TABLE II. The Glycolytic Reduction of TTC.

Enzyme	TTC reduction in terms of optical density × 10^3	Incubation time
Rat muscle ext. (Enzyme A type) (1)	0	30′ aerobic
Rat liver mitochondria (2)	46	30′ ″
Muscle ext. (1) + mitochondria (2)	500	15′ ″
Rat liver ext. (Enzyme A type) (3)	20	15′ anaerobic
Mitochondrion ext. (37°C) (Enzyme B) (4)	0	15′ ″
Rat liver ext. (3) + mitochondrion ext. (4)	720	15′ ″

Enzyme preparations: (1) = muscle ext. equivalent to 0.5 g fresh rat muscle (Enzyme A). (2) = rat liver mitochondria equivalent to 0.140 g fresh liver. (3) = rat liver ext. equivalent to 0.2 g fresh liver (Enzyme A). (4) = mitochondrion ext. equivalent to 0.3 g fresh liver (Enzyme B).

The glycolytic system is composed of: 60 μM HDP, 40 μM nicotinamide, 40 μM KF, 0.6 μM DPN and 3 μM TTC in a final volume of 3 ml made up with 0.1 M phosphate buffer. Temp. 37°C.

mental results. Essentially the same results were obtained when the triose phosphate dehydrogenase system of tissue extracts was replaced by crystalline yeast alcohol dehydrogenase(11). The reduction of TTC by the alcohol dehydrogenase system was measured anaerobically at pH 7.4, with semicarbazide hydrochloride (10^{-2} M) as carbonyl reagent. Here again, TTC reduction occurred only when mitochondria, or the extract of mitochondria (Enzyme B) were present in addition to the yeast enzyme, Table II.

Discussion. In animal tissues TTC can be reduced by a variety of dehydrogenases. Soluble flavoproteins, like aminoacid dehydrogenases, readily transfer electrons to TTC under anaerobic conditions. The retardation of TTC reduction by aminoacid dehydrogenases in air is probably due to a competitive mechanism between O_2 and TTC, particularly noticeable in homogenous systems (solutions). It was shown that an obligatory component of the glycolytic TTC-reducing system is present in mitochondria. This component appears to be a flavoprotein part of the cytochrome C reducing system(10). The fact that intact mitochondria mediate electrons to both cytochrome C and TTC, while the two reductase activities can be separated by extraction, requires further elucidation.

Summary. The conditions of the reduction of TTC by cytoplasmic fractions of rat tissue homogenates were determined. Rat kidney extracts, upon addition of aminoacids, reduce TTC anaerobically. Glycolytic enzymes of liver and muscle reduce TTC only when supplemented by mitochondria. An extract of mitochondria can be obtained which will supplement the glycolytic TTC reducing system.

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Retardation of Growth of Walker Rat Carcinoma 256 by Administration of Diethyl Riboflavin.* (19019)

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In recent years the effects of riboflavin deficiency on the growth of neoplasms have received increasing attention. Morris and Robertson(1) found that the growth rate of spontaneous mammary carcinomas in C3H mice was decreased by severe riboflavin de-

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1. Morris, H. P., and Robertson, W. v. B., *J. Nat. Cancer Inst.*, 1943, v3, 479.

ficiency. Stoerk and Emerson(2) have reported the regression of established lymphosarcomas (6C3H-ED) in mice rendered riboflavin deficient by either feeding a flavin deficient diet or by administering the riboflavin antagonists, isoriboflavin or galactoflavin. Another riboflavin analogue, 6,7-dichloro-9-(1′-D-sorbityl)-isoalloxazine, which has no antiriboflavin activity in rats, also causes regression of established lymphosarcomas(3).

Lambooy(4) has recently reported the synthesis of 6,7-diethyl-9-(D-1′-ribityl)-isoalloxazine. Lambooy and Aposhian(5) have shown that this new flavin has an "antiriboflavin" activity in the growing rat. The effects of this new flavin on the Walker Rat Carcinoma 256 are reported in this paper.

Methods. Twenty-four male rats of the Sprague-Dawley strain, weighing from 31 to 45 g were fed a riboflavin deficient diet consisting of sucrose 68%, vitamin test casein 18%, vegetable oil (hydrogenated) 10%, and salt mixture No. 2 USP 4%. Each 100 g of diet was supplemented† with 2 g USP cod liver oil, 5 mg each of vit. K_1 and nicotinamide, 1 mg each of thiamine and pyridoxine, 2.5 mg calcium pantothenate, 10 mg l-inositol, 100 mg choline chloride, 60 μg each of folic acid and biotin, 3 μg B_{12} and 30 mg para-aminobenzoic acid. A drop of mixed tocopherols containing 3 mg α-tocopherol per drop was administered orally to each animal once a week. On the thirteenth day the animals were paired into two groups according to weight and previous rate of growth and slice fragments‡ of the Walker Rat Carcinoma 256 were implanted aseptically by trocar into the subcutaneous tissue of the right inguinal region of the rats. Group A received 32.3 μg

TABLE I. Comparison of the Effects of Diethyl Riboflavin and Riboflavin on Walker Carcinoma 256.

Diethyl riboflavin Group A								Riboflavin Group B							
Final wt* (r + t)	Tumor wt* (t)	Carcass wt* (r—t)	Initial wt (i)	Net change (r—t—i)	Tumor size $\left(\frac{t}{r+t} \times 100\right)$	Net change	Δ Tumor size	Final wt* (r + t)	Tumor wt* (t)	Carcass wt* (r—t)	Initial wt (i)	Net change (r—t—i)	Tumor size $\left(\frac{t}{r+t} \times 100\right)$	Net change	Δ Tumor size
g	g	g	g	g	%	g	%	g	g	g	g	g	%	g	%
88	26	62	72	−10	30	− 3	− 3	98	26	72	79	− 7	27	− 3	− 3
90	11	79	76	+ 3	12	+ 8	+15	107	29	78	83	− 5	27	+ 8	+15
105	9	96	81	+15	9	+ 8	+18	120	32	88	81	+ 7	27	+ 8	+18
75	22	53	59	− 6	29	+20	+ 3	71	23	48	74	−26	32	+20	+ 3
100	20	80	70	+10	20	+ 7	+15	116	41	75	72	+ 3	35	+ 7	+15
90	12	78	73	+ 5	13	+10	+19	106	34	72	77	− 5	32	+10	+19
78	7	71	66	+ 5	9	+15	+12	70	15	55	65	−10	21	+15	+12
77	20	57	69	−12	26	− 3	+ 4	84	25	59	69	−10	30	− 3	+ 4
69	13	56	65	− 9	19	+10	+14	75	25	50	69	−19	33	+10	+14
78	12	66	66	0	15	+13	+17	90	29	61	74	−13	32	+13	+17
81	21	60	72	−12	26	− 3	0	88	23	65	74	− 9	26	− 3	0
74	23	51	60	− 9	31	+13	+ 2	73	24	49	71	−22	33	+13	+ 2
				Mean	20	+ 8	+10						30	+ 8	+10

* At time of sacrifice.

2. Stoerk, H. C., and Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1943, v70, 703.

3. Holly, F. W., Peel, E. W., Mozingo, R., and Folkers, K., *J. Am. Chem. Soc.*, 1950, v72, 5416.

4. Lambooy, J. P., *J. Am. Chem. Soc.*, 1950, v72, 5225.

5. Lambooy, J. P., and Aposhian, H. V., to be published.

† We are indebted to Merck and Co. for the generous gift of the vit. K_1 and B_{12} used in this study.

diethyl riboflavin§ and Group B received 30 μg riboflavin daily beginning with the day of tumor implantation. The flavins were administered by stomach tube as a gum acacia suspension.

Animals of Group A were allowed to eat freely. The food intake of each Group B rat was restricted to the food intake of its Group A pair mate. The weight of the animal on the day that the tumor was first palpable was designated as its initial weight. At the first sign of ulceration in any rat (no significant amount of fluid was lost) the pair of animals was decapitated, their tumors removed and weighed.

Results. See Table I. Net change (carcass weight—initial weight) is an expression of the change in weight of the animal carcass (rat weight—tumor weight) from the time the tumor was first palpable to the time of sacrifice. A positive net change indicates that while there was a tumor growing in the host, the animal carcass was gaining weight. A negative net change indicates the reverse.

By first calculating the net change of each animal, then finding the difference between the net changes of the two animals comprising each pair (Δ net change) and computing the average Δ net change of twelve pairs, the twelve rats receiving diethyl riboflavin had a highly significant average net change of 8 g more positive than the rats receiving riboflavin (S.E. $= \pm$ 2 g; p = 0.002).¶ This means that the diethyl riboflavin animals were able to retain 8 g more of their carcass tissue than the riboflavin animals.

By calculating first the size of the tumors of each animal expressed as per cent of total body weight $\left(\frac{t}{r+t} \times 100\right)$, then finding the difference between the size of the tumors of the two animals comprising each pair (Δ tumor size) and finally finding the average Δ tumor size of 12 pairs, it is concluded that the diethyl riboflavin group had tumors whose size, expressed as per cent of total body weight, averaged 10% less than the group of rats receiving riboflavin. This difference in tumor size is highly significant (S. E. $= \pm$ 2%; p = 0.001). However, if the average tumor size (20%) of the diethyl riboflavin group is compared to the average tumor size (30%) of the riboflavin group, it is seen that diethyl riboflavin caused a 33% inhibition in the growth of the tumor.

Although no daily quantitative measurements were made, the growth rate of the tumors of the riboflavin animals were strikingly greater than that of the diethyl riboflavin animals. That these changes have been brought about by the administration of unusually small amounts of the riboflavin analogue seems worthy of emphasis. The smallest amount of riboflavin analogue previously used in tumor studies has been 0.5 mg in mice(2).

The average time between implantation of these tumors and the sacrificing of the animals was 21 days. The time of sacrifice of a pair was determined by the ulceration of the tumor in the riboflavin animal in 11 of the 12 pairs.

Studies are now in progress to elucidate the mechanisms by which diethyl riboflavin is able to retard the growth of the Walker Rat Carcinoma 256.

Summary. 1. Rats with Walker Carcinoma 256 transplants showed an 8 g greater retention of their carcass tissue when receiving diethyl riboflavin than pair fed controls receiving an equimolar amount of riboflavin. 2. The average tumor size, expressed as per cent total body weight, of the diethyl riboflavin group was 20% while the average tumor size of the riboflavin group was 30%. This indicates a 33% inhibition of the growth of the tumors due to the administration of diethyl riboflavin.

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§ Equimolar with 30 μg riboflavin.

¶ Standard error; probability.

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