The Effect of a Vitaminlike Homolog of Riboflavin on Succinic Acid Dehydrogenase Activity of Brain[1] (34911)

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The riboflavin homolog 7-ethyl-8-methyl-10-(D-ribityl)isoalloxazine (7-ethyl-8-methyl-flavin; Fig. 1) (1) is able to serve as the sole flavin in the nutrition of the rat (2). Females receiving only 7-ethyl-8-methyl-flavin, produce litters of the usual size consisting of normal appearing young, but the young die during the first 3 days after birth (3).

Kim and Lambooy (4) have shown that 7-ethyl-8-methyl-flavin causes a reduction of succinic acid dehydrogenase (SDH) activity in tissues of healthy rats. The evidence (4) suggested that the coenzyme form of the homolog is able to replace riboflavin in the enzyme at a rate which is reasonably consistent with the half-life of the mitochondrion. These phenomena led to observations that the half-life of decay of rat liver SDH is approximately 14 days while that of the kidney is of the order of 100 days.

It might be argued that the large difference in the half-lives of SDH decay in the liver and kidney are not related to the reproductive cycle of the mitochondria in the tissues but rather that the homolog coenzyme forms a far more active holoenzyme with the apoenzyme of the kidney than with the apoenzyme of the liver.

The relatively slow rate of cell division in the brain reduces the need for new mitochondria to very low levels and it has been stated (5) that brain mitochondria grow and divide very slowly and may be stable for the lifetime of the cell. If the decay of SDH activity in the tissue of a rat receiving only the homolog is related to the rate of production of mitochondria, then the administration of the homolog as the sole flavin to rats and the determination of the SDH activity of the brains from weanling age well into adulthood should show an extremely slow rate of decay of the enzyme. The possibility that the mitochondria reproductive rate was very rapid and that the homolog coenzyme forms an extremely active holoenzyme with brain mitochondrial SDH apoenzyme could not be ruled out. However, if animals were fed the homolog-containing diet from the time of weaning until adulthood and then bred, the offspring from such animals would possess tissues all of whose SDH activity would be dependent on the homolog coenzyme. In this

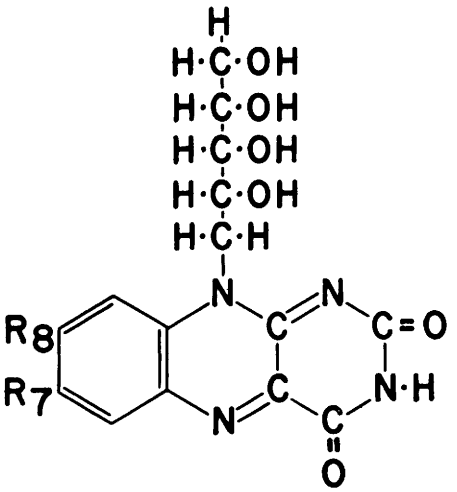

FIG. 1. Basic isoalloxazine or flavin structure:

		R_7	R_8
Riboflavin	(R)	CH_3–	CH_3–
7-Ethyl-8-methyl-flavin	(H)	C_2H_5–	CH_3–

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case, if the change in SDH activity is dependent on the mitochondrial turnover then two tissues such as the brain and liver should have the same activity. If the change in SDH activity is more dependent on the relative activity of the homolog coenzyme with liver apoenzyme or brain apoenzyme, then the SDH activity of the fetal brains should be many times higher than that of the fetal livers.

Materials and Methods. Time course of SDH activity decay in brain and liver. Female weanling rats of the Wistar strain,[4] weighing between 40 and 45 g, were distributed into two groups. Three rats were taken from each group for "day 0" enzyme analyses, and those remaining were fed the purified diet previously described (6), except that riboflavin (20 mg/kg) was added to the diet fed to Group R (riboflavin) rats, and 7-ethyl-8-methyl-flavin (20.8 mg/kg) was added to the diet fed to Group H (homolog) rats. The diets were fed *ad libitum*.

Three rats were taken from each group on days 0 (above), 30, 60, 120, 176, 200, and 221 for enzyme analyses. The rats were killed by decapitation, the livers and brains were removed immediately and placed in cold 0.25 *M* sucrose solution. The organs were converted into 10% homogenates in 0.25 *M* sucrose by the use of the Dounce (7) homogenizer. All homogenates were strained through a four-layer thickness of grade 80 cheesecloth. All operations were carried out in a cold (4–5°) room.

Succinic acid dehydrogenase activity was determined by means of the procedure developed by Arrigoni and Singer (8) with minor modifications; duplicate determinations were made on the tissues from each rat. The Lardy-Warburg respirometer was used and the determinations were made at 25°. All enzyme activities were expressed as μmoles of oxygen consumed/min/mg of protein (9).

SDH activity of maternal and fetal Livers and Brains. Forty-five female weanling Wistar rats, weighing 40–45 g, were distributed into three groups. The diets fed to two groups (Groups R and H) were the same as those described above for Groups R and H. The third group, Group M, received a diet supplemented with a mixture of the homolog (20.8 mg/kg) and riboflavin (1.6 mg/kg).[5] The rats were permitted to eat the diets *ad libitum* until they had reached 180 g of body weight. At this time they were bred with normal (riboflavin-containing diet) males and the time of sperm-positive vaginal smear was recorded as day "0." Twenty-one days later[6] the pregnant females were decapitated and the fetuses, maternal liver and brain were removed rapidly and placed in 0.25 *M* sucrose. The livers and brains of the fetuses of a single litter were removed and pooled in separate containers of 0.25 *M* sucrose. The enzyme activities of the maternal liver and brain were determined at the same time they were determined for the pooled livers and brains from the females' fetuses.

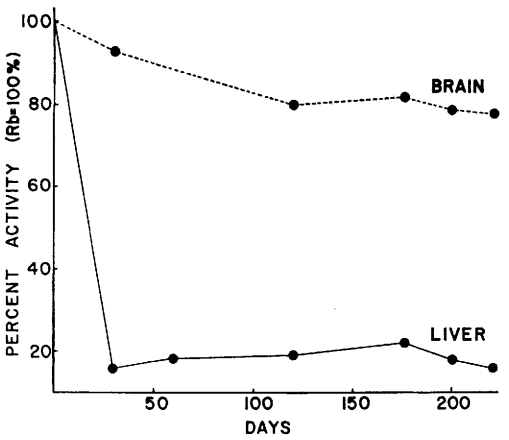

FIG. 2. Rate of decay of SDH activity in the brains and livers of Group H animals. Activity expressed as percentage of the activities for the corresponding tissues of Group R animals.

Results. The time courses of the decay of the SDH activities for the brain and the liver of the animals in Group H are shown in Fig. 2. The values shown for these tissues are

[4] CFN Rats, Carworth Incorporated, New City, New York.

[5] In studies to be reported at a later date this combination of the two flavins mixed with the diet was found to permit survival of the young born of females consuming the diet.

[6] Since all the females did not become pregnant at the same time and since the females of Group H did not always become pregnant the first time they were bred, the study extended over several weeks.

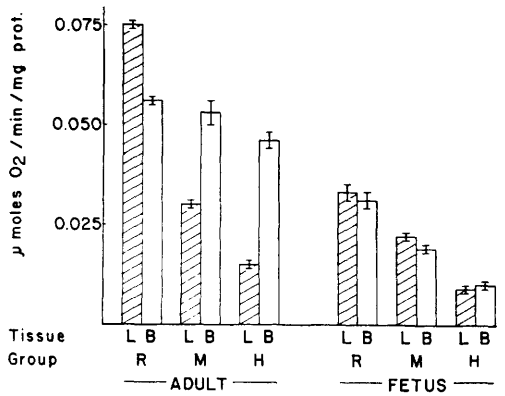

FIG. 3. SDH activities of livers (L) and brains (B) obtained from females and their fetuses. The females were fed diets containing riboflavin (R), a mixture of the homolog and riboflavin (M), or the homolog (H). The bars are shown with an estimate of the standard error of the mean. The number of mother–fetuses sets used were 9, 13, and 10 for groups R, M, and H, respectively.

μmoles of O_2 consumed per minute per milligram of protein for the particular tissue expressed as percentage of the μmoles of O_2 consumed per minute per milligram of protein by the same tissue determined at the same time from the animals in Group R. The rate of decay of the enzyme activity for the liver is rapid and extreme; the results are the same as those observed on an earlier occasion (4). The first determination made on Group H animals was made on day 30 because previous work had shown that at this time the liver SDH activity would have reached its minimum value and would thereafter remain relatively constant. The SDH activity of the brain undergoes a very slow rate of decay and it was reduced to 80% of normal after 200 days of consuming the homolog. The decay appears to be continuing beyond the experimental period.

The influence of the homolog on the SDH activity of the brains and livers of females and their fetuses is summarized in Fig. 3. In this instance the actual values (μmoles of O_2 consumed/min/mg of protein) are given because no single value would serve as an appropriate standard. The average age of the females when the fetuses were taken was 122 days (119–171) and the values for the tissues are in excellent agreement with those in Fig. 2 at the same time for Groups R and H. The preferential utilization of riboflavin by the liver mitochondria is illustrated by the SDH activity for Group M in which case 7% of the total flavin in the diet as riboflavin causes 100% increase in the enzyme activity.

The enzyme activities for the tissues of the fetuses show no differences between the tissues within a group. Although the standard errors of the mean values for the two tissues in Group M do not overlap, p value = 0.147. The very low SDH activity for the brains of the Group H fetuses is noteworthy. The preferential utilization of riboflavin by both tissues is emphasized by the increase of approximately 150% in liver activity and 100% in brain activity when only 7% of the dietary flavin is the vitamin.

Discussion. The administration of the homolog to the rat as his sole flavin causes a rapid loss of SDH activity from the liver. If the rate of fall is suitably corrected for the residual activity when all the riboflavin has been replaced by homolog in the mitochondria, it is found that the half-life of decay is 12 days. This is in excellent agreement with the value of 10 days (5) for the half-life of rat liver mitochondria. Previous evidence indicated that the half-life of brain mitochondria might be very long. If the decay of SDH activity in the brain as a result of the administration of the homolog is a real manifestation of mitochondrial half-life, then it appears that the half-life of rat brain mitochondria is approximately 500 days.

The possibility that the sustained high SDH activity by the brains of animals in Group H is due to an unusually active homolog–coenzyme brain apoenzyme combination is largely ruled out. This concept would be inconsistent with the findings on the fetuses where the liver and the brain had equal activities when all the flavin in both was homolog. The greatly improved SDH activity of the brains in Group M fetuses may well be a factor in the survival of these fetuses.

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1. Lambooy, J. P., J. Amer. Chem. Soc. **80**, 110 (1958).

2. Lambooy, J. P., J. Nutr. **75**, 116 (1961).

3. Lambooy, J. P., Biochim. Biophys. Acta **29**, 221 (1958).

4. Kim, Y. S., and Lambooy, J. P., Arch. Biochem. Biophys. **122**, 644 (1967).

5. Lehninger, A. L., "The Mitochondrion," p. 247. Benjamin, New York (1965).

6. Kim, Y. S., and Lambooy, J. P., J. Nutr. **91**, 183 (1967).

7. Dounce, A. L., J. Biophys. Biochem. Cytol. **1**, 139 (1955).

8. Arrigoni, O., and Singer, T. P., Nature (London) **193**, 1256 (1962).

9. Lowry, O. H., Rosenbrough, N. J., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

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