Enzyme Changes During Growth and Differentiation in the Tissues of the Newborn Rat*†

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A theory of cancer has been presented (10, 11) in which it was suggested that growth may be correlated with a lowering in the effective concentration of a hypothetical enzyme X, which was suggested to be a respiratory enzyme or a complex of respiratory enzymes (11). According to this theory, growth in specialized tissues is prevented by an intrinsic scarcity of factors essential to it. Available evidence as to the identity of these essential growth factors emphasizes the breakdown products of the reservoirs of phosphate-bond energy, and it was pointed out that the concentration of these products is the resultant of the balance between the energy-requiring processes of function and maintenance versus the activity of the energy-yielding processes of glycolysis and respiration (11).

A corollary of the theory is that tumor tissue and growing normal tissue should contain less of the respiratory enzymes than specialized adult tissues, and there is no reason, a priori, why tumor tissue should contain any less of these enzymes than growing undifferentiated tissue. However, growing tissue with concomitant specialized function might have a higher concentration of the energy-providing respiratory enzymes than tumor tissue, if at the same time the concentration of the energy-depleting functional enzymes were increased as well. The problem of identifying and testing for an enzyme of the latter class is a difficult one, but recent work has shown that the ATP-ase of muscle is one of the best representatives of this type of enzyme available. Although the function of ATP-ase in tissues other than muscle remains obscure, it is present in high concentrations in adult specialized tissues; and all the tissues examined contained ATP-ase, which was activated by calcium ions just as is the case in muscle (4).

In order to test the hypothesis of growth control it is necessary to have growing tissue as well as enzyme methods that can be applied to small samples of tissue. The tissues of the newborn rat seemed to offer possibilities as a source of growing tissue. Himwich and his co-workers (5, 6), as well as numerous others, have shown that the newborn rat is highly resistant to anoxia in comparison with the adult, and that in the course of 10 to 20 days it loses this resistance and becomes as susceptible as the adult. Resistance to anoxia was inversely correlated with the oxygen uptake rate of samples of cerebral tissue (5), and was suggested to be due to low functional activity. The newborn rat brain has been stated to be physiologically as young as that of the pig embryo between the 50 mm. and 100 mm. stage. Koch suggested that the newborn rat's brain is “as young nervous material as can conveniently be analyzed at present; and it forms, therefore, a convenient starting point for the study of the chemical differentiation of the central nervous system during growth” (7). The resistance of the newborn rat to anoxia suggests a relative independence from aerobic processes in comparison with adult specialized tissue. In this respect it is similar not only to embryonic tissue (9) but to cancer tissue as well (8). The fact that the newborn rat brain is embryonic in type, and that the conversion to the adult type of metabolism is compressed into the short space of about 15 days, facilitates the demonstration of the enzymatic changes that occur during this period of development.

The present study was carried out on both liver and brain, although the authors are aware of the limited interpretation to be placed upon the results with liver, owing to its hematopoietic function in the embryonic stage (2). The enzymes studied were succinic dehydrogenase and cytochrome oxidase as representative aerobic enzymes, and ATP-ase as a representative of the energy-depleting type of enzyme.

EXPERIMENTAL

Embryonic and newborn rats.—The rats were of the Sprague-Dawley strain, and the ages given for both the newborn rats and the embryonic rats are accurate to within 12 hours. The ages of the embryo rats were obtained from the time of conception, which was correct to within 12 hours on the basis of brief exposure to a male during estrus and the presence of spermatozoa in a vaginal smear taken within 12 hours from the time

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the male was placed in the cage. Newborn rats were used one or two at a time from any given litter, so that each litter provided data at several different points on the time curve between 1 and 25 days. Data for adult rats were taken from the results of previous assays for the respective enzymes (4, 13), which were carried out in this laboratory at about the same time.

Enzyme methods.—Succinic dehydrogenase was assayed by the homogenate technic described by Schneider and Potter (13), in which the measurement is based on the oxygen uptake of the succinoxidase system with conditions such that succinic dehydrogenase is the limiting factor. Cytochrome oxidase was determined by using ascorbate as the substrate, as previously described (13). The ATP-ase assay was that of DuBois and Potter (4). The methods are applicable to amounts of fresh tissue as small as 20 mgm., and are thus well suited for studies on embryonic and newborn rats.

RESULTS

The data for both brain and liver are presented in Fig. 1, in which are given the values for the total wet weight of the respective organs to show the extent of growth during the period from 5 days before birth to 25 days after birth. The time of birth, or zero on the abscissa, corresponds to an embryo age of 22 days. The values for the enzymes are given in terms of enzyme units. In the case of succinic dehydrogenase the data are expressed as the microliters of oxygen per mgm. of fresh tissue per hour. In the case of ATP-ase the data represent the micrograms of phosphorus split from ATP per mgm. of fresh tissue in 15 minutes for brain, while in the case of liver the actual values were multiplied by a factor of 2 for the purpose of graphing the results. Thus the value for ATP-ase in adult brain is 7.0 units, and for adult liver 12.9 units.

Fig. 1 shows that the concentration of both enzymes increased greatly during the 25 day period, and that by the end of this time the adult value had been reached. The conclusion that the increased enzyme concentration is not an artefact seems justified from the following considerations. According to Koch (7) the per cent dry weight of rat brains at birth was 10.42 and for adults 21.9, while Donaldson and Hatai (3) gave figures of 12.5 per cent at birth, 11.9 per cent at 4 to 8 days, 19.8 per cent at 25 days, and 21.5 to 22.5 per cent in the adult. Thus the threefold increase in succinic dehydrogenase in rat brain from birth to 30 days of age cannot be simply an expression of the increased dry weight. Similar considerations apply to ATP-ase, in which the increase was more than six-fold. Koch found that adult rat brains contained, in comparison with brains at birth, about the same amount of total sulfur, but about twice as much protein S, and 5 times as much lipid S. With respect to protein, adult brains contained nearly twice as much on the fresh basis as did the brains at birth (7). Thus the increased concentration of succinic dehydrogenase and ATP-ase during this period is not a result of the increase in either solids or protein, and it follows that during this period the quantity of some proteins must have decreased while the quantity of the enzymes studied increased, and that the balance between the various types of protein must have been altered. The values for cytochrome oxidase are not shown in Fig. 1, since the values in general paralleled the succinic dehydrogenase values throughout the period studied.

Of considerable interest is the relation between sur-

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![Graph](https://example.com/graph.png)
vival time under anoxic conditions and the enzyme concentrations shown in Fig. 1. Both succinic dehydrogenase and ATP-ase in brain remain at the same concentration for the last few days in utero and during the first 5 or 6 days of extrauterine life, and begin to rise between the seventh and 15th days. During the first period the anoxic survival time falls rapidly and reaches the adult level at about the 11th day. The anoxic survival time is thus more closely correlated with the oxygen uptake of minced fresh tissue and with the succinic dehydrogenase and ATP-ase concentrations than it is with the $Q_0$ values based on the dry weight of brain slices (6), suggesting that the molarity of a given enzyme in a tissue may be of greater significance than its concentration based on some other point of reference such as dry weight or protein phosphorus.

The findings in the case of liver present an interesting contrast to the brain data. In the case of brain, growth began at birth and the enzymes studied remained at embryonic concentrations for 5 or 6 days and then increased until the age of about 30 days. In liver this situation was reversed; the concentration of the enzymes rose rapidly during late uterine life, and continued after birth for about 15 days in the case of succinic dehydrogenase and about 30 days in the case of ATP-ase. Growth was slight in the period from birth to 10 days. Measurements on liver slices during this period of development show (2) that the anaerobic glycolysis decreases by one-half, while aerobic glycolysis increases and the $Q_0$ remains approximately constant.

**Discussion**

The results of this investigation show that there are notable increases in the concentration of the succinoxidase system in the brain and liver of the newborn rat during the period of greatest development, and that there are parallel increases in adenosine-triphosphatase.

Analyses for other biocatalysts in the tissues of newborn rats support the results of this investigation with respect to the increase of particular enzyme components. Thus Bernheim and Felsovanyi (1) determined the concentration of V-factor (coenzyme I + coenzyme II) in liver and kidney of embryos and newborn rats and found that the concentration in g per gram wet weight increased from 100 in 2 week embryos to 150 at birth, and to the adult level of 500 to 600 by 7 to 9 days. Tyler (14) studied the effect of malonate on the respiration of brain tissue from newborn rats and found that at birth the rat is relatively insensitive to malonate, but that the sensitivity of adult brain is reached by the tenth day of life.

It appears that differentiation and specialization are associated with the development of an increased capacity for energy mobilization. This involves an increase in biocatalysts that are necessary for the combustion of fuel, such as the succinoxidase system, as well as an increase in ATP-ase. The results support the idea that ATP-ase is somehow related to function in brain and liver, but do not prove this point. The results cannot be said to test adequately the theory that growth is inversely correlated with the concentration of aerobic enzymes, for while the data show that the concentration of aerobic enzymes is high in the fully differentiated nongrowing tissue and low in the less differentiated growing tissues of the newborn rat, the issue is confused during the transition period by the fact that function is being superimposed on growth during this period. The data probably have a greater bearing on the problem of differentiation than on growth per se, but complete differentiation in a cell is generally associated with a loss in reproductive capacity. According to the theory, growth is regulated not by the absolute concentration of any particular enzyme, but rather by the balance that results from the effective concentrations of the enzymes that esterify phosphate and those that break down the phosphate esters. At this point we can only point out that representatives of both types of enzyme were increasing during the period studied. It is also considered significant that the concentration of succinic dehydrogenase in various experimental tumors (12) is almost identical with that of the brain at birth. The newborn liver had a concentration of succinic dehydrogenase intermediate between hepatomas and adult liver, but it has a functional load that is probably greater than that of brain at this period.

**Summary**

1. Brain and liver from late embryonic and newborn rats up to the 30th day of postnatal life were compared with adult tissues in respect to their content of succinic dehydrogenase, cytochrome oxidase, and adenosine triphosphatase.

2. In brain the enzymes studied remained constant from about 3 days before birth until about 6 days after birth, and then increased rapidly so that the adult level was reached by the 30th day of life. The wet weight of the brains increased steadily throughout this period.

3. In liver the enzymes studied increased rapidly during late embryonic and early postnatal life, and the adult level was approximated within 10 to 15 days. Up to this time there was very little increase in the wet weight of the liver, but at this point its weight began to increase rapidly.

4. The results show that profound changes occur...
in the enzymatic makeup of the newborn rat during a relatively short time after birth. The enzymes studied increased from 300 to 600 per cent during this period.

5. The enzymes studied represented both the energy-yielding type (respiratory enzymes) and the energy-depleting type (ATP-ase). The studies show that during the period of increasing functional load and increased differentiation both types of enzyme increased, that is to say, the potential rate of energy mobilization was increased.

6. The results are discussed in relation to the theory of cancer put forth by Potter, according to which growth is considered to be governed by the effective balance between the classes of enzymes mentioned above.

REFERENCES

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