are maintained under these conditions and that the enzyme hence cannot be of hepatic origin.

Our data indicate that the increased serum alkaline phosphatase in obstructive jaundice, as in skeletal diseases, is phosphatase II (cyanide-sensitive) and therefore presumably not of hepatic cell origin. The results are compatible with the view that elevation of serum alkaline phosphatase in extra- or intrahepatic biliary tract obstruction is due to retention of serum alkaline phosphatase, largely of osseous origin. There is a great deal of clinical evidence for this view which was summarized elsewhere.[1]

The marked difference in the effects of cyanide on serum alkaline phosphatase and on liver tissue alkaline phosphatase indicates definite differences between these enzymes. However, the similarity between serum alkaline phosphatase and bone phosphatase with respect to cyanide does not prove their identity; all that can be said is that in this and other respects no significant difference is apparent. Absolute proof of identity of 2 enzymes from different sources is not possible at this time.

It may seem surprising that with so many alkaline phosphatases present in so many organs, the enzyme in the plasma should be so preponderantly of osseous origin. However, not only is the number of osteoblasts in the body very large and their alkaline phosphatase production great, but the secretion of the enzyme is extracellular for production of bone at the cell surface. In most cells, phosphatases operate intracellularly, often apparently within the confines of the nucleus to judge from histochemical evidence, and probably never reach the extracellular fluids.

Summary. Cyanide markedly inhibits the serum alkaline phosphatase of normal human subjects and the increased levels of patients with obstructive jaundice and skeletal diseases; no essenital differences were observed in these 3 categories. The evidence is consistent with the view that in all 3 categories the largest proportion of serum alkaline phosphatase is of osseous origin since bone phosphatase is inhibited by cyanide (Cloetens' phosphatase II) and liver phosphatases are, for the most part, cyanide-insensitive (Cloetens' phosphatase I). The data indicate that the rise in serum alkaline phosphatase in obstructive jaundice cannot be of hepatic origin but they are compatible with retention of serum alkaline phosphatase due to obstruction of the excretory biliary channels.

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17261. Intracellular Distribution of Vitamin B_6 in Rat and Mouse Livers and Induced Rat Liver Tumors.*

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In studies[1-4] from this laboratory the intracellular distribution of riboflavin was determined in normal rat livers, in the livers of rats fed various aminoazo dyes, and in liver tumors induced by 4-dimethylaminoazobenzene. Riboflavin is of particular interest since

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[2] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Research*, 1949, **9**, in press.

[3] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., manuscript in preparation.

[4] Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Research*, 1949, **9**, 96.

high dietary levels of this vitamin delay the induction of neoplasms by certain of the aminoazo dyes.[5] Furthermore, ingestion of any one of the various dyes lowers the level of riboflavin in the liver to a degree that is roughly proportional to its carcinogenic activity.[6,7] While the ingestion of 4-dimethylaminoazobenzene also decreases the level of vitamin B_6 in the liver this vitamin differs from riboflavin in that the various dietary changes which enhance or lower the carcinogenic activity of this dye do not alter the level of hepatic vitamin B_6 in a significant fashion.[7] Hence it was desirable to compare the intracellular distribution of vitamin B_6 with that of riboflavin in the livers of rats before and after the administration of 4-dimethylaminoazobenzene as well as in hepatic tumors induced by this dye. Similar data for the liver of the mouse, a species relatively resistant to the carcinogenic action of this dye,[5,8] are also presented.

Methods. The animals were fed a semisynthetic diet (diet 3[7]) containing 1.2 mg of riboflavin per kg. Male albino rats‡ were fed this basal diet with or without 0.06% 4-dimethylaminoazobenzene for 4 weeks. Female albino mice§ were fed the same diets but for 4 months. The animals were killed with ether and the livers perfused *in situ* with isotonic saline. The perfusion and all subsequent steps prior to the analytical procedures were carried out at 0 to 5°. The rat liver tumors were obtained from rats fed the basal diet plus the dye for 4 to 5 months. Nine mouse livers were pooled for fractionation No. 1 and 7 livers were combined for fractionation no. 2. Each fractionation of rat liver was made on pools from 2 (fractionations no. 3 and 5) or 3 (fractionations no. 4 and 6) animals. Small tumors which were grossly non-necrotic were pooled from 9 and 20 rats for fractionations no. 7 and 8, respectively. The selection of the tumors, which were obtained from non-perfused livers, was made as previously described.[4] The pooled tumors and livers were forced through a plastic tissue mincer and homogenized in 0.88 *M* sucrose solution as previously described.[1]

Differential centrifugation was used as described previously[1,2,4] to prepare the nuclear, large granule (mitochondria), small granule (microsome), and supernatant fluid fractions. The nuclear and large granule fractions were washed twice; the small granules were sedimented at 24,000 x *g* (at center of tube) for 3 hours and were not washed. In experiments where the small granules were washed the nuclear and large granule fractions were sedimented in the usual manner, but were washed only once. The supernatant fluid and washing from the large granules were combined and centrifuged for 36 minutes at 120,000 x *g* (at center of tube) in an air-driven ultracentrifuge. The small granules were then resuspended in the sucrose solution and, after aliquots had been taken for analysis, were brought to the volume in which they had previously been suspended. After recentrifugation under identical conditions the sediment was suspended in the sucrose solution for analysis.

Vitamin B_6 was determined with *Saccharomyces carlsbergensis* by the method of Atkin *et al.*[9] This yeast responds equally to the 3 known forms of vitamin B_6.[10] The intracellular distributions of protein, nucleic acids, and riboflavin were also determined as previously described.[1] The intracellular distributions of these constituents in the fractionations of the rat tissues agreed well with those previously published.[1,4]

Results. From Table I it is evident that most of the vitamin B_6 was in the large granules and the supernatant fluid in all of the tissues studied. The large granules con-

5 Rusch, H. P., Baumann, C. A., Miller, J. A., and Kline, B. E., in Moulton, F. R., A.A.A.S. research conference on cancer, Washington, 1945, 267.

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‡ Holtzman Rat Company, Madison, Wis.

§ A. Sutter, Springfield, Mo.

9 Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N., *Ind. and Eng. Chem., Anal. Ed.*, 1943, **15**, 141.

10 Snell, E. E., *Physiol. Rev.*, 1948, **28**, 255.

TABLE I.
Intracellular Distribution of Vitamin B_6 in Liver and Liver Tumor.

Fraction	Mouse livers		Rat livers				Rat liver tumors	
	Basal diet	Basal diet + dye	Basal diet		Basal diet + dye			
	Fractionation No.							
	1	2	3	4	5	6	7	8
	μg of vitamin B_6 per g of fresh tissue							
Whole homogenate	6.0	4.5	6.4	6.4	4.0	4.7	1.8	1.2
Nuclei	0.5	0.5	0.4	0.2	0.3	0.2	0.2	0.1
Large granules	2.0	1.4	2.3	2.9	1.6	1.9	0.5	0.4
Small "	0.3	0.2	0.3	0.4	0.2	0.2	0.2	0.1
Supernatant fluid	3.0	2.2	3.0	3.1	1.8	2.1	1.2	0.7
Recovery	5.8	4.3	6.0	6.6	3.9	4.4	2.1	1.3
	μg of vitamin B_6 per g of protein							
Whole homogenate	46	34	53	50	37	40	15	10
Nuclei	22	18	26	14	18	11	5	2
Large granules	57	57	60	73	60	58	46	29
Small "	19	16	18	21	17	13	12	9
Supernatant fluid	60	36	64	62	40	41	26	14

tained 28 to 45% and the supernatant fluid 45 to 64% of the total amount of vitamin B_6. The ratio of vitamin B_6 to protein was higher in the large granule and supernatant fluid fractions, and lower in the nuclear and small granule fractions than it was in the whole homogenate. When 4-dimethylaminoazobenzene was included in the diets of either rats (fractionations no. 5 and 6) or mice (fractionation no. 2) the amount of vitamin B_6 in the large granule and supernatant fluid fractions was considerably reduced. Since the protein content was also reduced in the large granule fraction[1,2] the ratio of vitamin to protein was unchanged in this fraction. On the other hand this ratio was decreased in the supernatant fluid since the protein content of this fraction remained unchanged.[1,2] In the liver tumor tissue the level of vitamin B_6 in the large granules and supernatant fluid was even lower than in the livers of rats fed the dye, and the ratio of the vitamin to protein was also much lower.

The small granules isolated from the livers of rats fed the basal diet after centrifuging at 120,000 x *g* for 36 minutes were entirely comparable in their contents of pentosenucleic acid, riboflavin and vitamin B_6 to those isolated from similar livers at 24,000 x *g* for 3 hours. A single washing reduced the level of vitamin B_6 in these particles by 50 to 80%. The pentosenucleic acid was reduced by 13 to 28% and in a single experiment no detectable loss of riboflavin occurred on washing. These changes are probably attributable in part to the removal of some residual supernatant fluid from the small granules.

Discussion. As in the case of riboflavin[1-4] the vitamin B_6 was found chiefly in the large granules and supernatant fluid in all the tissues studied. The nuclear fraction of normal rat liver contained 3 to 6% of the total vitamin B_6 whereas this fraction generally contains 5 to 9% of the total riboflavin. The greatest differences in the distribution of the two vitamins was found in the small granules. The washed small granules contained only 0.8 to 1.9% of the total vitamin B_6 but still contained 16% of the total riboflavin. It is probable that the values for the vitamin B_6 and riboflavin contents of the nuclear fraction are too high, since this fraction is contaminated with some whole cells and large granules.[1,11]

Claude[12] stated that he found most of the transaminase activity of rat and guinea pig liver in the supernatant fluid. The transa-

[11] Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, 1948, **172**, 619.

[12] Claude, A., in Moulton, F. R., A.A.A.S. research conference on cancer, Washington, 1945, 223.

minase activity was relatively low in the large granules and was completely absent from the microsome or small granule fraction. Subsequent to these observations it was found that vitamin B_6 is an integral part of this enzyme system.[13] Thus the data presented above support those of Claude since the small granules contain no transaminase activity and very little vitamin B_6 while the parts of the liver cell that contain transaminase activity are particularly rich in vitamin B_6. No information is available on the presence or distribution in these tissues of other enzymes which contain vitamin B_6.

The hepatic carcinogen 4-dimethylaminoazobenzene reduced the level of riboflavin[1,2] and vitamin B_6 in the large granules and supernatant fluid, but in both cases the reduction of protein in the large granules paralleled the decrease in vitamin content. This is probably the result of a reduction of the number of large granules in the liver cells.[3]

Summary. The intracellular distribution of vitamin B_6 in rat liver tumors induced by 4-dimethylaminoazobenzene and in the livers of rats and mice before and after administration of the azo dye was determined after differential centrifugation of the tissue homogenates in hypertonic sucrose solution.

In all cases vitamin B_6 was concentrated in the washed large granules or mitochondria (28 to 45% of total) and in the supernatant fluids (45 to 64% of total). The washed nuclear fractions contained 3 to 11% of the total vitamin B_6 present. The unwashed small granules or microsomes contained 4 to 8% of the total vitamin B_6; in the case of normal rat liver one washing reduced the level to about 1½%.

The ingestion of the azo dye reduced the levels of the vitamin in the large granule and supernatant fluid fractions of the livers in each species. The decrease in protein content of the large granules paralleled the decrease in vitamin B_6 content. The large granule and supernatant fluid fractions of the liver tumors contained even less vitamin B_6 than was found in the corresponding fractions from the livers of rats fed the dye and the ratio of the vitamin to protein was also much lower.

[13] Wynne, A. M., in *Ann. Rev. of Biochem.*, 1946, **15**, 58.

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17262. Healing of Tuberculous Pulmonary Cavities by Means of Skin Grafts.

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Giant tuberculous pulmonary cavities continue to present one of the most complex problems in the surgical treatment of pulmonary tuberculosis. Various types of collapse therapy have resulted in a high percentage of failures. Intracavitary drainage in tension or blocked cavities, combined with collapse therapy, has greatly increased the number of good results.

Intracavitary drainage without collapse therapy provides relief from toxicity but is of little value in cavity closure. In advanced pulmonary disease with multiple cavitation, collapse therapy is contraindicated.

We wish to present a somewhat different point of view. Let us consider that a tuberculous pulmonary cavity is a chronic lung abscess: The lung surrounding a chronic nonspecific lung abscess shows little disease. When such an abscess is drained, the surrounding lung tissues fill in the defect occupied by the abscess. In a tuberculous lung abscess, the surrounding lung tissues are usually diseased and cannot expand to fill in the space occupied by the abscess cavity. Drainage is of cleansing value in pulmonary tuberculous cavities, as in other types of pulmonary abscesses, but cavity obliteration does not occur without col-