficiency-reducing effect on gullet and peristome, or the holdfast (i.e., kinetosomes A). In mechanical injury, A kinetosomes may be removed bodily; in chemical injury or irritation, A kinetosomes may be rendered inoperative physiologically; in crowding or starvation, the organism does not obtain enough food, as if A kinetosomes were in effect injured. Also, if kinety I (kinetosomes C) is cut across so that the cuts cannot fuse (as in fission, and under certain experimental conditions), the posterior body parts are being physiologically separated from the anterior. Thus the terminal kinetosomes of the cut kinety I assume B status, as if A were not present at all, and a new, second A population develops: hence normal doubling of structures as in fission, or abnormal doubling as in monster formation.

It may be recognized generally that morphogenesis starts

when the A inhibition over B is removed, by external stimuli or by a particular (so far unspecifiable) internal condition leading to fission. Once B is deinhibited, the kinetosomes in this group begin dividing rapidly, utilizing the now unused A' materials from the nucleus (cf. Fig. 2). Newly formed kinetosomes are so numerous that they are "squeezed out" of kinety I and accumulate as an extrakinetal "anarchic field." Some unknown stimulus subsequently halts the growth of this anarchic field, and triggers its rearrangement into orderly double rows of kinetosomes. These rows are the "adoral zone," the foundations of the incipient gullet and peristome.

Experiments show that the anarchic field has a direct inhibiting influence on the kinesis of the nuclear apparatus. Specifically, the macronucleus is inhibited from contracting into a ball, and the micronucleus is inhibited from undergoing mitosis. As will be seen presently, macronuclear contraction is a later feature in all morphogenetic events. Micronuclear mitosis, presumably stimulated by the same internal metabolic condition which initiates the fission process as a whole, is prevented by the anarchic field from occurring too soon.

As the anarchic field converts into the adoral zone, its message to the nuclear apparatus changes in character: contrary to the anarchic field, the adoral zone exercises a positive contractive stimulus on the macronucleus, which overrides the inhibition by the anarchic field. (It may be noted that as the adoral zone increases in size, the anarchic

field decreases correspondingly; the same changing relation presumably holds for the strengths of inhibitory and stimulatory signals sent by these structures.)

This control over macronuclear contraction can be demonstrated as follows. If the adoral zone is excised as soon as it begins to appear, nuclear contraction never starts: anarchic field inhibition is now dominant. If the adoral zone is excised after nuclear contraction has begun, the contraction stops at the moment of excision. If the anarchic field is excised before nuclear contraction has begun and before conversion to adoral zone has started, contraction is never initiated (and morphogenesis does in fact not occur under these conditions). If the anarchic field is excised after nuclear contraction has begun (i.e., after some adoral zone is already present), contraction continues normally. Finally, if both anarchic field and adoral zone are excised, nuclear contraction also continues normally: the stimulating agency, although removed, has already been effective, and the inhibiting agency, also removed, can no longer be effective.

Concerning the micronucleus, it is doubtful if the adoral zone has a positive stimulating effect. Mitosis occurs precisely when all of the anarchic field has been converted to adoral zone, indicating that this process may simply occur as soon as an anarchic field inhibition has been removed. Clear cut evidence on this is not available. If there exist inhibitory effects of the anarchic field on the micronucleus,

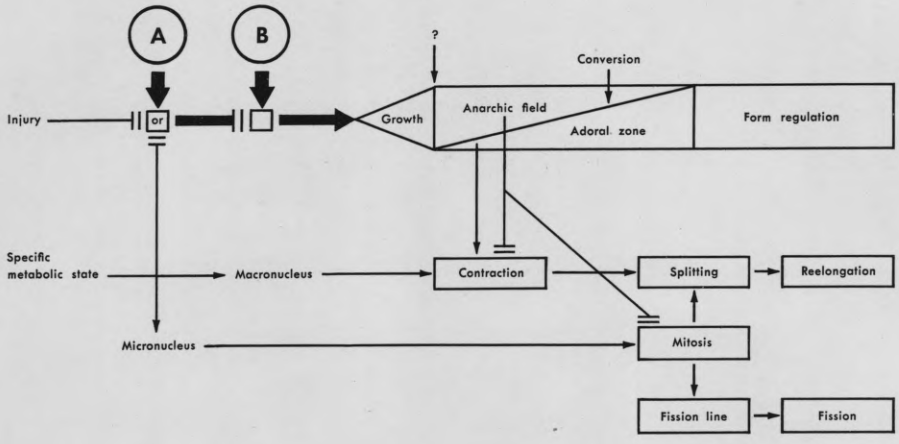


Fig. Control of morphogenesis in Stentor

then they would occur in regeneration and reorganization as well as in fission. But only in fission does mitosis take place. As has been noted, fission is distinguished from other developmental sequences by some unspecifiable internal metabolic state, which may make the micronucleus initially reactive. Such reactivity would be absent in regeneration and reorganization.

Once mitosis occurs, two other events follow more or less simultaneously. First, the macronucleus, at this time already past full contraction and beginning to reelongate, splits into two lengths, which represent the macronuclei of the presumptive daughter individuals. This splitting apparently depends on some stimulus from the dividing micronucleus. Second, a fission line becomes explicit visibly: all cortical structures dissolve along a thin line running around the "waist" of the already doubled organism. A "physiological" separation of the two presumptive daughters must have occurred at the very start of the whole fission process. The mechanical, visible separation occurs much later, apparently under a definite stimulus from the dividing micronucleus.

As the fission line is laid down, cytoplasmic constriction begins. Within a short time the morphologically double organism is pinched into two and fission is completed. Since, in morphogenesis other than fission, mitosis does not occur, macronuclear splitting and fission line formation also do not occur.

The above data are summarized in Fig. 3. From this diagram the number of signals required in the control of the course of morphogenesis may be estimated.

(1) One message is required for general morphogenetic stimulation, i.e., injury. Its address is "kinetosome A" and the instruction reads "inhibit." In the case of fission, the signal also goes to the micronucleus, reading "activate." B now is permitted to produce an anarchic field.

(2) The latter sends a signal with address "nuclear apparatus," and with the instruction "inhibit kinesis."

(3) An unknown signal arrives at the anarchic field reading "stop growth and start organizing."

(4) The adoral zone, newly forming, sends a signal to the macronucleus reading "contraction."

(5) The micronucleus, once activated and deinhibited, sends a signal for "splitting" to the macronucleus and for "fission line formation" to surface cytoplasm.

These amount to at least five different messages, which must be distinctly differentiated from one another.

Thus all aspects of morphogenesis in Stentor together involve 19 distinct signals - not too high a requirement for as vital a process as differentiation and development.

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EVOLUTION OF BIOLOGICAL CONTROL SYSTEMS

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In developing a Science of Organization, with its attendant body of knowledge and theory, different avenues of approach should be investigated. Evolution is one avenue which may add only little to the content of the science but could add appreciably to the conceptual basis of the science. The following tentative discussion along this line is divided into two distinct aspects: 1) the probably evolution of control systems; and 2) the use of the evolutionary approach in understanding why the systems have evolved in certain ways.

Evolution of Control Systems

The basic type of biological control system probably consists of essentially similar components which form a size-regulating system operating by a simple feedback mechanism as shown in Fig. 1.

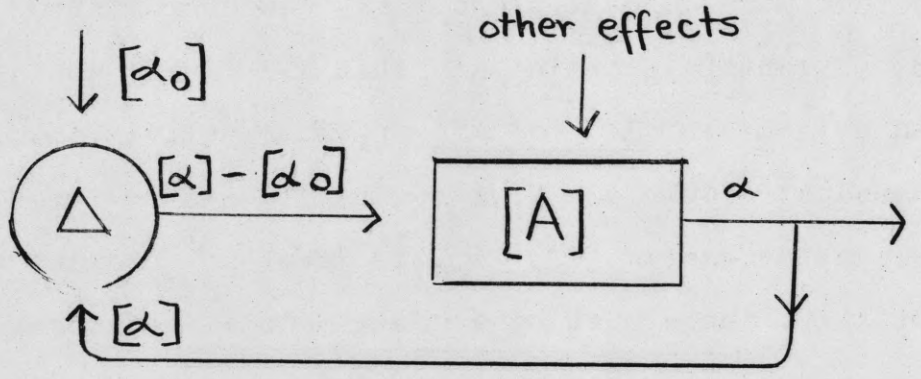

Fig. 1

A is a population of duplicating units (such as individuals, or cells, or molecules). The units A produce a substance α which is given off into the surroundings (possibly directly into a neighboring unit of the same type). The concentration of this substance will depend on the rates of production and removal; to each amount of A, $[A]$, there corresponds an equilibrium concentration, $[\alpha]$. Now α is inhibitory on A; the speed of growth decreases with $[\alpha]$ and stops completely at $[\alpha_o]$. If $[\alpha]$ grows faster than $[A]$, then the population A will reach an equilibrium size $[A_o]$ at which it yields an equilibrium concentration $[\alpha_o]$. If some units are removed (by death, migration, etc.), such that $[A] < [A_o]$, then automatically $[\alpha] < [\alpha_o]$, and the population will grow back to equilibrium size. Control systems of this type are postulated to regulate sizes of tissues, populations, organs, etc. (Weiss, 1952, 1953, Rose, 1952; Chase, 1952).

The orderly growth of a complicated structure is hardly possible without the existence of size-controlling systems. This does not at all imply that the need for such systems is the cause of their origin. Indeed, it is easy to show, by reference to some more general principles, how such systems must develop. The basic principle is that all living units are inhibited by some or all products of their activities. If any such product accumulates faster than it can be removed, then the feedback mechanism (cf. Fig. 1) develops automatically. Potentially, there must be a great many size-controlling substances associated with any unit A. The one among them which reaches the critical concentration earliest will assume

the function of size-controlling. It could also occur that changes in conditions could shift the control from one substance to another.

If several kinds of units form a complex system, various possibilities of interactions arise. For instance, a unit of one type, A, can be inhibited by some product of a unit of another type, B, before any of its own products reach critical concentration. On the other hand, B could also counteract some limiting substances of A, and thereby act as a stimulating agent. Thus, the development of a circulatory system raises the limiting size for tissues and organisms. As systems comprise more different kinds of components, a greater variety of interactions becomes possible. Thyroxine, for example, is probably not used in self-regulation of thyroid cells but is clearly an important substance in the regulation of other cells.

Control mechanisms exist, of course, which regulate functions and not the size of a population of components (cf. Fenton's examples on gastrointestinal functions in this volume). One example is of particular interest because it indicates the "accidental" nature of an evolved system. This is the case of respiratory control in mammals. The oxygen tension available to the cell is maintained by an elaborate system of respiratory movements, moist membranes and circulating blood. This system requires control, and one would naturally expect that the control mechanism responds to the oxygen concentration. This is not the case. The substance controlling oxygen intake is the waste product

carbon dioxide, which acts on the carotid and aortic bodies, and thence by nerve impulses on the respiratory center of the lower medulla of the brain. Ordinarily, the result is the same, but in exceptional situations when the oxygen tension can get dangerously low without CO_2 accumulation (e.g., high altitude, circulatory failure) the system fails to react properly.

Insects would appear to have more completely self-regulatory systems and fewer inter-related systems of control, i.e., are less unitized (as individuals) than are mammals, for instance. Returning to a consideration of size control, and insect society may be used as an example. In termites (Emerson, 1939; Castle, 1934) the proportion of soldiers, workers, and reproductive forms is maintained by the exchange of chemical exudates. When soldiers are destroyed and their exudates thus reduced, more young forms develop into soldiers until the normal balance is restored. As might be expected, extracts from soldiers can be introduced to the colony and thus prevent the formation of the normal proportion of soldiers. Perhaps self-regulatory mechanisms of similar components are basic and inter-dependent systems have evolved, when useful, by the extension of such mechanisms.

In the course of evolution, with more specialized divisions of labor, interactions and hierarchial control have modified or replaced self-control; but the total control mechanism becomes in turn essentially self-regulatory if viewed at a higher level of organization. Just as "self-duplication" is a misnomer but really means that a duplicate

can be formed if the correct substrates, enzymes, etc. are present, so is "self-regulation" a misnomer but nevertheless a useful expression, meaning that an element will exercise self-control if proper conditions are imposed. Diffusion rates, available substrates, decay and competition of anti-metabilites, are conditions imposed by the cell on its nucleoproteins, etc., by the organ on its tissues and cells, or by the termite colony on its proportion of reproductives, workers, and soldiers.

Regulation is not always manifest. Thus, in ecological succession the only true self-regulatory group is the climax stage, as the climax beech-maple forest. There would seem to be no self-regulation in most single species but rather regulation of a multi-species system, such as predator-prey or disease epidemics. There may, however, also be potential self-regulating mechanisms within the species due to stresses from excessive rates of reproduction. Broadly speaking, self-regulatory mechanisms operate only if substrate or food supply are not the limiting factor for size or number. Man, by cultivation, halts ecological succession for his non-climax domestic plants; by wild life management, attempts to restore normal population cycles; and by birth control, regulates his domestic animals at a level below the food supply limit, and presumably could regulate himself. His success is certainly less than spectacular but the degree to which he is successful depends on an awareness, if not complete understanding, of the nature of the organization of systems.

The Evolutionary Approach

Evolution means descent with change. In biological evolution, the critical process is change of gene frequency; the basic mechanisms are mutations, supplemented by natural selection and random drift. Such statements, however, do not constitute by themselves the evolutionary approach. Principles which are derived from the study of evolution give a better idea of the meaning of the evolutionary approach. These principles are generalizations derived from the study of biological material. To what degree they extend to evolution in general is not a subject of the present discussion.

The following principles will be discussed: 1) trial and error course of evolution; 2) rules of change; 3) co-operation vs. competition; 4) correlative adjustments; 5) replication of parts; 6) generalization vs. specialization; 7) evolution vs. "success." Most of the examples to follow will come from evolution among the vertebrates. Their fossil records are relatively complete since their evolution is all in post-Cambrian times.

(1) The Darwinian, or neo-Darwinian, interpretation of evolution is that of a random hunt with feedback. Various possibilities are tried out (by mutations) and tested; most will be rejected by natural selection; a few, accepted. The trial and error course of evolution is evident in the many "attempts" which are made and is exemplified by the asymmetry of evolution. In the very rapid evolution of the horse, there were many different combinations, especially during the Oligocene and Miocene. Some contemporary, or certainly non-

lineal, forms had reduced toes but still browsing teeth, others had complete 4 and 3 toes but grazing teeth, others gained elongated skulls but still had browsing teeth and complete toes, etc. Evolution does not generally show a straight line development but rather a series of trials, most of which become extinct, even though some may persist for several million years.

(2) Another set of principles can be described as the rules of change in evolution, the essence of which is that structures do not occur de novo. The rules are that there can be i) increase in complexity of structures already present, ii) change of function for structures present, and iii) degeneration or loss of structures. Increase in complexity is seen in the increased lobation of the lung, the increased compartmentalization of the heart, the increase of neurones in the telencephalon of the brain, etc. Change of function has been very important in evolution. Paired steering organs from lateral folds have become 5-toed feet for locomotion on land. The three middle ear bones of the mammal are derived from the cartilage bones involved in jaw suspension. The diverticulum from the pharynx which developed into a simple lung in some fish and amphibia became a closed hydrostatic organ, the swim bladder, in other fish. The food straining mechanism of primitive chordates became the respiratory gills of fish. The same gill arches became part of the voice box and tongue support in mammals. The food gathering, mucus-producing portion of the pharynx of the primitive chordate also accumulated iodine and produced

thyroxin. It lost its food-gathering function but is retained as the thyroid gland. The carotid and aortic bodies which are sensitive to the concentration of CO_2 in the blood are remnants of the earlier aortic arches. Feathers which are important for maintaining body temperature and for flight appear relatively suddenly in the Jurassic but are derived by certain modifications from reptilian-type scale follicles. Certain scutes of the skin over the head have become incorporated as the roofing bones of the skull in amphibians, reptiles, birds, and mammals.

A few cases where marked changes seemed to arise de novo have been reconciled with the rules of change by physiological genetics. For instance, there is a mutation in Drosophila the result of which is the appearance of a leg instead of an arista on the antenna. It has been shown that the mechanism is simply a gene-controlled disturbance in timing such that the anlage of the arista reaches the stage of differentation at about 2 days earlier than normal and at the time when foot parts are being differentiated.

(3) Cooperation is a principle to be found in the works of Darwin, although largely ignored since that time with the emphasis being placed on competition. Cooperative devices within and between organisms have had survival value. A single case will be cited here, namely, the symbiotic arrangement between certain cellulose-digesting protozoa and the wood roach. In the evolution of termite societies, this early symbiotic relationship has been maintained and perfected to the point that the protozoa increase only at the times of

termite moulting. Probably the control is the result of the increased amount of nitrogen available at this time which allows the protozoa to grow and divide (Cleveland).

(4) The principle of correlative adjustments is merely a statement that parts are inter-related in development, so that a change in one part results in changes in other parts. For example, the failure of the eye vesicle to form normally will result in changes in all structures (and functions) dependent on this development -- lack of optic nerve, loss of eye muscles and nerves, failure of areas of the brain to develop, etc. As part of the evolutionary approach, this principle emphasizes the fact that any observed changes may not necessarily be primary.

(5) Replication of parts implies in a sense a new part, but it can be achieved merely by extending the physiological conditions of development for a greater distance. Serially homologous structures are of this type. In a vertebral column there can be changes in the number of thoracic vertebrae (with ribs), of sacral vertebrae, of caudal vertebrae, etc. Serial replications give opportunities for new differentations without interfering with existing functions, as in the case of pronephros, mesonephros, and later metanephros. Bilateral replication may serve as a margin of safety by redundancy or may allow new possibilities such as binocular vision in the primates, but is basically only the result of the embryonic polarity. There is perhaps no more "need" for two kidneys than there would be for two intestines. In some cases, there occurs secondary reduction of one of a pair of

organs, as in the case of the bird in which only one ovary normally develops to its functional capacity.

(6) A useful dichotomy is generalized vs. specialized if the word "generalized" is used in a relative sense. Man is a relatively generalized animal compared with the horse or whale. Structurally man is close to the general "mammalian plan." Even his excessive development of the anterior end of the nervous system which gives him his dominance, is not a specialization in the usual sense. A general evolutionary rule is that new major adaptations arise from the more generalized forms, and not usually from "higher" specialized types. The stem forms of amphibians, reptiles and mammals (Devonian to Permian) were more alike than present day representatives of those classes.

(7) The last principle to be discussed here is the relation between evolution and "success." Evolution implies lack of success in a particular environment. Organisms which are not successful in a new and changed environment either become extinct or evolve. The brachiopods which changed little from the Ordovician are clearly successful within their range of environmental conditions, whereas the chordates evolved rapidly from that period. The mammals appearing in the Permian became nearly extinct during the 190 million years of the Mesozoic, but with the extinction of the major reptiles, they are having their turn at specializing and becoming extinct in the various environmental niches.

Conclusions

The generalizations described do not form a neat system of well-defined axioms. They are partly overlapping, they are not comprehensive; they are to be considered as present-day attempts to deal with an immensely complicated process. We wish to point out some consequences of the evolutionary approach; it implies an awareness that parts are made over, that some parts and functions are relics of past history, and that perfection is seldom acquired. It means that, although living systems may work, they are not necessarily the ultimate in perfection, and that they are to a large extent redesigns rather than new designs. Man, as an extremely recent and relatively unspecialized product of evolution, is not the organism most suited for the study of maximum refinements in most biolobical control mechanisms. His extensive cerebral development, possible only with such an undistinguished and unspecialized ancestry, is the most intricate mechanism of its sort yet attained in evolution on this earth. Again, however, it is not of de novo origin but rather an extension and elaboration of living structures and mechanisms already present.

Life has existed for one to two billion years through many vicissitudes and has thus obviously adopted certain rather efficient basic units and associated control mechanisms. Later and special modifications, however, may often be less efficient and certainly can be expected to carry more non-adaptive characteristics. Is the cerebrum of man an exception? The basic control mechanisms within a cell

probably represent more nearly the perfection of biological control systems than do the mechanisms which control gastrointestinal functions or control temperature regulation in man, for instance.

In comparing biological control systems with engineering control systems, caution must be observed. Nevertheless, an appropriate analogy is that of redesigning and extending earlier models rather than of producing a completely new design. Biological structures and mechanisms are essentially conservative and "make-shift;" they are adequate but not generally "fancy."

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