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CELL-CELL RECOGNITION AND INTERACTION

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A theory is proposed for the basic mechanism related to cell-cell interaction and cell recognition. A conceptual model-system is developed containing an enzyme system synthesized by a group of genes containing four operons. Activity of operons is controlled by regulatory metabolites derived from adjacent cells. Cellular recognition arises from 'metabolic decoding' external substrates produced by exoenzyme system. Mathematical formulation of the model-system is carried out and analyzed on analog computer. Kinetics of operational processes are presented for isotypic and macrophage type of cellular interactions.

1. INTRODUCTION

Within a complex biological system, a cell represents the basic functional entity. Formation and development of functional systems requires that cells should grow, differentiate, and multiply in selective and ordered patterns. Such process presumes high degree of cellular interactions. Interplay between various cell groups and maintenance of stable systems presupposes that cells should be able to 'recognize each other'. Furthermore, metabolic and synthetic activity of a cell would be influenced also by the adjacent cells, and there should be intercommunication (Loewenstein and Kanno, 1966). Cellular interactions could be cooperative, where homologous cell types are organized into a functional community, or these could be destructive where cellular disintegrations occur. The latter would be represented by the macrophage action as well as by some other cells which have lost their normal functional pattern. It is self-evident that cellular recognition phenomenon, combined with specific reactive patterns, presupposes that there is an interchange 'information' between the cells. At the present time cell-cell interactions are experimentally documented; however, how such interactions lead to cell-cell recognition and which are the underlying basic mechanisms in such processes are not known. The purpose of this paper is to develop a theory for basic mechanisms involved in cellular sensing, information exchange, and recognition. Furthermore, a *conceptual model-system* to simulate cell-cell interaction processes will be developed, formu-

lated mathematically and analyzed on analog computer. Thus kinetic data will be obtained describing specific functional processes during the cell-cell interactions. In order to develop a theory of 'cell recognition' many basic assumptions have to be made for which there is no *direct* experimental evidence. However, theory and model-system are derived on the basis of experimental information in the literature. In this process there is no need to develop new conceptual entities as being essential for 'information exchange'. Well-known cellular processes and basic functional entities organized into specific functional patterns are sufficient to provide mechanisms for cell-cell recognition and subsequent interactions. In order to set up basic postulates for the theory, a short literature review will be carried out dealing only with subject material considered to be pertinent for such process. Much of the experimental material will be used directly for development of the theory and the model-system. However, development of new concepts of information exchange and 'sensing' between the cells requires that metabolic reactions and processes, besides their role as considered conventionally, serve simultaneously as 'metabolic coding and decoding' process, thus providing means for the interacting cells to provide information about each other. While information exchange between the cells is instrumental, producing various reactions with the cells, the first reaction seems to be oriented response due to contact stimuli and locomotion of cells. These processes are of great significance, but the analysis of these mechanisms requires spe-

cial considerations and cannot therefore be dealt with here. However, within the framework of basic theory proposed here, a further elaboration of it could be carried out for development of a model-system for the cellular movement. Most detailed and informative experiments on cell-cell interactions have been carried out in tissue cultures (Moscona, 1962, 1963; Granger and Weiser, 1966; Dingle and Welb, 1965; Karnowsky et al., 1966; Daniel and DeOme, 1965; Gasic and Galanti, 1966). Moscona, who has contributed outstandingly in this field, has presented a thorough analysis and review on the subject (Moscona, 1965). The following summary can be made:

1) Restitution of organized aggregates from free cells can be subdivided into 3 phases: a) Initial 'clumping' of mixed cells into randomly orientated cluster represents 'primary aggregation'; cells are capable of moving in relation to each other and cohesive forces are weak; this probably leads to assortment of cells into more stable systems. b) In 'secondary aggregation' (24-36 hr), cells are arranged into histologically identifiable groupings comprising functionally matching cells; cell mobility is gradually reduced as structural stabilization sets in; cell patterns get complicated at the end of the phase due to onset of rapid cell multiplication. c) During 'tertiary aggregation' growth and histological differentiation proceed, leading to higher stages of development.

2) There is evidence that specific 'inductive' events and interactions between the cells play a role in their association and organization.

3) The behavior of self-aggregating cells reflects a complex interplay between cell-dependent and environmental factors; cells themselves contribute to the interphase within their function by releasing colloidal exudates surrounding the cell and leaving a 'trail' when cells migrate; the question has been raised whether exudate material might provide 'information' for 'self-recognition' between the cells; it was found by tracer studies that colloidal material appearing in aggregating cells was incorporated into the intracellular matrix.

4) Cell contact may be mediated either by 'surface coat-like' materials or by long cellular protrusions and filapoidal extensions; there is increasing evidence that cell surface extends both structurally and functionally outside the double membrane into an extracellular region consisting of mucoidal material; it has been suggested that extracellular materials may be involved in dynamics of cell interactions and their chemical and structural constituents may be re-

lated to cell adhesion and phenomenon of cell specificity.

5) It is considered that transition from non-selective attachment to selective attachment of cells in aggregates could be related to the sequence of synthesis of cell surface materials and their molecular ordering; such sequence would involve first 'initial precursor' products at the cell surface, followed by their arrangement into configurations that can make discriminatory, more stable linkages with corresponding molecular arrays; the characteristic properties of such extracellular products might be a function of biosynthetic pattern of cells and thus susceptible to modification by conditions affecting these patterns.

6) Cellular aggregation is dependent on chemical environment of cell; both puromycin and actinomycin D inhibit aggregation and the aggregation process is temperature dependent; both protein and RNA synthesis is required for the aggregation process.

7) Cells from the same tissue have to be in the same developmental and functional state. Otherwise, there is limited or no self-recognition; heterotypic aggregates in a mixed cell population reveal essentially tissue specific grouping.

Dingle and Webb (1965) have reviewed the mucopolysaccharide metabolism in tissue cultures. It appears that certain mucopolysaccharides (MPS) may be combined covalently with one protein and this mucoprotein (MP) then linked by ionic forces with another. Continuous MPS production occurs in tissue culture during the growth of human subcutaneous tissue. Histological evidence reveals in fibroblasts of intercellular fibrils containing MPS and MP. There is a turnover of MPS. Degradation occurs by enzymes, but these are not yet properly identified. Polysaccharide and MPS complex with the cell surface, bind water molecules and thus have an effect on ionic transport. Therefore, effective 'cell surface' might be at a considerable distance from the classical lipoprotein membrane of the cell. Isotypic cells form nonspecific complexes, pass through a recognition process, aggregate subsequently into stable cell groupings and then start to grow and multiply. In contrast, interaction between a macrophage and a foreign cell or a degrading cell also passes through the 'recognition phase' but subsequently instead of growth, degradative processes take place leading to destruction of both cells. It is of interest to note that the first step in specific adherence of immune macrophages to monolayers of target cells is also a passive phenomenon, but subsequent

mutually destructive interaction requires biosynthetic activities of the immune macrophage. Jacoby (1965) has presented a broad and extensive survey on macrophages. It appears that a positive chemotaxis is exerted on macrophages by injured cells, substances like starch and sugars or dead bacteria. This suggests that a macrophage is capable of 'sensing' its environment. Since macrophage activity represents a basic defense mechanism of biological systems and is in certain conditions also a cell-cell interaction phenomenon, we will attempt to analyse such processes in the framework of our theory and model-system.

2. DEVELOPMENT OF CONCEPTUAL MODEL-SYSTEM FOR CELL-CELL INTERACTION

2.1. Basic considerations

Model-system is developed for the cellular processes and reactions occurring after primary non-specific cell-cell contact leading to 'recognition' and permanent cell-cell bindings. We do not consider initial cellular movements nor cell multiplication. On the basis of our short literature review, the following summary outlines the cell-cell interaction processes:

a) Surface structures of the cell extend beyond membrane regions.

b) External cell boundary region contains various types of mucous materials such as: polymeric carbohydrates, proteins, lipids and cross-products of these; it contains also small degradation products of polymeric materials as well as cellular metabolites.

c) Primary nonspecific contact of isotypic cells leads, after definite delay, into formation of intracellular polymeric matrix and other structural elements, which lead to cellular binding; synthetic processes are required for this, but their initiation is preceded by cell 'recognition'.

d) There is a *turnover* of materials contained in intracellular matrix.

Any theory and model-system developed has to account for these phenomena. While underlying mechanisms of synthetic and degradating processes are in general understood, however, *how* these processes are linked together in a complex set of interactions in cellular aggregation is not known. This concerns especially cellular 'recognition' phenomena, on which subject *no* experimental information is available. Furthermore, there is *no proper concept* developed

in terms of biological or other processes as to what cell-cell 'recognition' means, besides phenomenological observations. Due to such circumstances, we propose to develop a theory as to how cell recognition arises and what is the 'meaning' of it. Furthermore, since basic cell-cell interaction processes are comprehended only in general, one can establish a functional model-system only on a *conceptual* basis. This means that a biological system is synthesized in which processes and mechanisms are organized in patterns and sets of interactions which yield an operational system capable of producing phenomena encountered in cell-cell interactions in the experimental field. Thus a deeper understanding of all important and complex behavior of cells and cellular systems may be obtained.

2.2. Outline of underlying processes

The basic operational characteristics of the model-system are based on the following basic concepts:

a) Cells are capable of 'sensing' each other via an exoenzyme system, which is either structure bound or loosely associated with mucoidal material situated at the peripheral region of the cell; enzymes which are projected out from the cell surface, being part of micro-filaments, are called 'extensor-enzymes' (ee).

b) Peripheral cellular region contains surface attached filament-type entities; or loosely associated mucoidal materials, as well as metabolites and degradation products; these serve as monomeric or polymeric substrates for the exoenzyme system; polymeric substrates extending out of the cell surface are called 'extensor-substrates' (es).

c) When two cells come into contact with each other, ee of one cell acts on the es of the other cell and *vice versa*.

d) Exoenzymes are linked to an enzyme system which transports enzyme reaction products into the cell, where these interact in certain pattern leading to information exchange; imported inducers and repressors exhibit control on synthetic processes.

e) Cell recognition arises basically from 'metabolic decoding' of these exoenzyme reaction products. (This problem will be analyzed specifically in a subsequent reaction.)

f) Intracellular adhesion-matrix is in a state of dynamic equilibrium; structural elements are synthesized and degraded continuously, thus providing constant turnover of materials; these are controlled at the genetic level via external stimuli.

g) Operational characteristics of synthetic systems are affected by metabolic products of cell-cell interactions; such 'information' can lead to cellular dissociation, aggregation or destruction.

3. MODEL-SYSTEM

The model-system (fig. 1) contains 4 basic operons, where each of these provides specific enzymes for the operational system. Exoenzymes are produced by operon O_1 system. Enzyme E_2^1 is non-bound and enzymes E_1^1, E_3^1, E_4^1 and E_5^1 are structure bound. Synthesis of these and other enzymes is represented only by general steps, since the system is too complex for detailed analysis. However, at the present level of information and theorizing, this presentation is considered adequate to reveal kinetic charac-

teristics of the system. Complimentary to the exoenzyme system are the respective 'extensor-substrates' which are surface bound. These are permanent cellular entities and their synthesis is not considered here. Metabolic products resulting from ee and es interactions, are transported into the cell, where they provide by metabolic degrading and rearrangement a decoding of the cell-cell 'recognition mechanism'. The end-products of the decoding-system regulate operon O_1 and O_2 activity. The latter provides enzyme system E_1^3, E_2^3 and E_3^3 to crosslink polymeric compounds P_1^2 and P_2^2 produced by operon O_2 system and thus form an intracellular 'adhesion matrix'. Operon O_3 becomes active only when cells recognize each other (formation of activator a_0^3). Enzyme E_3^3 produces a regulatory compound which increases operon O_4 activity above the ground level and at the same time reduces operon O_1 activity. De-

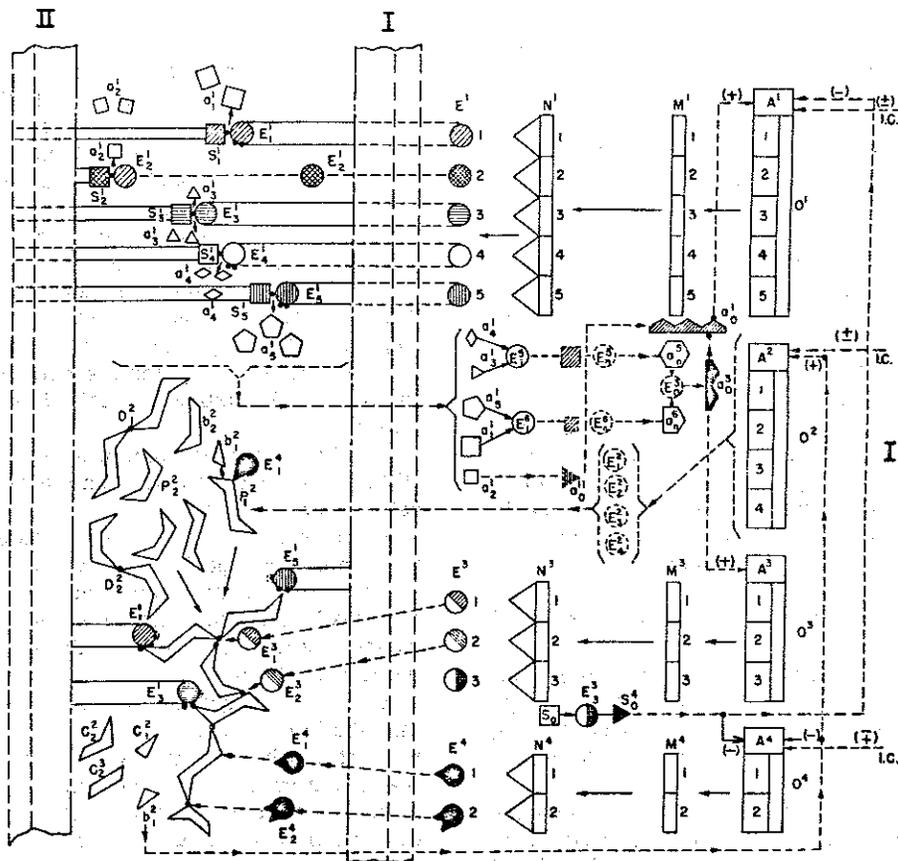


Fig. 1. Descriptive model-system for cell-cell interaction. Symbols are given in table 2.

grading enzymes E_1^4 and E_2^4 breakdown polymeric compounds P_1^2 and P_2^2 . Operon O^4 is controlled by a negative feedback loop. Breakdown products reduce O^4 and increase slightly operon O^2 activity. It is considered that activities of all operon systems are also controlled at a base level internally. These control mechanisms are not considered here. All functional entities are considered to be unstable and consequently degrade continuously.

All entities and respective symbols present in the model-system have been assembled into table 1. Interactions and activities of functional entities are described by flow schemes in table 2. Basic steps can be followed for understanding of operational characteristics of the system. We will outline the general operational procedure and sequence of events when two cells interact. In the normal state, operons O^1 , O^2 and O^4 are active and thus maintain respectively a steady-state level of sensory enzymes ee, polymeric entities P_1^2 and P_2^2 , and degrading enzymes E_1^4 and E_2^4 . When two cells (I and II in model-system, fig. 1) come into contact and recognize each other by a metabolic decoding process, there is an activation of operon O^3 . Interaction between isotypic cells leads to a cross-linked intracellular 'adhesion matrix' (indicated as a complex in eq. 8, table 2B) and permanent cell-cell association. Since the number of interaction steps (presented in table 2A) is large and hundreds of differential equations are required to describe it (which cannot be analyzed with computers available in the market), these have to be reduced by lumping several reaction steps together. This can be done without excessively modifying kinetic characteristics of the system. In addition 'metabolic decoding' is reduced into a single step operation. It provides means to control metabolic and synthetic activity of the system in states of *no*, *partial*, and *complete* recognition. Modification of the overall flow scheme for computer analysis is presented in table 2B. Basic reaction steps which provide the means to reduce the *level* and *activity* of extensor enzymes ee are presented in equations 2 and 8 in table 2B, and eq. 34 in table 2A. A mechanism which reduces the metabolic and synthetic activity of two adhering cells arises from the specific interaction in which ee enzymes are polymerized into a complex (i.e., equations 43-49, table 2A, or equations 2, 3 and 8 in table 2B) and there-

Table 1
Symbols and functional entities.

P_1^2, P_2^2	Polymeric compounds synthesized by cells
D_1^2, D_2^2	Linked polymeric compounds
$b_1^2, b_2^2, c_1^2, c_2^3$	Degradation products of P_1^2 and P_2^2
O^1, O^2, O^3, O^4	Operons for gene groups
N	Templates
M	Messengers
E_1^1, \dots, E_n^1	Surface enzymes attached to extended structures; here called 'extensor enzymes' (ee) which degrade 'extensor substrates' (es) of the attached cells
E_1^2, \dots, E_n^2	Enzymes converting a set of 'extensor enzyme' products (a_1^1, \dots, a_n^1) into gene activators (a_0^1, a_3^0).
E_1^3, \dots, E_3^3	Enzymes polymerizing sub-units (P_1^2, P_2^2) into intercellular 'adhesion matrix'
E^4, \dots, E_n^4	Enzymes degrading sub-units (P_1^2, P_2^2) into smaller fractions (b_1, b_2, c_1^2, \dots)
E_1^5, \dots, E_5^5	Metabolism of: a_1^1, \dots, a_5^1
E_1^6, \dots, E_n^6	
S_1^1, \dots, S_n^1	Extensor substrates (es) attached to, or part of extended surface elements of the cell
a_1^1, \dots, a_n^1	Products of enzyme system E_1, \dots, E_n^1
a_0^1, a_3^0	Products of a_1^1, \dots, a_n^1 metabolites which act as activators for operon O^1 and O^3 respectively
A_1, \dots, A_n	Operon activation sites; plus sign (+) indicates the increase of gene activity and minus (-) indicates reduction of gene activity
P_i	Internal pool for synthesis
I, C.	Indicates internal control of gene activation or repression
I, II	Cell boundary regions for two adjacent cells; both cells are assumed to be similar
*	Indicates the state of gene activity; it is increased when star is above the symbol ($\overset{*}{O}$) or decreased when below ($\underset{*}{O}$)
S_0	Normal metabolite present in the cell
S_0^4	Repressor for operons O^1 and O^4
$[E_n^1 D_1^2], [E_n^1 D_2^2]$	Enzyme-polymer complexes; enzyme in this state is functionally inactive
$[E_n^1 D_1^2 - E_n^1 D_2^2]$	Secondary complex results from interaction of primary complexes; called here 'super-complex' (sc); this represents symbolically polymeric network of 'adhesion matrix' which holds two cells attached together.

Table 2
Flow scheme for cell-cell interaction.

Conditions:

- 1) S_n and S_o are constants.
2) Operons O^1 , O^2 and O^4 are normally active: O^3 will be activated only when cell-cell interaction occurs.

A.

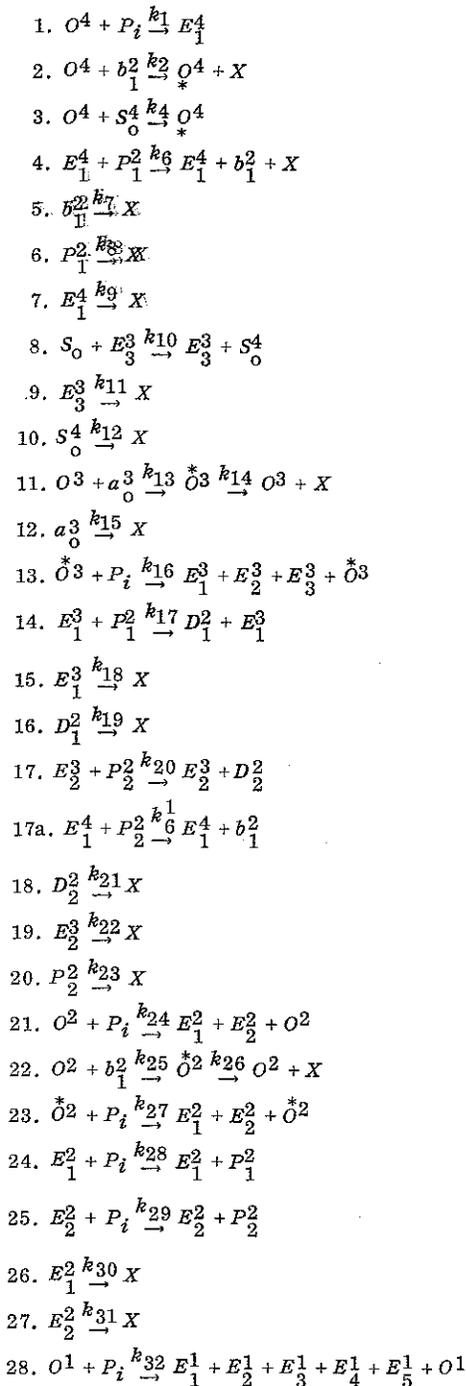
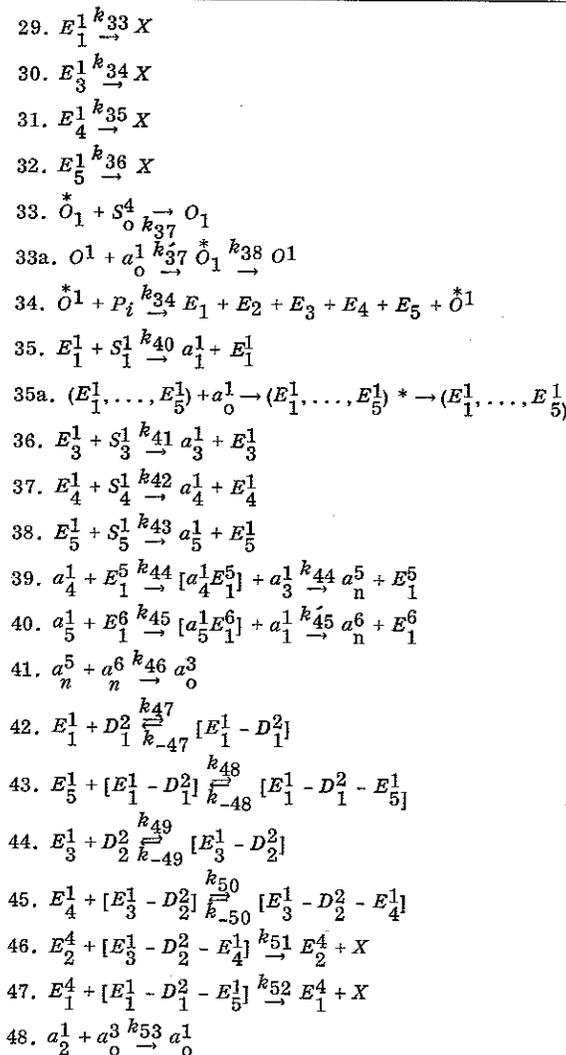


Table 2 (continued)



B.

In order to carry out computer calculations it is necessary to reduce the number of differential equations. Changes are made whereby several detailed steps in 'decoding' scheme are omitted and an overall description of kinetics is achieved by more general parametric interactions.

Following entities are introduced:

E^1 - General extensor enzyme system

S_n^1 - General extensor substrate system

Flow equations 29-33 and 36-50 are omitted. New reactions are:

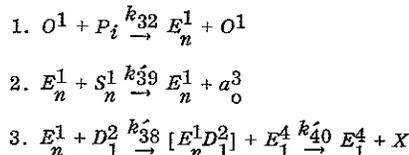


Table 2 (continued)

4.	$E_n^1 + D_2^2 \xrightarrow{k_{41}} [E_n^1 D_2^2] + E_1^4 \xrightarrow{k_{42}} E_1^4 + X$
5.	$a_0^3 \xrightarrow{k_{53}} a_0^1$
6.	$E_n^1 \xrightarrow{k_{36}} X$
7.	$\dot{O}_1 + P_i \xrightarrow{k_{39}} E_n^1 + \dot{O}_1^*$
8.	$[E_n^1 D_2^2] + [E_n^1 D_2^2] \xrightarrow{k_{55}} [E_n^1 D_2^2] - [E_n^1 D_2^2]$
9.	$[E_n^1 D_2^2] - [E_n^1 D_2^2] \xrightarrow{k_{56}} X$

Table 3

Differential equations for cell-cell interactions.

1.	$\dot{O}^4 = k_3 O^4 - k_2 b_1^2 O^4 - k_4 S_0^4 O^4$
2.	$\dot{O}^4 = -\dot{O}^4$
3.	$\dot{E}_1^4 = k_1 P_i O^4 - k_9 E_1^4$
4.	$\dot{b}_1^2 = k_6 P_1^2 E_1^4 - k_7 b_1^2 - k_2 b_1^2 O^4 + k_6^1 P_2^2 E_1^4 - k_25 b_1^2 O^2$
5.	$\dot{S}_0^4 = k_{10} S_0 E_3^3 - k_4 S_0^4 O^4 - k_{12} S_0^4 - k_{37} S_0^4 \dot{O}_1^*$
6.	$\dot{P}_1^2 = k_{28} P_i E_1^4 - k_6 P_1^2 E_1^4 - k_8 P_1^2 - k_{17} P_1^2 E_3^3$
7.	$\dot{E}_3^3 = k_{16} P_i \dot{O}_1^3 - k_{11} E_3^3$
8.	$\dot{O}_1^3 = k_{14} \dot{O}_1^3 - k_{13} a_0^3 O_1^3$
9.	$\dot{O}_1^3 = -\dot{O}_1^3$
10.	$\dot{a}_0^3 = k_{39} S_n^1 E_n^1 - k_{13} a_0^3 O_1^3 - k_{15} a_0^3 - k_{53} a_0^3$
11.	$\dot{E}_1^3 = k_{16} P_i \dot{O}_1^3 - k_{18} E_1^3$
12.	$\dot{E}_2^3 = k_{16} P_i \dot{O}_1^3 - k_{22} E_2^3$
13.	$\dot{D}_1^2 = k_{17} P_1^2 E_1^3 - k_{19} D_1^2 - k_{38} E_n^1 D_1^2$
14.	$\dot{P}_2^2 = k_{29} P_i E_2^2 - k_{20} P_2^2 E_2^3 - k_{23} P_2^2 - k_6^1 E_1^4 P_2^2$
15.	$\dot{D}_2^2 = k_{20} P_2^2 E_2^3 - k_{21} D_2^2 - k_{41} E_n^1 D_2^2$
16.	$\dot{O}_2^2 = k_{26} \dot{O}_2^2 - k_{25} b_1^2 O_2^2$
17.	$\dot{O}_2^2 = -\dot{O}_2^2$
18.	$\dot{E}_1^2 = k_{24} P_i O_2^2 + k_{27} P_i \dot{O}_2^2 - k_{30} E_1^2$
19.	$\dot{E}_2^2 = k_{24} P_i O_2^2 + k_{27} P_i \dot{O}_2^2 - k_{31} E_2^2$
20.	$\dot{E}_n^1 = k_{32} P_i O_1^1 - k_{36} E_n^1 + k_{39} P_i \dot{O}_1^1 - k_{38} E_n^1 D_1^2 +$ $-k_{41} E_n^1 D_2^2$

Table 3 (continued)

21.	$[E_n^1 D_2^2] = k_{38} E_n^1 D_1^2 - k_{55} [E_n^1 D_2^2] [E_n^1 D_2^2]$ $- k_{40} E_1^4 [E_n^1 D_2^2]$
22.	$[E_n^1 D_2^2] = k_{41} E_n^1 D_2^2 - k_{55} [E_n^1 D_2^2]$ $- k_{42} E_1^4 [E_n^1 D_2^2]$
23.	$\dot{O}_1^1 = k_{38} \dot{O}_1^1 - k_{37} a_0^1 O_1^1 + k_{37} S_0^4 \dot{O}_1^*$
24.	$\dot{O}_1^1 = -\dot{O}_1^1$
25.	$\dot{a}_0^1 = k_{53} a_0^3 - k_{37} a_0^1 O_1^1$
26.	$[E_n^1 D_1^2] - [E_n^1 D_2^2] = k_{55} [E_n^1 D_1^2] [E_n^1 D_2^2] -$ $k_{56} [E_n^1 D_1^2] - [E_n^1 D_2^2]$

fore become inactive. As a consequence, activity of operon O^1 which was initially increased (eq. 11 and 34a, table 2A) will be subsequently reduced (eq. 34, table 2A). Reduction of operon O^3 activity follows directly via operon O^1 system. Thus synthesis of the exoenzyme system is drastically reduced and consequently metabolic activity in the environment of the cell is lowered. Once a permanent intracellular matrix has been formed, it is maintained by balance of ground level synthetic and degrading processes. Quantitative aspects of this phenomenon become clear when results of computer analysis are presented.

4. RECOGNITION MECHANISM

How can cells recognize each other? What constitutes the phenomenon of recognition conceptually, and what are the underlying processes? These are basic questions which have to be answered before we proceed with the analysis. One can consider that two cells recognize each other when, after coming into contact, they perform certain functional and synthetic processes according to specifications acquired during cellular differentiation. For example, two isotypic cells adhere, heterotypic cells separate and macrophage cells destroy the foreign cells. The first phase of 'recognition' is represented by a chemotactic process which pertains to cellular 'sensing' by the exoenzyme system at a certain distance. This constitutes the first phase in some cells, but basic recognition arises when cells come into contact with each other. There is no specific experimental information in regard to

processes which take place during the cell-cell contact and as to how the recognition arises. Therefore, we propose a theory which is based on intuitive reasoning. This will provide basic mechanisms and processes essential for recognition phenomenon. Principal underlying features are: 1) Exoenzyme system, after cell-cell contact, due to its degrading activity 'samples', the neighbor cell and the products of enzyme activity are transported into the cell. 2) Metabolic products originating from the neighbor cell are transported via an existing enzyme system into the cell. There these are assembled into regulatory metabolites which control synthetic processes and thus determine cell-cell interaction proceedings. In the model-system 'extensor enzymes' of one cell act on the 'extensor substrates' of the other cell and metabolic products (a_1^1, \dots, a_5^1) are transported via the enzyme system (E_1^5, E_1^6). Finally these products are assembled into operon activators (a_0^3, a_0^1). Basically cell recognition arises from a process of comparison of both metabolic and synthetic patterns of two interacting cells. Only when these are complementary and similar can both cells initiate synthetic processes, leading to adhesion. How do two heterotypic cells 'recognize' each other? Exoenzymes of one cell act on the surface structures of the other cells and *vice versa*. This represents a symmetrical operation. Both cells, in order to produce respective regulatory compounds, have to metabolise exoenzyme products. The final regulatory products of both cells are similar only when all steps during these processes match each other. A series of enzymatic reaction steps represents essentially a decoding process. For example, a metabolite has to pass a certain number of structural and configurational transformations and provide an end-product. This in turn is assembled in subsequent processes with other products into a final regulator, which has to have a high degree of structural specificity. Thus a specific number of metabolites, after successive transformations, are assembled into a final regulatory entity. It is desirable to consider the probabilistic features of these processes, since it is essential that 'false recognition' should not arise.

Assume that probabilities for a series of enzymatic reaction steps are respectively $p_1, p_2, p_3, \dots, p_n$. Then the total probability for this chain reaction to proceed is:

$$P_1 = p_1 \cdot p_2 \cdot p_3 \cdot \dots \cdot p_n.$$

Since there are several reaction systems simultaneously in operation in linear and parallel pattern, then the final probability of producing the end-product is:

$$P = P_1, P_2, \dots, P_n.$$

Since P depends on multiplication of all probabilities, it is zero when any probability step is zero. The probability to produce a regulatory end product in conditions where one molecule *cannot* pass through certain reaction-steps is nil ($p_i=0$). However, when structural deviations in molecular structure are small (i.e. isomeric form of molecule is in the system), an end-product is produced which is active, but its activity level may be low. In practical terms, this may mean that when one of the two cells has suffered a minor change, there is a limited recognition, but strong adhesions between the cells are not formed. Consequently only cells which match each other metabolically and structurally can recognize each other and adhere together. Furthermore, cellular adhesion is maintained only so long as cells do not change their characteristics. For example, if metabolic processes of a cell are altered, perhaps by an external agent, a *mismatch* develops between the cells and dynamic synthetic balance is lost. Cells lose their adhesive structures and migrate apart.

In a macrophage system, where chemotactic phenomena play a great role, the contact information can be considered to operate differently. Synthesis of degradative enzymes will be augmented when macrophage cell meets foreign cell. This means that by 'metabolic sampling', a cell produces a regulatory metabolite only when another cell is foreign. Thus its surface structures contain molecules linked together by bonds, not present in native structures. This problem is too complex to be analyzed here, but a few comments can be added for clarification of the basic phenomenon of 'self-recognition'. First, macrophages constitute part of an immunological system, and these are special cells. It appears that 'self-recognition on macrophage' sense arises from the following:

1) The macrophage exoenzyme system (extensor enzymes) does not act on surface structures of own body cells since formation of enzymes specific to structures and bonds of own cells are repressed (for example, at operon O^1 level). Mechanisms behind such 'self-recognition' have been discussed in a previous publication (Heinmets, 1967). Consequently complexing macrophage and normal body cell cannot produce sta-

ble complexing, which is essential for degradative process.

5. COMPUTER RESULTS

In order to analyze interaction of two cells, it is assumed that both cells are metabolically in a stable state and their enzyme levels are constant. However, in order to achieve such a system, enzymes have to be synthesized on the computer from initial conditions. Therefore, there is a transient phase when the enzyme level increases, but finally a steady-state condition is established. This is shown in fig. 2, where en-

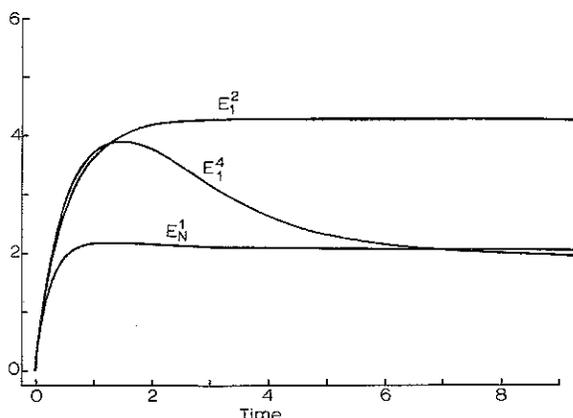


Fig. 2. Concentration of enzymes E_1^2 , E_1^4 and E_n^1 as a function of time. No cell-cell contact.

zymes E_1^2 , E_1^4 and E_n^1 , after initial transient, all reach a constant level. In this condition two cells are brought into contact. Such an operation is simulated on the computer by arrangement such that during the transient period the rate constant k_{39} (table 2B) is maintained at the zero level, but after constant enzyme level has been established k_{39} is switched in. This step makes the cellular interaction system operational. In practical terms it means that two cells have come close enough to a contact (non-specific primary contact) which permits enzyme systems of both cells to become active. Consequently, metabolic products resulting from 'extensor-enzyme' E_n^1 'extensor-substrates' s_n^1 interaction are metabolized. Therefore, regulatory substrates a_0^3 and a_0^1 (table 2B, eqs. 1 and 4) are produced and all the system becomes operational.

6. INTERACTION OF TWO ISOTYPIC CELLS

In order to gain insight as to how the model-system operates, we will follow the principal entities of the system as a function of the time. Since many enzymes of the same operon have similar values, only one will be represented on figures. These have been directly recorded on a computer. In the figures horizontal and vertical axes represent, respectively, time and relative concentration of entities. Total observation time on the computer is varied, depending on experiments. Maximum observation time was 700 sec and switching of k_{39} occurred at 100 sec. Data was recorded on a time compression scale 100:1 and for visual observations of solutions on scope time was compressed 1000:1. In order to organize and synthesize an operational model-system on an analog computer, it is essential that solution should be obtained fast enough to be observable on an oscilloscope. This requires high time compression, coupled with accurate repetitive computer solutions. The effect of isotypic cell-cell interaction on various functional entities has been explored. Fig. 3 shows that enzymes E_1^4 and E_n^1 are at a steady-state level when k_{39} is switched in. It is evident that the concentration of degrading enzyme E_1^4 starts to decline immediately, establishing itself finally at a relatively low concentration level. In contrast, extensor enzyme E_n^1 concentration increases rapidly, reaches a maximum, but finally declines to a low level. This means that after cell-cell contact there is an increased synthetic and metabolic activity result-

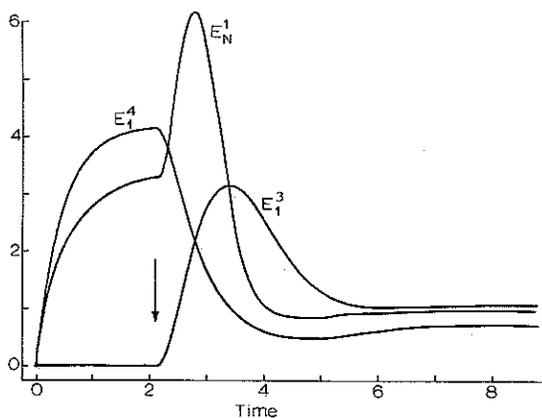


Fig. 3. Concentration of enzymes E_1^3 , E_1^4 and E_n^1 . Cells come to 'contact' at time indicated by the arrow (\downarrow). At the time interval from 0 to 2.2, $k_{39} = 0$; at the time interval 2.2 to 9, $k_{39}S_n^1 = 0.1555$.

ing from cell-cell recognition. There is formation of regulatory compounds a_0^3 and a_0^1 . Therefore operon O^1 is further activated (table 2A, eq. 34) and inactive operon O^3 will become active (table 2A, eq. 11). As a consequence, polymerizing enzyme E_1^3 is produced. As shown in fig. 3, it will start to increase after cell-cell contact reaches a maximum and subsequently declines to a constant value. Reduction of extensor enzyme E_n^1 results primarily from repression of operon O^1 by S_0^4 (table 2A, eq. 34) and removal of E_n^1 by complexing with polymers of D_1^2 and D_2^2 (table 2B, eqs. 2 and 3). Thus enzymes which were primarily required for cell recognition are drastically reduced after cellular identification.

According to the basic premises of the theory, the cellular adhesion is a dynamic state during which synthetic and degradative processes are in balance. Consequently there is a continuous metabolic turnover of materials associated with adhesive structures. Maintenance of such a dynamic steady-state requires continued presence of regulatory metabolites. Their behavior is demonstrated in figs. 4, 5 and 6. It is evident from fig. 4 that after cell-cell contact a_0^1 concentration is rapidly built up. It reaches a maximum but subsequently recedes to a very low value. Before a_0^1 reaches the maximum value, regulatory repressor S_0^4 starts to increase, the maximum occurs about at the time when a_0^1 reaches to a low value. While S_0^4 also starts to decline, a

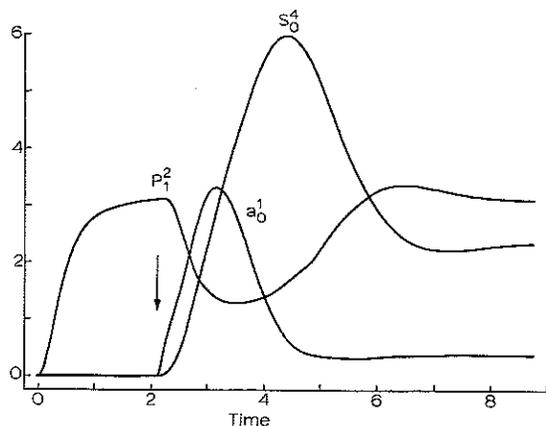


Fig. 4. Concentration of polymeric compound P_1^2 and interrelation of regulatory metabolites a_0^1 and S_0^4 in cell-cell interaction process.

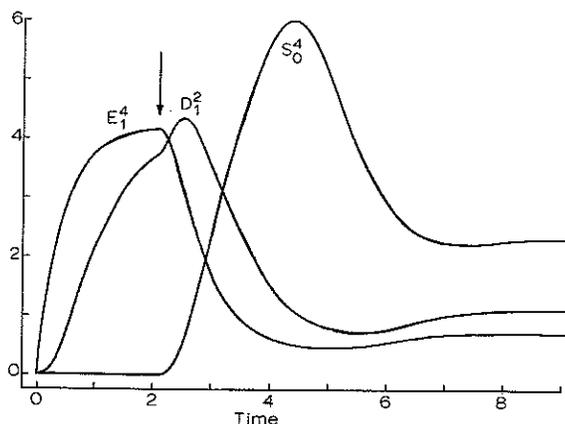


Fig. 5. The effect of regulatory metabolite S_0^4 on degrading enzyme E_1^4 .

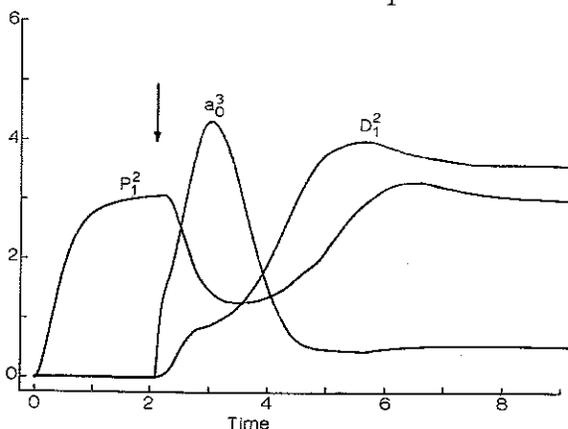


Fig. 6. The effect of regulatory metabolite a_0^3 on polymeric compounds D_1^2 and P_1^2 .

steady level is maintained at relatively high concentration. Thus a strong inhibitory effect is exerted on operons O^1 and O^4 after cells have established permanent contact. This effect reflects itself in enzyme E_n^1 and E_1^4 concentrations in figs. 5 and 7, respectively. In fig. 6 it is demonstrated that during the active phase of 'recognition metabolism' a_0^3 concentration also reaches maximum but then declines. There is a reduction of primary polymer P_1^2 , while secondary polymeric compound D_1^2 is built up. However, after the transition phase, P_1^2 and D_1^2 are both maintained in the system at a constant level. The final phase of cell-cell interaction is represented by formation of intermediate polymeric complexes leading to establishment of an 'adhesion matrix'. Fig. 7 shows that build-up of complex $E_n^1 D_1^2$ is

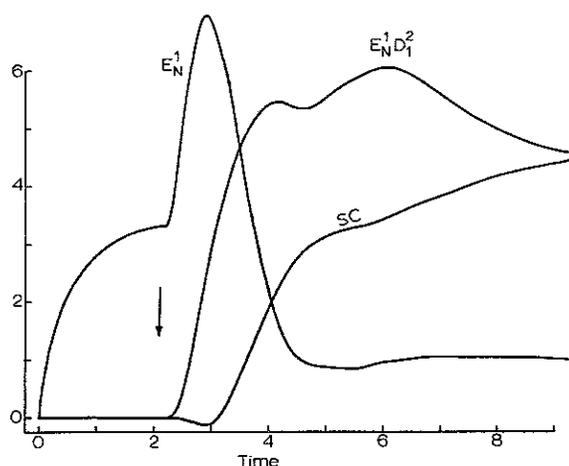


Fig. 7. Formation of enzyme-polymer complex $E_n^1 D_1^2$ and intracellular adhesion matrix sc after isotypic cell-cell contact.

associated with the reduction E_n^1 . After a short delay formation of 'adhesion matrix' sc starts reaching a constant level. At such a phase two cells can be considered to be linked together and such a state is maintained indefinitely, provided there is no interference with cellular processes. However, if for any reason there is interference and some functional entity is altered during linking of two cells, there will also be a change in the adhesive process.

On the other hand, there can also be a partial recognition phenomenon; for example, if geometry of the cells has been modified by some environmental factor and two cells cannot make a complete contact. Then the extensor enzyme system of one cell may not operate with full activity on the extensor substrate of the other cell. Consequently regulatory substrates, while properly coded, can be metabolized into final regulator compounds, but concentrations can be low. What is the effect of such conditions on cell-cell adhesion? This is shown in fig. 5, where aforementioned conditions are simulated by assigning normal and low values for rate constant k_{39} . It is evident that at low k_{39} value (0.02), there is no initial build-up of E_n^1 as in the normal case ($k_{39} = 0.156$). Formation of intracellular 'adhesion-matrix' takes place but concentrations are low. Consequently, these two cells are only weakly linked and such adhesion may not be adequate to hold cells at the proper distance to maintain the dynamic equilibrium. Such cells may move apart after a certain time interval.

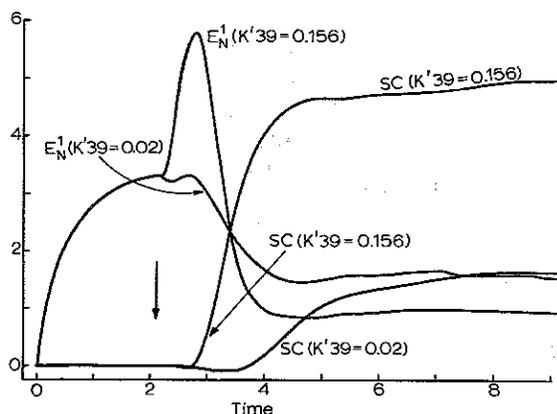


Fig. 8. Incomplete cellular recognition. In normal recognition ($k'_{39} = 0.156$) and partial recognition ($k'_{39} = 0.02$). E_n^1 and sc recorded in two conditions.

7. LOSS OF ADHESIVE BINDING BETWEEN TWO CELLS

It is a well-known growth phenomenon in cellular systems that cells which grow together in tissues and organs may become unattached and become migratory. This is related to the malignancy problem. It is of great importance to understand what are the underlying processes. Dissociation of two cells represents the reversal of metabolic and synthetic processes leading to adhesion. What could cause the reversal process? Basically two-cell interaction represents the phenomenon of mutual cellular interaction revealing that one cell can influence the metabolic and synthetic processes of the other cell. This means that organized cellular community, where each member affects neighbor cells, can be maintained only when cells have similar metabolic and synthetic patterns. There is no cellular 'independence' in such a community, since growth regulator and inhibitory compounds are derived from adjacent neighbor cells. The question is, what happens to the cellular community when one cell in the system is altered by some extrinsic or intrinsic factor. This phenomenon can be analyzed adequately considering only two-cell interactions. The model-system in fig. 1 can serve as a base for such an analysis. Conditions which were essential for leading to adhesion of two cells required that both cells had similar metabolic patterns. Therefore, if one of the cells alters its pattern, the dynamic binding equilibrium is altered when these changes are related to the processes involved in maintenance of cellular adhesion. Here we make a general assumption that at a certain time one of the cells has changed its

functional pattern. How does it affect the adhesion between two cells? This can be simulated on a computer by making k_{39} equal to zero in conditions where stable 'adhesion-matrix' has been already formed between two cells.

Experimental results simulating cellular dissociation are represented in figs. 9, 10 and 11.

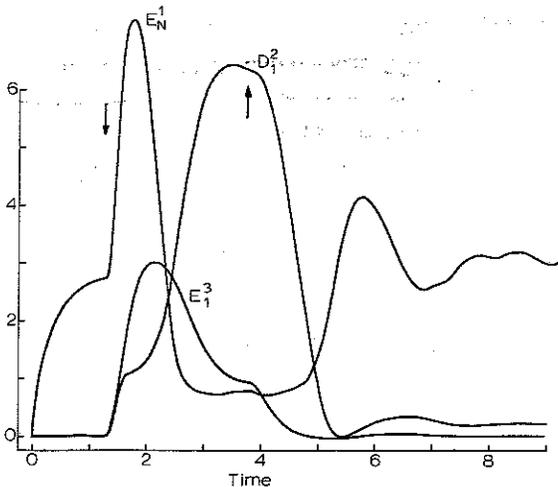


Fig. 9. The loss of cellular recognition. At time indicated by first arrow rate constant k_{39} acquires value 0.155 and at time indicated by second arrow k_{39} is made zero. Enzymes E_n^1 , E_1^3 and polymeric complex D_1^2 are recorded as a function of time.

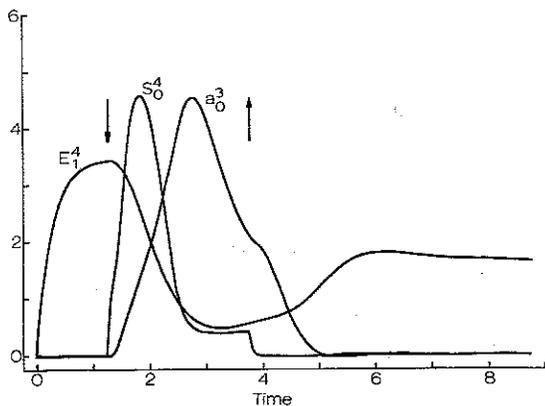


Fig. 10. Same experiment as in fig. 9. Enzyme E_1^4 , regulatory compounds S_0^4 and a_0^3 are recorded.

Only principal entities are presented here. Behavior of other functional entities is consistent with represented results. The first arrow (↓) on these figures indicates time when k_{39} has a value assigned and at the time of the second arrow (↑) it again becomes zero. This means cell recognition

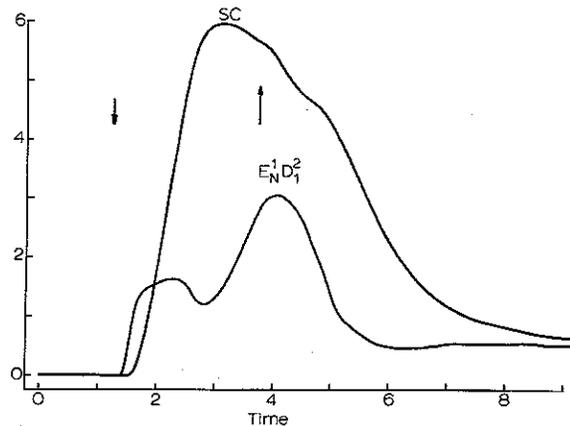


Fig. 11. Same experiment as in fig. 9. Complex $E_n^1 D_1^2$ and super complex sc recorded.

has been lost. It is evident in fig. 9 that enzymes E_n^1 and E_1^3 at the time of second arrow (↑) have reached a stable value, while the polymeric complex D_1^2 has reached the maximum. After reduction k_{39} to zero, extensor enzyme E_n^1 concentration is rapidly built up and after a transitory phase the concentration approaches a stationary value. Enzyme E_1^3 is reduced to zero level, while D_1^2 concentration slowly approaches zero. As seen in fig. 10, regulatory metabolites S_0^4 and a_0^3 are reduced to zero level after the second arrow. It should be noted that a_0^3 had not quite reached the steady state value as indicated in fig. 6 (at time of 5 time units), but this is immaterial. It is also evident that degrading enzyme E_1^4 , which is at relatively low level during normal adhesion, starts to increase and reaches its normal level as single cell (fig. 2). Fig. 11 shows that, after loss of 'recognition', polymer-enzyme complex $E_n^1 D_1^2$ and intracellular 'adhesion-matrix' sc rapidly start to decline and finally approach zero. At this phase cell-cell linking is lost and the two cells are independent.

8. DEGRADATIVE INTERACTION BETWEEN THE CELLS

We have seen that model-system operation provides conceptual interaction patterns and mechanism leading to adhesion of two isotypic cells coming into contact. We consider that the structure of the model-system contains potential

characteristics to provide means to interpret other types of cellular interactions. The basic four operon system can be linked by different regulatory interactions, thus providing new functional patterns. For example, flow schemes given in table 2 can be modified to obtain new operational systems. While the presented system dealt with cooperative behavior of two cells leading to cellular community, it would be of interest to analyze interaction when two cells come into contact and subsequently destroy each other or one cell degrades another one. Such interactions are well-known to be operative in cellular defense processes. Operation of macrophages represents a typical degradative interaction. We propose to show that on a conceptual basis, a model-system (fig. 1, table 2) can be modified to produce a degradative interaction pattern. In order to carry out detailed analysis, the model-system has to be modified. Degradative cell-cell interaction can be produced using the basic model-system presented in fig. 1. Some principal conceptual changes have to be made for such a purpose. It is assumed that for a degradative cell the operons for extensor enzymes being specific for cellular 'self' extensor enzymes are repressed. Consequently there are no interactions between 'self' and 'own' kind of cells. Furthermore, the cell surface contains sensor enzymes which are specific to 'foreign' structures and bonds. This problem has been dealt with in detail in a publication concerning antibody synthesis (Heinmets, in press) and not discussed here. It suffices to say that a degradative cell has the exoenzyme system (E_n^1 and E_1^4) which interacts with the surface structures of foreign cells. Consequently regulatory compounds are produced to activate the genetic system for the processes leading to degradation. Symbolically, enzyme systems E_n^1 and E_1^4 are degrading enzymes and can lead to destruction of other cellular entities. During isotypic cell interaction an 'adhesion-matrix' was formed which automatically reduced the concentration and activity of degrading enzymes. Here we propose a mechanism where two-cell interaction leads to increase of a degradative enzyme system. Polymerizing enzymes which build the 'adhesive-matrix' are maintained in the system, but these are kept at very low level. Consequently only a weak binding occurs between the two cells. It is considered that such binding is essential for the degrading process. The following modifications are carried out with rate constants represented in table 2:

a) Negative feedback inhibition at operon O_1 level is abolished: $k_{37} = 0$.

b) Operon O^4 activity is increased when cell-cell contact occurs; k_4 feedback loop is made positive.

c) k_{25} and k_{13} values are reduced. It has to be noted that cell-cell destruction per se is not carried out on the model-system. This would require extensive modifications which cannot be done here. However, 'symbolic-destruction' is characterized here by increased concentration of degrading enzymes and their activity coupled with weak adhesive linkage.

Figs. 12, 13, 14 and 15 show the principal entities after destructive cell contact. These have to be compared with figs. 3-7. It is evident that enzyme concentrations E_n^1 and E_1^4 (fig. 13) are maintained at high level, thus indicating high degrading activity. Adhesive binding is weak (fig. 15) and the level of degradation product (a_0^3 , fig. 14)

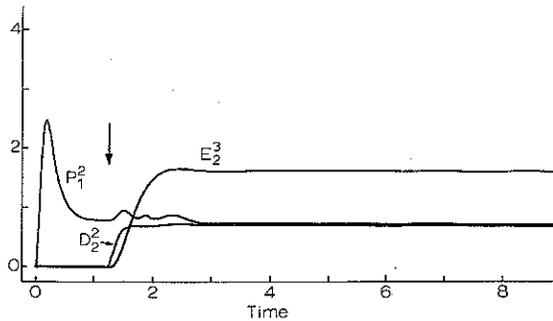


Fig. 12. Concentration of enzyme E_2^3 , polymeric compounds P_1^2 and D_2^2 during degradative cell-cell contact.

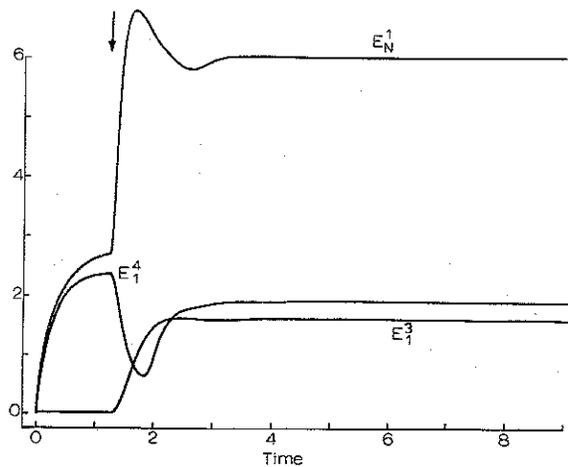


Fig. 13. Enzymes E_n^1 , E_1^4 and E_1^3 as function of time.

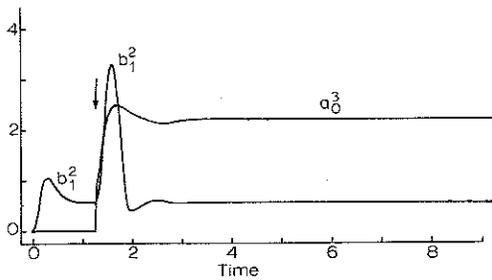


Fig. 14. Metabolic substrates a_0^3 and b_1^2 as function of time.

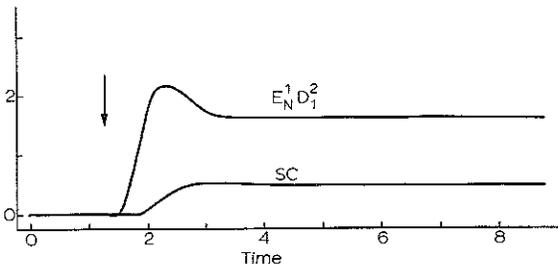


Fig. 15. Concentration of complex $E_N^1 D_1^2$ and super complex sc after cell-cell contact.

is high. If one considers that cell I is a degradative type and cell II is the 'victim', then the latter will be destroyed, since all entities have initially finite values.

9. DISCUSSION

Computer analysis of a model-system reveals that primary objectives of theory have been achieved where cell-cell interactions have been analyzed on the basis of postulated mechanisms. While we have considered only isotypic and macrophage types of interactions, there is no basic reason why the model-system could not be used after certain modification for other type cellular interactions. For example, sexual agglutination for opposite mating types of cells (Taylor, 1964) as well as fertilization processes in general can be analyzed. Furthermore, the cellular interaction model-system could be extended to include mechanisms for contact inhibition of cell division and release from such inhibitory state (Todaro et al., 1965). This, in turn, could help to relate cellular growth control in organized cellular communities. Here, functional behavior of cells could be correlated with cell surface structures and enzymes (Kalckar, 1965), and metabolic intercommunication (Loewenstein and Kanno, 1966) should emerge as the principal information

change mechanism between cells. It appears that the behavior pattern of individual cells emerges as a product of complimentary activity of intracellular as well as extracellular processes. When such cooperative characteristics are lost, cells become independent and social cooperation, therefore, will be terminated. The individual cell, when in varying environmental conditions, attempts to adjust to these; in so doing, it loses the cooperative characteristics. Thus a single cell acquires a migratory characteristic and its metabolism is geared for individual survival.

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