C00-3017-2

MASTER

THE PROTOPLASMIC PATTERNS OF TISSUE AND TUMORS

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JAN 10 1972

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Biochemists have long avoided the word protoplasm with its ancient vitalistic connotations. They undid its reference to a homogeneous material by busily enumerating the various chemical substances to be found in living cells. Now that most of these substances are known, and the various forms of life are found to conform to an essentially common chemical plan, the time may have come to resurrect this useful word. It is widely understood to mean the living cell substances. It could be well employed to name the patterns of chemical composition that are characteristic of each different kind of cell. Despite the common plan of living things, there are indeed very many such protoplasms. An immense variety of chemical compositions is one suggestion to account for the many different morphological structures and physiological functions which living cells assume. The enzyme proteins, because they embody the elementary cell functions, would seem particularly likely to differ among cells. Systematic comparisons of the compositions of different tissues in an animal now confirm this suggestion. The same comparisons open the way to new dimensions of resolution and inferences about living systems, because there is this great diversity of chemical composition within the biochemical unity of life. The method consists of using the many explicit things in its composition to characterize a tissue, and to compare these as patterns instead of as one thing at a time.

There are more sophisticated procedures used outside of biochemistry to analyze patterns than those described here (cf., Oxnard, 1969a and b; Sokal, 1963). Adequate chemical data to make effective use of known procedures could readily be accumulated by a little cooperative effort, and

the present purpose is to indicate by some simple examples that such effort would be worthwhile. The examples are ones chosen to eliminate some of the mystery surrounding neoplasms, and to some extent to clarify their natures. The effort should not be confused with more ambitious quests for the cause and cure of cancer. The emphasis is simply upon what are the compositions of some living systems, and the relation between normal animal tissues and their neoplasms, those lumps that the late Peyton Rous (1967) prophetically called "flesh of our flesh".

Machines that men build are generally regulated by increasing the rate at which a given part acts. An automobile accelerates because the motor turns faster. There was considerable surprise on finding that an important way of regulating the function of animal cells was to increase the amount of a molecular part (Knox, 1951; Knox et al., 1956), even though whole parts, like the arm of a blacksmith, were long known to hypertrophy with need. On the molecular scale, it was as if pressing down the accelerator pedal of a car brought into operation an uninterrupted sequence of more and more powerful engines. A cell, in contrast to the clumsier machines made by man, is exquisitely designed for just such transmutations. Its functional protein parts are made according to the DNA patterns stored in its genome. These genes are necessary but not sufficient to ensure the manufacture of the products. There is a continuous flux of gene products being made and unmade in the dynamic equilibrium of the bodily constituents that Schoenheimer (1942) first recognized. The sufficient stimulus for a gene's expression can therefore easily increase the manufacture of a gene product and cause its temporary accumulation by increasing the ratio of

its synthesis to its degradation. The composition and therefore the very nature of the cell is altered. The system works equally well in the opposite direction to diminish a part. The elegance of the system is brought home with realization that the sufficient stimuli on which the whole is poised are the concentrations of the metabolites to be changed in the system: the substrates to be gotten rid of and the products to be made. Elaborate translations or transductions are often interposed between cause and stimulus by information chains like the hormonal systems, but the principles are simple. As a result, the chemical composition of a cell faithfully reflects its functions at that moment. "Each cell, each living being, has a multipotential biochemical personality, but the physico-chemical environment determines the one under which it manifests itself" (Dubos, 1950).

Until recently this adaptive regulation of the cell's composition has been studied only by experimental interventions, measuring one thing at a time. Observations in animals were restricted mainly to liver tissue, whose peculiar attraction for biochemists may be related to its easy identification among the organs of an animal. A component, usually an enzyme, was measured in control animals, then after administration of some stimulus like a substrate or hormone to other animals, the component was remeasured. From the change in concentration of an enzyme produced by this experiment, various inferences were made about what controlled the enzyme, and what the enzyme controlled, i.e., about the chemical and physiological mechanisms involved.

Complete dependence upon these experimentally produced differences ended when it was eventually recognized that the concentration of an enzyme

in untreated animals also varied -- that nature was continuously producing the same kind of experimental differences for our edification -- we had only to observe them. Looming large among the natural differences in tissue composition produced by stress, age, nutrition and difference in sex were those produced during development. The several tissues differentiated into a variety of individual compositions, each nicely adapted to perform its particular function, but all representing compositions drawn by their needs, or sufficient stimuli, from the animal's library of genes.

The compositions of various tissues are not yet known on an appropriate scale to serve the many potential uses of such information, because its usefulness was not obvious. However, ~~contributing~~ quantitative and qualitative tissue analyses of animals in well-defined physiological states falls naturally within the competence and interest of most biochemists. Use of reference standards is increasing so that analyses from different laboratories can now sometimes be compared. This was less necessary when the greater emphasis was on an experimental difference produced in a given laboratory and not on the base line values. In lieu of stable preparations of the hundreds of enzymes needed to serve as standards, an animal itself can provide the norm. Some readily accessible living tissues, such as rat liver with which biochemists are already so familiar, are serving as reasonable reference standards. The chaos of expressing tissue analyses in terms of a variety of measures of tissue quantity (e.g., per cell number, protein, DNA, dry weight or ash) is subsiding in favor of rational use of the primary measure of weight as the tissue base. Such conventional cooperation is necessary simply because the number and variety of analyses to be accumulated are beyond the capacity and competence of

single laboratories. More analyses will inevitably accumulate, if only secondarily as control analyses for the more traditional kind of experimental interventions: they offer a hedge against the worthlessness of a negative experiment. When an idea doesn't work out, and this happens even to biochemists, properly expressed control values can still contribute significantly to our knowledge of living systems. An off-beat interest in controls instead of experimental results may seem like taking a seat in the stands instead of a position on the playing field, but it is a fact that the view from on high is incomparably better. The first substantial lists of tissue analyses are now being created or compiled from such perverse attention to the controls in published work (Knox, 1972). It is possible to begin to try on animal tissues some of the multivariant techniques that in other fields have created new insights about relations between things.

The pattern of composition of a tissue is a list of the kinds and amounts of the substances composing it. Any actual list we accumulate is only a sample of that tissue's pattern. One sample pattern is almost meaningless until it is compared with another one. Comparisons between whole patterns are somewhat more elaborate than the familiar comparisons between two items, one at a time, which biological science has mainly used until now. Comparison of patterns is analogous to the visual comparisons made by a trained morphologist. He distinguishes between visual images, real or remembered, in the same way that the rest of us recognize the face of a friend among a crowd or in a photograph -- usually without knowing explicitly what distinguishing features guide us.

The comparison of patterns is explicit and much simpler to describe. The differences between, and the developmental differentiation of, various

animal tissues can provide examples. Differentiation is a crucial process that should be more precisely defined than it now is. By this process we expect that a clump of primordial cells will change over a period of time into a mature tissue that is different from its primordial state. Another clump will change into a second mature ^{one} tissue, also different from the origin, but at the same time diverging from the first tissue. In this way, the two mature tissues are differentiated from their common origin and from each other. We will first compare liver and kidney, tissues which are available for ordinary biochemical purposes only from the late fetal stage. Morphological differentiation is by then nearly complete. Nevertheless, the minimal changes remaining to be transversed are very clear at the level of chemical resolution. The differences reside in the different amounts of each component in two tissues. If the amounts of all the components are normalized so they are expressed in comparable units (e.g., relative to adult liver), the differences between each pair of values can be averaged. Instead of a simple average it is most useful to employ one of those variants that statisticians call "distance". This eliminates certain complications and supplies as well measures of statistical significance. The answer is a number that expresses quantitatively the average difference in composition of the two tissues. For another pair of tissues that are more unlike than the first two, this average difference will be greater. There is almost no limit set to the degree of difference that can be resolved; limits are definitiely set by what a morphologist can observe and mentally compare. If the chemical difference observed is too small, additional components can always be included until the desired degree of precision is obtained for the difference between any two tissues.

When late fetal liver and kidney are compared in this way, with 25 components (Figure 1), the fetal tissues are indeed somewhat different. They have changed from the hypothetical embryonic tissue (with zero difference from itself) and in the process diverged from one another to an average difference of 0.769. The adult tissues each differ further from their fetal congeners, and have diverged still more from one another, to approach the maximum average difference of 2.5. Thus, these two tissues do undergo explicit chemical differentiation from their late fetal states and from each other in the process of maturation. The changes are readily detected by chemical means.

It is important to note that we have here a quantitative measure of differentiation. Heretofore this process has only been described in words or pictures. A statement by Lord Kelvin underlines the significance of this:

I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of science, whatever the matter may be.

-- Lord Kelvin (Wm. Thomson, 1891-94)

Average differences between other tissues were measured with the same analyses (Knox, 1972). Fetal brain and lung were relatively close to fetal liver and kidney, while all four adult tissues had diverged substantially

(Table 1). During differentiation the four tissue patterns diverged, spreading out like a cone. These patterns of tissue composition fit our ideas about morphological differentiation, but the chemical differentiation is quantitative and explicit and a good deal more sensitive than that based on morphology. Patterns of composition have provided factual confirmation for an important biological postulate: fetal tissues are more alike than are the adult tissues. I will return to this major theme shortly.

We must notice briefly the molecular nature of the changes occurring during tissue differentiation. Liver, which has been most closely studied, is convenient to show that the changes of differentiation do not flow smoothly. Differentiation is a lumpy, molecular process: the grains of it are stepwise changes in the components we used to establish the patterns. The steps are changes in gene expression. Some enzymes, like hexokinase, diminish from very high concentrations in the youngest liver to lower values after birth (Fig. 2). More often, as is the case with glucokinase, the responsible gene is not expressed until a particular age. Then the enzyme suddenly appears and soon reaches its adult level. Such changes are not programmed in time by the genome, but by the metabolic environment. The sufficient stimuli are the same kinds of adaptive stimuli, hormones and substrates, that were earlier recognized to induce elevations in preexisting enzymes in adult liver (Greengard, 1971). Widespread awareness of this problem of initial expressions of genes stems from the sequential expressions of different genes during human development that produce the molecularly different hemoglobins F and A. The effective stimuli controlling these particular genes are still not known, but we do know what particular substances in the chemical milieu of the organism control the expression of other genes.

Tyrosine aminotransferase, for example, first appears in liver suddenly after birth (Figure 3). Its level in later life is subject to ups and downs, and regulation of these have been very thoroughly studied. This knowledge helped to identify the initial, evoking stimulus, although we have learned that a stimulus effective in the adult need not be the one operating earlier on the same enzyme. For this enzyme it is glucagon, secreted by the newborn animal during the hypoglycemia that occurs when he is separated from his placental nourishment. Proof of this followed from prevention of the enzyme rise by prompt administration of glucose, and more dramatically by premature evocation of the enzyme when glucagon was given to the fetus in utero. Tryptophan oxygenase regulation in the adult liver has also been well studied. The cause of its first appearance early in the third week of life was not as easily identified. It required the successive action of two stimuli: hydrocortisone, followed by the substrate, tryptophan -- both of which influence the level of this enzyme in the adult (Figure 4). When both are given in the appropriate order, the enzyme can be produced long before its scheduled appearance in normal development.

A final example of the molecular steps in tissue differentiation concerns the appearances of an enzyme in two separate tissues (Figure 5). Ornithine aminotransferase normally appears in liver in response to glucocorticoids: its appearance can be avoided by adrenalectomy, or produced prematurely by earlier administration of hydrocortisone. The same enzyme in kidney is unaffected by these natural or artificial stimuli. The enzyme in kidney appears later than in liver. It appears during sexual maturation, and is partly under the influence of estrogen. Because of this it ultimately reaches a level twice as high in the female as in the

male kidney. This is, then, one of the numerous sexual "dimorphisms" produced during differentiation. That a sex difference should be found in kidney, not one of the most sexual of organs, simply testified to the sensitivity of the chemical method. There are many such instances.

Most of these stepwise changes in liver composition occur at certain critical periods in the life of the animal: in the late fetal period, immediately postpartum, late in the suckling period, and during sexual maturation. Changes cluster at these periods because of control of gene expression by substances, hormonal or metabolic in nature, present in the chemical milieu of the cells. These substances, representing the environment of the cell, fluctuate most at these critical times in the life of the animal. The sequence of gene expressions is therefore orderly, but not mechanically so like a clockwork. It is flexible, as shown by the ability to defer these changes, or to evoke them prematurely.

Molecular changes in composition of a tissue, like those that I have just described, were averaged together to give the average differences in composition that measured the chemical differentiation of a tissue. We can watch these differences accumulate by looking at patterns not only of fetal and adult tissues, but also at intermediate ages. We can, in fact, measure the rate of tissue differentiation. Fetal liver, for example, differentiates rapidly at first and later more slowly (Figure 6). With some imagination you can identify active periods, with the clustered changes occurring in the late fetal period, immediately after birth, and at later stages, each subsequent cluster adding less to the total differentiation as the tissue approaches its adult composition.

The quantitative, numerical description of chemical differentiation of tissues, especially of liver, indicates that tissue differentiation during its late phase observed here proceeds from a simpler to a more complex pattern of composition. More genes are newly expressed than are turned off. (Nevertheless, changes of either kind contribute equally to the average differences by which differentiation is measured.) The old familiar idea of a pluripotent embryonic tissue differentiating into a unipotent mature tissue does not actually conflict with the new facts, since the idea concerns only potentialities and not the actual expressions of genes. Embryologists have often confused these two aspects, and sometimes refer to differentiation as progressive loss of gene expression. The actual gene expressions are reciprocal to the "potentialities", so that differentiation increases as more genes, not fewer, are expressed.

I want to return now to the major theme. Consideration of the differentiation of fetal tissues can be extended to an analogous problem concerning the nature of neoplastic tissues. Jessie P. Greenstein in 1947 offered a conclusion, now relegated to footnotes of the history of cancer research, that was remarkably similar in form to the conclusion reached above about the differentiation of fetal tissues. Both were based on the study of the patterns of composition of tissues. Where we concluded that fetal tissues resemble each other more than do the adult tissues into which they will develop, Greenstein concluded that tumors resemble each other more than do the normal tissue from which they developed. Obviously, the method used to establish our conclusion could also be used to test his. However, we must pause to consider the fate of Greenstein's conclusion. It was based on about half as many analyses as the comparisons I have

shown, and it indicated that various tumors were indeed very similar in composition. One of his summarizing slides (Figure 7) is reproduced here (Greenstein, 1956): if you compare the tissues, component by component as you must when patterns are not given in numbers, you will reach his conclusion. This result created a paradox, because everybody who had some experience with neoplasms knew that the outstanding characteristic of tumors was not their uniformity but their diversity in nature and behavior. The known diversity of tumors would also settle the matter for me -- if I could not suggest a hypothesis to resolve the paradox. The hypothesis is that the great diversity we see among neoplasms comes from those tumors that are relatively well differentiated. These are tumors that possess some or many characteristics of their tissues of origin, characteristics that coexist with the neoplasm and contribute to it a highly unique and even bizarre nature. For example, we accept the obvious differences between breast and thyroid tissues. There is probably less difference between an adenocarcinoma of breast that forms milk and a thyroxine-forming thyroid carcinoma, but such a contrast exemplifies the diversity between tumors. On the other hand, the most highly undifferentiated carcinomas look alike and even behave in very much the same way, so that we are often uncertain of which tissue they come from. I am strengthened in my view that this is the basis of the paradox because Greenstein analyzed only highly undifferentiated tumors. We can test this hypothesis, as well as Greenstein's original conclusion, by another comparison between tissue patterns.

This time we compare two tissues, liver and mammary gland, and two tumors derived from each, one of which is morphologically well differentiated and the other undifferentiated (Figure 8). The average differences

in compositions show that within each tissue series the morphologically differentiated tumor is closer to the parent tissue than is the undifferentiated tumor. This relation further substantiates the parallelism between chemical and morphological differentiations. The crucial comparisons are between the two tissue series. The parent tissues are very different, as are most adult tissues; the intermediate tumors are less different; the undifferentiated tumors are quite similar -- as similar as some of the fetal tissues were to one another. Qualitatively unique components from the respective tissues of origin could easily persist in either of these intermediate tumors and make them appear and behave very differently, more than the average difference could seem to indicate. Such organ specific components, and consequently the great diversity between tumors, disappears in the undifferentiated tumors. Thus, these results confirm Greenstein's conclusion, and explain the diversity of intermediate tumors that caused the earlier rejection of his conclusion. The correctly stated generalization¹ is that tumors resemble each other more closely than do the tissues from which they developed, and this resemblance increases in proportion to the tumor's loss of (morphological and chemical) differentiation.

Patterns of composition have shown that there is an analogy between the differentiation of fetal tissues and its reverse, the loss of differentiation by tumors. If these changes are more than analogous -- if they are homologous -- important consequences would follow about the nature of the neoplasms. There is an a priori reason for taking the analogy at face value. If fetal tissues are similar because they have not yet undergone

¹ Historically, this general idea has been associated with the word "convergence". If it is used at all, it must be made clear that the data is based on analyses of discrete tumors which can be arranged in a convergent series. The generalization is independent of the possibility that a single tumor might "progress" from a differentiated to an undifferentiated state.

differentiation to adult tissues, and if tumors are similar because they have lost the same differentiation from adult tissues, both groups should be similar to one another -- unless, of course, we were willing to believe that some exotic composition of tumors would also be compatible with their efficient kind of life. However, the question is one of fact. It can be decided on the molecular scale of differentiation: are the enzymes that change during the differentiation of fetal tissues the same ones that undergo reverse changes in the dedifferentiation of tumors? Only about half of the 25 enzymes measured in the comparison of fetal tissues were among the 21 measured in the comparison of tumors, yet the small number common to both sets suggest that the same enzymes do change in differentiation and in dedifferentiation.

There is much more data available on this point, which can also illustrate another use for enzyme patterns of tissues. Biochemists have collected more analyses of hepatic tissues than of any others. No less than 82 different components have all been measured in fetal and adult livers and also in fast hepatomas of the rat (Knox, 1972). These have been compiled as values relative to each component in adult liver (= 1.0 L.U.), then classified according to whether they were low (< 0.2 L.U.) or high (> 0.9 L.U.) in fetal liver and hepatoma (Table 2). Those low in fetal liver would be identified as adult-type enzymes that have not yet developed. Thirty-two are in this class. Interestingly, the same number are in this class in hepatoma. Those high in fetal liver would be identified as fetal-type enzymes, being already present in that immature tissue. About the same proportion of these are in both tissues. The remaining ones are intermediate in concentration. It is now possible to see if particular enzymes are independently assorted into the classes, or whether the enzymes that are low in fetal liver are also the low ones in hepatoma, and the same for intermediate and high ones.

The null hypothesis of independent assortment must be firmly rejected. The same "adult" enzymes that are low in fetal liver are the low ones in hepatoma. The same "fetal" enzymes that are high in fetal liver are the high ones in hepatoma. The same enzymes are intermediate in both tissues. This means that the quantitative pattern of gene expressions in hepatomas very closely resembles that in fetal liver. This will inevitably have a major impact on our ideas about the nature of neoplasia.

What I have just presented is important enough to show these same patterns in another way. The enzyme values can be graphed on a scale with 1.0 as the level in adult liver, with the enzymes in hepatoma on one axis and those in fetal liver on the other axis. I have just shown that the low, intermediate and high groups of enzymes were generally the same ones in both tissues. When the individual values recorded for each enzyme are graphed, it is clear that most of the enzyme levels run parallel in the two tissues -- they are correlated with a high degree of significance (Figure 9). In the past, some hypothetical prominence was attributed to "deleted" enzymes in cancer. They are here in quantity -- those with low values in tumor (relative to adult liver). They can be immediately identified, because of their correlated low values in fetal liver, as "adult" type enzymes not yet developed in fetal liver and preferentially lost from tumors.

Important as this general relationship may be, it would be an onerous job to confirm each of these 82 measurements. (An even more weighty effort will be required to compare other kinds of tumors with other fetal tissues.) Still, there is reason for some confidence in the results as they stand. They were not done in double-blind studies, but at least the

measurements in the two tissues were usually made by different people, often oblivious of each other and certainly innocent of what I would contrive to do with their results. Correlations like this would not be produced, but destroyed, by analytical errors in any quantity. If we therefore accept most of the correlated measurements, this allows us to direct attention to the few aberrant values. These are either errors, as we have found by repeating some of them, or, as we have confirmed in most instances, they are real differences that distinguish the hepatoma from the normal tissue it most closely resembles! These confirmed but aberrant components show that chemical distinction is possible between particular tumors and normal tissues.

It will be some time before there are quantitative analyses on a sufficient scale to compare other kinds of tumors and other fetal tissues in the same way. A fast-growing hepatoma cannot yet be accepted as typical of tumors in general, and certainly fetal liver does not represent all fetal tissues. However, there is a logical basis for supposing that the resemblance extends to more than just hepatoma and fetal liver. We have seen that fetal tissues tend to resemble one another, and to that extent the resemblance of fetal liver to hepatoma indicates some resemblance of all fetal tissues to hepatoma. On the other hand, and neglecting the persistent characteristics carried over from the tissue of origin, (undifferentiated) tumors also resemble one another. To this extent also, the resemblance of hepatoma to fetal tissues indicates some resemblance of all tumors to all fetal tissues. This general tendency can be called the fetalism of tumors. Neoplasms become less mysterious as they are recognized to be similar to a familiar type of normal tissue and as they become susceptible to some logic.

There is another kind of pattern of tissue composition, the qualitative kinds of its proteins, that can be used efficiently to compare a whole variety of tumors and fetal tissues. Variants of some proteins, like the hemoglobins, occur in different forms in fetal and adult animals as a result of different genes being expressed at different ages. When a tissue contains such a protein, the form of the protein present could identify the ontological stage of the tissue in a very concrete way. The isoenzyme forms of many enzymes differ with age in this way. The logic of what has been learned from the quantitative patterns of tissues leads to the prediction that the same genes expressed in fetal tissues should be expressed in tumors, and this prediction can be tested by electrophoresis of the tissues. Isoenzyme forms in tumors had, of course, been examined before, but only individually, not systematically and not with the expectation of finding a particular pattern. Results on any single enzyme would, it turns out, be unconvincing. But ~~four~~^{three} different enzymes disclosed the clear pattern (Farron et al., 1972). Four adult tissues all differed from one another in the patterns of predominant isoenzymes of the ~~four~~^{three} enzymes that they contained. All of the related fetal and tumor tissues shared one pattern of predominant isoenzymes, and this pattern was not like that in any of the adult tissues (Table 3). These results are the expected ones, based on the differences in quantitative patterns examined earlier. Fetal and tumor tissues resemble each other more than do the related adult tissues, in qualitative isoenzymes as well as quantities. To the extent that the different isoenzymes do indeed represent products of different genes, the constellations of active genes are also similar in the several tissues. A large segment of the genome is apparently functioning in the same way in fetal and tumor tissues.

At present we suspect that much of the tumor's substance and metabolic machinery conforms to the pattern found in normal, immature and growing tissues. Since the same enzymes change during differentiation and dedifferentiation, stimuli similar to those found to produce the steps of chemical differentiation of immature tissues might also be used to reverse the steps of dedifferentiation in tumors. Attempts of this sort offer a wholly new therapeutic approach aimed at altering the nature of a tumor without necessarily "curing" or eradicating it. However, our current attempts are blind. Each of the known stimuli for differentiation acts strictly within the context of a definite tissue stage and milieu. At other ages or in other tissues the same stimulus is not effective -- this changed responsiveness is, after all, the point of differentiation. Unfortunately, this limitation applies most stringently to tissues in various disease and neoplastic states. Nevertheless, what is being learned adds substance to the belief that cancer, by its nature, is a disease of tissue differentiation.

In summary, analysis of the pattern of composition of a tissue (i.e., the kinds and amounts of substances making up a tissue), and comparison of such patterns of different tissues, can give chemical results of the kind we now depend upon morphological studies to provide. An outstanding virtue of the chemical method is the numerical form of its results, which can be combined with mathematical analyses of great power and range. Such patterns can distinguish between tissues, relate similar tissues to one another, and detect changes during development or disease. Other uses include ones based on more direct inferences of function and physiological state from the chemical components of a tissue than are now possible from its cytological structures.

A chemical pathology competent to do what cellular pathology also does can be realized in this way. The method will require tissue analyses to be done in such a way that they can be systematically accumulated on a larger scale than is now possible. Because of the need for many accumulated analyses, the chemical method will be of greatest usefulness in detecting either very broad relationships between tissues, as shown here between fetal tissues and tumor, or very fine distinctions between quite similar tissues.

Examples of the method's use demonstrated the differentiation of fetal tissues, the dedifferentiation of tumors, and the resemblances between these two processes.

Acknowledgment

This investigation was supported by United States Public Health Service Grants AM 00567 and AM-K6-2018, both from the National Institute of Arthritis and Metabolic Diseases, and by United States Atomic Energy Commission Contract AT(30-1)-3779 with the New England Deaconess Hospital.

(CNO-3779-50).

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Table 1. Average differences in patterns of 25 components between fetal tissues and between adult tissues of the rat.

Distances are expressed as in Figure 1 from data in Knox, 1972.

	Fetal tissues			Adult tissues		
	Liver	Kidney	Brain	Liver	Kidney	Brain
Kidney	0.769	--	--	1.947	--	--
Brain	0.926	0.425	--	1.701	1.170	--
Lung	0.750	0.293	0.374	1.402	1.177	0.938

Table 2. Assortment of enzymes in fetal liver and fast hepatoma by relative concentration.

Low (< 0.2 L.U.), intermediate, and high (> 0.9 L.U.) values (adult liver = 1.0 L.U.) are from Table XVIII, Knox, 1972.

Fetal liver	Fast hepatoma			Total
	Low	Intermediate	High	
Low	24	7	1	32
Intermediate	6	22	8	36
High	2	3	9	14
	—	—	—	—
Total	32	32	18	82

$$\chi^2 = 42.0; P < 0.001$$

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Table 3. Isoenzyme patterns of three enzymes in adult and fetal tissues and tumors of the rat. Significant secondary components in a tissue are placed in parentheses. Boxed components are those distinguishing the isoenzyme pattern of an adult tissue from the cognate fetal and neoplastic tissues.

	Liver	Kidney	Muscle	Mammary Gland
<u>Adult Tissues</u>				
Lactate				
dehydrogenase	M	H	M	M(H)
Aldolase	B	A(B)	A	A(C)
Pyruvate kinase	L	K	M	K
<u>Fetal and Tumor</u>				
<u>Tissues</u>				
Lactate				
dehydrogenase	M	M	M	M
Aldolase	A	A	A	A
Pyruvate kinase	K	K	K	K

Figure 1. Diagram of "distances" between enzymic compositions (25 unselected components) of fetal and adult rat liver and kidney. Values were standard deviates. Distance is Penrose's shape factor (C_2) on a scale running from 0.0 (identity) to ~ 2.5 for maximal difference (from Knox, 1972).

Figure 2. Changes in concentrations of hexokinase and glucokinase in rat liver during development, expressed as percent of adult values (from Knox, 1972).

Figure 3. Changes in concentration of tyrosine aminotransferase in rat liver during development, expressed as percent of adult value. Its explosive appearance in the hours after birth can be reproduced in utero by glucagon (---▲---) injections, and prevented by actinomycin treatment (X). (From Greengard, 1971)

Figure 4. Changes in concentration of tryptophan oxygenase in rat liver during development (adult value is 70 units/g). Premature formation (---▲---) occurs after hydrocortisone injection, followed six hours later by L-tryptophan injection (from Greengard and Dewey, 1971). It can be prevented by actinomycin treatment (X).

Figure 5. Changes in ornithine aminotransferase in rat liver (—) and kidney (---) during development, expressed as percent of the adult values in each tissue. Premature formation in liver but not kidney occurs with hydrocortisone treatment (filled symbols), and in kidney but not liver with estradiol treatment (open symbols) (from Herzfeld and Greengard, 1969).

Figure 6. Differentiation of rat liver at successive ages measured quantitatively by average distance (d , —) or shape (C_2 , ---) from 18 components. The same data is expressed as the increasing distances from fetal liver, or the decreasing distance to adult liver, with advancing age.

Figure 7. Relative enzyme activities in normal and neoplastic tissues of mice. Lengths of horizontal bars in a column represent levels of a given enzyme (from Greenstein, 1956).

Figure 8. Diagram of distances (cf. Figure 1) between enzymic compositions (21 unselected components) of adult liver and virgin mammary gland, differentiated hepatoma (Sl. Hep.) and mammary adenocarcinoma (7A), and undifferentiated hepatoma (F. Hep.) and mammary carcinoma (5A). Distances between tumors of the two tissues are significantly less than between the parent tissues (P values). Mammary glands of pregnancy or lactation show the same relationships.

Figure 9. Comparison of 82 components in fetal rat liver and fast hepatoma (concentrations in adult liver = 1 L.U.). Components which distinguish between the tissues by diverging most from the approximate correlation (---) are identified (from Knox, 1972, Table XVIII).

AVERAGE DIFFERENCES IN PATTERNS OF 25 COMPONENTS

CONVERGENT PATTERNS OF UNDIFFERENTIATED TUMORS (21 COMPONENTS)