

AN ABSTRACT OF THE THESIS OF

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Title: FACTORS AFFECTING ERYTHROCYTE TRANSAMINASE ACTIVITY
IN PRESCHOOL CHILDREN

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Erythrocyte glutamic-pyruvic transaminase (EGPT) and glutamic-oxaloacetic transaminase (EGOT) activities reflect vitamin B₆ status in humans (Baysal, Johnson, and Linkswiler, 1966). Pyridoxal phosphate (PALPO), an active form of vitamin B₆, serves as the coenzyme for these transaminases. Compared to other methods of vitamin B₆ assessment, transaminase measurement has the advantage of dealing with a single enzyme requiring PALPO and reflecting the subject's vitamin B₆ status over a long period of time (Sauberlich et al., 1970).

Although studies on transaminase activity in adults have been reported, information on EGPT and EGOT activities in children is not available. This study was undertaken to determine the activities of EGPT and EGOT in normal preschool children. In addition, factors affecting transaminase activities were considered. The storage stability of EGPT and EGOT was also reported.

Participating in this study were 109 subjects, aged from 21 to 126 months. The activities of EGPT and EGOT were expressed as μg pyruvate/mg hemoglobin (Hb)/hr and mg pyruvate/ml red blood cells/hr. The basal activity indicates the level of holoenzyme. The stimulated activity with added in-vitro PALPO shows the level of holoenzyme plus apoenzyme. The percent stimulation represents the degree of saturation of apoenzyme with the coenzyme (Cavill and Jacobs, 1967).

For EGPT, the basal activity and percent stimulation were 1.20 ± 0.44 μg pyruvate/mg Hb/hr and 11.70 ± 7.00 percent, respectively. Those of EGOT were 23.30 ± 5.77 μg pyruvate/mg Hb/hr and 69.90 ± 23.3 percent. The two different ways of expressing basal activity of EGPT and EGOT were highly correlated with one another.

A significant positive correlation was found between the basal activities of EGPT and EGOT ($p < 0.01$). However, the positive relationship between their corresponding percent stimulation was not significant. The stimulated and basal activities for both EGPT and EGOT were closely correlated ($p < 0.01$), which indicated that the level of holoenzyme is largely dependent on the amount of apoenzyme available. A significant inverse relationship ($p < 0.01$) existed between the basal activity and percent stimulation of EGOT, which meant that the high enzyme activity level is usually associated with a high degree of saturation of the

apoenzyme with PALPO. The similar inverse relationship for EGPT was not statistically significant.

In the subjects whose diet was supplemented with multivitamins containing pyridoxine, the transaminase activities appeared to be higher and the corresponding percent stimulation lower than in those receiving no supplementation. However, the difference was only significant for basal EGPT, using the Student's t test ($p < 0.01$).

The subjects with high basal activities or low percent stimulation of EGPT or EGOT also tended to have higher plasma vitamin B₆ levels. But these relationships were not significant.

As the age of the subjects increased, the basal and stimulated activities of both EGPT and EGOT declined, accompanied by the corresponding increase in percent stimulation. The correlations for basal and stimulated activities, as well as percent stimulation of EGOT, but not EGPT, with age were significant ($p < 0.05$).

The differences in transaminase activities due to sex were not significant. But in general, the girls had a lower basal activity and a higher percent stimulation for both EGPT and EGOT than the boys.

The average hemoglobin level of the subjects was 12.95 ± 0.77 g percent. The hemoglobin levels increased significantly with age ($p < 0.01$).

Finally, experiments with two hemolysate samples showed that no loss of EGPT or EGOT activities occurred with freezing and storage within 13 days.

Factors Affecting
Erythrocyte Transaminase Activity
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FACTORS AFFECTING ERYTHROCYTE TRANSAMINASE ACTIVITY IN PRESCHOOL CHILDREN

INTRODUCTION

The importance of vitamin B₆ in metabolism has been recognized since György established the nutrient as a dietary essential for animals (György, 1934). In recent years, attention has been directed towards human requirements and nutritional status regarding vitamin B₆.

The need for vitamin B₆ by the human became evident when Snyderman and associates (1953) observed convulsions in one mentally retarded infant and anemia in another resulting from an ingestion of a vitamin B₆-deficient diet. Both of the manifestations were corrected by the administration of vitamin B₆. In later years, a few cases of anemia and mental disturbance in humans which responded to pyridoxine treatment were reported (Linkswiler, 1967). The human body has only a small reserve of vitamin B₆ and can be quickly depleted of the vitamin (Sauberlich et al., 1963). The antagonism of certain drugs and hormones to the vitamin was also discovered (Brown, 1972).

Since pyridoxal phosphate, the active form of vitamin B₆, is involved in numerous enzyme reactions, deficiency of the vitamin causes many biochemical changes which can be measured. Various techniques have been successfully developed to detect subclinical vitamin B₆ deficiency.

However, these methods have many limitations, such as lack of sensitivity, specificity, or of practicability (Woodring and Storvick, 1970). Most of these techniques involve the determination of the vitamin levels, the metabolites of the products, abnormal metabolic products caused by low intake of the vitamin, or alterations in blood enzyme activities (Sauberlich et al., 1963).

Among these techniques are the measurements of glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase. Both of these enzymes require pyridoxal phosphate as a coenzyme. Their activity is reduced in vitamin B₆-deficient tissues and the erythrocyte transaminase activities have been employed to assess the state of vitamin B₆ nutrition in man (Raica and Sauberlich, 1964; and Cheney, Sabry and Beaton, 1965a).

The reduction of transaminase activity is accompanied by a corresponding increase in the in-vitro stimulation by pyridoxal phosphate. The ratio of these values has been found particularly indicative of vitamin B₆ status by many authors (Jacobs, Cavill, and Hughes, 1968; and Cinnamon and Beaton, 1970).

Although information on the transaminase levels in adults is currently available, no study has been reported on the activity of these enzymes in children. The purpose of this thesis is to gather information about the activities of erythrocyte glutamic-pyruvic and glutamic-oxaloacetic

transaminases in preschool children. In addition, factors affecting the transaminase activities such as age, sex, level of plasma vitamin B₆, and the consumption of multi-vitamin supplements containing pyridoxine were examined. Finally, the stability of transaminase through storage was also studied.

A REVIEW OF LITERATURE

Chemistry and Biochemical Functions of Vitamin B₆

Since György (1934) established the essentiality of vitamin B₆ in animal metabolism, many studies have been done to recognize the various biochemical functions of this vitamin, which participates in over sixty enzyme systems (Sauberlich et al., 1970).

Chemistry of Vitamin B₆

The term "vitamin B₆" refers to the group of interconvertible compounds shown in Figure 1. Pyridoxine was purified by Lepkovsky in 1938. In the following year, its structure was determined by Harris and Folkers (György, 1971).

Among the vitamin B₆ compounds, pyridoxal phosphate and pyridoxamine phosphate are the coenzymatically active forms. In transamination, the aldehyde group of pyridoxal phosphate is capable of reversibly forming a Schiff's base, or ketimine, with an amine. On the other hand, pyridoxamine phosphate can form a Schiff's base by donating its amino group to an α -keto acid (Figure 2). Thus by oscillating between the aldehyde and amine forms, the coenzyme acts as carrier of amine groups from an α -amino acid to an α -keto acid (Snell and Di Man, 1970).

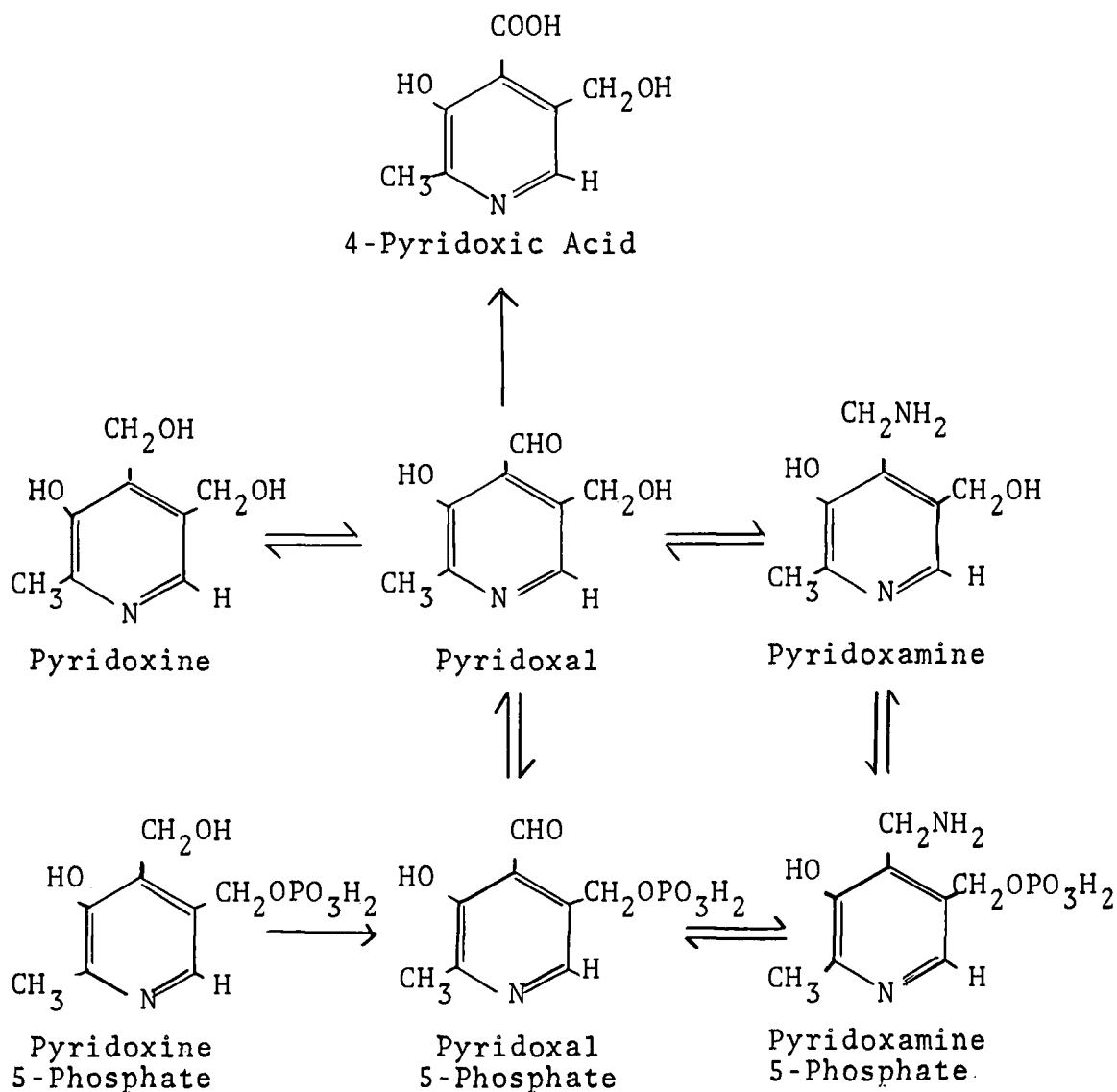


Figure 1. Metabolic interconversions of vitamin B₆ and the formation of 4-pyridoxic acid (adapted from Snell, 1964).

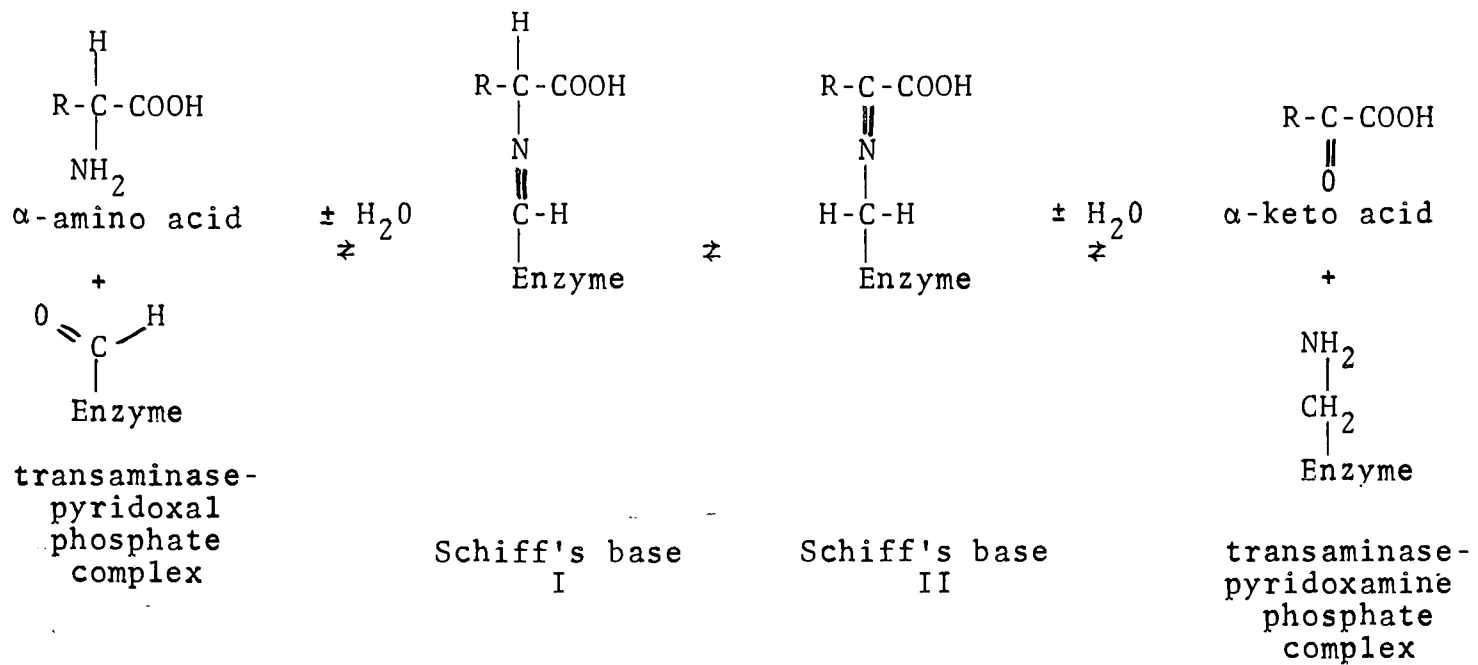


Figure 2. Steps in the transaminase reaction (adapted from Snell and DiMan, 1970).

Biochemical Functions of Vitamin B₆

The reactions involving amino acids that are catalyzed by vitamin B₆-dependent enzymes include transamination, decarboxylation, desulfhydration, racemization, cleavage, synthesis, and dehydration. The major reaction among these is transamination (Guirard and Snell, 1964).

The pyridoxal phosphate-dependent decarboxylases are necessary for dopamine formation from dihydrophenylalanine (Dopa), serotonin from 5-hydroxytryptophan, γ -aminobutyric acid from glutamic acid, and many others. A number of reactions catalyzed by vitamin B₆-dependent enzymes are required for tryptophan metabolism, including the conversion of this amino acid to nicotinic acid (Coon and Nagler, 1969).

Some other reactions that involve vitamin B₆ include the conversion of cysteine to pyruvic acid, oxalate to glycine, and synthesis of σ -aminolevulinic acid. Phosphorylase needs pyridoxal phosphate for its binding capacity (Snell and DiMan, 1970).

Role of Vitamin B₆ in Blood Glutamic-Pyruvic and Glutamic-Oxaloacetic Transamination

The two most abundant transaminases in mammals are glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT). These two enzymes are also referred to as alanine aminotransferase and aspartate aminotransferase, respectively (Searcy, 1969). See Figure

3. Snell (1970) noted that the 5-phosphate forms of vitamin B₆ are not an absolute requirement for transamination. The reactions shown in Figure 3 are actually composed of two

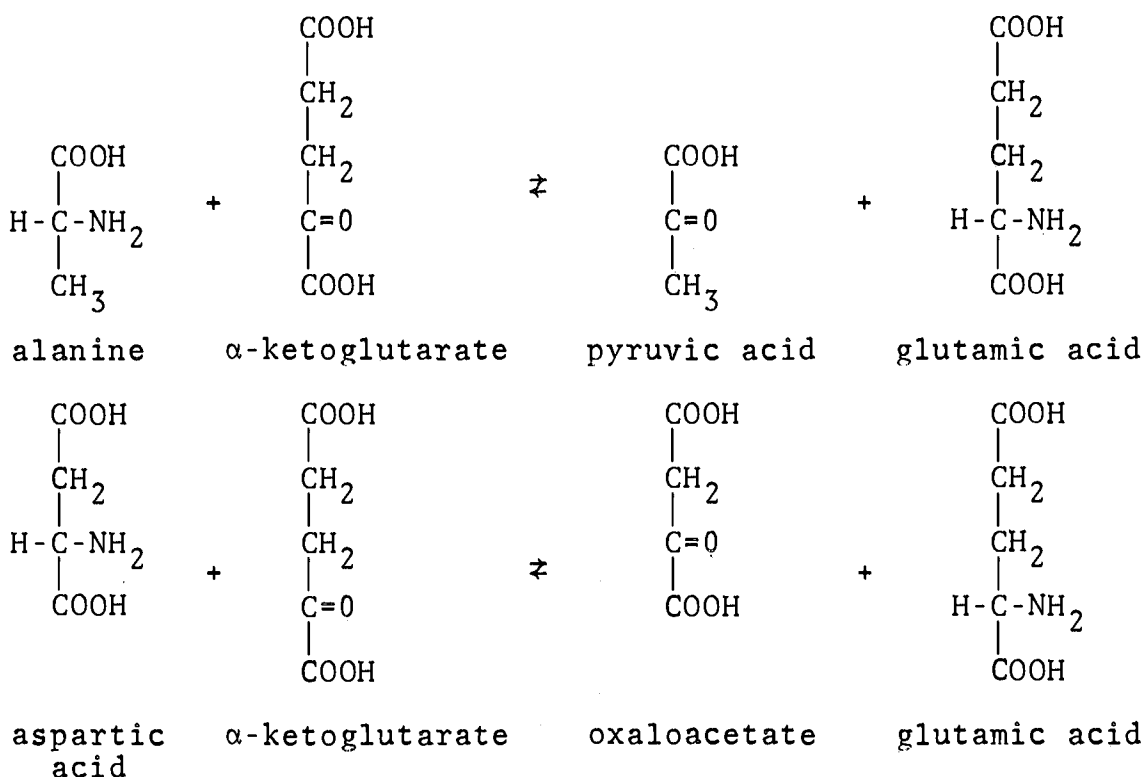
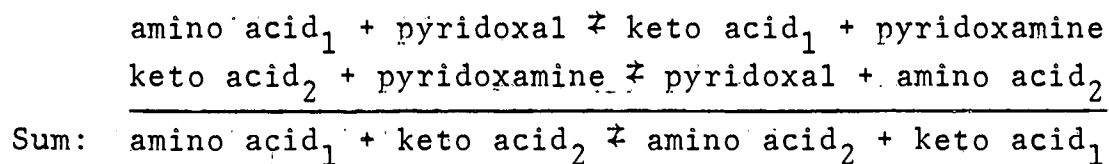


Figure 3. Reactions catalyzed by GPT and GOT.

coupled reactions. They could also be catalyzed nonenzymatically in-vivo as well as in-vitro by pyridoxal and pyridoxamine as follows (Guirard and Snell, 1964):



Transaminase Activity in Assessing Vitamin B₆ Status

Transaminase Assay Method

Levels of GPT and GOT in serum, plasma, leukocytes, erythrocytes, and whole blood are useful in providing a biochemical functional test regarding the state of vitamin B₆ reserve. In this method, transaminase in the sample is allowed to act on a substrate by incubation at 37C. The product formed indicates the basal activity of the enzyme and can be measured colorimetrically, spectrophotometrically, or fluorometrically (Searcy, 1969). When the enzyme is stimulated in vitro with added pyridoxal phosphate then the amount of product formed represents the stimulated level of enzyme activity. The ratio of the difference between these two values over the basal level is the percent stimulation, which implies the degree of saturation of apoenzyme with the coenzyme (Woodring and Storvick, 1970).

Although serum transaminases have been investigated more extensively in the past, erythrocyte transaminases appear to be better indicators of vitamin B₆ status. Sauberlich et al. (1972) reported that the red blood cells contain far more transaminase activity than does serum. Karmen, Wroblewski, and LaDue (1955) stated that GOT activity in whole blood is twenty times that in the serum. In addition, less individual variation in the erythrocyte GOT and GPT (EGOT and EGPT) measurements makes

these values more meaningful than those of serum GOT and GPT (Baysal, Johnson, and Linkswiler, 1966). Cheney and associates (1965b) also found that both EGPT and EGOT are better reflectors of vitamin B₆ intake than plasma GOT and GPT.

Compared to other methods for vitamin B₆ assessment, transaminase measurement has the advantage of dealing with a single enzyme requiring vitamin B₆ rather than a metabolic pathway such as the conversion of tryptophan to niacin in which pyridoxal phosphate is required by several enzymes. Moreover, transaminase activity reflects the subject's vitamin B₆ status over a long period of time (Sauberlich et al., 1972). For example, Cinnamon and Beaton (1970) reported that in subjects depleted of vitamin B₆, the level of xanthurenic acid excreted in response to the tryptophan load test returned to normal 24 to 48 hours after vitamin B₆ supplementation. On the other hand, the activity of EGOT and EGPT continued to be low until after three to four weeks of vitamin B₆ supplementation. Woodring and Storvick (1970) noted that the enzyme activity may be related to red cell turnover as its response to vitamin B₆ supplementation is slower in erythrocytes than in plasma.

Human Erythrocyte Transaminase Activity

Table I outlines the levels of percent stimulation of EGPT and EGOT reported in literature. The methods employed in these studies were modified from the colorimetric method

Table 1. Human EGPT and EGOT percent in vitro stimulation¹ with pyridoxal phosphate².

Investigator	Description of Subjects	Diet and Drug Treatment	Result (Average) Percent Stimulation
Cheney <u>et al.</u> , ³ 1965	7 men and women, 30-35 years	None	EGPT=25 EGOT=80
Rose <u>et al.</u> , 1973	50 women	None	EGPT=18±14
	30 of the 50 above	None	EGOT=77±15
	20 healthy women	Estrogen containing oral contraceptives for at least 6 months	EGPT=22±17 EGOT=67±17
	above 20 women	Same as above +40 mg pyridoxine HCl for 8 weeks	EGPT=8±5 EGOT=22±5
Standall <u>et al.</u> , 1974	15 men, 21-50 years	None	EGPT=14±4 EGOT=101±16
	9 men and women, healthy, 20-30 years	300 mg INH/day for 21-371 days	EGPT=8±5 EGOT=121±13
	10 tuberculosis patients	10 mg INH/Kg/day +15 mg ethambutal/Kg + 50 mg pyridoxine/day	EGPT=0 EGOT=23±5
Woodring and Storvick, 1970	6 women	None	EGPT=5.1±7.5
		After 7 days of 50 mg pyridoxine HCl/day	EGPT=3.1±3.5
		35 days after above supplementation period	EGPT=11.8±4.7

¹ Percent stimulation + (stimulated activity-basal activity) (100)/basal activity.

² 100 µg pyridoxal phosphate was added to the assay medium.

³ Used only 50 µg PALPO to stimulate.

of Tonhazy, White, and Umbreit (1950) and Wroblewski and Cabaud (1957). The general procedure is to incubate the hemolysate with either alanine or aspartate as substrate for one hour at 37C. After the enzyme is inactivated, the product is measured photometrically after being reacted with dinitrophenyl hydrazine to form a colored compound. The absorbance of the product is proportional to the level of transaminase activity.

Due to the various ways of expressing enzyme activity, it is only possible to compare the percent stimulation (Table 1). The results show greater stimulation for EGOT than EGPT in all cases. Even in healthy subjects, the apo-enzyme is not saturated with the coenzyme as the enzymes are stimulated with PALPO added in vitro. In addition, the percent stimulation is reduced further when pyridoxine supplement is administered to these subjects. The antagonistic effect of certain drugs such as isoniazid is also evident. Unfortunately, information on the level of transaminase activity in children is not available.

Expression of Enzyme Activity

The amount of pyruvate or oxaloacetate formed by the action of EGPT on alanine or EGOT on aspartic acid, respectively, indicates activity of the enzymes.

The basal activity of the transaminases which is expressed differently by various authors in the literature

causes difficulty in comparing the data. Cavill and Jacobs (1967) expressed their results as micromoles of pyruvate or oxaloacetate produced per 10^{12} erythrocytes per hour of incubation at 37C. Krishnawamy (1971a) employed a similar expression, but based his calculation on 10^8 rather than 10^{12} erythrocytes. The most common way of expression found is mg or μ g of pyruvate or oxaloacetate per ml of red blood cells per hour (Standall et al., 1974; Cheney et al., 1965b; and Woodring and Storvick, 1970). This latter expression is convenient as it eliminates cell counting.

Recently, Rose et al. (1973) used the expression of μ g of sodium pyruvate or oxaloacetate per mg hemoglobin per hour. Beutler (1971) favors this method of expression as it would be subject to less error when the hemoglobin determination is done on the hemolysate. When "per ml red blood cells" is used, he stated the errors involved as due to the remaining washing medium, varied length and force of centrifugation, swelling of red cells in storage or from the anticoagulant as well as errors in pipetting and diluting the hemolysate. However, results expressed as per mg hemoglobin would be falsely high for subjects suffering from anemia. Thus hematocrit and hemoglobin data of subjects would be helpful.

Comparison of EGPT, EGOT, and In Vitro Stimulation in Reflecting Vitamin B₆ Status

Raica and Sauberlich (1964) reported that the activity of GOT is about twenty times as much as that of GPT in erythrocytes and leukocytes. This means that a smaller sample of red blood cells is required for EGOT assay.

Reports on the sensitivity of EGPT and EGOT in assaying vitamin B₆ status are conflicting. Cavill and Jacobs (1967) noticed that EGPT is more sensitive than EGOT as the level of the former decreased sooner in vitamin B₆ deficiency. Baysal et al. (1966) observed similar results and noticed that upon vitamin B₆ repletion, serum GOT activity was restored more slowly than was serum GPT. These observations were supported by Cinnamon and Beaton (1970) and Jacobs et al. (1968). The latter researchers suggested that GOT is more avid for the coenzyme pyridoxal phosphate than GPT. On the other hand, Standall et al. (1974) observed that EGOT is better than EGPT in revealing vitamin B₆ inadequacy.

However, all of the above authors agreed that due to wide individual variation, the basal activity of either GOT or GPT is not as useful as the ratio of this value to in vitro stimulation of the enzyme. Woodring and Storvick (1970) noted that individual variation appeared too much for erythrocyte GPT per se to reflect vitamin B₆ status of normal women. While the basal value measures the level of

holoenzyme alone, the stimulation with pyridoxal phosphate shows the level of holoenzyme plus apoenzyme, and their ratio indicates the degree of saturation of apoenzyme with coenzyme (Cavill and Jacobs, 1967).

Nevertheless, a low percent of stimulation does not necessarily mean a healthy situation. If the coenzyme is lacking over a long period of time, then the formation of apoenzyme could be arrested, resulting in a lower basal value but normal percent stimulation (Coursin, 1964; and Sauberlich, 1972).

Factors Affecting Blood Transaminase Activity

The blood transaminase levels in clinically normal humans can be affected by various factors discussed below. In some diseases, particularly those of the heart and liver, the activity of blood transaminases may be altered appreciably (Searcy, 1969).

Vitamin B₆ Intake

A dietary supplement of vitamin B₆ clearly affects transaminase activity in various blood components (Linkswiler, 1967). Baysal et al. (1966), who fed adult men with purified diet containing only 0.16 mg vitamin B₆ daily, found that both serum GPT and GOT activities decreased. These enzyme activities increased with pyridoxine supplementation. Similar findings were reported by Cinnamon and

Beaton (1970), who studied EGOT and EGPT activities in men.

Linkswiler (1967) also noted that vitamin B₆ deficiency induced by diet resulted in a decrease in EGOT activity in human subjects. Rose and associates (1973) argued that the elevation of EGOT activity in their pyridoxine treated women was due to enzyme stabilization and reduced degradation rather than to an increased synthesis of enzyme protein which is not possible in the mature erythrocyte.

Deficiency State of Other B Vitamins

Cheney et al. (1965a) found that blood GOT and GPT levels were specific indicators of vitamin B₆ status in rats, even when the animals suffered from induced single or multiple deficiencies of other B vitamins. In this connection, Krishnaswamy (1971a) reported that pellagrins responded to pyridoxine therapy. In his other paper, Krishnaswamy (1971b) reported that six out of thirteen patients suffering from severe oral lesions responded more favorably to vitamin B₆ treatment than to riboflavin. Besides the possibility that these patients were more deficient in vitamin B₆ than riboflavin, Krishnaswamy mentioned that riboflavin plays a role in the formation of pyridoxal phosphate and thus could condition a pyridoxine deficiency when the level of riboflavin itself is low. The study by Wada and Snell (1961) showed that flavin mononucleotide

serves as a prosthetic group for the pyridoxine phosphate oxidase which oxidizes both pyridoxine phosphate and pyridoxamine phosphate to pyridoxal phosphate.

Iron Nutritional Status

Vitamin B₆ is required for the formation of protoporphyrin and heme through the synthesis of glycine and σ -aminolevulinic acid (Baker et al., 1969). Otherwise, the relationship between vitamin B₆ and iron metabolism is not clear. Cavill and Jacobs (1967) found a significantly lower basal activity and higher in vitro stimulation in EGPT, but not in EGOT, in patients with iron deficiency anemia than in controls. They commented that the correlation could merely be a coincidence as anemic patients usually have a poor diet in general. Patwardhan (1958), experimenting on purified GOT prepared from fresh green beans, found that FeSO₄ increased the activity of the enzyme while HgCl inhibited it. Metals such as Al, Cu, Ni, Mg, Mn, Co, and Fe in ferric state had no effect. The relationship between the metabolism of vitamin B₆ and iron awaits further study.

Age

Although the relationship between the transaminase activity and age is not well established, Jacobs et al. (1968) reported a decrease in both basal and stimulated

EGPT with age, possibly due to diminishing levels of co-enzyme and apoenzyme in older subjects. Ranke and associates (1960) studied serum GOT in two groups of people having average ages of 25 and 76. They found lower basal level and higher in vitro stimulation in the older group compared to the younger group. However, after the older subjects had received a supplement of 15 mg of pyridoxine daily for three weeks, their basal serum GOT rose to the same level as that of the younger subjects. The authors mentioned that the results indicated a normal level of apoenzyme but a decreased level of coenzyme in the older subjects.

Searcy (1969) noted that serum and erythrocyte GOT activity is much higher at birth than during adulthood. Within the first three days of life, the activity of serum GOT diminished rapidly whereas EGOT levels remain elevated during the neonatal period.

Genetics

Genetic factors may play a role in the diversity of GPT and GOT activity in human populations. Chen and Giblett (1971) found in humans three genetic polymorphisms of GPT which represent the homozygous and heterozygous expressions of two alleles. The frequency of these alleles was reported to vary from one population to another. GOT was also reported to be polymorphic in a similar pattern with GPT (Davidson, Cortner, and Rattazzi, 1970). Aebi (1969)

discussed that the individual variation in the enzyme level of isozyme pattern may be due to the diversity of the enzyme characteristics itself or to the differences in rates of synthesis or degradation.

Stability of Erythrocyte Transaminase in Storage

Beutler (1971) noted that red cell constituents are much more stable in whole blood than in washed and frozen red cells. Woodring and Storvick (1970) found that EGPT activity is lost when stored frozen, thus the samples should be assayed as quickly as possible after a consistent period of time. Uniformity of storage time for the samples was also considered by Standall et al. (1974), who assayed all samples four weeks after the blood was drawn. Rose et al. (1973) indicated that assaying on the same day as blood is drawn may be important. The latter researchers found that after 10 days of storage at -20C, the activity of EGPT and EGOT decreased 48 percent and 28 percent, respectively.

Babcock, Brush, and Sostman (1960), however, found no change in serum GOT after two months of frozen storage. Beutler (1971) also reported that whole blood stored with anticoagulants showed less than ten percent loss of GOT activity in five days at 25C and twenty days at 4C.

Vitamin B₆ in the Diet and Human Requirement

Information on the level of vitamin B₆ in foods is very limited. Orr (1969) provided the best list of vitamin

B₆ in various foods. Among baby products, meat with 200 µg to 800 µg of vitamin B₆ per 100 g and cereals with 200 µg to 400 µg of vitamin B₆ per 100 g are good sources while fruits are low. One liter of fresh cow milk contains only 400 to 580 µg of vitamin B₆. Polansky (1966) reported on the methods of assay and level of vitamin B₆ in fruits and nuts. Banana and avocado with levels of 450 µg and 500 µg per 100 g, respectively, are rich sources of vitamin B₆.

In animal food products, pyridoxal and pyridoxamine predominate, while vegetables contain vitamin B₆ largely as pyridoxine (Orr, 1969). While pyridoxine is relatively stable, pyridoxal and pyridoxamine are not. This is the reason for the lowered level of vitamin B₆ in sterilized milk, which contains pyridoxal and pyridoxamine (Woodring and Storvick, 1959). These authors also reported that storage lowered vitamin B₆ content of commercial evaporated milk, although pasteurization or evaporation per se do not appreciably destroy vitamin B₆.

Schroeder (1971) pointed out losses of vitamin B₆ in food processing. Frozen and canned vegetables, as well as canned fish, seafood, meats, and poultry lose from 36 percent to 77 percent vitamin B₆. In view of the fact that the American diet could easily be low in vitamin B₆ and other trace elements, Schroeder suggested an enrichment program and an increased consumption of unprocessed foods. Standall and associates (1974) also considered the diets of

their normal volunteer subjects as inadequate (1.3 mg to 1.6 mg vitamin B₆ per day).

The National Academy of Sciences (1974) has a list of vitamin B₆ levels recommended for each age group. For the preschool child, the daily vitamin B₆ intakes recommended are 0.6 mg and 0.9 mg for the age groups of 1 to 3 and 4 to 6 years, respectively. However, it is suggested that further studies need to be done on the human requirement of vitamin B₆.

Clinical Symptoms of Vitamin B₆ Deficiency

Vitamin B₆ Deficiency in Animals

The essentiality of pyridoxine to animal metabolism has been established for a long period of time. The most noticeable symptoms in vitamin B₆-deficient animals are dermatitis, convulsions, and microcytic hypochromic anemia accompanied by an elevated level of serum iron (Linkswiler, 1967). For example, Babcock and associates (1960) reported a decrease of GOT activity and retarded growth in rats fed a purified diet deficient in vitamin B₆. Sifri, Dagher, and Asmas (1972) observed growth retardation and alteration of plasma free amino acids in young broiler-type chickens when the pyridoxine level in the diet was not adequate.

Vitamin B₆ Deficiency and Dependency in Infants and Children

In 1953, Snyderman et al. reported convulsions in a mentally retarded eight-month old infant and anemia in a two-year old child. These disease manifestations were the result of a controlled vitamin B₆-deficient diet and were corrected with a pyridoxine supplement. Bessey, Adam, and Hansen (1957) observed convulsions in seven infants aged one to four months who were receiving a milk mixture containing less than 0.1 mg of vitamin B₆ daily. At the early stage of the deficiency, irritability was noticed in the infants. Two other babies who consumed human milk providing 0.067 mg of vitamin B₆ daily developed both convulsions and iron-deficiency anemia at eight to ten months of age. All of these infants responded well to an intake of 0.26 mg vitamin B₆ daily (Bessey et al., 1957).

It is important to note that a marked increase of γ -aminobutyric acid level in brain after birth occurs to exert an inhibitory effect on neuronal excitability of the newborn (Watson and Lowrey, 1969). Vitamin B₆ is required for the formation of γ -aminobutyric acid from glutamic acid (Searcy, 1969).

Despite the above observations, symptoms of vitamin B₆ deficiency are usually mixed with the manifestations of other B vitamin deficiencies (Krishnaswamy, 1971a). The cause of vitamin B₆ deficiency is rarely traced back to

dietary inadequacy but rather to vitamin B₆ dependency or an inborn error of metabolism in infants (Krishnaswamy, 1971a). The pyridoxine dependency syndrome is characterized by a persistent need for a large amount of vitamin B₆ supplementation to control seizures (Searcy, 1969).

Waldinger and Berg (1963) reported three cases of familial disorder where the neonates suffered from seizures within three hours of birth and responded only to large doses of vitamin B₆. At five months of age, one of the infants required 12.6 mg of pyridoxine HCl per day to control seizures.

Other inborn errors of metabolism involving vitamin B₆ are cystathionurea and Down's syndrome. A relative deficiency of vitamin B₆ may occur when metabolic activity is elevated like in pregnancy, fever, or certain other disease manifestations (Brown, 1972).

Level of Vitamin B₆ in Blood of Children

The level of vitamin B₆ in the blood corresponds to the dietary intake of the vitamin (Sauberlich et al., 1972; and Donald et al., 1971). The blood is hemolyzed, hydrolyzed with acid, and then assayed with Saccharomyces uvarum (carlsbergensis), Streptococcus faecium, or Lactobacillus casei (Storvick et al., 1964) as the test microorganism.

The level of vitamin B₆ in plasma is useful as plasma has three times as much B₆ as do erythrocytes (Sauberlich

et al., 1972). These authors mentioned a decrease of plasma vitamin B₆ with age and reported a plasma level of 50 ng/ml as normal and 25 ng/ml as deficient for adults. However, Baker et al. (1967) studied over 600 normal children 10 to 13 years old and found a mean serum B₆ level of only 36 ng/ml by using a protozoological assay.

Iron Status in Preschool Children

Extensive studies on nutritional status indicated the prevalence of iron deficiency among American infants and children (Burroughs and Huenemann, 1970; Department of Health, Education and Welfare, 1968-1972; and Owen, Lubin, and Garry, 1971).

Criteria for Assessment of Iron Status

Hemoglobin and hematocrit values have been employed widely to detect anemia. Levels lower than 11 g percent for hemoglobin and 33 percent for hematocrit were considered indications of anemia in infants and young children (Theuer, 1974).

However, to discover the stage of iron deficiency with anemia yet to develop, it is necessary to measure the percent transferrin saturation with iron. Hunter and Smith (1972) noted a direct relationship between this measurement and the amount of iron that transferrin can supply to bone marrow. The Committee on Nutrition of the American Academy

of Pediatrics as cited by Theuer (1974) suggested levels less than 15 percent transferrin saturation as indicative of iron deficiency.

Unfortunately, the assessment of transferrin saturation which involves measuring serum iron and serum total iron binding capacity is still expensive and impractical due to the large blood volume needed (Hunter and Smith, 1972). Brigety and Pearson (1970) suggested the use of increased levels of hemoglobin and hematocrit following a period of iron therapy as indication of iron deficiency in clinically normal children.

Iron Deficiency in American Preschool Children

Using the above criteria, Theuer (1974) stated that iron deficiency is the most serious nutritional problem in American infants. Burroughs and Huenemann (1970) reported that of 48 healthy children of low socio-economic background aged from 12 to 24 months, 53 percent had hemoglobin values below 10 g percent. However, among 2,000 preschool children studied by Owen et al. (1971), only five percent were anemic, with their hemoglobin levels below 10 g percent and 11 g percent for the age groups 1 to 2 and 2 to 6 years, respectively. These authors observed that although anemia was more prevalent among preschool children of low income families, iron deficiency is found in all segments of population.

Due to rapid growth, the preschool child has a great need for iron to avoid hypochromic microcytic anemia (Watson and Lowry, 1969). The National Academy of Sciences (1974) recommended levels of 15 mg and 10 mg of daily iron intake for the age groups 1 to 3 and 4 to 6 respectively. However, studies indicated that few children met this suggested level (Burroughs and Hueneman, 1970; and National Dairy Council, 1972). The study of the Department of Health, Education and Welfare (1968-1970) indicated a low correlation for iron intake and hemoglobin. However, this relationship is not always established (National Dairy Council, 1972). The latter paper also mentioned the cases of infants consuming less iron than recommended but nevertheless maintaining satisfactory hemoglobin levels.

The effect of iron nutritional status on the blood transaminase activity was mentioned earlier in this report.

MATERIALS AND METHODS

Subjects

This study is a part of a larger nutritional investigation entitled Nutritional Health, Food Intake, Socio-Environmental Profiles in Oregon Preschool Children. The Oregon counties that participated in this study were Multnomath, Harney, Morrow, and Columbia. The subjects attended the Well Child and Multiphasic Screening Clinics set up by the individual counties in collaboration with the State Health Division and the Cooperative Extension Service.

For the research reported in this thesis, 109 children aged from 22 to 126 months with a mean of 57.7 months were studied. Of these, 54.5 percent were boys. Some vital statistics of the subjects are presented in Table 2. The children were largely Caucasian and were apparently healthy.

Blood Drawing

Venous blood was drawn by a medical technician from the non-fasting subjects and then was transferred to vacuum tubes containing heparin. The blood was used for the determination of EGPT and EGOT, hemoglobin, hematocrit, plasma vitamin B₆, vitamin A, and folic acid. Results of the latter assays will be reported elsewhere.

To avoid loss of vitamin B₆, the blood samples were placed on ice in the dark immediately after drawing. The

Table 2. Some vital statistics for 97¹ of the 109 subjects participating in this study.

Age Group (Months)	Number of Subjects			Average Height (cm)	Average Weight (Kg)	Number of subjects below 10th percentiles ²	
	Boys	Girls	Total			Height	Weight
0-23	1	1	2	83.9	10.6	0	0
24-35	11	5	16	88.4	13.1	1	2
36-47	4	7	11	97.0	14.5	3	4
48-59	8	6	14	102.5	16.6	4	3
60-71	17	13	30	110.8	19.4	2	1
72-83	8	10	18	117.6	21.1	1	2
84-95	3	2	5	118.9	21.4	0	1
126	1	0	1	124.5	24.9	1	1

¹ The data on height and weight were available only for 97 subjects.

² The percentiles are derived from the anthropometric charts for boys and girls from 2 to 12 years of age based on original data of Stewart and Meredith for Children's Medical Center, Boston, Massachusetts.

dietary history of the subjects was obtained by a nutritionist who interviewed the mothers.

Due to the larger sample size required for EGPT than for EGOT assay and the uncooperativeness of some children, only 101 samples were available for EGPT assay.

Procedure

As soon as possible after the blood was drawn, the red cells were separated from plasma. The plasma was saved for vitamin B₆, vitamin A, and folic acid determinations. The erythrocytes were washed with 0.85 percent saline and frozen until the hemolysates for EGPT and EGOT assays were prepared on the next day. The hemolysates were then stored frozen from one to 28 days before the EGPT and EGOT assays were made.

Methods

The procedure for EGPT assay was that of Woodring and Storvick (1970). To determine EGOT, the method for EGPT was modified by using aspartate as the substrate. In addition, the oxaloacetate formed was converted to pyruvate by reacting with aniline citrate. The basal activity of the enzymes was determined without the addition of pyridoxal phosphate (PALPO) to the assay medium, while in vitro simulation was measured by the addition of 100 µg of PALPO. Each enzyme activity level was measured in triplicate. The

basal activity of EGPT and EGOT was expressed as μg pyruvate/ml red blood cells (RBC) per hour and μg pyruvate/mg hemoglobin (Hb) per hour. For the latter expression, the amount of hemoglobin in the hemolysate was determined by the standard cyanomethemoglobin method (Henry, 1964).

Vitamin B₆ status of subjects was determined by the basal activity mentioned above and by the percent stimulation with added PALPO, which was calculated as follows:

$$\frac{\text{EGPT (or EGOT) with PALPO} - \text{EGPT (or EGOT) without PALPO (100)}}{\text{EGPT (or EGOT) without PALPO}}$$

For vitamin B₆ determination, the plasma was deproteinized with ten percent trichloroacetic acid. Vitamin B₆ was measured by assay with the test organism Saccharomyces uvarum (carlsbergensis) (Storvick et al., 1964), which responds to pyridoxal, pyridoxamine, and pyridoxine. Hemoglobin and hematocrit of the subjects were determined by the standard cyanmethemoglobin (Henry, 1964) and microhematocrit method (Davidsohn and Nelson, 1969), respectively.

Correlations were determined among the basal activities and percent stimulation of EGPT and EGOT, plasma vitamin B₆, hemoglobin, hematocrit, and age of subjects. The Student's paired and unpaired t tests were used to determine the statistical significance of vitamin supplementation and sex of the subjects on the enzyme activities.

Stability of Transaminase in Storage

The stability of EGPT and EGOT activity with storage was also tested. The hemolysates prepared from red blood cells of two adult women were dispensed in plastic tubes in appropriate amounts and stored frozen until assayed. The EGPT and EGOT assays were done every two to three days within thirteen days.

RESULTS AND DISCUSSION

EGPT and EGOT Activities in Preschool Children

The mean basal activity, the in vitro stimulated activity with added PALPO, and the corresponding percent stimulation of EGPT and EGOT in the preschool subjects are summarized in Table 3. The basal and stimulated activities are expressed in two different ways, as mentioned earlier. While the basal level indicates the amount of holoenzyme present, the stimulated level represents the level of holoenzyme plus free apoenzyme available. The basal activity and the percent stimulation, which implies the degree of saturation of apoenzyme with the coenzyme, are more indicative of vitamin B₆ status than the stimulated activity. For this reason, these two sets of data will be considered in detail.

The wide variation in EGPT and EGOT activities found in this study has been noted by many other authors in their normal healthy adult subjects (Sauberlich et al., 1972). These variations were partly due to factors such as vitamin supplementation, age, and sex of the subjects, which will be discussed later. Genetic diversity among individuals may also play a role (Aebi, 1969). Furthermore, the blood used for this transaminase study was not drawn at a specified time, thus diurnal variation in enzyme activity may be another factor. The apparently wider variation in EGOT

Table 3. Basal activity and in-vitro stimulated activity with added PALPO of EGPT and EGOT and the corresponding percent stimulation level in preschool children. The basal and in-vitro stimulated activities are expressed in two different ways.

		Number of subjects	Mean \pm SD	Range
EGPT	Basal activity, (μ g pyruvate/mg Hb/hr)	101	1.20 \pm 0.44	0.44 - 2.65
	Basal activity, (mg pyruvate/ml RBC/hr)	101	0.42 \pm 0.14	0.18 - 0.87
	Stimulated activity, (μ g pyruvate/mg Hb/hr)	101	1.33 \pm 0.48	0.54 - 2.84
	Stimulated activity, (mg pyruvate/ml RBC/hr)	101	0.48 \pm 0.16	0.22 - 0.93
	Percent stimulation	101	11.70 \pm 7.00	0 - 33.00
EGOT	Basal activity (μ g pyruvate/mg Hb/hr)	109	23.30 \pm 5.77	9.70 - 41.60
	Basal activity, (mg pyruvate/ml RBC/hr)	109	7.86 \pm 1.77	3.50 - 12.40
	Stimulated activity, (μ g pyruvate/mg Hb/hr)	109	38.97 \pm 7.64	21.8 - 53.70
	Stimulated activity, (mg pyruvate/ml RBC/hr)	109	13.08 \pm 2.52	7.15 - 17.88
	Percent stimulation	109	69.90 \pm 23.3	13.0 - 156.0

data than those of EGPT may be due to the higher magnitude in the basal and stimulated EGOT activity levels and percent stimulation.

Figures 4 to 7 present the distribution of basal EGPT and EGOT activities and the corresponding percent stimulation in the preschool children. Except for the percent stimulation of EGPT (Figure 5), all the other data followed a normal distribution pattern. The abnormal distribution of the percent stimulation of EGPT was due to the preponderance of low values obtained with this assay, which is normal.

Since the information on the activity levels of transaminases in children are not reported in the literature, no comparison may be made with results from other laboratories for this age group. Reports on the activity level of EGPT and EGOT in adults are available. However, due to the difference in the methods and the various ways of expressing the basal activities, it is only possible to compare data on the percent stimulation.

It appears that the children in this study generally had a lower percent stimulation of EGPT and EGOT than did the adults as summarized in Table 1. However, after a period of supplementation, the percent stimulation of EGPT and EGOT in the adults (Table 1) declined to levels lower than those of the subjects in this study (Table 3) as expected. The relationship between age and activity level of transaminase will be discussed later.

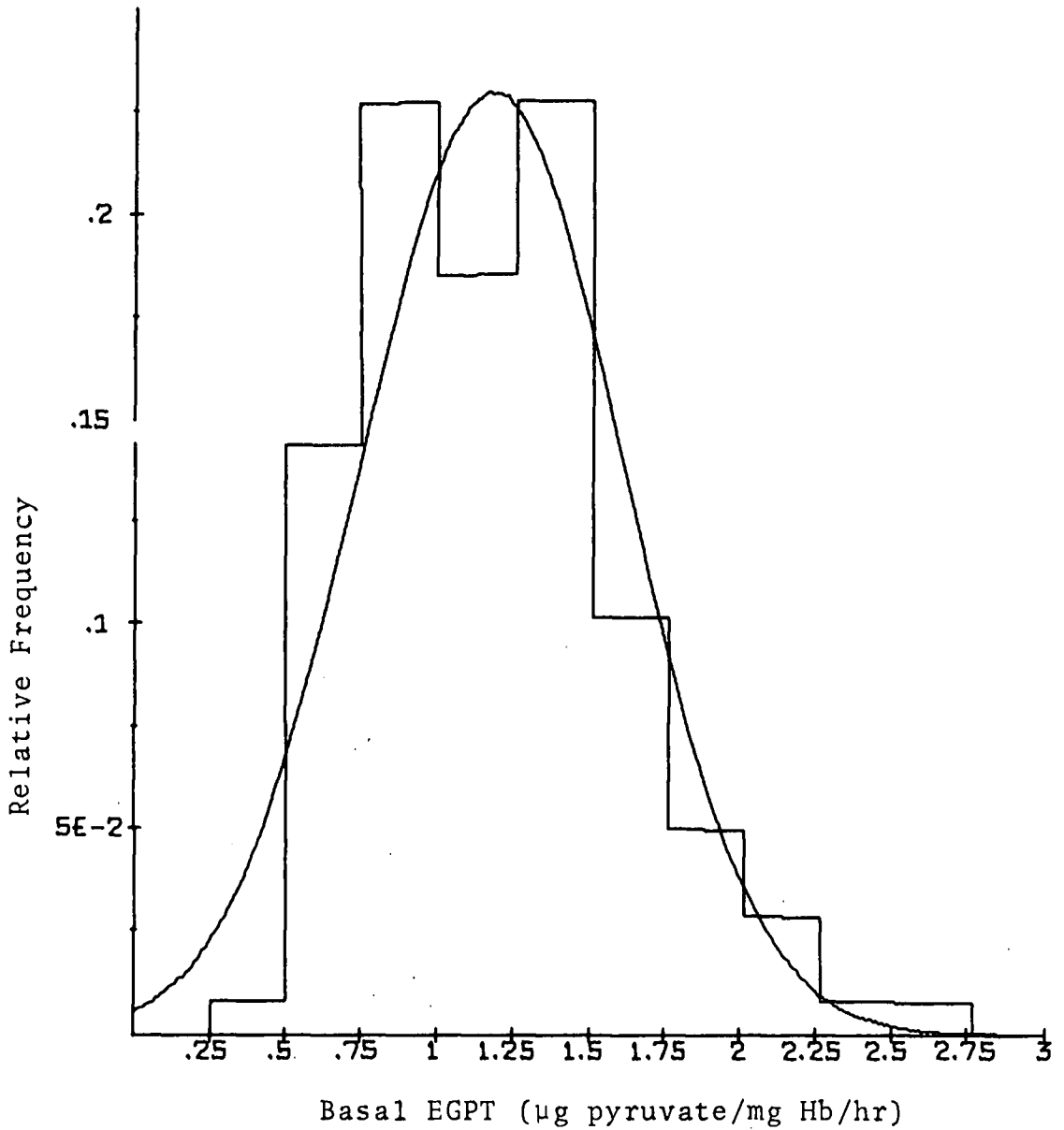


Figure 4. The relative frequency distribution of basal activity of EGPT for 101 children aged from 22 to 126 months. The graph was drawn mechanically.

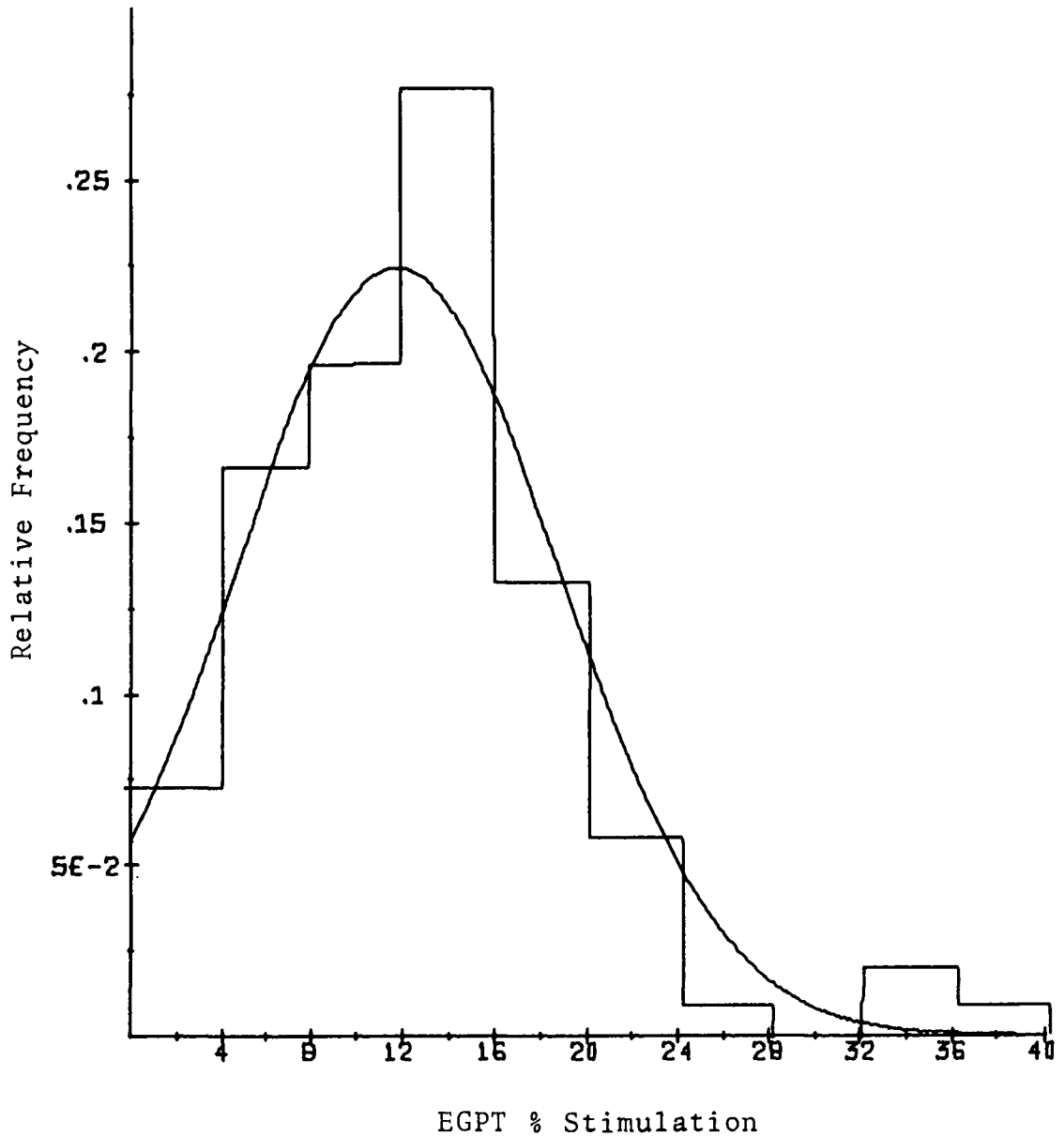


Figure 5. The relative frequency distribution of EGPT percent stimulation of 101 children aged from 22 to 126 months. The graph was drawn mechanically.

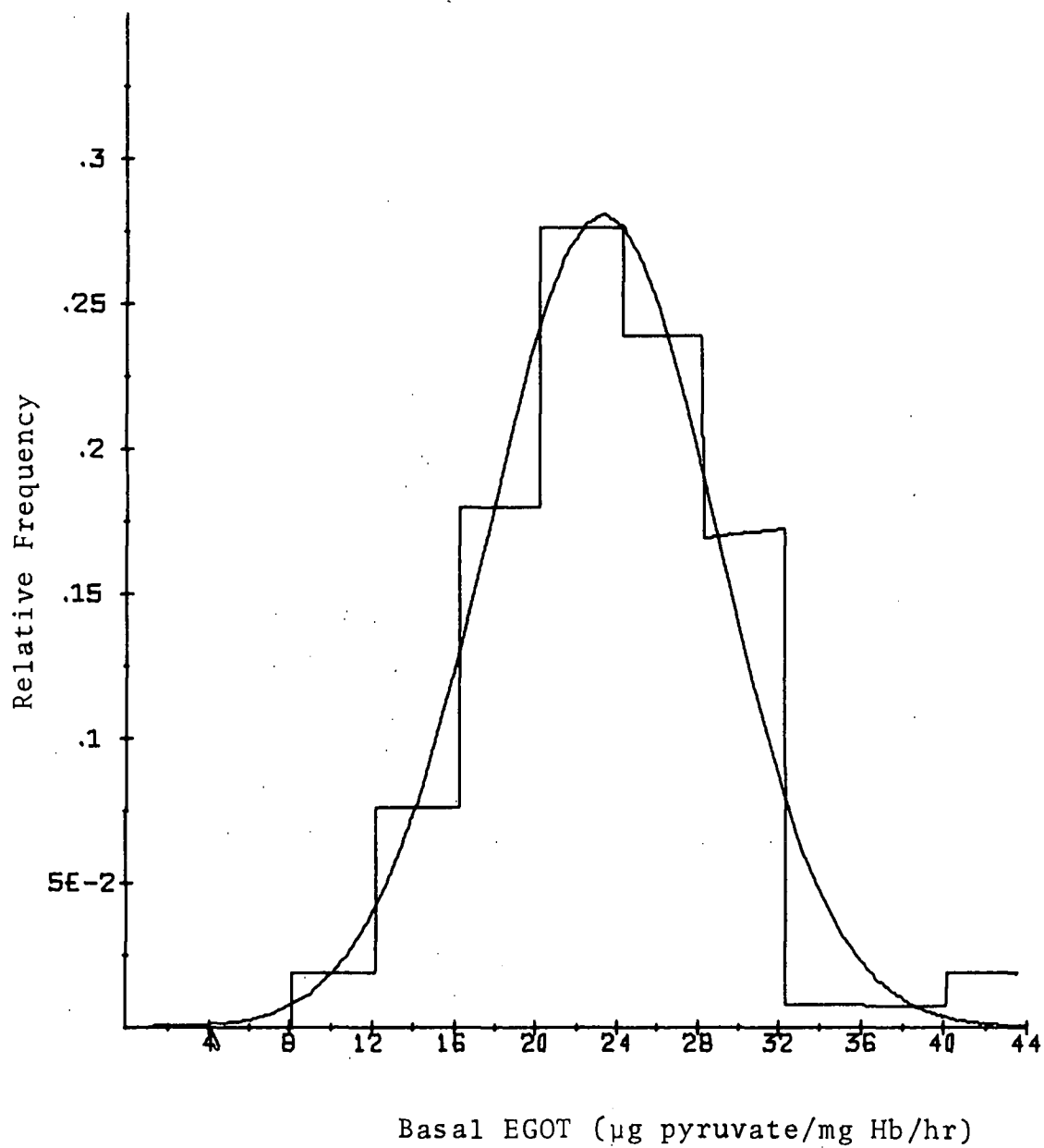


Figure 6. The relative frequency distribution of basal activity of EGOT for 109 children aged from 22 to 126 months. The graph was drawn mechanically.

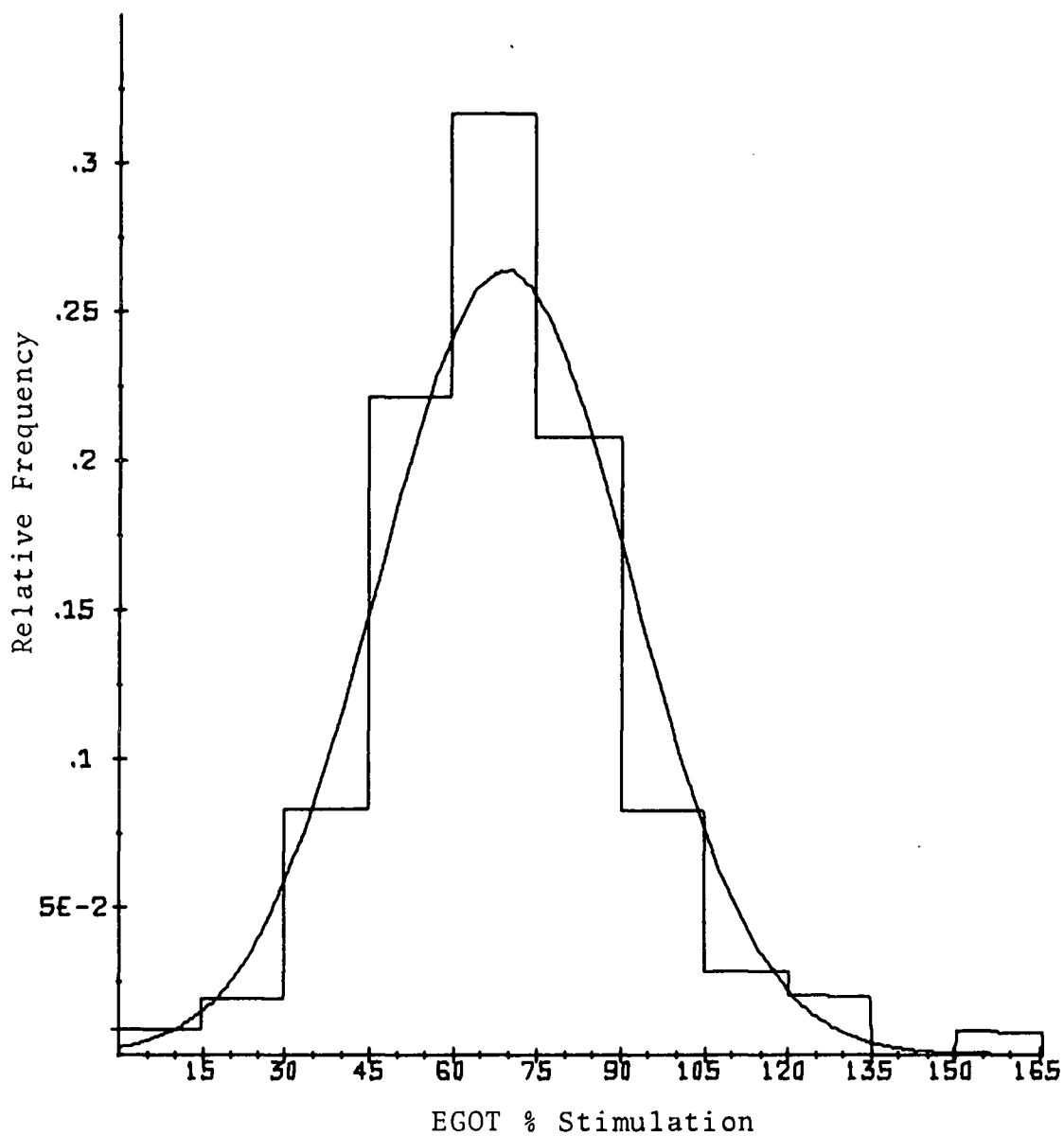


Figure 7. The relative frequency distribution of EGOT percent stimulation of 109 children aged from 22 to 126 months. The graph was drawn mechanically.

The relatively large number of subjects participating in this study makes the results a valuable information for future studies on transaminase activity in children.

Expression of Results

The two different ways of expressing basal activity of EGPT and EGOT were highly correlated with one another. For EGPT basal activity data, the correlation coefficient (r) was $+0.970$ ($p < 0.001$) while that for EGOT was $+0.930$ ($p < 0.001$). Thus, not much difference in the two result expressions was found.

The expression, μg pyruvate per mg Hb per hour, required an extra step to determine the amount of hemoglobin in each hemolysate prepared for EGPT or EGOT assay. Moreover, it can be falsely high or low for subjects suffering from anemia or polycythemia, respectively. Thus, information on hemoglobin and hematocrit are necessary. It will be seen in the discussion later that the subjects of this study were virtually free from these conditions.

On the other hand, measuring the volume of red blood cells may involve many errors due to remaining washing medium, varied length and force of centrifugation, swelling of red cells in storage or from the anticoagulant, as well as errors in pipetting and diluting the hemolysate (Beutler, 1971). Unfortunately, these errors seem very likely to happen. In this study, the same anticoagulant was used, the

cells were washed, pipetted, and measured by the same person, and were centrifuged in the same machine for the same length of time. This consistency may account for the close correlation between the two different ways of expressing the basal activity of transaminases.

Since high correlations between the two different ways of expressing basal transaminase activities were found, only the basal activity expressed as "per mg Hb" will be discussed from this point on.

Comparison of EGPT and EGOT Activities and Corresponding In-Vitro Stimulation

The results were analyzed by seeking correlation among basal and stimulated activities and percent stimulation of both transaminases. A correlation between basal EGPT and EGOT activities was found ($r = +0.306$, $p < 0.01$). The relationship between the basal activities of EGPT and EGOT was not available in the literature. Nevertheless, it is expected as these activity levels reflect the vitamin B₆ status of the subjects on the same principle, assuming that the pyridoxal phosphate in the body is available for EGPT activity as much as it is for EGOT activity. A knowledge of the K_m values of EGPT and EGOT for PALPO would also be helpful. Unfortunately, these figures are not available.

On the same line of reasoning, a positive correlation would be expected between percent stimulation values of

EGPT and EGOT. Unfortunately, this correlation was not significant.

It is interesting that close correlations were found between the stimulated and basal activities of both EGPT and EGOT ($r = 0.841$, $p < 0.01$ and $r = 0.978$, $p < 0.01$, respectively). These relationships indicate that the holoenzyme activities of EGPT and EGOT are largely dependent on the levels of apoenzymes available.

The reason for the low level of apoenzyme in some of the subjects is not clear, but it seemed to correlate with age as will be discussed below. Individual variation in the formation of apoenzyme in the body due to genetic factors may also play a role. The formation of apoenzyme can be arrested when the coenzyme is lacking over a period of time (Coursin, 1964; and Sauberlich et al., 1972), but this seemed unlikely in the apparently healthy subjects of this study.

A significant negative correlation was obtained between basal activity of EGOT and the corresponding percent stimulation ($r = -0.558$, $p < .01$). This result indicated that a low level of the holoenzyme probably means a reduced degree of saturation of the apoenzyme with PALPO (an increase in percent stimulation), i.e., a lack of PALPO in addition to the low level of the apoenzyme itself.

A similar correlation was sought for the basal activity and percent stimulation of EGPT, but the negative

relationship found was not statistically significant. In this respect, the EGPT activity levels appeared not as good a measurement for vitamin B₆ status as EGOT activity. However, since in this research, the activity levels of transaminase were not compared before and after oral supplementation with vitamin B₆, it was inconclusive whether EGOT was actually better than EGPT in reflecting vitamin B₆ status.

It is still a controversial matter as to whether EGPT or EGOT activity is a better indicator of pyridoxine nutritional status than the other. Studying the transaminase activity during the depletion and repletion of vitamin B₆ in subjects, Standall et al. (1974) and Donald et al. (1971) found that EGOT is better than EGPT in revealing vitamin B₆ inadequacy while other authors such as Cinnamon and Beaton (1970) and Jacobs et al. (1968) observed the opposite. The basal activity of either EGOT or EGPT is not as useful as the percent stimulation due to wide individual variation in the basal activity (Woodring and Storvick, 1970; and Sauberlich et al., 1972). In an attempt to give a guideline for the transaminase activity method, Sauberlich and associates suggested percent stimulation levels over 25 percent and 100 percent as indicators of vitamin B₆ deficiency for EGPT and EGOT, respectively. Using the above criterion for EGPT, the data for this study showed that 4 out of 101 subjects were deficient in vitamin B₆; while employing the standard for EGOT, 9 out of 109 subjects were deficient.

As mentioned above, the correlation between the percent stimulation of EGPT and EGOT was too small to be significant, thus it is anomalous, but not surprising that the four subjects deficient by the standard set for percent stimulation of EGPT were different from the nine subjects deficient by that of the EGOT. The four subjects who were deficient by EGPT standards had percent stimulation of EGOT from 53 percent to 89 percent, while the nine children deficient by EGOT standards had percent stimulation of EGPT from 7 percent to 21 percent. Furthermore, additional studies with known vitamin B₆-deficient and normal subjects are necessary before the validity of the standards set by Sauberlich et al., may be established. The authors also noted that the use of these criteria depends upon standardized methods.

Therefore, it is suggested that other methods of assessing vitamin B₆ nutritional status be done on those children who were found deficient by either standard above to confirm their deficiency in vitamin B₆.

No reasonable explanation may be given for some abnormal results of this study except the fact that variation exists among the triplicates of each blood sample in the assay. Thus, the transaminase assay method currently used appears to lack precision.

Factors Relating to EGPT and EGOT Activities

Due to the unavailability of certain data, the results discussed below are based on between 97 and 106 samples only.

Multi-Vitamin Supplement Containing Pyridoxine

In subjects whose diet was supplemented with multi-vitamins containing pyridoxine, transaminase activities appeared to be higher than in those receiving no supplementation (Table 4). The increase in basal activities of EGPT and EGOT was accompanied by a corresponding decrease of the percent stimulation. However, of the four comparisons made by the Student's t tests, only the basal EGPT showed significant difference ($p < .01$). The data on the dietary intake of the subjects are not available at the moment this thesis is written.

The vitamin B₆ intake of an individual affects his transaminase activity level in various blood components (Linkswiler, 1967). Rose and associates (1973) reported a significant increase in EGOT activity in pyridoxine-treated women. Standall et al. (1974) and Woodring and Storvick (1970) observed similar results. However, in all of these studies, the subjects were treated with a high dosage of pyridoxine HCl (40 mg to 50 mg per day). On the other hand, most of the multi-vitamins consumed by the children in this study contained only one milligram of pyridoxine per

Table 4. The mean values of basal activity and percent stimulation of EGPT and EGOT in subjects with and without vitamin supplementation.

	Vitamin Supplementation	Number of Subjects	Basal Activity (μg pyruvate/mg Hb/ hr)	Percent Stimulation
EGPT	No	75	1.13 ± 0.39^1	12.16 ± 6.88
	Yes	22	1.34 ± 0.52	10.45 ± 7.87
EGOT	No	79	22.98 ± 5.88	71.42 ± 23.18
	Yes	26	24.27 ± 4.99	63.69 ± 20.30

¹ Mean value \pm SD.

tablet. Furthermore, the extent and duration of vitamin supplementation were not investigated fully to conclude that multi-vitamin supplementation including pyridoxine in preschool children actually improves their vitamin B₆ status as assessed by the increase of basal EGPT activity.

Plasma Vitamin B₆

The results indicated that the plasma vitamin B₆ values tend to increase with an increase in basal activities of EGPT and EGOT and decrease with an increase in the corresponding percent stimulation. But the above relationships were not significant. This was not expected because both plasma vitamin B₆ and transaminase activities are indicators of vitamin B₆ nutritional status (Sauberlich et al., 1972).

The plasma vitamin B₆ of 99 subjects studied had a mean of 18.42 ± 12.21 ng/ml and a range of 5 ng/ml to 99 ng/ml. The subjects receiving multi-vitamins containing pyridoxine supplements had a higher mean value than the ones without supplementation, 28.22 ± 19.64 ng/ml versus 15.40 ± 6.60 ng/ml. The 13 subjects classified as deficient in vitamin B₆ by Sauberlich's standards (1972) set for either EGPT or EGOT had blood vitamin B₆ values ranging from 6 to 44 ng/ml. The data had a wide range and the mean is low compared to the data obtained by other authors. Sauberlich et al. (1972) reported a normal plasma vitamin

B₆ level of 50 ng/ml and a deficient plasma vitamin B₆ level of 25 ng/ml for adults. Baker et al. (1967) reported a mean serum B₆ level of 36 ng/ml for children 10 to 13 years old, using a protozoological assay. The kind of microorganisms and the hydrolyzing procedure employed in the assay may make a difference in the values of plasma vitamin B₆ obtained (Benson, E. M., personal communication).

Age

Inverse relationships were found between age and the basal activities of EGPT and EGOT, i.e., as age increases, the basal values decline. This observation was accompanied by positive correlations between age and the percent stimulation of EGPT and EGOT. However, only the correlations between basal activity and percent stimulation of EGOT and age were significant ($r = -0.503$, $p < .01$, and $+0.237$, $p < .01$, respectively).

The distribution of the basal activity and percent stimulation of EGOT with different age groups are presented in Table 5. From this table, it appears that the older age groups had lower basal activity and higher percent stimulation of EGOT than the younger age groups.

Negative correlations between stimulated activity of EGPT and EGOT with age were also found. The inverse relationship between stimulated EGOT activity and age was significant ($r = -0.436$, $p < 0.01$). Since the stimulated

Table 5. The distribution of the mean values of the basal activity and percent stimulation of EGOT with age.

Age Group (Months)	Number of Subjects	Basal Activity, EGOT (μg pyruvate/ mg Hb/hr)	Percent Stimulation
21-23	3	26.77 \pm 0.25	69.00 \pm 18.33
24-35	17	29.60 \pm 5.13	55.18 \pm 15.37
36-47	11	24.31 \pm 5.79	66.18 \pm 15.21
48-59	17	22.12 \pm 3.58	75.65 \pm 20.29
60-71	31	22.10 \pm 4.37	75.42 \pm 23.55
72-83	19	21.58 \pm 4.03	63.89 \pm 23.88
84 and over	6	16.92 \pm 5.76	90.00 \pm 24.79

activity represents the level of apoenzyme, it appears that apoenzyme levels decline with increase in age, especially for EGOT.

The above findings suggested that the transaminase activities represented by the basal activities in the subjects of this study diminish with age. This decline in the enzyme activities of EGPT and EGOT may be due to the decreases both in the apoenzyme levels and the amounts of available coenzyme as seen in the decrease of stimulated activities and increase of percent stimulation with age, respectively. All these relationships are statistically significant for EGOT, but not for EGPT.

It is interesting to compare the EGPT and EGOT activity levels of this research with those of an older group obtained by Miller et al. (1975) as both sets of data came from the same laboratory. In the study by Miller et al. (1975), the 11 control women (aged 20 to 29 years, mean 23.6) had mean values of basal activities of EGPT (0.32 ± 0.09 mg pyruvate/ml RBC/hr) and EGOT (6.52 ± 1.60 mg pyruvate/ml RBC/hr) lower and percent stimulation of EGPT (12 ± 5 percent) and EGOT (89 ± 41 percent) higher than those of the children in this study. Thus, this comparison is also suggestive of a tendency for transaminase activity to decline with age.

Although the decrease in basal transaminase activities with age was never established in children, Searcy (1969)

noted that serum and erythrocyte GOT are much higher at birth than in adulthood. He pointed out that while serum GOT diminishes rapidly within the first three days of life, EGOT remains elevated throughout the neonatal period. Jacobs and associates (1968) found a decrease in basal and stimulated levels of EGPT with age among a large group of adults aged 18 to 94. They proposed that the levels of coenzyme and apoenzyme diminish with age. Ranke et al. (1960) studied similar age groups and reported a lower basal level and higher in vitro stimulation of serum GOT in the older subjects. Since the basal serum GOT of the older subjects rose to the same level as that of the younger ones after three weeks of supplementation, the authors suggested that the level of coenzyme was reduced, but that the apoenzyme level remained normal with the advancement of age.

Whether the decline of transaminase activity with age indicates a change in body metabolism or dietary intake of vitamin B₆ remains to be investigated.

Sex

Table 6 provides the mean values of basal activity and percent stimulation of EGPT and EGOT for male and female subjects. For both EGPT and EGOT, girls have a lower basal activity and higher percent stimulation than the boys. However, these interesting differences were not statistically significant.

Table 6. The mean values of basal activity and percent stimulation of EGPT and EGOT according to sex.

	Sex	Number of Subjects	Basal Activity (μg pyruvate/mg Hb/hr)	Percent Stimulation
EGPT	Girls	43	1.17 ± 0.45^1	12.35 ± 7.80
	Boys	54	1.19 ± 0.42	11.31 ± 6.54
EGOT	Girls	48	22.47 ± 5.35	70.77 ± 18.62
	Boys	57	24.00 ± 5.91	68.43 ± 25.68

¹ Mean \pm SD.

A question was raised as to whether or not girls were generally older than boys in this study, thus tending to have lower transaminase levels as found in the correlation between age and transaminase activity as described above. Nevertheless, a look at the distribution of sex of subjects within each age group in Table 2 indicated that this is not the case.

Searcy (1969) noted that males have a slightly higher transaminase activity than females. Otherwise, information on the sex difference of the enzyme activity is not available. This factor deserves further study.

Hemoglobin and Hematocrit

The average hemoglobin and hematocrit levels of 106 subjects studied was 12.95 ± 0.77 g percent and 37.72 ± 3.43 percent, respectively. Only four children had either hematocrit or hemoglobin levels or both slightly lower than the acceptable standards given by Sauberlich, Dowdy and Skala (1974). The standards are 10 g percent, 11 g percent, and 11.5 g percent hemoglobin and 31 percent, 34 percent, and 36 percent hematocrit for the age groups 0-2, 2-5, and 6-12 years of age, respectively.

Whether the children were deficient in iron or not requires the measurements of serum iron and the percent transferrin saturation. However, the hemoglobin and hematocrit data of the subjects suggested that iron deficiency

was probably not a widespread problem. Iron deficiency has been noted by many authors as the most serious nutritional problem in American children (Theuer, 1974; Owen et al., 1971; and Burroughs and Huenemann, 1970). A statistically significant correlation was found between hemoglobin level and age ($r = 0.443$, $p < 0.01$). The fact that hemoglobin concentration increases with age until adulthood is reached was well established by the National Nutrition Survey (Sandstead and Pearson, 1973).

The healthy hemoglobin status of the children also implied that none of them have been seriously deficient in vitamin B₆ over a long period of time. When this is the circumstance, anemia develops since vitamin B₆ is required for the formation of protoporphyrin and heme (Coursin, 1964).

No correlation was found between the basal activities and percent stimulation of either EGPT or EGOT and hemoglobin or hematocrit levels. This result was anticipated since hemoglobin formation is only affected by vitamin B₆ deficiency at a severe level. Reports on the relationship between hemoglobin concentration and transaminase activity are limited. Cavill and Jacobs (1967) found a significantly lower basal and higher percent stimulation in EGPT, but not in EGOT, in patients with iron-deficiency anemia than in controls. But they commented that the correlation could merely be a coincidence as the anemic patients usually have

a poor diet in general. Donald et al. (1971) found that the hematocrit and hemoglobin level of their subjects remained unchanged through periods of depletion and repletion of vitamin B₆, even though the EGOT activity did correspond to the level of vitamin B₆ intake.

Storage Stability of EGPT and EGOT

Experiments with two hemolysates indicated that no loss of EGPT or EGOT activities occurred with freezing and storage within 13 days. Figure 8 showed that the fluctuation of transaminase activity levels with time followed no definite pattern and appeared to be due to the variation caused by the method itself.

Stability of GOT activity in blood compartments was noted by other authors. Babcock et al. (1960) reported no change in serum GOT after two months of freezing and storage. Beutler (1971) also found less than 10 percent loss of GOT activity in whole blood stored with anticoagulant for 20 days at 4C.

On the other hand, Woodring and Storvick (1970) indicated that EGPT activity is lost when stored frozen. Rose and associates (1973) further reported that after 10 days of storage at -20C, the activity of EGPT and EGOT decreased 48 percent and 28 percent, respectively.

When a large number of samples must be assayed as in this study, uniformity of storage time is usually precluded.

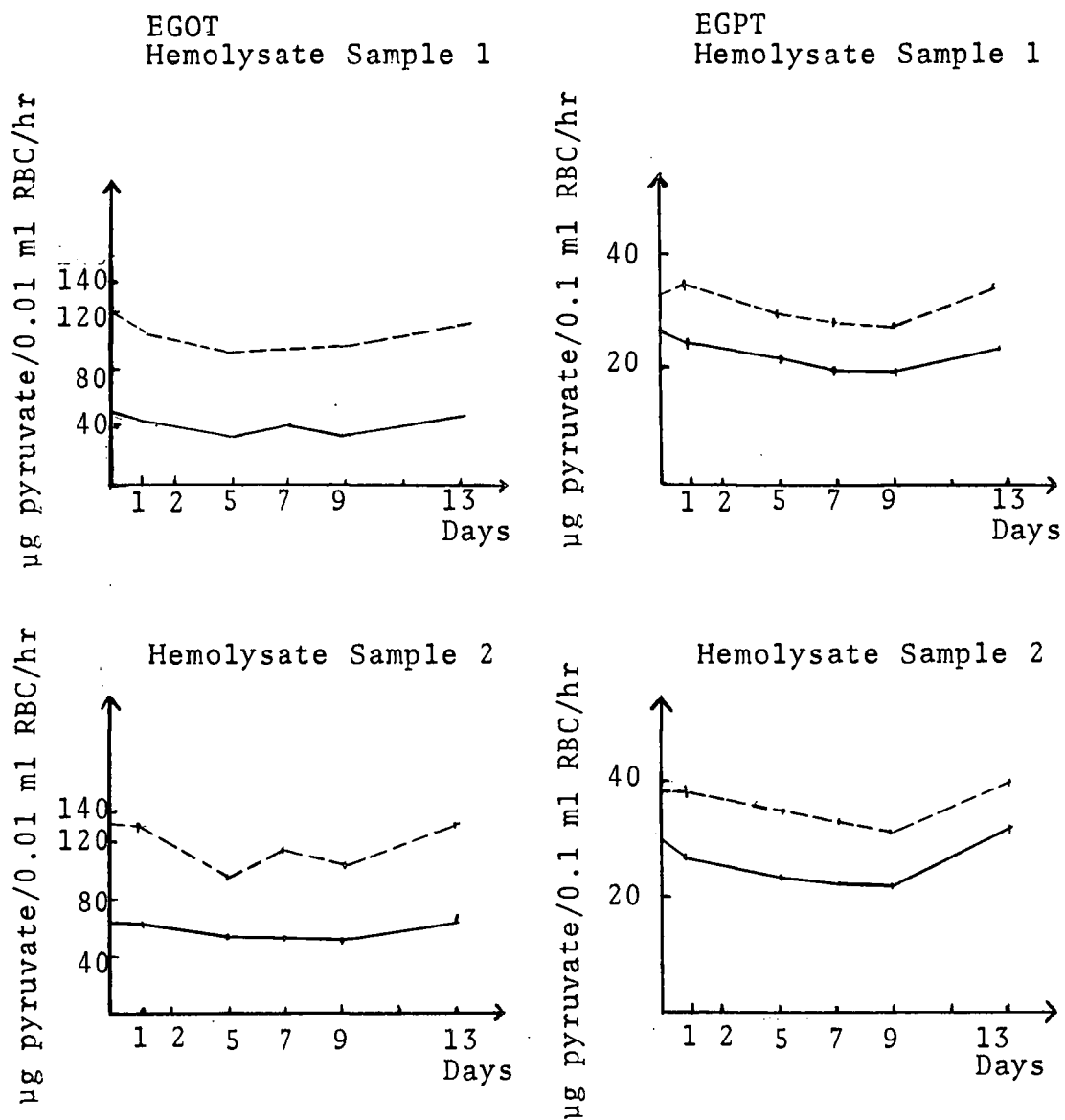


Figure 8. Basal activity of EGPT and EGOT (—) and in-vitro stimulated activity of EGPT and EGOT (-----) for two samples during thirteen days of storage.

Thus the stability of the enzyme activity with storage and freezing becomes of significant importance.

Although most of the samples in this research were assayed for transaminase activity within 13 days, some had to be delayed beyond 13 and up to 28 days. Thus, an extended study on the activity of the transaminases may be needed to ascertain their stability through a prolonged period of storage.

SUMMARY AND CONCLUSION

From this study on transaminase activities in preschool children, the following conclusions can be drawn:

1. For EGPT, the basal activity and percent stimulation were 1.20 ± 0.44 μg pyruvate/mg Hb/hr and 11.70 ± 7.00 percent, respectively. Those of EGOT were 23.30 ± 5.77 μg pyruvate/mg Hb/hr and 69.90 ± 23.30 percent.

2. The two ways of expressing transaminase activities, as μg pyruvate/mg Hb/hr and μg pyruvate/ml RBC/hr, were highly correlated with each other.

3. A significant positive correlation was found between the basal activities of EGPT and EGOT ($p < 0.01$). However, the positive relationship between their corresponding percent stimulation was not significant. The stimulated and basal activities of both EGPT and EGOT were closely correlated ($p < 0.01$). A significant inverse relationship ($p < 0.01$) existed between the basal activity and percent stimulation of EGOT, but not of EGPT.

4. In the subjects whose diet was supplemented with multi-vitamins containing pyridoxine, the transaminase appeared to be higher and the corresponding percent stimulation lower than in those receiving no supplementation. But the difference was only significant for basal EGPT ($p < 0.01$).

5. The subjects with high basal activities or low percent stimulation of EGPT or EGOT also tended to have higher plasma B₆ levels. However, these relationships were not significant.

6. As the age of the subjects increased, the basal and stimulated activities of both EGPT and EGOT declined, accompanied by corresponding increases in percent stimulation. The correlations for basal and stimulated activities, as well as percent stimulation of EGOT, but not EGPT, with age were significant ($p < 0.01$).

7. The differences in transaminase activities due to sex were not significant. But in general, the girls had a lower basal activity and a higher percent stimulation for both EGPT and EGOT than the boys.

8. The average hemoglobin level of the subjects was 12.95 ± 0.77 g percent. The hemoglobin levels increased significantly with age ($p < 0.01$).

9. Experiments with two hemolysate samples showed that no loss of EGPT or EGOT activities occurred with freezing and storage.

BIBLIOGRAPHY

- Aebi, H. 1969. In Protein-Calorie Malnutrition, von Muralt, A., Ed. Springer-Verlag, New York. 194 pp.
- Anderson, B. B., C. E. Fulford-Jones, J. A. Child, M. E. Beard, and C. J. T. Bateman. 1971. Conversion of vitamin B₆ compounds to active forms in the red blood cell. *Journal of Clinical Investigation* 50:1901-1969.
- Babcock, M. J., M. Brush, and E. Sostman. 1960. Evaluation of vitamin B₆ nutrition. *Journal of Nutrition* 70:369-376.
- Baker, H., O. Frank, S. Feingold, G. Choristakis, and H. Ziffer. 1967. Vitamins, total cholesterol, and triglycerides in 642 New York city school children. *American Journal of Clinical Nutrition* 20:850-856.
- Baker, H., O. Frank, A. D. Thomson, and S. Feingold. 1969. Vitamin distribution in red blood cells, plasma, and other body fluids. *American Journal of Clinical Nutrition* 22:1469-1475.
- Baysal, A., B. A. Johnson, and H. Linkswiler. 1966. Vitamin B₆ depletion in man: Blood vitamin B₆, plasma pyridoxal-phosphate, serum cholesterol, serum transaminase, and urinary vitamin B₆ and 4-pyridoxic acid. *Journal of Nutrition* 29:19-23.
- Bessey, O. A., J. D. Adam, and A. E. Hansen. 1957. Intake of vitamin B₆ and infantile convulsions: A first approximation of requirements of pyridoxine in infants. *Pediatrics* 20:33-44.
- Beutler, E. 1971. *Red Cell Metabolism. A manual of biochemical methods.* Grune and Stratton. New York and London, 145 pp.
- Brigety, R. E. and H. A. Pearson. 1970. Effects of dietary and iron supplementation on hematocrit levels of pre-school children. *Journal of Pediatrics* 76:757-760.
- Brown, R. R. 1972. Normal and pathological conditions which may alter the human requirement for vitamin B₆. *Journal of Agricultural and Food Chemistry* 20:498-505.

- Burroughs, A. L. and R. L. Huenemann. 1970. Iron deficiency in rural infants and children. *Journal of American Dietetic Association* 57:122-128.
- Cavill, I. A. J. and A. Jacobs. 1967. Erythrocyte transaminase activity in iron deficiency anemia. *Scand. Journal of Hematology* 4:249-256.
- Chen, S. H. and E. R. Giblett. 1971. Polymorphism of soluble glutamic-pyruvic transaminase: A new genetic marker in man. *Science* 173:148-149.
- Cheney, M. C., P. M. Curry, and G. H. Beaton. 1965a. Blood transaminase activities in vitamin B₆ deficiency: Specificity and sensitivity. *Canadian Journal of Physiology and Pharmacology* 43:579-589.
- Cheney, M., A. I. Sabry, and G. H. Beaton. 1965b. Erythrocyte glutamic-pyruvic transaminase activity in man. *American Journal of Clinical Nutrition* 16:337-338.
- Cinnamon, A. D., and J. K. Beaton. 1970. Biochemical assessment of vitamin B₆ status in man. *American Journal of Clinical Nutrition* 23:696-702.
- Coon, W. W., and E. Nagler. 1969. The tryptophan load as a test for pyridoxine deficiency in hospitalized patients. *Annals of New York Academy of Science* 166:30-43.
- Coursin, D. B. 1964. Vitamin B₆ metabolism in infants and children. *Vitamins and Hormones* 22:755-786.
- Davidsohn, I. and D. A. Nelson. 1969. In *Todd-Sanford Clinical Diagnosis by Laboratory Methods*, Davidsohn, I. and B. B. Wells, Ed. W. B. Saunders Co. 14th Edition. pp. 416-418.
- Davidson, R. G., J. A. Cortner, and R. C. Rattazzi. 1970. Genetic polymorphism of human mitochondrial glutamic-oxaloacetic transaminase. *Science* 169:391-392.
- Department of Health, Education and Welfare. 1968-1970. *Ten State Nutrition Survey*. Publication No. (HSM) (72-8132). pp. V8-9.
- Donald, E. A., L. D. McBean, M. H. W. Simpson, M. F. Sun, and H. E. Aly. 1971. Vitamin B₆ requirement of young adult women. *American Journal of Clinical Nutrition* 24:1028-1041.

- Gailani, S. D., J. F. Holland, A. Nussbaum, and K. B. Olson. 1968. Clinical and biochemical studies of pyridoxine deficiency in patients with neoplastic diseases. *Cancer* 21:975-988.
- Guirard, B. M., E. E. Snell. 1964. Vitamin B₆ function in transamination and decarboxylation reactions. In *Comprehensive Biochemistry*, Vol. 15, Florkin, M. and E. H. Stotz, Ed., Chapter 5, Elsevier Publishing Company, Amsterdam.
- György, P. 1934. Vitamin B₂ and the pellagra-like dermatitis in rats. *Nature* 133:498-499.
- György, P. 1971. Developments leading to the metabolic role of vitamin B₆. *American Journal of Clinical Nutrition* 21:1250-1256.
- Henry, R. J. 1964. *Clinical Chemistry*. New York: Harper and Row, 78-83 pp.
- Hunter, R. E., and N. J. Smith. 1972. Hemoglobin and hematocrit values in iron deficiency in infancy. *Journal of Pediatrics* 81:710-713.
- Jacobs, A., I. A. J. Cavill, and J. N. P. Hughes. 1968. Erythrocyte transaminase activity. Effect of age, sex, and vitamin B₆ supplementation. *American Journal of Clinical Nutrition* 21:502-507.
- Karmen, A., F. Wrobluvski, and J. S. LaDue. 1955. Transaminase activity in human blood. *Journal of Clinical Investigation* 34:126-131.
- Krishnaswamy, K. 1971a. Erythrocyte transaminase activity in human vitamin B₆ deficiency. *International Journal of Vitamin and Nutrition Research* 41:240-245.
- Krishnaswamy, K. 1971b. Erythrocyte glutamic-oxaloacetic transaminase activity in patients with oral lesions. *International Journal for Vitamin and Nutrition Research* 41:247-252.
- Linkswiler, H. 1967. Biochemical and physiological changes in vitamin B₆ deficiency. *American Journal of Clinical Nutrition* 20:547-557.
- Miller, L. T., A. Johnson, E. M. Benson, and M. J. Woodring. The effect of oral contraceptives and pyridoxine on the metabolism of vitamin B₆, plasma tryptophan, and α -amino nitrogen. Accepted for publication in *American Journal of Clinical Nutrition*.

- National Academy of Sciences. 1974. Recommended Dietary Allowances. Eighth Ed. 28 p.
- National Dairy Council. 1972. Iron-deficient anemia in infants and preschool children. Dairy Council Digest 43, N1. 1-6.
- Orr, M. L. 1969. Home Economics Research Report No. 36. Pantothenic Acid, Vitamin B₆, and Vitamin B₁₂ in Foods. Agricultural Research Service. U.S.D.A. 53 p.
- Owen, G. M., A. H. Lubin, and P. J. Garry. 1971. Preschool children in the United States: Who has iron deficiency? Journal of Pediatrics 79:563-368.
- Patwardhan, M. W. 1958. Role of ferrous ion in enzymatic transamination. Nature 181:187.
- Polansky, M. M. and E. W. Murphy. 1966. Vitamin B₆ components in fruits and nuts. Journal of American Dietetic Association 48:109-111.
- Raica, N. and H. E. Sauberlich. 1964. Blood cell transaminase activity in human vitamin B₆ deficiency. American Journal of Clinical Nutrition 15:67-72.
- Ranke, E., S. A. Tauber, A. Horonick, B. Ranke, R. S. Goodhart, and B. F. Chow. 1960. Vitamin B₆ deficiency in the aged. Journal of Gerontology 15:41.
- Rose, D. P., R. Strong, J. Folkard, and P. W. Adams. 1973. Erythrocyte amino-transferase activities in women using oral contraceptives and the effect of vitamin B₆ supplementation. American Journal of Clinical Nutrition 26: 48-52.
- Sandstead, H. H. and W. N. Pearson. 1973. In Modern Nutrition in Health and Disease, Goodhart, R. S. and M. E. Shils, Ed. Lea and Febiger, 5th edition, p. 586.
- Sauberlich, H. E., J. E. Canham, E. M. Baker, N. Raica, and Y. F. Herman. 1970. Human vitamin B₆ nutriture. Journal of Scientific and Industrial Research 29:pS28-pS36.
- Sauberlich, H. E., J. E. Canham, E. M. Baker, N. Raica, and Y. F. Herman. 1972. Biochemical assessment of the nutritional status of vitamin B₆ in the human. American Journal of Clinical Nutrition 25:629-642.

- Sauberlich, H. E., R. P. Dowdy, and J. H. Skala. 1974. Laboratory Tests for the Assessment of Nutritional Status. CRC Press, Inc. 136 pp.
- Schroeder, H. A. 1971. Losses of vitamins and trace minerals resulting from processing and preservation of foods. American Journal of Clinical Nutrition 24:562-573.
- Searcy, R. L. 1969. Diagnostic Biochemistry. McGraw-Hill Book Co. pp 510-516. 659 p.
- Sifri, M., N. J. Dagher, and J. A. Asmar. 1972. Serum aminotransferase activities and plasma free amino acid concentration as criteria for pyridoxine nutritional status of chicks. British Journal of Nutrition 28:173-182.
- Snell, E. E. 1964. Summary of session I and some notes on the metabolism of vitamin B₆. In Vitamins and Hormones, 22:485-494.
- Snell, E. E. 1970. Analogs of pyridoxal or pyridoxal phosphate: Relation of structure of binding with apoenzymes and to catalytic activity. In Vitamins and Hormones, Harris et al., Ed. Vol. 28:265-290.
- Snell, E. E. and S. J. Di Man. 1970. Schiff base intermediates in enzyme catalysis. In The Enzymes, Boyer, P. D. Ed., Vol. 2:335-362, Chap. 7, 584 p.
- Snyderman, S. E., L. M. Holt, R. Carreters, and K. Jacobs. 1953. Pyridoxine deficiency in human infants. American Journal of Clinical Nutrition 1:200-207.
- Standall, B. P., S. M. Kao-Chen, G. Y. Yang, and D. F. B. Char. 1974. Early changes in pyridoxine status of patients receiving isoniazid therapy. American Journal of Clinical Nutrition 27:479-484.
- Stuart, H. C. and H. V. Meredith. 1946. Use of body measurements in the school health program: Part I. General considerations and the selection of measurements; Part II. Methods to be followed in taking and interpreting measurements and norms to be used. American Journal of Public Health 36:1346-1386.
- Storvick, C. A., E. M. Benson, M. A. Edwards, and M. J. Woodring. 1964. Chemical and microbiological determination of vitamin B₆. In Methods of Biochemical Analysis, Glick, D., Ed. New York: Wiley, Vol. XII, 183 pp.

- Theuer, R. C. 1974. Iron undernutrition in infancy. *Clinical Pediatrics* 13:522.
- Tonhazy, N. E., N. G. White, and W. W. Umbreit. 1950. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Archives of Biochemistry and Biophysics* 28: 36-44.
- Wada, H. and E. Snell. 1961. The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. *The Journal of Biological Chemistry* 236:2089-2095.
- Waldinger, C., and R. B. Berg. 1963. Signs of pyridoxine dependency manifest at birth in siblings. *Pediatrics* 32:161-168.
- Watson, E. H. and G. H. Lowrey. 1969. *Growth and Development of Children*. Year Book Medical Publishers, Inc. Fifth Ed. 463 p.
- Woodring, M. J. and C. A. Storvick. 1959. Vitamin B₆ in milk: Review of literature. *Oregon Agriculture Experimental Station*. No. 85.
- Woodring, M. J. and C. A. Storvick. 1970. Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women. *American Journal of Clinical Nutrition* 23:1385-1395.
- Wroblewski, F. and P. Cabaud. 1957. Colorimetric measurement of serum glutamic pyruvic transaminase. *American Journal of Clinical Pathology* 27:235-239.