phosphate oxidase and aldehyde oxidase. Although the total body half-life of administered ^{3}H-PN has been calculated (Contractor and Shane, 1968), there is no data on turnover of vitamin B_6 in brain or liver. However, techniques are now available for this work to be done. The work on interconversion and distribution of vitamin B_6 in liver, brain, and carcass of normal mice (Colombini and McCoy, 1970b) has laid the foundation for further work of the overall effects on B_6 metabolism of drugs such as amphetamines or reserpine known to alter single steps in the metabolism of this vitamin. It is hoped that soon data will be available on the turnover of each of the B_6 vitamers and of the factors which control B_6 turnover in brain, liver, and carcass.

LITERATURE CITED

Bain, J. A., Williams, H. C., "Inhibition of the nervous system and gamma-aminobutyric acid," Pergamon Press, New York, N.Y., 1960, pp 275–293.
Bilodeau, F., *J. Neurochem.* **12**, 671 (1965).
Booth, C. C., Brain, M. C., *J. Physiol.* **164**, 282 (1962).
Brain, M. C., Booth, C. C., *Gut* **5**, 241 (1964).
Braunstein, A. E., Pyridoxal phosphate in: The Enzymes, Vol. 2, Academic Press, New York, N.Y., 1960, pp 113–181.
Brown, R. R., Rose, D. P., Price, J. M., Wolf, H., *Ann. N.Y. Acad. Sci.* **166**, 44 (1969).
Colombini, C. E., McCoy, E. E., *Anal. Biochem.* **34**, 451 (1970a).
Colombini, C. E., McCoy, E. E., *Biochemistry* **9**, 533 (1970b).
Contractor, S. F., Shane, B., *Clin. Chim. Acta* **21**, 71 (1968).
Ebadi, M. S., Russell, R. L., McCoy, E. E., *J. Neurochem.* **15**, 659 (1968).
Ebadi, M. S., *Pharmacology* **3**, 97 (1970).
Greenberg, L. D., *Vitam. Horm.* **22**, 677 (1964).
Harris, J. W., Horrigan, D. L., *Vitam. Horm.* **22**, 722 (1964).
Henn, S., McCoy, E. E., unpublished data, 1971.
Johansson, S., Lindstedt, S., Register, U., Wadstron, L., *Amer. J. Clin. Nutr.* **18**, 185 (1966).
Marino, Y., Wada, H., Morisue, T., Sakamoto, Y., Ichihara, K., *J. Biochem.* **48**, 18 (1960).
McCormick, D. B., Gregory, M. E., Snell, E. E., *J. Biol. Chem.* **236**, 2076 (1961).
Scriver, C. R., *Pediatrics* **26**, 62 (1960).
Snell, E. E., Ayling, J. E., in: Symposium on Pyridoxal Enzymes, Maruzen Company, Tokyo, 1968, pp 5–12.
Vilter, R. W., *Vitam. Horm.* **22**, 797 (1964).
Wada, H., Snell, E. E., *J. Biol. Chem.* **236**, 2089 (1961).
White, R. S., Dempsey, W. B., *Biochemistry* **9**, 4057 (1970).

Received for review July 9, 1971. Accepted October 26, 1971. Presented at the Division of AGFD 161st Meeting, ACS, Los Angeles, California, March–April 1971.

Normal and Pathological Conditions Which May Alter the Human Requirement for Vitamin B_6

R. R. Brown

Factors which may affect the production and assessment of vitamin B_6 deficiency in humans are presented. Although the tryptophan load test has been widely used to assess vitamin B_6 deficiency, this test used alone is difficult to interpret because interrelated hormonal and metabolic factors play an important role in affecting the results obtained. Other methods of measuring vitamin B_6 nutrition, such as the determination of blood and tissue levels of the vitamin B_6 vitamers, and the measurement of erythrocyte aminotransferases, should be used in conjunction with the tryptophan load test. Although the abnormal tryptophan metabolism observed in pregnant women and in women using oral contraceptives is probably only in part due to a vitamin B_6 deficiency, it seems reasonable at this time to suggest that such women receive supplemental pyridoxine. However, serial studies will be necessary to establish the long-term effects of such supplementation.

Since the codiscovery of vitamin B_6 by György, Edgar, and Chick (Robinson, 1951) numerous functions for this versatile nutrient have been defined. The chief function of this vitamin is in the metabolism of amino acids (Williams, 1964), but it is also involved in lipid and carbohydrate metabolism (Wiss and Weber, 1964; Mueller, 1964). It is beyond the scope of this report to acknowledge the many investigators who have made significant contributions to the knowledge of requirements and functions of this important vitamin; these have been reviewed in recent years (Robinson, 1951; Wiss and Weber, 1964; Snell, 1958; Storvick and Peters, 1964; Harris *et al.*, 1964).

Conditions Which May Lead to Vitamin B_6 Deficiency or Dependency. In discussing conditions which may alter the human requirement for this vitamin, it will be helpful to consider a number of conditions which might possibly lead to vitamin B_6 deficiency or dependency. Some of these factors are listed in Table I. Inadequate dietary intake is the first and most obvious possible cause for a deficiency. Although this vitamin is widely distributed in a variety of foods, the content of foods eaten by a weight conscious population may be rather marginal. That is, foods such as beans, peas, cabbage, nuts, cereals, etc., contain reasonable amounts of this vitamin while meats, eggs, and milk are not such good sources. Hence, someone eating a high protein diet, which actually increases the need for the vitamin (Natl. Acad. Sci., 1968), may have a marginal intake.

Even with adequate intake, there may be a tissue deficiency if there is impaired delivery of the vitamin. We may list defective intestinal absorption, defective cellular and intracellular transport, as well as impaired activation of the vitamin by oxidation to pyridoxal and phosphorylation to the active coenzyme, pyridoxal phosphate (PLP). Examples of these mechanisms are rare, and in some instances not clearly established.

Conditions of deficiency may also arise if there is excessive loss of the vitamin, such as enhanced renal clearance of the vitamin due to impaired renal function. Also, if there is

Division of Clinical Oncology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

excessive oxidation of the vitamers to 4-pyridoxic acid, the major urinary metabolite of the vitamin, a deficiency may ensue. Excessive loss of the vitamin through inactivation with chemicals and drugs has also been well recognized. Schiff's base formation between the aldehyde form of the vitamin and a variety of amines, hydrazines, and hydrazides has been demonstrated; an example of this is the deficiency produced by the drug isonicotinyl hydrazide (Isoniazid) used in the treatment of tuberculosis. If this drug is used for prolonged periods without concomitant administration of pyridoxine, both metabolic and clinical evidence of vitamin B_6 deficiency may appear (Biehl and Vilter, 1954). A similar deficiency may appear in patients treated with penicillamine (Jaffe *et al.*, 1964).

A relative deficiency may occur if the primary intake of the vitamin is inadequate relative to the metabolic demands for the vitamin. Such would be the case in pregnancy, where the growing fetus elicits an increased requirement in the mother not only because of the vitamin used by the fetus, but because of the increased metabolic activity of the mother resulting from the altered hormonal environment during pregnancy. Fever, infection, and other similar conditions may also increase requirement for the vitamin temporarily (Rapoport and Beisel, 1971). Related to this mechanism of producing a deficiency is the condition in which induction of apoenzyme formation results in an increased number of binding sites for the coenzyme. If the induced, newly formed enzymes have a stronger affinity for pyridoxal phosphate than do certain other enzymes, the latter group of enzymes may lose their cofactor, and biochemical or metabolic evidence of a vitamin B_6 deficiency may be found. This may be the situation, in part, in the production of apparent metabolic deficiency with high doses of cortisol, or in the stress of certain diseases.

In recent years evidence has appeared suggesting that in certain patients a defect in the binding of PLP to the apoenzyme may be present. In this case, the enzyme may be essentially nonfunctional at usual concentrations of cofactor, but may have activity restored in the presence of much higher concentrations of the cofactor. Several well recognized clinical entities may fall into this category of defect. For example, about half of the children with a specific type of convulsion will be well controlled by administration of large amounts of pyridoxine (Scriver, 1967). Similarly, there is a growing number of anemia patients whose disease is specifically responsive to amounts of vitamin B_6 above the normal intake levels (Harris and Horrigan, 1964). Cystathioninuria is another condition where a defective enzyme may be stimulated to function essentially normally by massive amounts of the cofactor (Frimpter *et al.*, 1969). Not all of the cystathioninuria patients respond to vitamin B_6 dosages, suggesting that in some cases the defect in the enzyme cystathionase is not correctable by excess cofactor. Vitamin B_6 metabolism and retention are apparently altered in patients with Down's Syndrome, but a specific defect has not been identified (McCoy *et al.*, 1969).

Table I. Factors Which May Lead to Vitamin B_6 Deficiency or Dependency

Inadequate dietary intake
Impaired delivery of vitamin (intake adequate)
- Defective intestinal absorption
- Defective cellular and intracellular transport
- Impaired oxidation of pyridoxine
- Impaired phosphorylation

Excessive loss of vitamin
- Enhanced renal clearance
- Excessive oxidation
- Chemical or drug inactivation (*e.g.*, Isoniazid therapy)

Relative deficiency (primary intake inadequate relative to demand)
- Increased metabolic activity (pregnancy, fever, etc.)
- Apoenzyme induction
- Competition by other metabolites (*e.g.*, estradiol conjugates)

Apoenzyme defects which alter binding of cofactor
- Vitamin B_6 responsive convulsions
- Vitamin B_6 responsive anemias
- Cystathioninuria responsive to vitamin B_6
- Homocystinuria
- Down's Syndrome

Methods of Assessing Vitamin B_6 Nutrition. With the above brief and entirely inadequate review of vitamin B_6 deficiency problems, I would like to review briefly methods of assessing vitamin B_6 deficiency, since some discussion of the methods is essential to the interpretation of the results of these methods. The most obvious and direct evaluation of vitamin B_6 nutrition is the direct measurement of the B_6 vitamers in tissues or body fluids of the subject. Thus, for example, it is possible to assay directly for pyridoxal, pyridoxamine, and pyridoxine and the corresponding phosphorylated derivatives in blood, urine, and even in tissues (Storvick and Peters, 1964; Contractor and Shane, 1968; Brin, 1970; Coursin and Brown, 1961). Such assays, while they may be tedious or unavailable in many laboratories, provide direct information about the levels of the vitamin B_6 vitamers in the tissue measured. However, it is not convenient or possible to sample many human tissues, so that information about liver, brain, etc., levels cannot be obtained. Similarly, this direct assay does not measure the functional activity of the coenzyme or the functional capacity of specific PLP-requiring enzyme systems.

Evidence of vitamin B_6 levels may be obtained indirectly by measurement of selected enzymes which require PLP. For example, plasma and red cell amino transferases are often measured as indices of vitamin B_6 deficiency. The degree of stimulation of these enzymes by PLP added *in vitro* is an additional reflection of the degree of saturation of the enzyme *in vivo* (Cinnamon and Beaton, 1970).

A number of *in vivo* functional tests for vitamin B_6 deficiency have been developed based on changes in blood or urinary levels of metabolites which require vitamin B_6 for their production or further breakdown. These include measurement of urinary taurine, oxalate, cystathionine, tryptophan metabolites, and other substances, the metabolism of which involves vitamin B_6 directly or indirectly. The most commonly used of such metabolic tests is the tryptophan load test which is based on the early observation that xanthurenic acid was excreted in elevated amounts by animals and humans deficient in the vitamin (Greenberg *et al.*, 1949). The test for xanthurenic acid has been extended to other urinary metabolites of tryptophan, and numerous analytical methods have been used for the assay of these metabolites (Price *et al.*, 1965; Musajo and Benassi, 1964). The ease and simplicity of the tryptophan load test have been overshadowed in recent years by the realization that the interpretation of the results of the tryptophan load test is difficult, and in many cases is influenced by a variety of conditions.

The pathway involved in the tryptophan load test is shown in Figure 1. The initial step in this pathway, that of the tryptophan pyrrolase (tryptophan oxygenase) reaction, is an inducible enzyme which will increase appreciably in activity in response to high levels of tryptophan or to cortisol (endogenous or exogenous) (Knox and Greengard, 1964; Knox, 1965). In a condition of vitamin B_6 deficiency, the enzyme

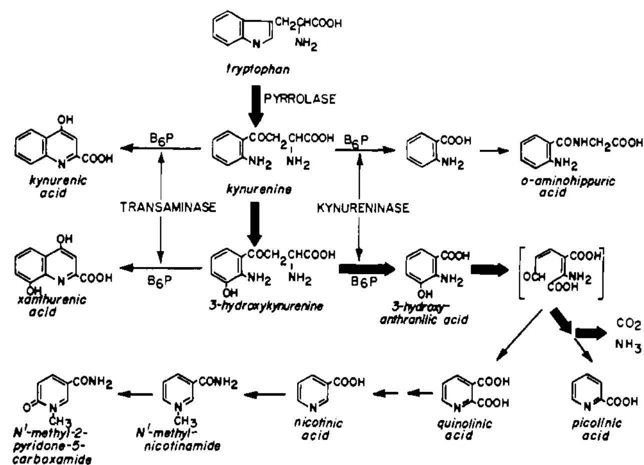

Figure 1. Metabolic pathway for the degradation of tryptophan through the kynurenine–niacin pathway. The heavy arrows indicate the major pathway from a quantitative standpoint. B_6P indicates pyridoxal phosphate

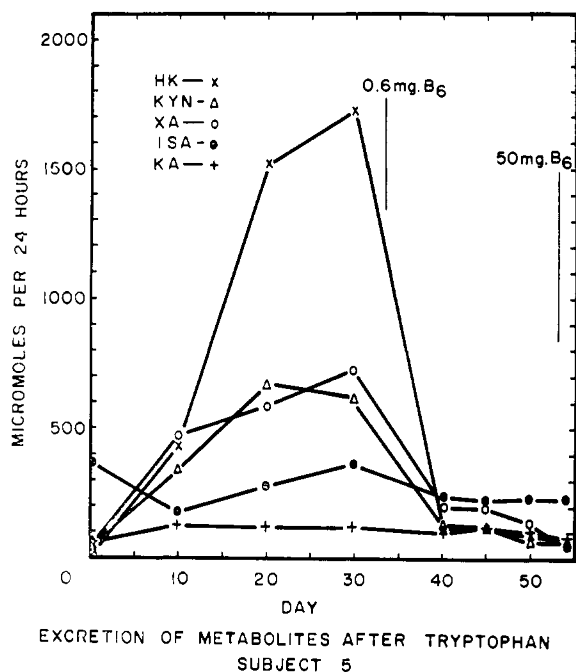

Figure 2. The excretion of tryptophan metabolites by a subject maintained from day 5 on a diet containing 0.16 mg of vitamin B_6 per day. Values are after a 2.0 g loading dose of L-tryptophan. At the indicated points the subject was supplemented with 0.6 and 50 mg per day of pyridoxine hydrochloride. The abbreviations are HK, 3-hydroxykynurenine; KYN, kynurenine; XA, xanthurenic acid; ISA, indoxylsulfuric acid (indican); KA, kynurenic acid. Data from Yess *et al.* (1964). Reprinted with permission from *Journal of Nutrition*

kynureninase is the first to lose its cofactor (Ogasawara *et al.*, 1962; Ueda, 1967) resulting in a metabolic block at this step which allows hydroxykynurenine and kynurenine to accumulate. This in turn leads to enhanced levels of xanthurenic acid, kynurenine, and hydroxykynurenine. Other rate-limiting steps in this pathway probably exist, since elevated excretion of quinolinic acid, a metabolite beyond the kynureninase block, has been demonstrated in vitamin B_6 deficient men (Brown *et al.*, 1965). The accumulation and excretion of these metabolites are only readily demonstrable after a loading dose of tryptophan is administered. Doses

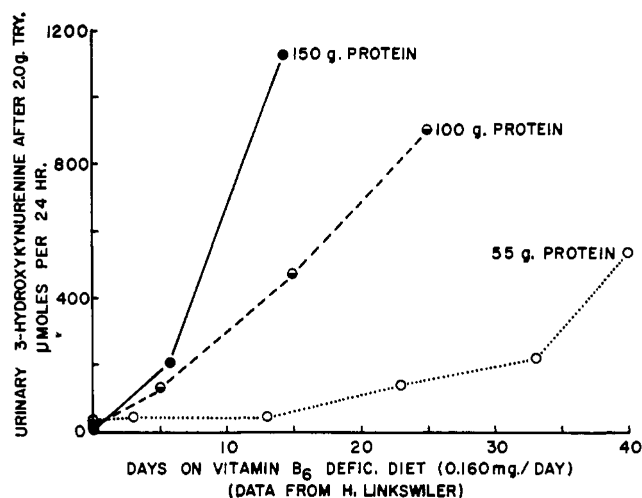

Figure 3. The excretion of urinary 3-hydroxykynurenine after a 2.0 g load of L-tryptophan in subjects maintained on a vitamin B_6 deficient diet containing varying levels of protein. Data from Miller and Linkswiler (1967). Reprinted with permission from *Journal of Nutrition*

of from 1 to 10 g of tryptophan have been used by various investigators, but a dose of 2.0 g of L-tryptophan is quite adequate for most purposes and is to be preferred to the higher doses (Price *et al.*, 1965; Coursin, 1964).

The analytical methods used in the tryptophan load test range from a simple colorimetric method for xanthurenic acid in raw urine, which is not very specific but which is adequate when high levels are present (Price *et al.*, 1965), to more complex and tedious methods for other tryptophan metabolites which may include paper or thin-layer chromatography, ion-exchange column chromatography, electrophoresis, gas chromatography, or automated procedures (Price *et al.*, 1965; Musajo and Benassi, 1964; Toseland and Price, 1969; Arend *et al.*, 1970).

The tryptophan load test has been used in several studies of experimentally induced vitamin B_6 deficiency in man (Yess *et al.*, 1964; Baker *et al.*, 1964; Canham *et al.*, 1969) and indeed is a sensitive method of detecting the deficiency. For example, studies in our laboratory showed that tryptophan metabolism became altered as early as 5 days after initiation of a vitamin B_6 deficient diet in healthy young men (Figure 2) (Yess *et al.*, 1964). Baker *et al.* (1964) have reported similar findings using a test load of 10 g of DL-tryptophan. The minor differences between these two studies are probably due to the use by Baker *et al.* (1964) of the DL-mixture of tryptophan, since unusual metabolites of the D isomer are known to be excreted by humans and will interfere with some of the analytical methods used.

The protein content of the diet is important in determining the rate of appearance of deficiency. Baker and collaborators (1964), Canham *et al.* (1969), and Miller and Linkswiler (1967) showed that the appearance of abnormal tryptophan metabolism was much earlier in subjects on a high protein diet (Figure 3). The mechanism for enhanced need for vitamin B_6 in high protein diets is not clearly understood; presumably it is due to enhanced breakdown of the cofactor used in the metabolism of the excess protein ingested. However, the high level of ingested protein may also maintain tryptophan oxygenase at an elevated level which would insert tryptophan into the pathway at an increased rate, thus overloading kynureninase and producing evidence by the tryptophan load test of vitamin B_6 deficiency even though tissue

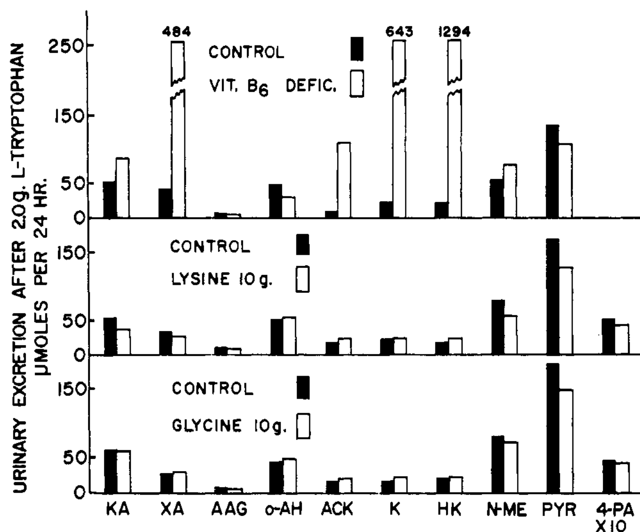

Figure 4. The excretion of tryptophan metabolites by four normal subjects given oral doses of 10 g of L-lysine or 10 g of glycine at the same time as a 2.0 g load of L-tryptophan. Control values are from the same subjects studied 4 days earlier with only a 2.0 g load of tryptophan. Data from subjects ingesting a vitamin B_6 deficient diet are shown for comparison. Metabolites were measured by the methods described by Price *et al.* (1965). Abbreviations are KA, kynurenic acid; XA, xanthurenic acid; AAG, anthranilic acid glucuronide; *o*-AH, *o*-aminohippuric acid; ACK, acetylkynurenine; K, kynurenine; HK, hydroxykynurenine; *N*-ME, *N*-methylnicotinamide; PYR, *N*-methyl-2-pyridone-5-carboxamide; 4-PA, 4-pyridoxic acid

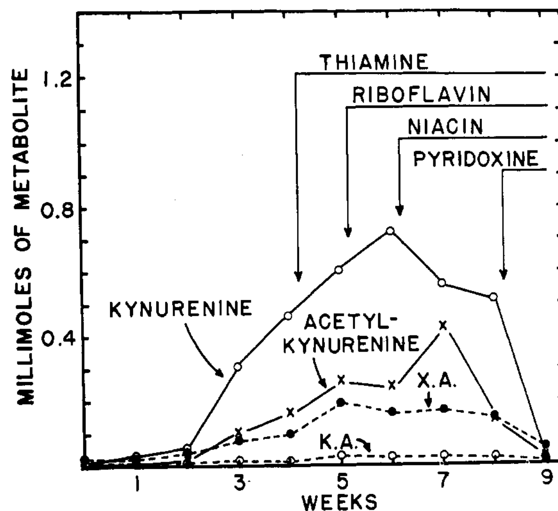

Figure 5. Urinary excretion of tryptophan metabolites after a 2.0 g load of L-tryptophan in a subject treated with Isoniazid. Abbreviations are X.A., xanthurenic acid; K.A., kynurenic acid. Data from Price *et al.* (1957). Reprinted with permission from *Journal of Clinical Investigation*

levels of PLP are not changed. In addition, if the high protein diet induces high levels of enzymes which require PLP, this would tend to deplete kynureninase of its cofactor, resulting in an abnormal tryptophan load test.

It has been suggested that the tryptophan load test itself may generate a vitamin B_6 deficiency (Hughes and Raine, 1966) by a mechanism in which PLP combines with the amino group of the excess tryptophan administered. If this is true, then excesses of other amino acids should also form such combinations and produce a deficiency. To evaluate this possibility we administered 10 g of glycine or 10 g of L-lysine at the time of a tryptophan load test given to normal subjects and found no change in their tryptophan metabolite excretion (Brown, 1971) (Figure 4). However, this kind of depletion mechanism has been shown to occur in patients treated with isonicotinyl hydrazide (Isoniazid) (Biehl and Vilter, 1954; Price *et al.*, 1957). Patients treated with this drug for prolonged periods of time may develop biochemical and clinical evidence of vitamin B_6 deficiency unless supplemented with pyridoxine (Figure 5). Similarly, penicillamine will also produce evidence of a vitamin B_6 deficiency (Jaffe *et al.*, 1964).

Cinnamon and Beaton (1970) compared the xanthurenic acid test with erythrocyte aminotransferase studies in subjects ingesting a vitamin B_6 deficient diet. They found that both tests for deficiency gave comparable results during the induction phase in their study, but differences appeared during repletion with pyridoxine. The xanthurenic acid excretion returned to normal within 1 to 2 days of pyridoxine supplementation (2.0 mg per day), whereas the erythrocyte aminotransferase levels did not return to normal until after 3 to 4 weeks of supplementation. They also measured the *in vitro* response of these aminotransferases to PLP and found that the erythrocyte glutamic–pyruvate aminotransferase was a more sensitive measure of the vitamin B_6 status than was the

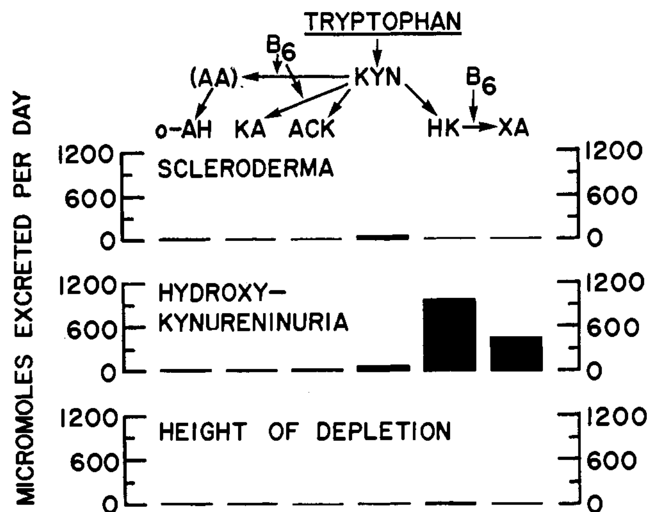

Figure 6. Excretion of tryptophan metabolites by a patient with hydroxykynureninuria (Komrower and Westall, 1967). For comparison, data from a patient with scleroderma and subjects at the height of a vitamin B_6 depletion are shown. These values are basal, unsupplemented values. The abbreviated metabolic pathway above the bars identifies the metabolites measured. Abbreviations are AA, anthranilic acid; KYN, kynurenine; B_6, pyridoxal phosphate. Other abbreviations as in Figure 4

erythrocyte glutamic–oxalacetate aminotransferase, particularly in mild deficiency.

The Significance of the Tryptophan Load Test in Disease. Abnormal tryptophan load tests suggestive of a vitamin B_6 deficiency have been observed in a variety of diseases by many investigators (Price *et al.*, 1965; Musajo and Benassi, 1964). These diseases include Hodgkins' disease, rheumatoid arthritis, schizophrenia, porphyria, tuberculosis, aplastic anemia, scleroderma, and cancers. The question is whether these abnormalities represent a vitamin B_6 deficiency or some other effect of disease. In most instances the abnormality is corrected

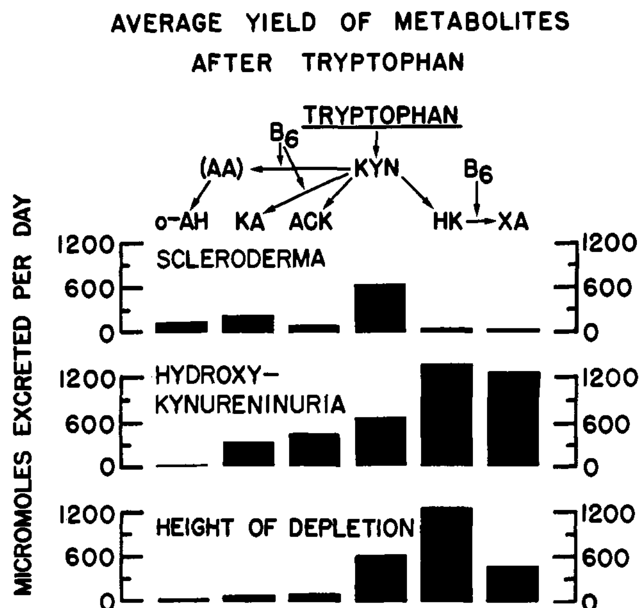

Figure 6A. Excretion of tryptophan metabolites by the subjects described in Fig. 6, after a 2.0 g loading dose of L-tryptophan. Note that the patient with hydroxykynureninuria had the marked excretion of all metabolites except *o*-aminohippuric acid (*o*-AH). Not shown is the inability to convert tryptophan to niacin metabolites. These data suggest that kynureninase function is severely impaired or absent

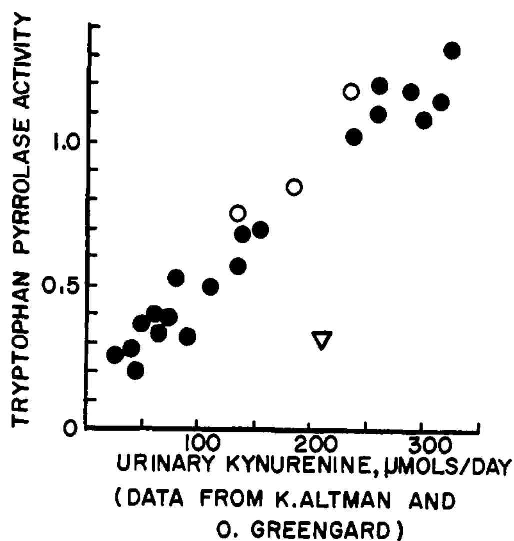

Figure 7. Graph of liver tryptophan oxygenase activity *vs.* urinary kynurenine in a variety of patients (closed circles). The open circles represent three subjects receiving hydrocortisone, the triangle represents one subject with a nutritional deficiency of vitamin B_6. Data from Altman and Greengard (1966). Reprinted with permission from *Journal of Clinical Investigation*

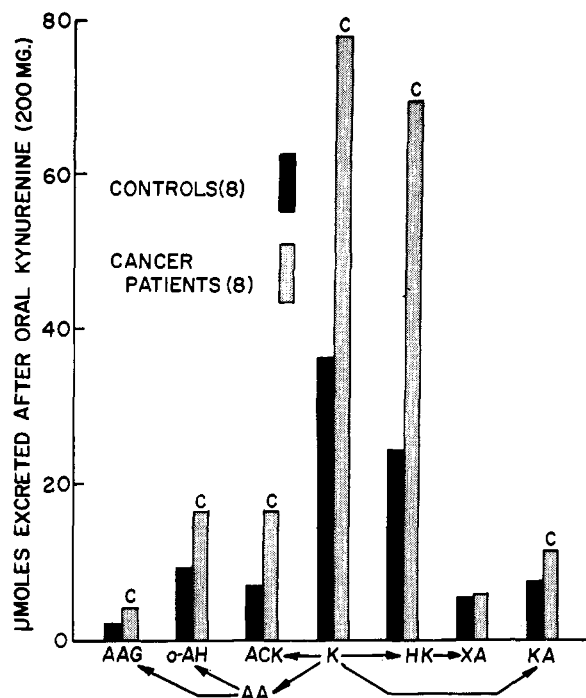

Figure 8. Excretion of metabolites in the 8-hr period after an oral load of 200 mg of L-kynurenine sulfate, given to eight cancer patients (six breast cancer, one each of Hodgkins' disease and bladder cancer), and eight age and sex matched control subjects. The letter C over a bar denotes a significant difference from control value with $p \leq 0.05$. Abbreviations are as in Figure 4

wholly or in part by supplemental pyridoxine, although in some diseases, it is not (Scriver, 1967; Price *et al.*, 1967b; Komrower and Westall, 1967). In some instances, the abnormality is found only during the acute phase of the disease and disappears when the disease is in a remission or quiescent phase (Rapoport and Beisel, 1971; Price *et al.*, 1959; Flinn *et al.*, 1964; DeVita *et al.*, 1971). This does not seem to be the case with the abnormalities observed in bladder cancer patients (Price and Brown, 1962). In this case, we have observed consistently abnormal tryptophan metabolism in patients who have been free of bladder tumors for prolonged periods. These abnormalities still respond to relatively small doses of vitamin B_6 (5 to 10 mg per day) (Brown, 1971). In comparison, the unique case of hydroxykynureninuria described by Komrower and Westall (1967) exhibited very abnormal tryptophan metabolism (Figures 6 and 6A), even without a tryptophan load. Administration of pyridoxine had little effect on this pattern. In this case, there is good evidence to believe that the enzyme kynureninase is defective or congenitally absent.

Undoubtedly, the abnormal tryptophan load tests found in many ill patients are not the result of an actual vitamin B_6 deficiency, but rather may be explained by the findings of Altman and Greengard (1966) who showed an excellent correlation between activity of liver tryptophan oxygenase and urinary kynurenine excretion in a variety of patients (Figure 7). It is known that this enzyme is inducible by elevated cortisol levels (endogenous or exogenous) (Knox and Greengard, 1964; Knox, 1965). Thus, Altman and Greengard suggest that the stress of a disease increases the adrenal secretion of cortisol which, in turn, induces higher levels of tryptophan oxygenase. In such a case, the tryptophan load may enter the kynurenine pathway at such a rate that subsequent enzymes, particularly kynureninase, become rate-limiting, thus allowing the accumulation and excretion of intermediates in elevated amounts. Supplemental pyridoxine then more fully saturates the kynureninase to allow it to operate more efficiently, relieving the rate-limiting step and decreasing urinary excretion of metabolites. This explanation undoubtedly holds for many of the abnormalities of tryptophan metabolism observed in various diseases. However, in some conditions, the problem seems to be more complicated. We have attempted to circumvent the inducible step of tryptophan oxygenase by giving loads of kynurenine to control subjects and cancer patients. If adaptation of the

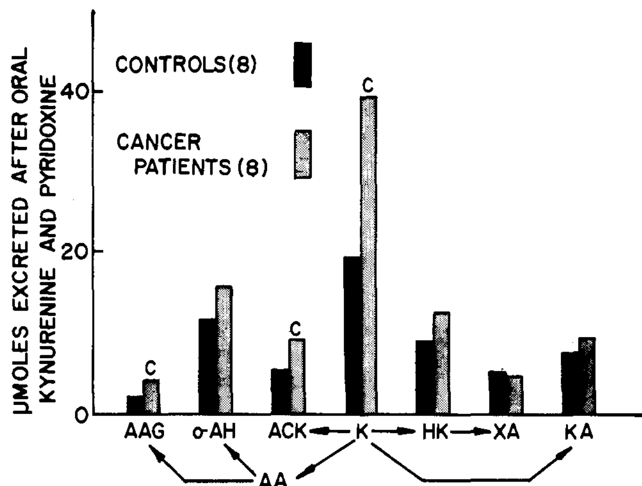

Figure 9. The experiment described in Figure 8 was repeated with all subjects receiving 75 mg of pyridoxine hydrochloride the day before the kynurenine load, and another 50 mg of pyridoxine at the time of the load. Abbreviations are the same as in Figure 8. Pyridoxine administration resulted in appreciable reduction of excretion of most metabolites in both control and patients. However, three of the metabolites are still elevated in the patients. The somewhat elevated levels of anthranilic acid glucuronide and *o*-aminohippuric acid (the products of kynureninase action) in the cancer patients suggests that impairment of kynureninase is not the explanation for the elevated levels of metabolites in the cancer patients

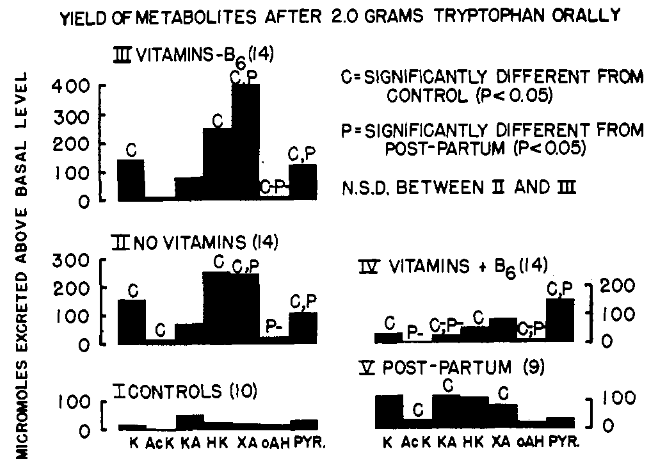

Figure 10. The yield (posttryptophan level minus basal level) of tryptophan metabolites by pregnant women after a 2.0 g load of L-tryptophan. Pregnant women in late second trimester or early third trimester were studied first without supplemental vitamins, then with all vitamins except pyridoxine, and again with all vitamins including 6 mg of pyridoxine per day. Two weeks elapsed between each study. The number of subjects is shown in parentheses. Data from Brown *et al.* (1961)

tryptophan oxygenase is the chief reason for abnormal tryptophan metabolism in cancer patients, such patients should handle kynurenine in a normal manner. This was not the case (Figure 8). Cancer patients excreted significantly higher levels of the kynurenine load than did age-matched control subjects. Administration of pyridoxine lowered the excretion of metabolites by both groups (Figure 9), and while the response to pyridoxine was somewhat more in the patients, they still remained significantly higher than the controls in their metabolite levels after pyridoxine. These data indicate that factors other than induced pyrrolase and vitamin B_6 deficiency play a role in the abnormal tryptophan metabolism observed in these patients. These factors may include reduced apokynureninase levels due to a catabolic state in the patient, reduced binding capacity of the apoenzyme for coenzyme, or altered absorption, transport, or excretion rates of metabolites (Spiera, 1966; Hansson, 1968) and perhaps other factors not yet considered.

Tryptophan Metabolism in Pregnancy and in Users of Oral Contraceptives. Pregnancy was one of the first conditions in which tryptophan metabolism was found to be abnormal (Sprince *et al.*, 1951; Vandelli, 1951; Brown *et al.*, 1961) and the response to supplemental pyridoxine (Figure 10) suggested that this abnormality was due to a vitamin B_6 deficiency. That the pregnant woman has low levels of vitamin B_6 has been shown by several investigators (Hamfelt and Hahn, 1969; Contractor and Shane, 1970; Brin, 1971). Hamfelt and Hahn (1969) showed a reciprocal relationship between xanthurenic acid excretion and blood PLP levels. Contractor and Shane (1970), using chromatographic and chemical methods for assay of the vitamers of vitamin B_6 and the phosphorylated metabolites (Figure 11), found lower levels of B_6 vitamers in the blood of mothers relative to the levels in fetal umbilical cord blood. Brin (1971) reported similar findings. The low levels of the vitamin in the pregnant women are probably the result of the increased needs arising from the developing fetus. However, the findings that the levels are significantly higher in cord blood indicate that cord

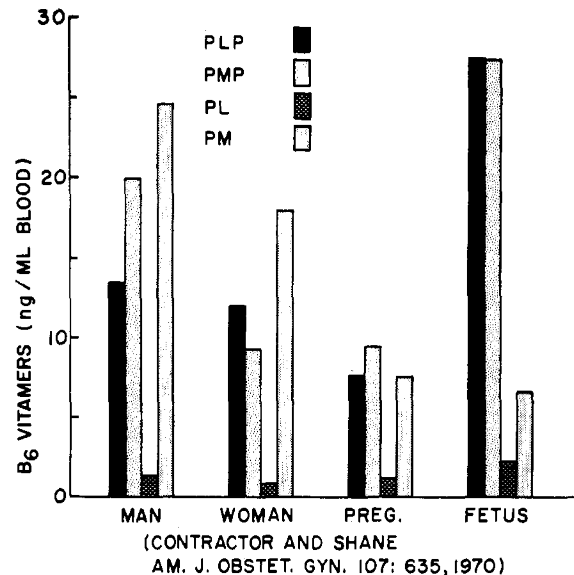

Figure 11. Levels of vitamin B_6 vitamers in blood of men, women, pregnant women, and fetal umbilical cords. Abbreviations are PLP, pyridoxal phosphate; PMP, pyridoxamine phosphate; PL, pyridoxal; PM, pyridoxamine. Data from Contractor and Shane (1970)

and mothers' blood are not in equilibrium, and suggests that the placenta must have a mechanism for actively concentrating the vitamin in the cord blood against a concentration gradient.

Since supplemental pyridoxine did not completely normalize the pattern of tryptophan metabolites in pregnant subjects, it was suggested that the hormonal changes of pregnancy may play a role in producing the metabolic picture found (Brown *et al.*, 1961). The changes in hormonal levels associated with the normal menstrual cycle were found to produce small but consistent changes in the tryptophan load test (Brown *et al.*, 1961; Rose, 1967), with elevated urinary tryptophan metabolites appearing at the time of maximum estrogen production. Subsequent studies in women taking oral contraceptives demonstrated remarkably altered tryptophan metabolism

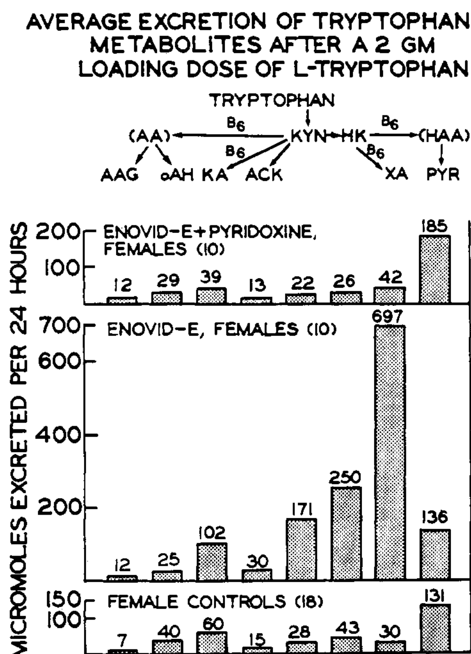

Figure 12. Excretion of tryptophan metabolites by women using Enovid E as an oral contraceptive. Abbreviations are the same as in Figure 4. Enovid E contains 0.1 mg of mestranol and 2.5 mg of norethinodrel. Data from Price *et al.* (1967a)

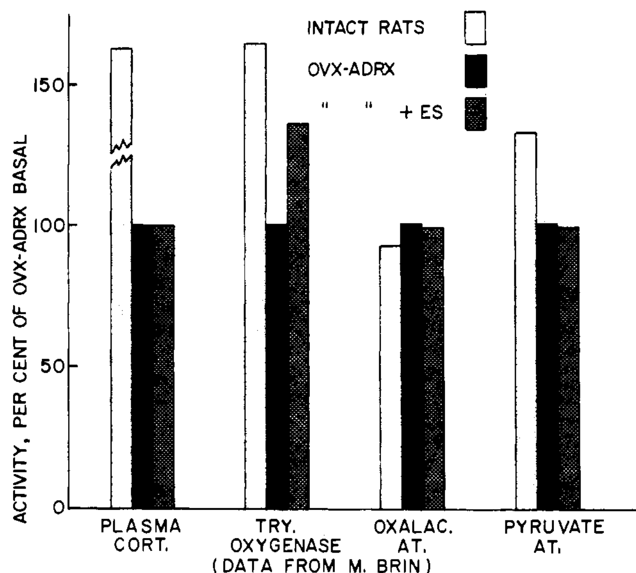

Figure 13. Activity of tryptophan oxygenase, oxalacetic aminotransferase, and pyruvate aminotransferase as related to plasma corticosterone levels in intact, ovariectomized–adrenalectomized rats and in these rats treated with ethinyl estradiol (ES). Ethinyl estradiol administration (8 μg orally per day for 4 days) produced a significant increase in liver tryptophan oxygenase but did not affect the two aminotransferases measured. Data from Brin (1971)

(Price *et al.*, 1967a; Rose, 1966), characterized by an excretion pattern of metabolites like that seen in pregnancy (Figure 12). The effect of the oral contraceptives on tryptophan metabolism could be reproduced by the estrogen component of the pill with the progestogen component having little effect (Rose, 1966; Wolf *et al.*, 1970). The effect was not restricted to women, since men treated with estrogens showed a similar change in their tryptophan metabolism (Rose, 1966; Wolf *et al.*, 1970). As in pregnancy, the tryptophan metabolism was responsive to pyridoxine supple-

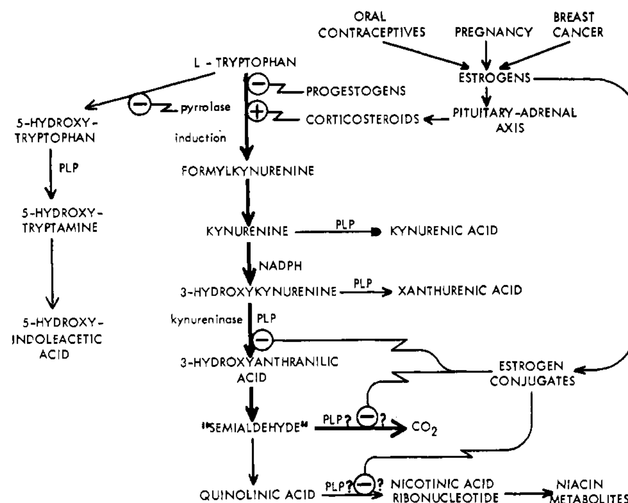

Figure 14. Tryptophan metabolic pathway showing probable regulatory factors. Zigzag lines with circles containing + or − indicate enhancement or suppression of the reaction

mentation (Price *et al.*, 1967a; Rose, 1966). Luhby *et al.* (1971) have done a careful study to determine the amount of pyridoxine necessary to correct the excretion of xanthurenic acid in women taking oral contraceptives. They suggest that as much as 30 mg per day may be required.

The mechanism by which estrogens affect tryptophan metabolism has received considerable attention, and a complex picture is beginning to emerge. Estrogens have been shown to enhance plasma corticosteroid levels in man (Sandberg and Slaunwhite, 1959) which would lead to elevated activity of tryptophan oxygenase. Indeed, estradiol benzoate administration significantly increased liver tryptophan oxygenase activity in intact rats (Rose and Braidman, 1971). Adrenalectomy resulted in a decrease of tryptophan oxygenase from intact values, but estradiol benzoate also increased the tryptophan oxygenase activity of the adrenalectomized rats. Also, estradiol increased this enzyme activity in adrenalectomized–ovariectomized rats (Figure 13) (Brin, 1971). Thus, in addition to the cortisol-mediated explanation for regulation of tryptophan metabolism offered by Altman and Greengard (1966), estrogens probably have a direct effect on the liver tryptophan oxygenase activity independent of adrenal activity. The induction of enzymes may play yet another role in this complex story. It is known that cortisol will induce a number of enzymes, among which are several which require PLP as a coenzyme. Thus, synthesis of new PLP-requiring apoenzymes will tend to deplete existing noninducible enzymes, such as kynureninase, of their cofactor and thus create a condition of PLP inadequacy. Yet another mechanism probably plays a part. Mason *et al.* (1969) have shown that conjugates of estrogens, such as estradiol disulfate, may act as competitive inhibitors of PLP binding to apoenzyme. Thus, if estrogen conjugates displaced PLP from kynureninase, an effective block in the kynurenine pathway of metabolism would ensue, even though tissue levels of PLP remained unchanged. In all likelihood, all of these mechanisms are acting in concert to produce the altered tryptophan metabolism resulting from estrogen administration. These interactions are presented schematically in Figure 14.

An important question arises from these considerations of tryptophan metabolism in pregnant women and in users of oral contraceptives: Should this altered tryptophan metabolism be corrected by pyridoxine administration? It should be pointed out that this altered metabolism is evident

mainly after the administration of a tryptophan load test. However, basal levels of tryptophan metabolites are also probably somewhat elevated since Toseland and Price (1969) have reported elevated levels of hydroxyanthranilic acid in basal urine of women on the pill. In the case of pregnant women, there seems little doubt that additional pyridoxine should be taken (Natl. Acad. Sci., 1968). In the absence of evidence of harmful effects of pyridoxine, the recent report of numerous birth defects in offspring of rats with deoxypyridoxine-induced vitamin B_6 deficiency (Davis *et al.*, 1970) dictates that reasonable amounts of pyridoxine be given pregnant women. In the case of women using oral contraceptives, again there seems to be no evidence to contraindicate supplementation with pyridoxine. Indeed, it has been reported that mental depression, which occurs in about 5 to 10% of subjects on the pill, will respond to supplemental pyridoxine (Baumblatt and Winston, 1970). In view of these and other metabolic changes observed in women using oral contraceptives (Salhanick *et al.*, 1969), it would seem prudent at this time to suggest supplementation with pyridoxine. However, long term, serial metabolic studies must be conducted in women using oral contraceptives to determine the effects of pyridoxine, not only on tryptophan metabolism, but on other metabolic, psychological, and physiological changes which result from the use of these agents.

SUMMARY

Factors affecting the production and assessment of vitamin B_6 deficiency in humans were discussed. Although the tryptophan load test has been widely used to assess vitamin B_6 deficiency, this test used alone is difficult to interpret since interrelated hormonal and metabolic factors play a major role in affecting the results obtained. Other methods of assessing vitamin B_6 nutrition, such as the determination of blood and tissue levels of the vitamin B_6 vitamers and the measurement of erythrocyte aminotransferases, should be used in conjunction with the tryptophan load test. Although the abnormal tryptophan metabolism observed in pregnant women and in women using oral contraceptives is only in part due to a vitamin B_6 deficiency, it seems reasonable to suggest that such women receive supplemental pyridoxine. However, serial studies will be necessary to establish the long-term effects of such supplementation.

LITERATURE CITED

Altman, K., Greengard, O., *J. Clin. Invest.* **45,** 1527 (1966).
Arend, R. A., Leklem, J. E., Brown, R. R., *Biochem. Med.* **4,** 457 (1970).
Baker, E. M., Canham, J. E., Nunes, W. T., Sauberlich, H. E., McDowell, M. E., *Amer. J. Clin. Nutr.* **15,** 59 (1964).
Baumblatt, M. J., Winston, F., *Lancet* **1,** 832 (1970).
Biehl, J. P., Vilter, R. W., *Proc. Soc. Exp. Biol. Med.* **85,** 389 (1954).
Brin, M., *Amer. J. Clin. Nutr.* **24,** 699 (1971).
Brin, M., *Methods Enzymol.* **18,** 519 (1970).
Brown, R. R., Thornton, M. J., Price, J. M., *J. Clin. Invest.* **40,** 617 (1961).
Brown, R. R., Yess, N., Price, J. M., Linkswiler, H., Swan, P., Hankes, L. V., *J. Nutr.* **87,** 419 (1965).
Brown, R. R., unpublished observations (1971).
Canham, J. E., Baker, E. M., Harding, R. S., Sauberlich, H. E., Plough, I. C., *Ann. N.Y. Acad. Sci.* **166,** 16 (1969).
Cinnamon, A. D., Beaton, J. R., *Amer. J. Clin. Nutr.* **23,** 696 (1970).
Contractor, S. F., Shane, B., *Amer. J. Obstet. Gynecol.* **107,** 635 (1970).
Contractor, S. F., Shane, B., *Clin. Chim. Acta* **21,** 71 (1968).
Coursin, D. B., *Amer. J. Clin. Nutr.* **14,** 56 (1964).
Coursin, D. B., Brown, V. C., *Amer. J. Obstet. Gynecol.* **82,** 1307 (1961).
Davis, S. D., Nelson, T., Shepard, T. H., *Science* **169,** 1329 (1970).
DeVita, V. T., Chabner, B. A., Livingston, D. M., Oliverio, V. T., *Amer. J. Clin. Nutr.* **24,** 835 (1971).
Flinn, J. H., Price, J. M., Yess, N., Brown, R. R., *Arthritis Rheum.* **7,** 201 (1964).
Frimpter, G. W., Anderson, R. J., George, W. F., *Amer. J. Clin. Nutr.* **22,** 794 (1969).
Greenberg, L. D., Bohr, D. F., McGrath, H., Rinehart, J. F., *Arch. Biochem.* **21,** 237 (1949).
Hamfelt, A., Hahn, L., *Clin. Chim. Acta* **25,** 91 (1969).
Hansson, O., *Acta Soc. Med. Upsal.* **73,** 11 (1968).
Harris, J. W., Horrigan, D. L., *Vitam. Horm.* **22,** 721 (1964).
Harris, R. S., Wool, I. G., Loraine, J. A., *Vitam. Horm.* **22,** 1 (1964).
Hughes, P. A. M., Raine, D. N., *Clin. Chim. Acta* **14,** 399 (1966).
Jaffe, I. A., Altman, K., Merryman, P., *J. Clin. Invest.* **43,** 1869 (1964).
Knox, W. E., *Advan. Enzyme Regul.* **4,** 287 (1965).
Knox, W. E., Greengard, O., *Advan. Enzyme Regul.* **3,** 247 (1964).
Komrower, G. M., Westall, R., *Amer. J. Dis. Child.* **113,** 77 (1967).
Luhby, A. L., Brin, M., Gordon, M., Davis, P., Murphy, M., Spiegel, H., *Amer. J. Clin. Nutr.* **24,** 684 (1971).
Mason, M., Ford, J., Wu, H. L. C., *Ann. N.Y. Acad. Sci.* **166,** 170 (1969).
McCoy, E. E., Colombini, C., Ebadi, M., *Ann. N.Y. Acad. Sci.* **166,** 116 (1969).
Miller, L. T., Linkswiler, H., *J. Nutr.* **93,** 53 (1967).
Mueller, J. F., *Vitam. Horm.* **22,** 787 (1964).
Musajo, L., Benassi, C. A., *Advan. Clin. Chem.* **7,** 63 (1964).
Natl. Acad. Sci., "Recommended Dietary Allowances (7th rev. ed.), Natl. Res. Council Publ. 1694 (1968).
Ogasawara, N., Hagino, Y., Kotake, Y., *J. Biochem. (Tokyo)* **52,** 162 (1962).
Price, J. M., Brown, R. R., *Acta Unio Int. Contra Cancrum* **18,** 684 (1962).
Price, J. M., Brown, R. R., Larson, F. C., *J. Clin. Invest.* **36,** 1600 (1957).
Price, J. M., Brown, R. R., Peters, H. A., *Neurology* **9,** 456 (1959).
Price, J. M., Brown, R. R., Yess, N., *Advan. Metab. Disorders* **2,** 159 (1965).
Price, J. M., Thornton, M. J., Mueller, L. M., *Amer. J. Clin. Nutr.* **20,** 452 (1967a).
Price, J. M., Yess, N., Brown, R. R., Johnson, S. A. M., *Arch. Dermatol.* **95,** 462 (1967b).
Rapoport, M. I., Beisel, W. R., *Amer. J. Clin. Nutr.* **24,** 807 (1971).
Robinson, F. A., "The Vitamin B Complex," Chapman & Hall, London, 1951, p 688.
Rose, D. P., *Clin. Chim. Acta* **18,** 221 (1967).
Rose, D. P., *Clin. Sci.* **31,** 265 (1966).
Rose, D. P., Braidman, I. P., *Amer. J. Clin. Nutr.* **24,** 673 (1971).
Salhanick, H. A., Kipnis, D. M., Vande Wiele, R. L., "Metabolic Effects of Gonadal Hormones and Contraceptive Steroids," Plenum Press, New York, 1969.
Sandberg, A. A., Slaunwhite, W. R., Jr., *J. Clin. Invest.* **38,** 1290 (1959).
Scriver, C. R., *Amer. J. Dis. Child.* **113,** 109 (1967).
Snell, E. E., *Vitam. Horm.* **16,** 77 (1958).
Spiera, H., *Arthritis Rheumat.* **9,** 318 (1966).
Sprince, H., Lowy, R. S., Folsome, C. E., Behrman, J. S., *Amer. J. Obstet. Gynecol.* **62,** 84 (1951).
Storvick, C. A., Peters, J. M., *Vitam. Horm.* **22,** 833 (1964).
Toseland, P. A., Price, S., *Brit. Med. J.* **1,** 777 (1969).
Ueda, T., *Nagoya J. Med. Sci.* **30,** 259 (1967).
Vandelli, I., *Acta Vitaminol. (Milan)* **5,** 55 (1951).
Williams, M. A., *Vitam. Horm.* **22,** 561 (1964).
Wiss, O., Weber, F., *Vitam. Horm.* **22,** 495 (1964).
Wolf, H., Price, J. M., Brown, R. R., Madsen, P. O., *Scand. J. Clin. Lab. Invest.* **25,** 237 (1970).
Yess, N., Price, J. M., Brown, R. R., Swan, P. B., Linkswiler, H., *J. Nutr.* **84,** 229 (1964).

Received for review July 9, 1971. Accepted October 4, 1971. This investigation was supported by USPHS Research Grant No. 5 RO1 CA-11331 from the National Cancer Institute; Recipient of Career Development Award No. CA-K3-18,404 from the National Cancer Institute, U.S. Public Health Service. Presented at the Division of Agricultural and Food Chemistry, 161st Mtg. ACS, Los Angeles, Calif., March–April 1971.