CHEMOTHERAPY OF CANCER DISSEMINATION AND METASTASIS

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EDITORS:

S. Garattini, M.D.

Director,
Mario Negri Institute for
  Pharmacological Research,
Milan

G. Franchi, M.D.

Mario Negri Institute for
  Pharmacological Research,
Milan

Preface

Cancer chemotherapy has, until recently, focused mainly on efforts to block cell proliferation. This has certainly proved to be of value, and has led to the development of suitable models, the development of many drugs, and considerable progress in the entire field of cell proliferation and cell kinetics. However, the hundreds of drugs developed up to the present time show a high degree of toxicity since they do not distinguish between cancerous and normal cells, most probably because the basic proliferation steps in these two types of cells may be quite similar.

Other approaches to cancer chemotherapy should therefore be considered. The theory has been advanced in recent years that the heart of this disease is represented not by the growth of the tumor itself but by its dissemination and formation of metastases invading the entire body. Accordingly, it has been suggested that cancer chemotherapy should be aimed at confining and controlling the metastatic process.

A number of targets may be considered in trying to achieve control of metastases. These are leakage of cancer cells from the primary tumor, their transport by blood or lymph, their "take" in normal tissues, and the establishment of conditions which favor the growth of a metastatic tumor. Since these various processes are possibly unique for cancer cells, they may offer the opportunity for a selective attack.

A combined, multidisciplinary effort is necessary to achieve this goal because of the complexity of the problems inolved. This is why we have sought to gather here scientists representing diverse disciplines, in the hope that we may learn of the most recent developments concerning subjects of interest to us, such as the properties of the cell surface, the intricacies of cell organization, and the interactions between the host and cancer cells. We also hope to learn what new models have been devised which will facilitate investigations of the processes of cancer dissemination and the formation of metastasis.

New types of drugs will be described here which may for the present be of only theoretical interest, but which may represent the vanguard of new therapeutic means to fight cancer. If these drugs offer a new and valid approach, they may greatly inflience screening programs. Thus, instead of

searching only for cytotoxic action, we may consider more carefully what effects old or new chemical agents have on cancer cell adhesiveness, motility, migration, cohesion, and other kinetic aspects.

The meeting on which this volume is based was organized and held in collaboration with the International Union Against Cancer on May 24–26, 1972. We wish to express our gratitude to Dr. Delafresnaye, Director of the Geneva office of the Union, for having made this conference possible.

I am confident that this meeting will provide the stimulus that will challenge us to search for new ways and means of controlling cancer.

S. Garattini
Director,
Mario Negri Institute
for Pharmacological Research,
Milan, Italy

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EFFECTS OF DRUGS

The Structural Basis for Membranes as Sites for Complex Metabolic Functions

Fritiof S. Sjöstrand

Department of Biology, University of California, Los Angeles, California 90024

I. EFFICIENCY IN UTILIZATION OF CELLULAR ENZYMES

There are certain features that characterize living systems in the normal state on which I would like to focus attention. The complex mixture of chemical reactions that is the basis for cell metabolism is catalyzed by enzymes in a most efficient way. The efficiency of utilization of these enzymes at a low overall enzyme concentration applies in an astonishing degree to complex enzyme systems, catalyzing sequences of chemical reactions by a corresponding number of enzymes. The efficiency of these multienzyme systems must be due to a coordination of the catalysis by the various components of the system, and this coordination in some cases will prevent labile intermediaries from being lost through spontaneous modification of the molecules.

It is obvious that this efficient utilization of the enzymes is unlikely to occur in an uncontrolled, random system. In contrast, one of the most basic features of living systems is the high degree of control of the metabolism. This control involves a control of the rate of chemical reactions, a coordination of sequences of chemical reactions, and a balancing of various chemical reaction rates to establish a steady-state situation.

This characterizes the normal cell. The cancer cell can be looked upon as a cell in which this control has been lost at certain points. It therefore

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appears justifiable to point to a conceivable basis for the control of cell metabolism at an ultrastructural level.

II. MEMBRANES AS METABOLICALLY ACTIVE STRUCTURAL COMPONENTS OF CELLS

Electron microscopy has contributed some very basic discoveries. In 1948, it was shown that the outer segments of the photoreceptor cells of the retina consist of a pile of double membrane disks (Sjöstrand, 1949). Since this is the only structural component of the outer segments, it was reasonable to associate these disks with the function of the outer segments, which is to transduce the energy of absorbed photons into some form of chemical energy. This discovery made it justifiable to associate membranous structures with a complex metabolic function. Later, similar observations were made on the grana of chloroplasts (Steinmann, 1952), and in 1952, with the development of suitable thin-sectioning techniques, the mitochondria were shown independently by Palade (1952) and by Sjöstrand (1953) to contain double membranes forming incomplete septa, mostly oriented roughly perpendicular to the long axis of the mitochondrion. Electron microscopists proposed that the components of the respiratory chain would be located in these membranes, which would constitute the "structural factor" that had been considered necessary for the respiratory function. This assumption has been verified by biochemical studies on submitochondrial particles.

We have, then, three examples of complex metabolic functions involving energy transduction that are associated with membranous structures. In fact, any complex metabolic function involving multienzyme systems is likely to be associated with membranes. Membranes therefore represent a basic principle of structural organization that can be correlated with complex metabolic functions. These functions are severed if the membrane structure is disrupted or damaged. A good example of this is the respiratory chain in inner mitochondrial membranes. This chain involves dehydrogenases that pick up hydrogen from the substrates NADH and succinate, NADH dehydrogenase, and succinate dehydrogenase. While hydrogen ions are released in connection with oxidation of the dehydrogenases, electrons are fed into the cytochrome chain consisting of cytochromes b, c_1, c, and the $a + a_3$ complex, and are eventually transferred to oxygen (Fig. 1).

The electron transport is coupled to phosphorylation of ADP at three coupling sites along the chain. Normally, electron transport is controlled by the presence of ADP and inorganic phosphate in such a way that respira-

M.W. 96,000

SUCCINATE ⟶ SUCCINATE DEHYDROGENASE

M.W.30,000   M.W.51,000   M.W.12,400   M.W.360,000

cyt. b ⟶ cyt. c_1 ⟶ cyt. c ⟶ cyt. a a_3 ⟶ O_2

NADH ⟶ NADH DEHYDROGENASE
M.W. 1,000,000   M.W. 280,000

COUPLING FACTOR 1   COUPLING FACTOR 2
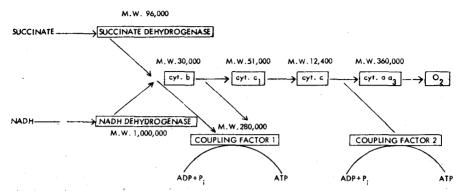
ADP + P_i ATP   ADP + P_i ATP

FIG. 1. The respiratory chain with the molecular weights of the components indicated. The molecular weights are known with a high degree of accuracy for cytochrome c and succinate dehydrogenase, but not for other components. This scheme illustrates the great differences of the molecular weights of these components.

tion is blocked when either or both of these components is lacking. This is referred to as respiratory control.

When isolating mitochondria for biochemical analysis, utmost care must be taken not to modify the mitochondria, because exposure of the mitochondria to slightly adverse conditions leads to an impairment of the function of the respiratory chain. The respiratory control is the most sensitive function and is usually only partially retained in isolated mitochondria. The next function that is affected is oxidative phosphorylation, while respiration may be maintained. Eventually, respiration is stopped.

These functional changes are likely to be caused by structural changes of the inner mitochondrial membranes. The structural lability of these membranes is very obvious. Even aging, in what has been established as an appropriate medium to retain mitochondrial function when preparing mitochondrial fractions, is sufficient to produce extensive structural changes. The membranes break up spontaneously to form various kinds of modified structures and eventually disintegrate into multimolecular particles.

An intact membrane structure, therefore, appears to be essential for the coordination of the complex sequences of chemical events associated with the respiratory chain, and this is likely to apply also to other multienzyme systems.

The metabolic function of cellular membranes makes us focus our attention on the enzyme molecules, that is, the protein molecules of membranes; we want to find the background for the membrane as a necessary structure for the coordination of complex sequences of chemical reactions.

We therefore approach membranes from a different point of view than that of most researchers involved in permeability properties of the cell membrane. In that case the emphasis is on the barrier function of the membrane, and the interest usually becomes focused on the lipid components of membranes, the lipid molecules representing a very useful material with which to build a barrier against ion diffusion.

III. THE MOLECULAR STRUCTURE OF METABOLIC MEMBRANES

How can a membrane structure facilitate catalytic activity of complex multienzyme systems? We do not know for sure, but we can develop a concept which is based on both structural and biochemical information.

Let us first look at the structural information. Special techniques had to be developed in order to make it possible to observe the membrane proteins. These proteins are globular proteins, and in order to be seen they must retain their globular shape. Conventional preparatory procedures denature the proteins, which means that the peptide chains are unfolded randomly and the globular shape of the molecules is lost. The proteins can now be observed only when many unfolded peptide chains are aggregated. This happens in connection with conventional preparatory procedures. The aggregated, unfolded peptide chains of membrane proteins then form a new membrane structure that has structurally little to do with the native, original membrane structure. It appears in electron micrographs as a layered structure with three continuous layers, reflecting a layering of nonpolar structures such as nonpolar amino acid side chains and the hydrocarbon tails of lipid molecules in a middle layer sandwiched between two layers of predominantly polar structures. It is the latter structures that appear stained in the electron micrographs. This "unit membrane" pattern is thus an artefact due to extensive denaturation of the membrane proteins. This explains why this pattern is observed whether one is looking at an artificial lipid bilayer, the myelin sheath with 20% proteins and 80% lipids, the plasma membrane with 60% proteins and 40% lipids, the mitochondrial membranes with 75% proteins and 25% lipids before or after extraction of more than 95% of the lipids, artificial protein membranes containing only traces of lipids, or membranes containing no lipids.

When avoiding extensive denaturation of the membrane proteins (Figs. 2–5), the membranes appear to consist of particles with diameters that range from below 40 Å to about 100 Å (Sjöstrand and Barajas, 1968). This range of dimensions corresponds to what would be expected from our knowledge of the molecular weights of membranes such as those in the respiratory chain.

FIG. 2. Mitochondria in proximal convoluted tubule cells in the rat kidney. The tissue was treated according to a new procedure aiming at reducing conformational changes in protein molecules. The inner mitochondrial membranes appear lightly stained against the more intensely stained matrix. A particulate substructure can be seen in these membranes. Magnification: 96,000×. From the work of Sjöstrand and Barajas (1968). Picture published in Sjöstrand (1971).
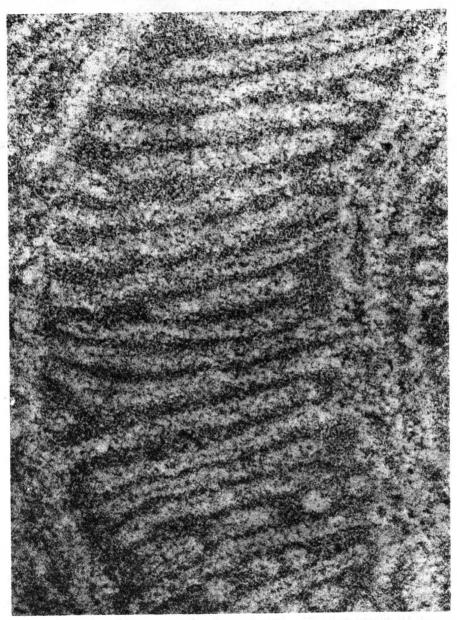
FIG. 3. Higher magnification of part of Fig. 2 to show the irregular particulate substructure of the inner mitochondrial membranes. Magnification: 192,000×.
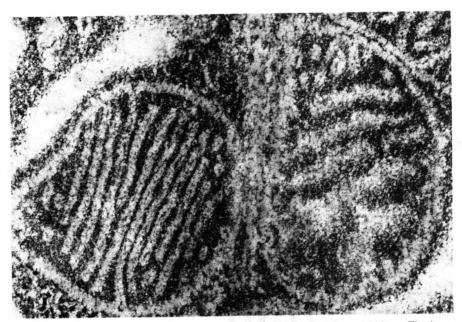
FIG. 4. Two mitochondria in proximal convoluted tubule cell of the rat kidney. The inner mitochondrial membranes in the mitochondrion to the left are oriented perpendicular to the plane of the section and are viewed in profile, while the inner membranes in the other mitochondrion are oriented obliquely to the plane of the section. This oblique orientation makes it possible to observe the particles of the inner membrane with minimum confusion due to superposition. Magnification: 86,000×. From the work of Sjöstrand and Barajas (1968). Picture published in Sjöstrand (1969).

Furthermore, the thickness of the inner mitochondrial membranes was estimated to be 150 Å, while the average diameter of the particles was found to be 70 Å. This means that the membrane particles are present in a three-dimensional arrangement.

Intentional denaturing of the tissue by heat or low pH made the membranes appear layered as after conventional treatment of tissues for electron microscopy (Fig. 6). This fact and the correspondence in dimensions between the observed particles and protein molecules that are known to be present in these membranes make it justifiable to conclude that we observe the proteins of the membranes and that the membrane is a three-dimensional condensation of globular proteins with lipids.

From a biochemical point of view, it has been justified to assume that the components of the respiratory chain form a complex in which the component molecules are closely aggregated. This concept dates back to the