Succinic Acid Dehydrogenase Activity of Walker Rat Carcinoma 256 When Utilizing Riboflavin Homologs[1] (37503)

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Only two homologs of riboflavin (Fig. 1, I) 7-ethyl-8-methyl-flavin [7-ethyl-8-methyl-10-(1′-D-ribityl)isoalloxazine] (Fig. 1, II), and 7-methyl-8-ethyl-flavin [7-methyl-8-ethyl-10-(1′-D-ribityl)isoalloxazine] (Fig. 1, III) (1) are able to serve as replacements for riboflavin in the metabolism of Wistar (2) and Sprague–Dawley (3) rats with respect to growth, survival, optimal physical appearance and efficiency of food utilization. Derivatives of these homologs are able, therefore, to function as pseudoflavoprotein coenzymes in mammalian tissues.

In spite of the indistinguishability of these three flavins by the above criteria, when 7-ethyl-8-methyl-flavin is the only metabolically active flavin in the animal's tissues (4), the succinic acid dehydrogenase (EC 1.3.99.1) (SDH) activities of the kidney, heart and liver are reduced to 56, 36, and 10%, respectively, of the activities shown by the same tissues in animals utilizing riboflavin (5).

That the influence of some analogs of riboflavin might be due to a considerable degree of tissue selectivity was suggested by the findings that while 7-ethyl-8-methyl-flavin possessed the activities cited above, the isomeric 7-methyl-8-ethyl-flavin possessed between 56 and 75% of the activity of riboflavin for the heart, kidney and liver (Dombrowski and Lambooy, unpublished data) while still another analog, 7-chloro-8-methyl-flavin [7-chloro-8-methyl-10-(1′-D-ribityl)isoalloxazine] (Fig. 1, IV) (6), was found to cause the specific displacement of riboflavin from the kidney, which loss in turn, caused a precipitous loss of SDH activity (7).

These findings, coupled with our observation that 7-ethyl-8-methyl-flavin was unable to support normal growth of the Walker rat carcinoma 256, while 7-methyl-8-ethyl-flavin was nearly equivalent to riboflavin as a stimulus for growth of this tumor (3), prompted us to investigate the influence of these two homologs of riboflavin on the SDH activity of the viable tumor tissue. Since reduced flavoprotein enzyme activity may be due to inadequate tissue flavin or inadequate coenzyme catalysis, a partial answer to these questions was sought by also determining the flavin concentrations of the tissues. This information made it possible to express the SDH activities of the tissues in terms of the flavin present.

The SDH activity and flavin concentration was determined for the liver of each animal at the same time that these measurements were made on the tumors. This was done so that the values for the liver could be used as an additional standard of comparison.

Materials and Methods. Thirty female weanling rats of the Sprague–Dawley strain weighing between 40 and 45 g were used in groups of 10 but the members of the groups were started at different times to provide the necessary time for assays. One group (Group R) was fed the riboflavin-deficient diet described before (7), to which had been added 5 mg[5] of riboflavin/kg; a second

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[5] The equivalence of these quantities of the three flavins was demonstrated in Ref. (2).

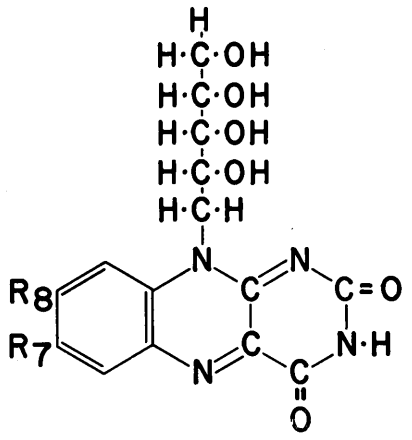

Fig. 1. Basic flavin structure.

No.	R_7	R_8	Trivial name
I	CH_3–	CH_3–	Riboflavin
II	C_2H_5–	CH_3–	7-Ethyl-8-methyl-flavin
III	CH_3–	C_2H_5–	7-Methyl-8-ethyl-flavin
IV	Cl–	CH_3–	7-Chloro-8-methyl-flavin

group (Group 7-Et) was fed the same deficient diet to which had been added 11.1 mg[5] of 7-ethyl-8-methyl-flavin/kg and a third group (Group 8-Et) was fed the same deficient diet to which had been added 14.3 mg[5] of 7-methyl-8-ethyl-flavin/kg. All animals were fed *ad libitum*. They were caged individually and were maintained as described before (8) and every effort was made to reproduce the experimental conditions which prevailed during Study V, of the earlier related report (3).

After the animals had been fed their respective diets for 31 or 32 days, all were given a fragment implant of the tumor as described (3). The tumors were permitted to grow for 22 days. At the end of this time the animals were killed in groups of 3 by decapitation, exsanguinated and then taken immediately into a cold room. All subsequent steps for the preparation of the tissue homogenates were carried out rapidly in the cold room at 5°. Approximately 1 g of the right median lobe of the liver was suspended in 9.0 ml of fresh 0.25 *M* sucrose/g and converted into 10% homogenates as described before (4) for the liver. One gram portions of the viable tumor tissue was converted into homogenates by the same procedure. From each homogenate 6.0 ml was transferred to a 30 ml screw-capped culture tube and frozen for later flavin analysis. The remainder of the homogenate was used for the determination of the SDH activity (9) and protein concentration (10) as described before (11).

To each of the 6.0 ml[6] samples of the homogenate reserved for flavin analyses was added 2.0 ml of 0.4 *N* hydrochloric acid. The culture tube was sealed tightly and the contents of all were hydrolyzed by autoclaving for 20 min at 1.06 kg/cm^2. These sterile samples were stored in a frozen state until needed.[7] The amounts of flavin, whether riboflavin, 7-ethyl-8-methyl-flavin or 7-methyl-8-ethyl-flavin, were determined by the usual microbiological procedure (12) using *Lactobacillus casei* 7469 (American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD), for which the three flavins have equal activity (2).

Results. The SDH activities for the tumor and the livers expressed as oxygen consumption based on protein are given in Table IA. The absolute values observed for the tumors, although approximately only ⅓ of those shown for the livers, when expressed as relative values, mimic to a considerable degree the relative values observed for the livers.

The flavin concentrations for the tumors and the livers are given in Table IB. The absolute values for the tumors are also approximately ⅓ of those for the livers. It is noteworthy that the tumor accumulates 7-ethyl-8-methyl-flavin to a greater degree than it does riboflavin.

The SDH activities for the tumors and the livers expressed as a function of the tissue flavin concentrations are given in Table IC. When expressed in this way and when, as was true in this case, an adequate supply of flavin is available, it is clear that the tumor utilizes riboflavin more efficiently than either of the homologs and that 7-ethyl-8-methyl-flavin is used with the least efficiency.

Discussion. SDH and electron-transport

[6] It was found that if necessary, 3.0 ml samples provided enough material for flavin analysis.

[7] The preparation of the unknown and the assay solutions as well as all of the microbiological assay procedure was performed in subdued light.

TABLE I. Succinic Acid Dehydrogenase and Flavin Concentrations of Walker Carcinoma 256 and Livers of Rats Utilizing Different Flavins.

	Group[a]		
	Rb	7-Et	8-Et
A. Succinic acid dehydrogenase			
Tumor	0.034 ± 0.001[b]	0.008 ± 0.001	0.014 ± 0.002
Relative value[c] (%)	100	23	41
Liver	0.081 ± 0.001	0.025 ± 0.002	0.042 ± 0.002
Relative value (%)	100	31	52
B. Flavin concn			
Tumor	0.032 ± 0.001[d]	0.041 ± 0.002	0.028 ± 0.002
Relative value (%)	100	128	88
Liver	0.146 ± 0.002	0.137 ± 0.002	0.113 ± 0.004
Relative value (%)	100	95	78
C. Succinic acid dehydrogenase as a function of the tissue flavin concn			
Tumor	1.060 ± 0.065[e]	0.195 ± 0.034	0.500 ± 0.107
Relative value (%)	100	18	47
Liver	0.558 ± 0.015	0.187 ± 0.018	0.372 ± 0.031
Relative value (%)	100	33	67

[a] Rb, riboflavin; 7-Et, 7-ethyl-8-methyl-flavin; 8-Et, 7-methyl-8-ethyl-flavin.

[b] The SDH activity given as μmoles O_2/min/mg protein ± SE of the mean.

[c] The value expressed as percentage of the value for the riboflavin group which is rated as 100%.

[d] Flavin concentrations given as μg/mg protein ± SE of the mean.

[e] The SDH activity given as μmoles O_2/min/μg flavin ± SE of the mean.

linked diphosphopyridine nucleotide dehydrogenase (EC 1.6.99.3) (DPNHD) are both required at critical stages in energy production. Since we had observed that the 7-ethyl-8-methyl-flavin was used as successfully as riboflavin by the DPNHD of the heart, kidney and liver (4) but that it caused a severe and probably lethal depression of SDH in embryonic tissues (4, 13), it seemed appropriate to study the activity of the latter enzyme in the metabolism of Walker rat carcinoma 256. If we confine our attention to the question of why the rate of tumor growth is retarded when the tissue utilizes 7-ethyl-8-methyl-flavin (3), the finding that the flavin is used with poor efficiency by SDH when compared with riboflavin or with 7-methyl-8-ethyl-flavin (Table IC) is impressive.

The possibility that a critical flavoprotein enzyme operating at approximately 50% efficiency with reference to the flavin available can still provide a tissue with sufficient metabolic capacity to carry on its role (Table IC; 8-Et) while a reduction to 18% does not meet these needs (Table IC; 7-Et) appears to be consistent with other observations we have made. We have found (4) that when 7-ethyl-8-methyl-flavin is being utilized by an adult rat the oxygen consumed (SDH) per unit flavin by the heart is 49% of normal. This animal thrives. An infant rat which is also utilizing the same flavin has an oxygen consumption (SDH) per unit of flavin by the heart of 15% of normal, and this animal dies (4).

The accumulation of 7-ethyl-8-methyl-flavin by the tumor (Table IB) appears to be more a matter of tissue selectivity than the quantity of flavin available in the diet. While it is true that approximately twice as much 7-ethyl-8-methyl-flavin was available to the 7-Et animals as there was riboflavin available to the Rb animals, thrice as much of the 7-methyl-8-ethyl-flavin was available to the 8-Et animals yet they accumulated less flavin than the Rb animals.

When an adequate quantity of any of the three flavins is available to the rat, the efficiency of utilization of the flavin for SDH activity by the tumor is greater than it is by the liver (Table IC).

It is probable of course, that other factors in addition to the SDH activity may play a role in the suppression of the growth of Walker carcinoma 256 when 7-ethyl-8-methyl-flavin is the metabolically active flavin. The striking parallelism between the reduced growth of the tumor and the drastically reduced activity of SDH in the tumor, strongly suggests that the relationship is a significant one.

Summary. When biologically equivalent amounts of riboflavin, 7-ethyl-8-methyl-flavin and 7-methyl-8-ethyl-flavin are being utilized by rats bearing Walker carcinoma 256, 7-ethyl-8-methyl-flavin is accumulated by the tumor in higher concentration than are the other two flavins. The SDH activity for the tumors utilizing 7-ethyl-8-methyl-flavin is reduced to approximately 20% of the value for the animals utilizing riboflavin, whether expressed in absolute units or in units per unit of tissue flavin. The tumor utilizes any one of the flavins for SDH activity more efficiently than the liver utilizes them for this enzyme.

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