

the exception of the disulfate are approximately equal to that of the standard, 2-methyl-1,4-naphthoquinone. The disulfate is about one-third as potent on a molecular basis, but owing to the much larger molecular weight its activity per milligram is somewhat less than one-sixth that of 2-methyl-1,4-naphthoquinone.

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#### **Riboflavin Determinations on Normal Liver and Liver Tumor.**

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It was decided to use a fluorometric method in making quantitative estimations of riboflavin from fresh liver tissue after several checks had been run against the Snell and Strong method.\* The fluorometric method here used is first to determine the total fluorescence, then eliminate the riboflavin by raising the alkalinity to pH 11 and determining the "interfering" fluorescence. The difference between these two values gives the approximate riboflavin value. The fluorescence was measured with a photocell using suitable optical filters.

Liver tumors from Osborn-Mendel rats which had been fed 2-amino 5-azo toluene for a long period were used. These animals were kindly put at our disposal by Dr. E. Emmart. Samples of liver which had been perfused with saline were ground with  $n/10$  HCl, autoclaved for 15 min. and clarified by precipitation at pH 5-6, followed by filtering through No. 42 paper. Recovery of added riboflavin was about 97% by this fluorometric method.

Some of the material was given a short low temperature drying by the lyophile process eliminating most of the water. The data in the tables indicate that the difference between normal and tumor liver is not due to water content.

Comparisons were made between normal livers from normal rats, nontumor bearing liver from dye-fed rats, liver tumors from dye-fed rats, residual liver from which the tumor had been excised, fetal liver, and leg muscle. In the case of a general riboflavin deficiency it would be expected that lower riboflavin values for the muscle would be ob-

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\* Made by Drs. Isbell and Wooley of the National Institute of Health.

RIBOFLAVIN DETERMINATIONS ON LIVER AND LIVER TUMOR 605

TABLE I.  
Riboflavin Concentration in Micrograms per Gram of Fresh Tissue.

Rat No.	Histologic diagnosis on liver	$\gamma/g$		Leg muscle, $\gamma/g$
		Tumor	Liver residue	
1	Tumor (no diagnosis)	8	16	
2	" " "	12	15	
3	Carcinoma	11	15	
4	Pooled carcinoma, hepatoma	16	16	
5*	Carcinoma, Region	A 15.7		2.52
		B 13.2		
		C 18.6		
6*	Hepatoma	19.2	20.7	
7	"	21.3	21.3	
8	"	16.9	24.7	
9	" , carcinoma	22.2	22.2	2.80
10	" , cirrhosis	25.7	29.8	2.95
11	" , carcinoma			
	+ cirrhosis	26.8	27.95	2.56
	Dye fed; no tumors	$\gamma/g$		
12	Cirrhosis	26.14		2.1
13	" (slight)	27.84		2.9
14	" "	29.8		3.3
15	"	31.04		
16	"	34.0		2.1
17	Essentially normal	35.7		
	Normal rats, normal diet			
18	Normal	25.0		
19		25.5		
20		25.85		
21		28.6		
22		30.8		
23		30.9		
24		39.0		
25	Normal fetal liver	4.7		
26	" " "	6.3		

\*Large amount of blood in tissue.

TABLE II.  
Riboflavin Concentration in Micrograms per Gram of Dried Tissue.

Rat No.	Histology	$\gamma/g$ dry wt	$\gamma/g$ wet wt (calc.)
27, 28	Pooled material, carcinoma		
29, 30	and hepatoma	58.9	14.9
31	Pooled hepatoma, carcinoma and cirrhotic liver	59.4	15.0
32	Normal	106.6	29.0
33	"	106.8	29.0

tained. A value of 1.4  $\gamma$  is reported<sup>1</sup> for adult rat muscle on a deficient diet.

<sup>1</sup> Fraser, H., Topping, N., and Isbell, H., *Public Health Rep.*, 1940, **55**, 280.

In the absence of any other criteria the estimation of "malignancy" was based solely on histologic grounds. The term hepatoma was used to denote growths which reproduced the normal liver structure so closely that there was no histologic indication of "malignancy". On the other hand certain of these tumors manifested such extreme degrees of deviation from normal liver cell structure and architecture that they were termed carcinomas to denote their malignant histologic appearance.

The following conclusions may be drawn from these results:

1. The average value for normal and cirrhotic livers of 30.1  $\gamma$  (age 256 to 550 days) agrees with Fraser, Topping and Isbell's<sup>1</sup> values for considerably younger rats of 33  $\gamma$  (age 130 days).
2. Continuously dye fed rats with cirrhosis not developing gross tumors gave normal riboflavin content for liver.
3. Livers in which tumors had developed gave a lower value per gram of fresh tumor tissue and per gram of residual tissue than normal liver.
4. On the basis of lyophilized dry weight normal liver is considerably higher in riboflavin concentration than tumor material.
5. The muscle values indicate that the animal is not suffering from a general riboflavin deficiency.

These results are consistent with the earlier findings<sup>2</sup> that lactic acid accumulates in various tumors especially following large sugar injections. According to current views<sup>3</sup> pyruvic acid which is formed from sugar break down is normally oxidized to CO<sub>2</sub> and H<sub>2</sub>O through molecular oxygen and the flavoprotein-cytochrome enzymes. With a deficient oxygen and/or enzyme concentration the pyruvic acid is converted to lactic acid giving tumors a low pH. In this connection see the recent finding of low coenzyme in tumor<sup>4</sup> and the high ratio of reduced to oxidized cozymase in Jensen sarcoma.<sup>5</sup>

The histologic diagnoses were made by Drs. H. L. Stewart and Hugh G. Grady.

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<sup>2</sup> Voegtlin, C., Fitch, R., Kahler, H., Johnson, J. M., and Thompson, J. W., *Nat. Inst. Health Bull.*, No. 164, 1935.

<sup>3</sup> Eric Ball, *Johns Hopkins Hosp. Bull.*, 1939, **65**, 253.

<sup>4</sup> Bernheim, F., and Felsovanyi, A. V., *Science*, 1940, **91**, 76.

<sup>5</sup> Euler, H. V., Schlenk, F., Heiwinkel, H., and Högberg, B., *Z. f. physiol. Chem.*, 1938, **256**, 208.