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

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W. Eugene Knox

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NEW ENGLAND DEACONESS HOSPITAL
CANCER RESEARCH INSTITUTE
185 Pilgrim Road
Boston, Massachusetts 02215

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An atlas of chemical anatomy of rat tissues was published this year (8). It also contained investigations of multivariate analyses that can use this data to identify and classify tissues and to infer their functions. Studies of the latter kind revealed the quantitative similarity between compositions of high grade tumors and fetal tissues, and this relationship has already been confirmed by finding qualitative identities of isozymic variants in the two kinds of tissues. The active genomes of fetal and neoplastic tissues are apparently very similar. A third major advance was the introduction of cytomorphometry, a quantitative type of histology, in parallel with biochemical analyses of tissues so that actual correlations of structures and volumes of specific cell types can be made with chemical components.

Developmental formations of two significant enzymes were explained as inductions by hormones, both combined with substrates as necessary but not sufficient stimuli. The emerging picture of chemical differentiation of tissues by sequential enzyme inductions was reviewed.

The retrospective study of the treatment of phenylketonuria in the United States during the decade 1960-1969 was completed. It showed that the treatment with low phenylalanine diet is effective but not completely so. Several possibilities for improving the results emerged from this study (5).

This year saw the fruition or consolidation of several different aspects of our work. These now represent solid attainments upon which we can now base new experiments. All are related to the overall problem of the control of tissue function by enzyme concentrations, specifically as this is seen in differentiation, cancer and irradiation of tissues. The book described below is one product of six years of support by the AEC that helps to crystallize the nature of the work we are doing.

Statement of Time or Effort of the Principal Investigator

During the current year of the project, the Principal Investigator, W. Eugene Knox, M. D., Senior Research Associate at the Cancer Research Institute, has spent 50% of his time devoted to the research of this project.

It is expected that during the coming year, Dr. Knox will continue to spend 50% of his time on this project.

The major limitation in the study of enzyme physiology, even in the rat upon which the most biochemical work has been done, was until now the lack of systematic information about tissue compositions, especially of nonhepatic tissues. This situation has been remedied to a certain extent by the publication of our own analyses and those from 50 years of biochemical literature that can be expressed in comparable units. It comprises values for more than 150 chemical components, mostly enzymes, in 17 and more different rat tissues and tumors (8). It will eventually have a considerable effect on the science of measurement in biochemistry, since it makes clear that most biochemical work has been of a qualitative kind which contributed little to the corpus of knowledge about tissue compositions. It also provides a much needed yardstick for evaluating tissue analyses, many of which are now published based on inadequate methods or containing decimal errors without this being noticed. The major purpose of the compilation, however, is to provide data of sufficient breadth so that answers could begin to be sought to some fundamental problems. These include the possibility that the different functions of tissues are paralleled by different chemical compositions, that tissues in health and disease can be identified chemically even more precisely than by morphology, and that generic relationships between certain tissues and their reactivities can be found.

A variety of methods of multivariate analysis were tried out on this first compilation that is of sufficient size for such applications. The results show that it will be worthwhile to invest the effort needed to extend and improve this nuclear compilation. Experts in numerical analysis are already trying new methods on it. Even this imperfect compilation gave results of interest: normal tissues are chemically distinct from one another, fetal tissues are more alike than adult tissues, and tumors do tend to resemble one another. New classifications to be useful should lead to predictions that can be tested.

Of special interest in this connection was the similarity found between tumors and fetal tissues, a phenomenon referred to as the fetalism of tumors. In addition, a quantitative numerical way of measuring the degree of chemical differentiation of tissues was devised. This will have the large effect that always follows replacement of descriptive by quantitative information.

Isozymes in Fetal, Neoplastic and Adult Tissues

Similarity between the quantitative patterns of enzymes in fetal and tumor tissues indicates that the composition of neoplasms is not disorderly, but, in fact, of a familiar type. The apparent relation of dedifferentiated neoplasms to undifferentiated embryonic tissues, which morphologists cannot certify as being more than apparent, would seem on this chemical evidence to be real. For these and other reasons it was important to test the validity of the relationship further. Particularly concrete evidence of similarity would be qualitative identities, i.e., the occurrence of the same chemical entities in fetal and tumor tissues, when these were not characteristic of the cognate adult tissues.

Isozymes that exist in different forms in fetal and adult tissues were examined (10). In three cases limited to hepatic tissues the same isozyme form predominated in fetal liver and hepatoma, with a different form in adult liver (ATP:glucose phosphotransferase, glutaminase and carbamylphosphate synthetase). Special search of tissues and tumors turned up three other cases that could be compared in nonhepatic tissues and tumors. The same pattern in fetal, tumor and adult forms of kidney, muscle and mammary gland was followed by one or more of the isozymes of lactate dehydrogenase, aldolase and pyruvate kinase. Fetal forms of some enzymes persisted in all three kinds of a tissue, which is consistent with but not positive evidence for the rule. In no case did the isozyme occurrence run counter to the rule. It can be concluded that a significant part of the genome active in tumors is that part also active

during immaturity of the tissues. This will necessarily affect our view of the nature of neoplasms.

Cytomorphometry

This technique of calculating three-dimensional forms from two-dimensional microscopic sections has been used by geologists for more than 50 years, but never by histologists until very recently. Biochemical measurements are quantitatively related to the weight and volume of tissue structures, and do not fit together well with the usual descriptive and enumerative data of histologists. Cytomorphometry has, however, already given us quantitative measures of the volumes of the various cell types in developing liver which we can compare directly to the chemical measurements per gram that we made on the same material (6). Volumes of organelles and other structures, and changes of the morphology by induced differentiation, are currently being measured as part of the integration of chemical differentiation of tissues as we see it into its morphological counterpart that has been known for a longer time.

Chemical Induction of the Molecular Steps in Tissue Differentiation

New instances of prematurely induced enzyme formations both occur in the late suckling stage. As predicted, different stimuli are responsible for these nearly simultaneous differentiations. Malate-NADP dehydrogenase which is normally absent up to the 18th day of life can be evoked at the age of 9 days with thyroxine treatment (3). The levels of ATP-citrate lyase and pyruvate kinase can be raised in the suckling rats by glycerol or glucose administration (3). These results, together with previous observations on glucokinase (which requires hydrocortisone and glucose for premature induction), support the hypothesis that in the normal course of development the almost simultaneous formation of different groups of enzymes is evoked by different

physiological stimuli: towards the end of the suckling period increased pituitary-adrenocortical activity, thyroid hormone secretion and dietary factors are the important stimuli. Both glucokinase and tryptophan oxygenase can be caused to develop in rat liver well before the scheduled times by an injection of hydrocortisone followed within a definite period by injection of the appropriate substrate (glucose or tryptophan, respectively). Both the hormone and the substrate are obligatory, and so is the particular schedule of dosage (4).

Biochemical Definitions of Tissue Enzymes

A considerable amount of our work has consisted of developing and verifying an assay for an enzyme in crude tissue extracts, and then surveying its distribution in tissues in various physiological states. Almost every enzyme studied in this way reveals problems. Glucosamine synthetase was measured in many tissues (7). It persists in all tumors, but its assay needs improvement. Aspartate aminotransferase has been widely studied, but no distribution study of both its mitochondrial and soluble isoenzymes had been done with precautions to measure the full activities. This was done (2), with the result that the relative isozyme concentrations in mammary gland and muscle were found to fit the rule previously described: fetal and tumor tissues have the same isozymes predominating, but adult tissues may (and do in these tissues) have a different isozyme form. The two isozymes of glutaminase, one of which is characteristic of neoplastic tissues, was further characterized by showing that their different requirements for phosphate were retained in soluble extracts and were not simply properties of the different mitochondria in which they occur (9). A more elaborate study failed to find any chemical differences between the kidney and liver forms of ornithine aminotransferase which are independently induced by estrogen and cortisol, respectively (1). Apparently, controls can be quite

different when acting on the same genes but in the differing milieus of two tissues. This is consistent with all we have learned about regulation of specific enzymes in animal tissues.

Diagnosis and Treatment of Phenylketonuria

Analysis of the National Registry of phenylketonuria patients treated during the 1960's was completed and a report submitted to the Children's Bureau, which has contractual relations with most state programs for caring for these patients. The report and its preliminary findings have already dampened down unfounded criticism of the treatment of this first genetic disease to be ameliorated on a national scale. The results show that the treatment used is effective and that the patients are reasonably well identified. Treatment is neither bad, as some charged, nor completely preventive of mental deficiency in all cases. Possible improvements in diagnosis and handling become apparent from the study. One follows from the evidence that phenylalanine need be reduced only to moderate levels, not to normal, for the full effect of treatment.

PUBLICATIONS SUPPORTED BY

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LECTURES AND TALKS BY SCIENTISTS SUPPORTED BY
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W. E. Knox	5/6/71	PKU Program Analysis, New York Medical Center, New York, N. Y.
O. Greengard	5/11/71	"Enzyme Differentiation in Liver" Methodist Hospital, Brooklyn, N.Y.
W. E. Knox	10/5/71	Phenotypes of developing and neoplastic tissues. Boston Cancer Meeting
F. Farron	10/5/71	Fetal isoenzymes in rat tumors. Boston Cancer Meeting
W. E. Knox	10/11-12/71	Patterns of Enzymes in Tissues (2 lectures) University of North Carolina Chapel Hill, North Carolina
W. E. Knox	11/7-17/71	Chairman of session; participant 2nd PAABS Symposium "Biochemistry of Glycosidic Linkage", San Carlos de Bariloche, Argentina
W. E. Knox	11/23/71	Phenylketonuria, Harvard Medical School, Biochem. 709
W. E. Knox	11/30/71	Cystinuria, Harvard Medical School, Biochem. 709
W. E. Knox	1/19/72	Phenylketonuria, Oregon State University, Nutrition Research Institute, Corvallis, Oregon
W. E. Knox	2/16/72	Control of enzyme phenotypes in tissues. Harvard Medical School, Department of Physiology
W. E. Knox	3/21 and 3/23/72	Enzyme induction in mammalian tissues. Harvard Medical School, Biochem. 716

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