Summary. In an *in vitro* test system at pH 7.4 it was found that degradation of serotonin by ceruloplasmin was markedly inhibited by sodium metabisulfite, ascorbic acid, glutathione, BAL, isoniazid, iproniazid, hydralazine, an analog of hydralazine (Ba-20523), isocarboxazid, thiocarbohydrazide, hydrazine hydrate, syrosingopine (Su-3118) and its isomer, Su-5171. Lesser effects were produced by reserpine, tetraethylammonium chloride, and a thiazide diuretic (Su-7075). A number of other sulfhydryl containing, chelating, anti-hypertension, and psychopharmacologic agents were without effect in the test system employed. It was not possible to correlate reaction inhibition with simple presence of sulfhydryl groups, the hydrazine nucleus, or with monoamine oxidase and diamine oxidase inhibition.

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Pyridoxal Phosphate in Plasma and Leukocytes in Patients with Leukemia and Other Diseases.* (26177)

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The concentration of pyridoxal phosphate, the coenzymatically active form of Vit. B_6, can be estimated in leukocytes and plasma using a modification of the manometric method of Umbreit *et al.*(1). Previously, this has been done in the blood of normal (2,3,4) and pregnant subjects(3,4) and in the cord blood of new born babies(3,4). In addition, load tests were performed in normal controls and in pregnant women with measurement of the B_6 content of plasma and leukocytes following oral administration of pyridoxine hydrochloride(4). The present communication deals with estimation of pyridoxal phosphate in the blood and plasma of patients with chronic and acute leukemia and various other diseases.

Method. For simultaneous estimation of pyridoxal phosphate in leukocytes and plasma, 5 ml of venous blood were collected in a tube containing about 0.002 g of sodium versenate. The assay method is based on coenzyme function of pyridoxal phosphate in a tyrosine decarboxylase system according to Boxer *et al.*(2) with minor changes as previously described(4). Streptococcus fecalis was obtained from the Dept. of Bacteriology, Rutgers Univ., New Brunswick, N. J. Leukocytes were isolated using freeze-dried phytohemagglutinin prepared from navy beans. The phytohemagglutinin does not contain any measurable B_6. The samples were usually

* Supported by Vitamin Fn.

TABLE I. Pyridoxal Phosphate Values in Plasma and Leukocytes in Normal Controls and Various Disease Groups.

Group	No. of cases	Plasma, mγ/ml Avg	Plasma, mγ/ml Range	Leukocytes, mγ/million cells Avg	Leukocytes, mγ/million cells Range	% of cases below 4 mγ/ml plasma	% of cases below 0.13 mγ/mil leuko.	P Plasma	P Leukocytes
Normal	40	9.6 ± 2.8	5.2-33	.22 ± .05	.14-.36	0	0		
Fibroid uterus	12	6.0 ± 5.2	1.1-19.4	.22 ± .10	.05-.44	42	8	0	0
Cancer	23	4.3 ± 4.1	.0-16.2	.21 ± .02	.07-.68	50	26	0	0
Cardiac	26	7.1 ± 3.8	2.0-16.5	.25 ± .08	.05-.38	19	8	0	0
G.I. pathology	25	6.2 ± 6.2	.7-23.1	.26 ± .11	.08-.62	44	12	0	0
Respiratory diseases	20	7.5 ± 7.6	.7-30.2	.24 ± .10	.14-.54	35	0	0	0
Diabetes mellitus	21	6.3 ± 3.8	1.1-30.2	.25 ± .11	.10-.54	33	9	0	0
Liver cirrhosis	11	4.6 ± 3.9	.7-12.5	.22 ± .13	.08-.55	50	18	0	0
Obstructive jaundice	22	5.0 ± 4.8	.0-16.2	.18 ± .14	.02-.68	60	41	0	0
Infectious hepatitis	9	6.6 ± 5.4	1.6-19.7	.24 ± .22	.00-.59	33	44	0	0
Nutritional anemia	4	2.4 ± 3.0	1.1- 3.8	.21 ± .20	.07-.52	100	50	0.10	0
Pernicious "	5	3.7 ± 3.2	.0- 7.2	.22 ± .11	.04-.31	50	20	0	0

obtained in the morning. The subjects were either fasting or had received a light breakfast. It was ascertained in control experiments that breakfast had no influence on Vit. B_6 level of plasma or leukocytes. No vitamin preparation had been taken by any of the individuals studied for at least 2 months. Determinations were done in duplicate within 12 hr after samples had been obtained. Even at 4°C some loss of Vit. B_6 occurred after 24 hr in plasma and after 48 hr in leukocytes. Duplicate determinations for plasma showed an error of ± 0.5 mγ/ml and for leukocytes of ± 0.03 mγ/million cells.

Results. Table I shows the results obtained in normal controls and in hospital patients excluding those suffering with leukemia. In normal controls, pyridoxal phosphate concentration ranged between 5.2 and 33 mγ/ml with an average of 9.6 mγ in plasma, and between .14 and .36 mγ/million cells with an average of .22 mγ in the leukocytes. Patients were divided, for convenience, into various groups as listed in the Table. There were wide variations of B_6 content of plasma and leukocytes in all groups and, therefore, standard deviations were great. Average values for leukocytic pyridoxal phosphate were close to those of the normal group. On the other hand, average plasma values were considerably lower in several of the patient groups, particularly those with nutritional anemia, pernicious anemia, cancer of various sites and liver cirrhosis. However, a statistically significant difference was found only in patients with nutritional anemia.

The Table also lists the percentage of cases in each group in which plasma contained less than 4 mγ/ml and leukocytes less than .13 mγ/million cells. These amounts were lower than the lowest found in the normal group. In patients with obstructive jaundice, infectious hepatitis and nutritional anemia, between 40 and 50% of the patients had such low leukocyte values. Plasma estimations revealed values of less than 4 mγ/ml in many of the patients regardless of the type of disease for which they had been admitted to the hospital.

Table II lists findings in cases of leukemia as well as those of normal bone marrow and lymphocyte suspensions from surgically removed tonsils. Leukemias were grouped into chronic myeloid, chronic lymphatic and acute. Total white counts in the patients with chronic myeloid leukemia varied between 28,000 and 500,000, with chronic lymphatic, between 5,200 and 900,000, and with acute leukemia, 3,200 and 125,000 cells per cc. Average leukocyte values were between .06

TABLE II. Pyridoxal Phosphate Values in Plasma and Leukocytes in Leukemias and in Normal Bone Marrow and Tonsils.

Group	No. of cases	Plasma, mγ/ml Avg	Plasma, mγ/ml Range	Leukocytes, mγ/million cells Avg	Leukocytes, mγ/million cells Range	% of cases below 4 mγ/ml plasma	% of cases below 0.13 mγ/mil leuko.	P Plasma	P Leukocytes
Leukemia									
Chronic myeloid	7	3.1 ± 2.2	1.5-6.3	.07 ± .04	.03-.16	60	87	.05	.01
" lymphatic	11	4.9 ± 2.6	1.9-8.8	.06 ± .04	.00-.14	66	91	.10	.01
Acute	15	3.5 ± 2.7	.0-7.2	.09 ± .04	.03-.16	60	80	.05	.01
Normal bone marrow	5			.20 ± .11	.09-.38				
Tonsils	10			.19 ± .07	.09-.31				

and .09 mγ/million cells and for plasma, between 3.1 and 4.9 mγ/ml. The difference between the patients suffering from leukemia and the normal control group is statistically significant. Correspondingly between 80-91% of all cases showed leukocytic values lower than .13 mγ/million cells and two-thirds showed plasma values lower than 4 mγ/ml. In 5 bone marrows of subjects either healthy or with minor illnesses, the white cells contained between 0.09 and 0.38, average .20 mγ/million cells of pyridoxal phosphate. Lymphocytes obtained from homogenates from tonsils from 10 otherwise healthy children contained between .09 and .31, average .19 mγ/million cells of pyridoxal phosphate. Thus, in both instances, considerably greater amounts of B_6 were found in normal bone marrow cells and tonsillary lymphocytes.

Comment. These findings show that the average amount of B_6 in leukocytes and plasma is not statistically different in patients with various diseases as compared to those of normal controls with the exception of patients with leukemia and in the case of the plasma, those with nutritional anemia. A considerable number of nonleukemic patients, however, had lower amounts of B_6 than the lowest found among controls. This may be due in individual cases either to a decreased intake or an increased need for this essential vitamin. Similarly, Boxer *et al.*(2) found in 7 out of 36 patients with manifest arteriosclerotic disease very low amounts of leukocytic B_6.

The consistent depression of leukocytic B_6 of leukemic patients cannot be due to the relative immaturity of the leukemic cells since many of the leukocytes in the blood of patients with chronic myeloid and most with lymphatic leukemia were mature. Furthermore, cells from normal bone marrow contain a significantly higher amount of B_6 than those from patients with chronic myeloid leukemia. Likewise, lymphocytes obtained from tonsils contain more pyridoxal phosphate than do leukemic lymphocytes. It is interesting to note that so far only leukocytes in pregnant women at term revealed a comparable depression of leukocytic B_6 attributable to depletion of the maternal tissues by the growing fetus(3,4). A marked abnormality in B_6 metabolism of a different, as yet unknown, etiology has been recently described in several neoplastic processes. Thus total Vit. B_6 content of hepatomas induced by butter yellow in rats is considerably lower in the tumor as compared to the normal liver (5). Haggerty and Sullivan(6) found not only relatively small values of B_6 in a trans-

planted sarcoma in CAF_1 mice, but in addition, a progressive decrease in Vit. B_6 content of the tumor during its growth with a simultaneous accumulation of kynurenin. In human subjects, Musajo *et al.*(7) isolated kynurenin and 3-hydroxykynurenin from the urine of patients with myeloid and lymphatic leukemia and Hodgkin's disease.

Summary. The amount of coenzymatically active Vit. B_6 was determined in plasma and leukocytes in patients with various diseases. Values for plasma and leukocytes varied widely in all groups examined. A statistically significant decrease of plasma B_6 was found only in patients with nutritional anemia and various forms of leukemia. However, the amount of pyridoxal phosphate was very low in individual patients with various other diseases. This may indicate a decreased intake of vitamin in some hospitalized patients or an increased need for it. B_6 values in leukocytes were consistently decreased in patients with various forms of leukemia. In contrast, cells from normal bone marrow and from tonsils showed significantly higher amounts of pyridoxal phosphate. The findings are discussed with reference to a specific disturbance in B_6 metabolism in some neoplastic conditions.

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Liver Ketogenesis (3). High Fat Feeding on Acetoacetate Production in Liver Slices, Utilization by Kidney Slices. (26178)

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Nath *et al.*(1-3) have reported that continued injection of acetoacetate to normal animals causes disturbances in carbohydrate metabolism and it has been postulated that gradual accumulation of acetoacetate in blood and tissues is one of the factors responsible for development of diabetes. Such accumulation may result either from overproduction of acetoacetate in the liver or its decreased utilization by the extra-hepatic tissues.

The authors(4) have reported that the hydrolyzed product of glucose cycloacetoacetate (GCA), a crystalline substance obtained by condensation of glucose with acetoacetate, prevents overproduction of acetoacetate by liver slices and increases its utilization by kidney slices when fed to rats along with a high carbohydrate diet. High fat diet has also been known to cause metabolic changes in rats, including diminished rate of lipogenesis (5), glycogenesis(6) and glucose utilization (7). Therefore, the effect of high fat feeding on liver ketogenesis and the influence of hydrolyzed GCA feeding with high fat diets is reported here. Desiccated thyroid and thiouracil were also mixed in the diet of 2 groups of animals to study their additional influence on liver ketogenesis and acetoacetate utilization by kidney slices.

Methods. In view of the relatively rapid response of young rats to thyroid and thiouracil feeding(8), male albino rats weighing 75-100 g and growing steadily on a standard diet were used. These rats were divided into 8

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