REGULATION OF ENZYMES IN ANIMALS:
EFFECTS OF DEVELOPMENTAL PROCESSES, CANCER AND RADIATION

Six Year
Summary Progress Report
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Objectives and Accomplishments

Metabolic controls that operate by changing the intracellular concentrations of enzymes were known at the outset of the period, mainly from this kind of regulation of two enzymes in liver that had been worked out here under earlier Atomic Energy Commission contracts. This kind of regulation in response to hormones and substrates produced concrete changes in concentrations that could be associated with functional changes in vivo. This made it more credible than other possible types of regulation. Its study was therefore taken up by many laboratories around the world. We then concentrated on a less obvious aspect of this phenomenon, one that became the new objective of the contract period under review: Regulation by changing the concentration of an enzyme in a cell changes the nature of that cell. The nature of a cell is determined by its chemical composition. Cells become different when modified by regulation, and the extreme of this chemical difference occurs between cells from different tissues or cells in health and disease. Our objective was to extend to other enzymes and tissues the principal of the regulations that occur by changing enzyme concentrations. We hoped in this way to come to grips with the diversity shown by cells within one animal and with a common genome, as well as the alterations of cells that occur in disease. We chose tissue differentiation, neoplasia and irradiation as areas to examine. The choices were happy ones, because we have made satisfying progress in several directions.

Detailed reviews of progress have been submitted annually, including this year (1971-1972), and a 3-year summary for the period 1966-1969. With
the present 6-year summary a complete bibliography of the 61 papers published
during the period is appended. None are abstracts, and 10 are books or chap-
ters of books. In view of this documentation, an effort is made in the
following three-part summary to identify the major advances in understanding
at the expense of fascinating but repetitious detail. The perspective gained
is invaluable to us for choosing our continuation plans. The strands of work
most deserving of emphasis are 1) chemical inductions of enzymes as molecular
steps in tissue differentiation, 2) characterization of the unique patterns of
chemical composition of tissues; and 3) the discipline of quantitative enzyme
analysis of tissues upon which much of the above is based.
1. Molecular Steps of Tissue Differentiation

Starting from the known hormonal and substrate inductions of tyrosine aminotransferase and tryptophan oxygenase in adult liver, an effort was made to find out what accounted for the first appearance of enzymes during tissue development. Techniques were perfected to inject and observe in situ rat fetuses as well as neonatal rats, and enzyme assays were scaled down to fit the small amounts of tissue available. Appropriately, success came first with tyrosine aminotransferase, but the developmental formation of the equally well known tryptophan oxygenase resisted explanation for several years. Tyrosine aminotransferase appears explosively in liver for the first time in the few hours after birth. This was traced to induction by glucagon that is secreted in the immediately postnatal period of hypoglycemia. Appearance of the enzyme could be prevented by avoiding the postnatal hypoglycemia by administration of glucose. Proof was obtained that glucagon was the effective agent by its induction of the enzyme prematurely in fetuses in utero as early as several days before birth. The induction was prevented by actinomycin.

It is of interest that the normally scheduled development of this enzyme is irreversible, since it thereafter persists in liver, but when it is prematurely formed it disappears when the glucagon stimulus stops. The secret of differentiation, that changes persist after removal of the causative stimulus, is nearly exposed here. Presumably the enzyme that appears on its normal schedule is caught up in a nexus of regulatory influences that at an earlier age are not yet capable of supporting it. The nature of this nexus is not known.

A large number of other enzymes have been investigated in the same manner and with similar results. Detailed time curves of the appearances of the enzymes were first determined. Then treatments with appropriate agents were
tried at an age well before the enzyme normally appeared. At first these agents were all hormones and the choices were empirical. Gradually a rough sequence of the various hormonal and nutritional actions in the fetus and neonatal rat was worked out, and more informed guesses could be made. Enzymes were found to emerge in clusters associated in time with major changes in the state of the organism; i.e., in late fetal, immediate postnatal, late suckling and pubertal periods.

The most sophisticated accomplishment was the premature formation of tryptophan oxygenase. It long remained refractive because, as we eventually found, it required two stimuli (cortisol and tryptophan) given in a particular normal schedule. With the correct formula, the enzyme's appearance after the 12th day could easily be anticipated by a week or more. Another dual stimulus (of cortisol and glucose) also prematurely formed glucokinase.

There are many possible extensions of these findings, but the major advance has been the recognition that the same kind of chemical inductions that modulate enzyme concentrations in adult liver also cause enzymes to appear for the first time in tissues during development. Summation of these individual enzyme formations accounts on a molecular scale for the chemical differentiation of a tissue, and thus explaining the varied compositions arrived at by the different adult tissues.

2. Unique Tissue Patterns of Enzyme Composition

In the same way that the concentration of a particular enzyme at successive times during development had to be measured in order to identify the time and then the inducer of its appearance, so must the whole pattern of enzymes in a tissue be measured to find out its final, differentiated state. This can be recognized as characteristic only by comparison with the pattern of a second, and a third tissue, and so on. In fact, the whole panoply of tissues
should ideally be compared to see how any one is differentiated from the others. The different enzymes and amounts of each that make up the pattern of composition of a tissue represent the end result of all the inductions occurring during its development, but they also have a more practical aspect. These enzymes are the elements which express the function of a tissue. We are dealing here directly with information of the kind that first prompted investigations to be made into the biochemistry of cells: What are their functions? How do they work? That we now know something about the genesis of the differences does not diminish the practical significance of the differences themselves.

It has been a major achievement to systematize the analyses of enzymes in tissues so these results could be accumulated, and to multiply analyses so as to include a representative sample of components and tissues. In this way the first nucleus of information was collected about how the chemistries of tissues relate to one another. Much of the knowledge about the varied substances present in living systems cannot yet be included in such a description, but a critical mass has been collected that will grow—because it is already producing new insights about classical biological problems.

It is easiest to understand this new genre of biochemistry (called enzyme physiology and pathology) after considering the classical roles of the sister sciences in biology. Physiology concerns itself with the functions of the tissues, each tissue representing a subspecialty. Anatomy considers the structures of each tissue, mainly emphasizing comparisons or contrasts between them but also inferring functions from the arrested poses it finds. Pathology combines the two concerns about structure and function in reference to those departures from the normal seen in disease states. A list of the chemical components in a tissue, each labeled by its concentration, identifies
the structure of a tissue uniquely and also relates it to others constituted from the same ground plan. Since these components, especially the enzymes, are also the elements that function, the behavior of the tissue can be inferred from them more reasonably and in greater detail than from its grosser morphology.

When the tissue is diseased, its dysfunction must be manifested by these altered chemical parts. Patterns of chemical composition of tissues therefore offer precise and detailed guides to the identification, classification and inference of functions among the tissues. It is truly a basic science of physiology, anatomy and pathology. A strength of this approach is its dependence upon numbers and not upon observational opinions.

Investigation of transplanted neoplasms of the rat along these compositional lines has proceeded farther than our concurrent studies of differentiation and irradiation and will serve to provide examples. The results already make a meaningful picture out of what has sometimes seemed a chaos of mutually unrelated facts about cancer. We find that normal tissues are chemically diverse. Tumors from these tissues are more similar. Tumors from any one tissue can be graded in terms of the degree to which they express the cardinal characteristics of neoplasticity, i.e., growth and anaplasia. They can be graded in a parallel way by their chemical compositions. The highest grades of neoplasms from any source are chemically very alike. This objective recognition that there are type examples of neoplasms, i.e., purest forms of the breed, will affect our approach to the whole problem of the nature of cancer. Furthermore, these most similar, high grade tumors are chemically very similar to the normal fetal tissues, both quantitatively and qualitatively. The embryonic tendencies of tumors are indeed evidences of dedifferentiation. A sizable portion of the genome that is active in tumors is part of the genome that is normally active in immature tissues. These findings have no immediate relation to the cause or cure of cancer, but they are important for the essential recognition of what is the nature of a tumor.
3. **Quantitative Analysis of Tissue Enzyme Concentrations**

We have recognized that the basic observations upon which we depend represent the special discipline of quantitative chemical analysis. It is a novel area only because it involves enzymes, which in tissues have not generally been treated as quantifiable substances. Once extracted from their source and subjected to purification, of course, quantitative analysis of them is usual, essential and reproducible. The difficulties of analysis of enzymes by their catalytic activities are many, but systematic studies show that such results can be generally reproducible and reliable—about in proportion to the extent of knowledge that is available about any given system. The acid test of any set of results is its confirmation by other workers using other methods. The analytical systems we have devised anticipate such corroborative studies by incorporating reference standards into each series of assays. Relative concentrations can then be compared even between assays that give quite different absolute activity rates in the same tissues. The compiled tissue analyses referred to in the previous section represent a published nucleus that can be completed and emended by other biochemical studies. The whole compilation can be used for investigation of the fine chemical details of particular living systems.

**Plans for Continuation with Unchanged Objectives**

Detailed plans for the coming year are presented in the proposal accompanying this summary. These are specifics that fit in tactically with our present efforts and capabilities. In a strategic sense the direction of our work will adhere to the original objectives because these have been so very rewarding. We can now emphasize certain accomplishments, and fill in other areas that up to now have been less well studied. The plan is to
broaden and deepen what we have learned, repeatedly supporting the same principles with new examples whenever possible so that these principles cannot be ignored or forgotten. The effort can be summarized as continuation in each of the three areas described above.

It is mandatory that new examples of chemical differentiation in non-hepatic tissues be added to the very few of these we have already studied. For reasons connected with a decision to use our capabilities to find out new relationships instead of only corroborating known ones, we will concentrate on the somewhat unlikely choices of spleen and thymus. In these organs changes in the cell populations occur, and we must demonstrate that the combination of chemical analyses of whole organs with quantitative cytomorphology, possibly aided by histochemistry, can handle the complexities of composition that are expected in these organs.

The patterns of composition of tissues will be extended by adding both new components and additional tissues, because this compilation forms the atlas that orients our work. It is already sufficiently detailed so that informative comparisons can be made. We expect to utilize this capability first in the description of liver in tumor-bearing rats, and second in comparisons between the different states of spleen and thymus in a variety of physiological situations. Irradiation will be one of these, used as a precise kind of injury which we have not yet been able to exploit fully. The variety of changes expected in these organs is relatively great.

Comparisons between tumors and normal tissues and especially with fetal tissues must be extended because these have produced the greatest insights from our work. We are mindful in this connection of the surprisingly similar patterns found between spleen and high-grade tumors, as well as between tumors and fetal tissues. Other emphases on spleen are also intended to investigate
this relationship.

A continuing emphasis can also be expected on fundamental biochemical problems dealing with the identification and definition of substances and processes. Our general approach continually turns up new phenomena in apparently well studied areas, and these must be described sufficiently well so they can be added to the store of knowledge of the science.

Opinion of the Present State, Significance and Needs of this Area of Investigation

The general area of interest can be called enzyme physiology and pathology. It is only inadvertently concerned with the discovery and qualitative identification of enzymes in living systems. That is the province of enzyme chemistry. Only the advanced state of the latter makes it possible to ask what its enumerated lists of substances are doing in the various kinds of cells. The point of biochemical investigation, in biological terms, is realized only by studies in this area of physiology and pathology. The import of this shows up most clearly when the earlier results of biochemical work in embryology, and even more strikingly in cancer, are evaluated. The results we have achieved provide the first coherent and meaningful biochemical bases for understanding, or at least for further unraveling, the mysteries of tissue differentiation and the nature of neoplastic tissues.

The breadth and depth of the approach we are using must be realized. We are creating a detailed science analogous to cellular pathology. The latter, by attention to a multitude of details in many tissues, has over the period of a hundred years shaped our knowledge of living things. Our new information is similar, but it is objective, numerical and concerns functional elements whose significance is immediately apparent. It can become a powerful
extension of cellular pathology, likened perhaps to an electron microscopy that reveals minute, labeled parts. Obviously, one laboratory cannot do all of this alone.

The needs of any developing area are only too obvious. What this one needs is continued support, exposure and some practical successes. Then in the marketplace of scientific progress it will be judged more realistically than if it is emphasized by artificially contrived dramatization or over-confident promises to explain all. The need for widespread concerted work can be met this way.
Publications Supported by Atomic Energy Commission

May 1966 - April 1972


Postdoctoral tenures completed:

G. C. Tremblay, Ph. D., assumed teaching and research responsibilities 9/66 as Assistant Professor at the University of Rhode Island, Department of Biology and Chemistry, Kingston, Rhode Island.

W. D. Denckla, M. D., left 12/66 to continue research at the National Heart Institute, Nation Institutes of Health, Bethesda, Maryland.

Maria Linder, Ph. D., left 6/67 for a second postdoctoral year at the Massachusetts Institute of Technology.

Agnes Tan, Ph. D., left 8/68 to continue her training at the University of Minnesota.

H. Z. Kupchik, Ph. D., left 5/69 to accept a position at the Mallory Gastroenterology Laboratory, Boston City Hospital.

Louise P. Liu, Ph. D., left 8/69 to continue her training at the University of Missouri.

S. D. Jamdar, Ph. D., left 8/70 to accept a teaching position at the University of North Carolina, Department of Surgery, School of Medicine.

M. C. M. Yip, Ph. D., left 8/70 to accept a research position with Merke, Sharp and Dohme, Rayway, New Jersey.

H. H. T. Hsu, Ph. D., left 6/71 to accept a research position with Rockefeller University, New York.

Francoise Farron, Ph. D., completed her postdoctoral work 9/71 and is remaining in this department as a Research Associate.

Graduate Students Trained

Marta M. Piras returned 10/66 to the University of Buenos Aires, Argentina, to complete her Ph. D. requirements.
Paul Goodyer, Harvard College undergraduate, did his Senior Honors Thesis here during 1968.

Freddy J. Hendler, a medical student at the State University of New York, Downstate Medical Center, spent three months in 1969 in fulfillment of his fourth year elective.

Jeanne E. Li completed her Ph. D. requirements and was awarded her degree from Harvard Medical School, the Division of Medical Sciences, Harvard University.

Margaret McGee returned 10/71 to the University, Dundee, Scotland, to complete her Ph. D. requirements.

Vas Mezl started his research work in this laboratory toward a Ph. D. degree in the Division of Medical Sciences, Harvard Medical School, 6/71.

During 1966-72 for short periods of time, medical students and graduate students from Harvard Medical School have joined this department in either research work or attending seminars as part of their course work in the Department of Biological Chemistry.