and penicillin-resistant strains of viridans streptococci tested, but not in the parent strains. The aureomycin and penicillin-resistant strains had also increased in sulfathiazole resistance. No glutamic acid was present in 5 trained streptomycin-resistant strains which had not increased appreciably in sulfathiazole resistance, but was found in one strain which had increased in sulfathiazole resistance. Glutamic acid appears to accompany sulfathiazole resistance regardless of how this resistance is acquired. The possible relation of the free glutamic acid to the increased resistance to sulfathiazole is discussed.

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Levels and Intracellular Distribution of Coenzyme A and Pantothenic Acid in Rat Liver and Tumors.* (18232)

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The relative inability of tumor homogenates to oxidize oxalacetic acid(1,2) and the participation of coenzyme A (CoA) in the enzymatic condensation of oxalacetate and C_2 units to citrate(3,4) suggest that tumors might be relatively deficient in this coenzyme. Earlier work pointed to a low level of pantothenic acid (PA) in tumors(5), but the methods available at that time for liberating the vitamin from bound forms were probably inadequate(6). Therefore the contents of coenzyme A and pantothenic acid were determined in several types of transplantable rat tumors. Since the enzymes involved in the Krebs tricarboxylic acid cycle appear to be relatively concentrated in the mitochondria of normal liver(7) a comparison was also made of the intracellular distribution of these factors in normal rat liver with that in azo dye-induced rat liver tumors.

Experimental. The transplantable tumors were collected from multiply-inoculated rats† bearing the Walker carcinosarcoma, the Jensen sarcoma, or the Flexner-Jobling carcinoma. Only firm tumors less than 1 cm in diameter were analyzed. The minced tumors were homogenized in ice-cold isotonic saline in the Potter-Elvehjem apparatus to give a final tissue concentration of 10%. The liver tumors were taken from rats fed for 5 months on a low riboflavin, semi-synthetic diet(8, diet 3) containing 0.06% 4-dimethylaminoazobenzene. The liver tumors were perfused *in situ* with ice-cold isotonic saline by injection into the aorta at the thoracic level; the aorta below the coeliac artery was clamped off and the fluid allowed to escape by severing the vena cava above the liver. This perfusion route was necessary since liver tumors, unlike liver, are supplied only by arterial blood

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1. Potter, V. R., LePage, G. A., and Klug, H. L., *J. Biol. Chem.*, 1948, v175, 619.

2. Potter, V. R., Pardee, A. B., and Lyle, G. G., *J. Biol. Chem.*, 1948, v176, 1075.

3. Ochoa, S., and Stern, J. R., *J. Biol. Chem*, 1949, v179, 491.

4. Novelli, G. D., and Lipmann, F., *J. Biol. Chem.*, 1950, v182, 213.

5. Taylor, A., Pollock, M. A., and Sortomme, E. L., *Univ. of Texas Publication No.* 4237, 1942, 56.

6. Neilands, J. B., and Strong, F. M., *Arch. Biochem.*, 1948, v9, 287.

7. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1949, v177, 893.

† Holtzman Rat Co., Madison, Wis. We are indebted to Dr. G. A. LePage for the transplantable rat tumors.

8. Miller, E. C., Miller, J. A., Kline, B. E., and Rusch, H. P., *J. Exp. Med.*, 1948, v88, 89.

(9). Firm tumors 1 cm or less in diameter were carefully dissected from the livers, pooled, and washed in ice-cold saline. All of the normal livers were perfused with saline via the vena cava as described by Price *et al.*(10). The normal rats (160-200 g) and the rats (120-150 g) bearing the transplantable tumors were maintained on a grain diet (11). The preparation and differential centrifugation of the liver and liver tumor homogenates were carried out in 0.88 M sucrose essentially as previously described(10, 12). The nuclei, mitochondria, and microsome fractions were collected at 600 g for 10 minutes with two washings, 18,000 g for 10 minutes with one washing, and 120,000 g for 36 minutes without washing, respectively. The tube and pellet containing the latter fraction were rinsed with the sucrose medium and the washings added to the supernatant fraction. A refrigerated International centrifuge with a high-speed attachment was used to separate the first two fractions; the microsome fraction was sedimented in an air-driven ultracentrifuge.

All of the homogenates and 10% suspensions of the intracellular fractions in the sucrose medium were heated for 10 minutes in a boiling water bath to preserve the bound forms of pantothenic acid and suitable dilutions were made with distilled water for analysis. CoA was determined by the sulfanilamide acetylation method of Kaplan and Lipmann(13). Total PA was determined microbiologically with *L. arabinosus*(14) after enzymatic digestion(6). Free PA values were obtained by applying the same method to undigested aliquots. Duplicate PA and CoA assays were conducted on each sample and agreed within 15% for the more concentrated samples. With some of the lower potency tumor fractions, a 30-50% variation occurred and the average of the two values is reported unless additional determinations were made.

TABLE I. Coenzyme A and Pantothenic Acid Content of Certain Transplantable and Induced Tumors in the Rat.

Tumor	Sample No.	Coenzyme A*	Pantothenic acid†	
			Total	Free
Walker carcinosarcoma	1	9.8	5.7	.5
	2	12.0	15.3	.1
	3	7.2	5.3	1.9
Jensen sarcoma	1	11.2	4.5	.0
	2	8.4	6.8	.4
	3	8.4	7.1	.0
Flexner-Jobling carcinoma	1	11.9	12.3	.0
	2	16.8	19.7	.2
	3	7.6	7.0	.0
Induced liver tumor	1	18.8	15.2	.6
	2	28.8	22.2	1.1

* Lipmann units per g fresh tissue.
† μg per g fresh tissue.

Results and discussion. From the data in Table I it appears that the transplantable tumors contained roughly similar amounts of coenzyme A and pantothenic acid, although fairly wide variations were observed with some of the samples. The induced liver tumors (Tables I and II) on the other hand contained appreciably more of these factors than the transplantable tumors. In comparison with several normal rat tissues analyzed by similar methods, however, all of these tumors contain only about 1/2 to 1/10 as much of these factors(13, and Table II). The PA values in Table I are about 20 to 30% higher than those previously reported for similar rat tumors (5). This is undoubtedly a result of a more complete release of PA from bound forms(6). The low amount of coenzyme A in these tumors thus parallels the finding of Potter *et al.*(1,2) that homogenates of these tissues contain low amounts of the enzymes that oxidize oxalacetic acid.

The intracellular distribution of CoA and PA in normal rat liver and in azo dye-induced rat liver tumors is given in Table II. In gen-

9. Breedis, C., and Young, G., *Fed. Proc.*, 1949, v8, 351.

10. Price, J. M., Miller, E. C., and Miller, J. A., *J. Biol. Chem.*, 1948, v173, 345.

11. Miller, E. C., Miller, J. A., Sandin, R. B., and Brown, R. K., *Cancer Research*, 1949, v9, 504.

12. Price, J. M., Miller, J. A., Miller, E. C., and Weber, G. M., *Cancer Research*, 1949, v9, 96.

13. Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 1948, v174, 37.

14. Skeggs, H. R., and Wright, L. D., *J. Biol., Chem.*, 1944, v156, 21.

TABLE II. Intracellular Distribution of Coenzyme A and Pantothenic Acid in Normal Rat Liver and Induced Rat Liver Tumor.

	Coenzyme A*				Pantothenic acid†							
					Total				Free			
	Liver		Tumor		Liver		Tumor		Liver		Tumor	
Sample No. Fraction	1	2	3	4	1	2	3	4	1	2	3	4
Whole homogenate	174	166	30	15	126	118	17	14	—	1.9	4.8	5.2
Nuclei	39	40	4.2	2.9	33	24	3.0	2.9	—	.2	1.4	.8
Mitochondria	95	82	9.4	4.4	63	42	2.6	1.8	—	.3	1.0	.8
Microsomes	3.8	8.6	0.0	0.3	5.3	5.9	.4	1.2	—	.1	.4	.0
Supernatant	33	57	12	6.3	30	32	5.5	5.7	—	.5	2.1	.9
Recovery	171	188	26	14	131	104	12	12	—	1.1	4.9	2.5

* Lipmann units per g fresh tissue.
† μg per g fresh tissue.

eral, the distribution of these two components paralleled one another closely. As expected from previous work on the distribution of the enzymes involved in the Krebs tricarboxylic acid cycle(7) a large share of the CoA was found in the mitochondrial fraction of each tissue. In the normal livers this fraction contained about half of the total amounts of the CoA and PA present. The remaining amounts of these substances were divided almost equally between the supernatant and nuclear fractions. As in the case of vitamin B_6(15) the microsomes contained little of these factors; it is quite possible that the small amounts found were due to contamination by supernatant fluid since the microsomes were not washed by resuspension. The tumors, on the other hand, not only contained much less CoA and PA but the distribution of these factors among the morphological constituents appeared to differ from that of normal liver. Thus the supernatant fraction accounted for nearly half of the vitamin and coenzyme present in the tumor and the mitochondria and nuclei contained the remaining amounts in the order named. As in the normal liver only very low amounts were found in the tumor microsome fractions.

While few conclusions can be drawn from the free PA values given in Table II, it would appear that there was a much higher content of free pantothenic acid in the liver tumor than in the normal liver. However, the data in Table I indicate that when these liver tumors are analyzed quickly after death of the animal the content of free PA is considerably less although it is still higher on a percentage basis than in normal liver. This discrepancy is probably due to the fact that the fractionation procedure required several hours to complete. Even though the homogenates and fractions were held at 3 to 5° throughout this time it is quite probable that some enzymatic liberation of PA occurred. Similarly, the low recoveries of free PA shown in Table II may have been due to a greater liberation of PA in the whole homogenate than in any of the isolated fractions.

The recoveries of CoA and total PA in the fractionations were reasonably good (89 to 113%) in the case of normal liver but they tended to be low (71 to 93%) with the liver tumor samples. The reason for this difficulty is not known.

Summary. The intracellular distribution of pantothenic acid and of coenzyme A have been determined in normal rat liver and in azo dye-induced rat liver tumors. These substances were also determined in three kinds of transplantable rat tumors. All of the tumors were very much lower in these components than normal liver. The majority of each factor in the liver was contained in the mitochondria; however, in the liver tumor the supernatant fraction contained the largest amounts. The remaining amounts were distributed between the nuclear and supernatant fractions of the liver and the mitochondrial

15. Price, J. M., Miller, E. C., and Miller, J. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 575.

and nuclear fractions of the liver tumor. The microsomes from each tissue contained only small amounts of the vitamin and coenzyme. The finding that these tumors contain low levels of coenzyme A is in harmony with the observation of Potter and his associates that homogenates of such tumors contain low amounts of the enzymes that oxidize oxalacetic acid.

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A Complement Fixation Test for Equine Virus Abortion.* (18233)

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Equine virus abortion (E.V.A.) was first described as a clinical entity, of unknown etiology, by Dimock and Edwards(1). Subsequent reports(2-8) established the viral etiology, and more fully characterized this disease. E.V.A. is an epizootic contagious disease of Equidae characterized by abortion, usually in the late stages of pregnancy, without signs of infection of the mare before or after parturition. The most striking pathological lesion noted in the aborted fetus are small foci of necrosis in the liver. Other changes worthy of note are hemorrhages in the abdominal and thoracic organs, edema and congestion of the lungs, and pleural effusion. Necrosis and intranuclear inclusions are the chief histopathological features. E.V.A. has been reported(6,9) from 12 states in this country, and five foreign countries. Although data regarding the incidence of this disease in the United States are incomplete, the majority of cases appear to occur in central Kentucky, principally on thoroughbred farms. abortion rates ranging from 10% to 85% in exposed groups of animals indicate the extreme degree of contagiousness of the virus. Little, however, is known concerning the mode of transmission of E.V.A.; apparently it is not venereal, since the stallion has not as yet been implicated. The disease has been induced artificially by feeding pregnant mares unfiltered infected placenta or fetal tissue, and by the injection of bacteria-free filtrates of infected tissue. The incubation period of such infected animals is from 18-30 days.

The host range of the virus appears to be narrowly limited. Small success has attended the efforts of those who have attempted to grow this virus in animals other than the natural host. Dimock, Edwards, and Bruner (6) were unable to demonstrate infection in mice, rabbits, guinea pigs and chick embryos. Serial passage through guinea pig foetuses(10) has failed to maintain the virus. Histopathological evidence of infection in newborn Syrian hamsters has been adduced by Goodpasture and Anderson(11,12). Other investigators (9,10) have not succeeded in demonstrating

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1. Dimock, W. W., and Edwards, P. R., *Ky. Agric. Exp. Sta.*, Suppl., 1933, Bull. 333.

2. Dimock, W. W., and Edwards, P. R., *Cornell Vet.*, 1936, v26, 231.

3. *49th Ann. Rep. Director Ky. Agric. Exp. Sta.*, 1936, part 1, 18.

4. *52nd Ann. Rep. Director Ky. Agric. Exp. Sta.*, 1939, part 1, 19.

5. Dimock, W. W., *J. Am. Vet. Med. Assoc.*, 1940, v96, 665.

6. Dimock, W. W., Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta.*, 1942, Bull. 426.

7. Westerfield, C., and Dimock, W. W., *J. Am. Vet. Med. Assn.*, 1946, v109, 101.

8. Dimock, W. W., Edwards, P. R., and Bruner, D. W., *Ky. Agric. Exp. Sta.*, 1947, Bull. 509, 4.

9. Bruner, D. W., Doll, E. R., and Hull, F. E., *The Blood Horse*, 1949, v58, 31.

10. Doll, E. R., Personal communication.

11. Anderson, K., and Goodpasture, E. W., *Am. J. Path.*, 1942, v18, 555.