



REVIEW ARTICLE

A REVIEW OF METHODS FOR INDUCING VITAMIN B12 DEFICIENCY IN ANIMAL MODELS

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ABSTRACT

Vitamin B12 (cobalamin) deficiency is a significant global health issue, particularly impacting older adults, vegetarians, and those with gastrointestinal disorders. It is linked with a broad spectrum of clinical symptoms, including megaloblastic anaemia, neurological impairments, and cardiovascular problems. To explore the pathophysiology of this deficiency and assess potential therapeutic approaches, animal models are essential. This review concentrates on various established techniques for inducing vitamin B12 deficiency in animal models, particularly rodents. Three main methods are examined: the nitrous oxide-induced functional deficiency model, the restricted diet model, and the modified diet model that omits vitamin B12. The nitrous oxide model is swift and can reproduce the neurological and hematologic symptoms of deficiency; however, it carries risks such as hypoxia and neurotoxicity. The restricted diet model consists of feeding animals a diet devoid of B12 for a prolonged period, which is safer but requires more time. The modified diet model incorporates pectin, which binds to intrinsic factor and obstructs B12 absorption, providing an alternative way to mimic issues with bioavailability. Each method presents unique advantages and drawbacks regarding safety, induction duration, reproducibility, and clinical relevance. Additionally, this review outlines the physiological pathways for B12 absorption and emphasizes the diagnostic tools utilized to evaluate deficiency, including serum B12

levels, methylmalonic acid, homocysteine, and holotranscobalamin. Gaining insight into these models and diagnostic methods is crucial for progressing research on vitamin B12 deficiency and crafting more effective prevention and treatment options. This review acts as a guide for researchers in choosing suitable models to investigate the clinical and biochemical dimensions of vitamin B12 deficiency.

Keywords: *Absorption pathway of Vitamin B12, Deficiency, Haematological Test, Screening models*

INTRODUCTION

Vitamin B12 is a water-soluble nutrient which is synthesized by few bacteria. In human's bacterial synthesis occurs in the large intestine, but absorption from large intestine is not possible. Therefore, dietary intake of Vitamin B12 becomes essential. Vitamin B12 plays vital role in growth, DNA synthesis, cellular metabolism, bone marrow synthesis, as metabolic co-factor for gastrointestinal tract microbes, and in regeneration of myelin sheath in nervous tissue [1]. The main forms of cobalamin are cyanocobalamin (most stable form), methylcobalamin, adenosylcobalamin, and hydroxycobalamin. In the body, cyanocobalamin converts to methylcobalamin (a coenzyme for methionine synthesis) and adenosylcobalamin (involved in amino acid metabolism, including valine, leucine, and threonine) [2, 3].

One of the most captivating and intriguing molecules in the realms of science and medicine is vitamin B12, which was extracted from liver in 1955 and found to be effective in treating pernicious anaemia. Cobalamin is the official name for vitamin B12, as it contains the heavy metal cobalt, which imparts a red hue to this water-

soluble vitamin. The chemical formula for vitamin B12 is $C_{63}H_{90}CoN_{14}O_{14}$ [1]. This nutrient is vital for maintaining the health of the body's nerve and blood cells, and it also plays a role in the synthesis of DNA [2]. Vitamin B12 is a crucial growth factor and is important in the metabolism of cells, particularly those found in the gastrointestinal tract, bone marrow, and nervous tissue. Furthermore, vitamin B12 has been utilized in contemporary research as a key element in the medium for cell line growth [1, 3].

Vitamin B12 (molecular weight = 1355.4) is part of the "corrinoids" category, which includes compounds that have a corrin macrocycle. The label "Vitamin B12" typically refers specifically to cyanocobalamin, the most stable and non-natural form of cobalamin, although it can also mean any biologically active cobalamins. Cyanocobalamin is commonly found in human dietary supplements and is easily converted into the coenzyme forms of cobalamin. For example, methylcobalamin acts as a coenzyme for methionine synthase, which plays a role in methionine synthesis, while 5'-deoxyadenosylcobalamin serves as a coenzyme for methylmalonyl-CoA mutase,

which is involved in the metabolism of amino acids and odd-chain fatty acids in mammalian cells [4].

Vitamin B12 is produced exclusively by specific bacteria, and it mainly accumulates in the organisms at the top of

the food chain. It is widely recognized that Vitamin B12 is the only vitamin that is either missing or found in very small quantities in plant-based foods. Animal-derived foods such as meat, milk, eggs, fish, and shellfish are the primary sources of Vitamin B12 in the diet [5] (Table 1).

Table 1: The recommended dietary allowance (RDA) of Vitamin B12 depending upon age [5, 6]

Sr.no	Life stages	Recommended amount (mcg)
1.	Birth to 6 months	0.4mcg
2.	Infants 7-12 months	0.5mcg
3.	Children 1-3 years	0.9mcg
4.	Children 4-8 years	1.2mcg
5.	Children 9-13 years	1.8mcg
6.	Teens 14-18 years	2.4mcg
7.	Adults	2.4mcg
8.	Pregnant teens and women	2.6mcg
9.	Breastfeeding teens and women	2.8mcg

EPIDEMIOLOGY

The primary indicators of Vitamin B12 deficiency include pernicious anaemia and neuropathy. The prevalence of vitamin B12 deficiency depends on its underlying causes. Research in the general population has indicated that among individuals with anaemia, roughly 1% to 2% is attributed to B12 deficiency. Similarly, other investigations found that among patients presenting with clinical macrocytosis (characterized by an MCV greater than 100), approximately 18% to 20% were

found to have a deficiency in vitamin B12. This deficiency is particularly prevalent among the elderly, irrespective of its origin. Furthermore, vitamin B12 deficiency resulting from pernicious anaemia is more frequently observed in individuals of Northern European descent. Conversely, the occurrence of pernicious anaemia is less common among individuals of African descent or those from various regions of Europe [6, 7].

A deficiency in Vitamin B12 (cobalamin) is a frequent contributor to macrocytic

anaemia and has been associated with a range of neuropsychiatric conditions. The link between B12 deficiency and hyperhomocysteinemia, as well as its potential role in the advancement of atherosclerosis, is currently being investigated [8].

A lack of vitamin B12 can impact people of any age, but it is especially prevalent among the elderly. Babies, children, teenagers, and women of reproductive age are also at elevated risk for deficiency in groups where the consumption of B12-rich animal foods is limited. This deficiency can result from insufficient intake, poor bioavailability, or issues with absorption. Additionally, problems with B12 transport in the bloodstream, as well as hindered cellular uptake or metabolism, can lead to a deficiency at the cellular level [9].

Diagnostic indicators for vitamin B12 status consist of reduced concentrations of circulating total B12 and transcobalamin-bound B12, along with elevated levels of homocysteine and methylmalonic acid. Nonetheless, the precise thresholds for defining clinical and subclinical deficiency are still a matter of debate. Treatment involves B12 supplementation, which may be administered through high-dose oral methods or via injections [10].

ABSORPTION OF VITAMIN B12

Peripheral absorption of Vitamin B12 in humans

The primary types of cobalamins include cyanocobalamin, methylcobalamin, deoxyadenosylcobalamin, and hydroxycobalamin, with cyanocobalamin being the most stable variant. Within the body, cyanocobalamin is transformed into methylcobalamin and adenosylcobalamin. Methylcobalamin serves as a coenzyme crucial for methionine synthase, which plays a role in the synthesis of methionine, while adenosylcobalamin acts as a coenzyme essential for methylmalonic-CoA mutase, which is involved in the synthesis of amino acids. Mammals such as cats, rodents, humans, and bats are unable to produce vitamin B12, with only certain bacteria capable of its synthesis. In humans, bacterial production occurs in the large intestine, but absorption from that area is not feasible, resulting in a complete reliance on dietary sources for vitamin B12. The uptake of vitamin B12 from food happens through four distinct phases [11].

- **Intragastric events:** Vitamin B12 is detached from its binding protein and subsequently attaches to haptocorrin (also referred to as R-protein or transcobalamin-I), which is produced by the salivary glands. This protein has a strong affinity for vitamin B12 and shields the acid-sensitive vitamin B12 from the stomach's acidic environment. The binding affinity of R-protein for vitamin B12 is 50 and three times

greater than that of human intrinsic factor at pH levels of 2 and 8, respectively. The haptocorrin-B12 complex then moves through the stomach via the pylorus to the duodenum [12].

- Duodenal and jejunal events: In the duodenum, haptocorrin is broken down by pancreatic proteases and pepsin (which is produced as pepsinogen by the parietal cells of the stomach and activated into pepsin in the presence of HCl), releasing vitamin B12 in its unbound form. The unbound vitamin B12 quickly attaches to intrinsic factor (IF), a glycoprotein secreted by the parietal cells of the stomach, forming the IF-B12 complex. This complex then travels through the small intestine [7].
- Ileal events: In the small intestine, the complex reaches the terminal part known as the ileum, which is the longest segment of the small intestine compared to its other sections. The ileum contains specialized membrane receptors known as cubilin receptors. These receptors are highly selective, allowing only the IF-B12 Complex to pass through and rejecting vitamin B12 that is bound to different carriers. Vitamin B12 has various binding sites to connect with both the cubilin receptor and intrinsic factor. The interaction between the IF-B12 Complex and the cubilin receptor occurs without the need for energy, relying instead on the presence of

calcium ions and a neutral pH. Cubilin receptors are located at the base of pits situated between the microvilli in the small intestine. The extent of vitamin B12 absorption is influenced by the quantity of vitamin B12 present in the diet. The transport mechanism of vitamin B12 remains partially unclear. Intrinsic factor does not enter the bloodstream; instead, it may be taken up by the cell surface or transported into the cells along with vitamin B12. Following the absorption of vitamin B12 from the small intestine, there is a delay of several hours before its detection in the bloodstream. This delay is attributed to the intracellular processes involved in the absorption of vitamin B12. After its absorption into the cells, there is a notable increase in mitochondrial radioactivity, which diminishes as hepatic activity rises. This delay is due to the metabolism of vitamin B12 within the mitochondria. Subsequently, vitamin B12 exits the enterocyte and enters the portal plasma. In the portal plasma, vitamin B12 attaches to transcobalamin-II, which is crucial for the proper absorption of vitamin B12. Transcobalamin-II facilitates the circulation of vitamin B12 throughout the bloodstream [5] [11].

- Enterohepatic circulation: The enterohepatic circulation of vitamin B12 is known as such because the quantity of vitamin B12 expelled in bile exceeds that found in urine and faeces.

Approximately 4µg of vitamin B12 released from the liver is bound to R-binder. In the presence of pancreatic enzymes, the bile R-binder is broken down, allowing the transfer of vitamin B12 to intrinsic factor in the upper small intestine. Around half of the hepatic vitamin B12 is reabsorbed, while the portion not attached to intrinsic factor is eliminated from the body. Additionally, the bile is regarded as the primary pathway for eliminating vitamin B12 analogues. Nonetheless, bile is deemed crucial for vitamin B12 absorption, potentially due to the dissociation of the vitamin B12-intrinsic factor complex or the uptake of vitamin B12 by receptors in the ileum [5] [12]

Cellular absorption of vitamin B12 in humans

At the cellular level, only the IF-Vitamin B12 Complex is absorbed. This complex binds to the CD320 (Cluster of Differentiation) receptor. The CD320

receptor is a substrate-specific protein that permits only the IF-Vitamin B12 Complex to enter the cell. From the CD320, the IF-Vitamin B12 Complex moves into the lysosome, where it is broken down in the presence of two lysosomal proteins: the lysosomal cobalamin transporter (LMBD1) and the lysosomal membrane transporter ABCD4. Subsequently, within the cytoplasm and aided by two chaperones—methylmalonic aciduria and homocystinuria type C protein (MMACHC) and (MMADHC)—vitamin B12 is directed towards the mitochondria. Inside the mitochondria, the enzyme methylmalonyl-CoA (MUT) along with two mitochondrial proteins (cblA and cblB) play a role in transferring and maintaining the adenosyl group to adenosylcobalamin, with mutations in these proteins leading to methylmalonic aciduria cblA and cblB types (MMAA and MMAB), respectively; plus the two corresponding target enzymes for methyl B12 [methionine synthase reductase (MTRR) and methionine synthase (MTR)] [9, 7, 11] (Table 2) (Figure 1).

Table 2: Phases of vitamin B12 absorption [5, 8, 7, 11, 12]

Sr. No.	Phases of vitamin B12 absorption	
	Absorption events	Steps of Absorption Events
1	Gastric events	i. Detachment of vitamin B12 from dietary proteins. ii. Binding of vitamin B12 by R-protein.
2	Duodenal and jejunal events	i. Breakdown of R-protein. ii. Attachment of vitamin B12 to intrinsic factor. iii. Creation of the IF-Vitamin B12 complex.
3	Ileal events	i. Attachment of IF- Vitamin B12 COMPLEX to receptor. ii. Transport of vitamin B12 across enterocytes. iii. Release of vitamin B12 into blood plasma.
4	In blood plasma	i. Binding of the IF- Vitamin B12 COMPLEX to the receptor. ii. Movement of vitamin B12 through enterocytes. iii. Transfer of vitamin B12 into blood plasma.
5	Cellular absorption	i. The binding of transcobalamin-II-vitamin B12 to the CD320* receptor on the cell membrane. ii. The transcobalamin-II-vitamin B12 complex is internalized into the lysosome. iii. The degradation of the transcobalamin-II-vitamin B12 complex. iv. The transformation of vitamin B12 into methylcobalamin and adenosylcobalamin.
6	Enterohepatic circulation	i. Elimination of B12 and its analogues bound to R-protein through bile. ii. Breakdown of R-protein by pancreatic enzymes leading to the creation of the B12-Intrinsic factor complex. iii. Reabsorption of B12 in the ileum and the excretion of B12 analogue in faeces.

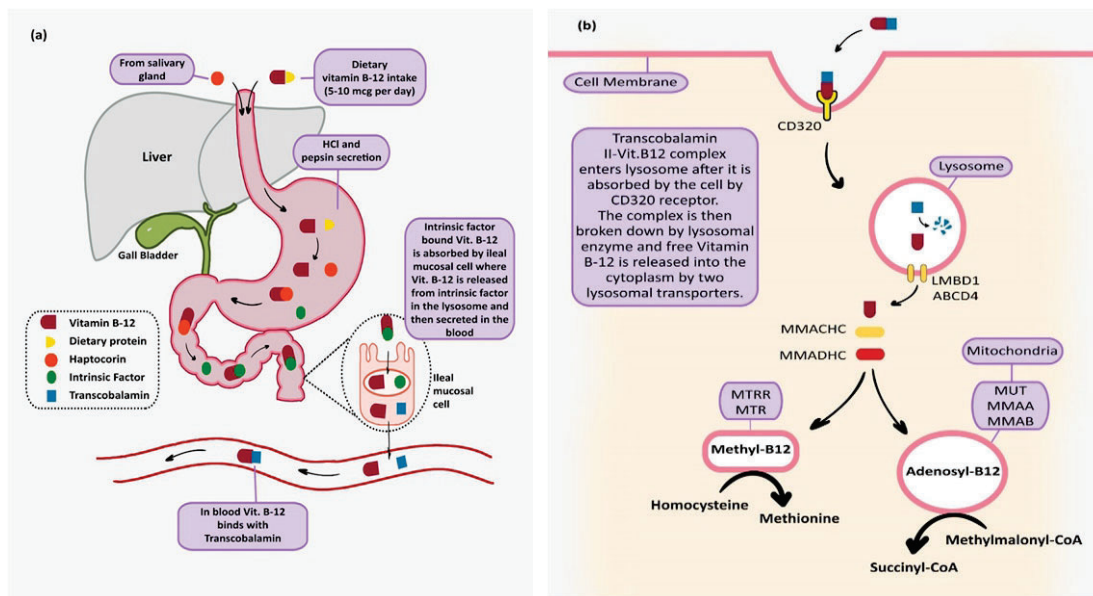


Figure 1 (a) Absorption of vitamin B1, (b) Cellular uptake of vitamin B12

CD320 Receptor – Cluster of Differentiation 320 receptor; *LMBD1*- lysosomal cobalamin transporter; *ABCD4*- lysosomal membrane transporter; *MMACHC*- methylmalonic aciduria and homocysteine type-c protein, *MMADHC*-methylmalonic aciduria and homocysteine type-D protein, *MUT*- mitochondrial enzyme methylmalonyl- CoA, *cblA* and *cblB*- two mitochondrial proteins, *MMAA*- methylmalonic aciduria *cblA*, *MMAB*- methylmalonic aciduria *cblB*, *MTRR*- methyl-B12 (methionine synthase reductase), *MTR*- methionine synthase .

VITAMIN B12 DEFICIENCY

Vitamin B12 deficiency is a significant public health concern, especially among older adults and those who follow vegetarian diets. Typically, Vitamin B12 is found in animal-based foods, although certain plant foods do provide meaningful amounts of this vitamin. The lack of Vitamin B12 is linked to hematologic, neurologic, and psychiatric issues. It is a frequent cause of macrocytic (megaloblastic) anaemia and, in severe instances, pancytopenia. Neurological

complications resulting from Vitamin B12 deficiency can include tingling sensations, peripheral nerve damage, and demyelination of the corticospinal tract and dorsal columns (subacute combined degeneration).

MAIN CAUSES OF VITAMIN B12 DEFICIENCY

- Autoimmune causes: Pernicious anaemia is an autoimmune disorder characterized by the production of antibodies against intrinsic factor. These

anti-intrinsic factor antibodies attach to intrinsic factor and prevent it from functioning properly, leading to a failure in the absorption of vitamin B12 in the terminal ileum [13].

- **Malabsorption causes:** Intrinsic factor, produced by parietal cells in the stomach, is essential for B12 absorption. Consequently, individuals with a history of gastric bypass surgery may be susceptible to B12 deficiency since their altered alimentary pathway circumvents the area where intrinsic factor is generated. For patients who produce intrinsic factor normally, any damage to the terminal ileum—such as that resulting from surgical resection due to Crohn's disease—can hinder B12 absorption, leading to a deficiency. Additionally, other forms of small intestine damage, including inflammation caused by Celiac disease or infection with the tapeworm *Diphyllobothrium latum*, may also lead to a deficiency in B12 [14].
- **Nutritional causes:** Vitamin B12 is retained in large amounts in the liver; however, individuals who have adhered to a strict vegan diet for around three years could experience a B12 deficiency due to insufficient dietary consumption [13].
- **Other GI Causes:** Other causes of vitamin B12 deficiency, while less frequent, are worth noting. Individuals exhibiting signs of vitamin B12

deficiency along with long-term gastrointestinal issues like dyspepsia, recurrent peptic ulcers, or diarrhoea may need to be assessed for conditions such as Whipple's disease (an uncommon bacterial infection that disrupts absorption) or Zollinger-Ellison syndrome (a gastrinoma responsible for peptic ulcers and diarrhoea). Those with a background of intestinal surgery, strictures, or blind loops could experience bacterial overgrowth that competes for dietary vitamin B12 in the small intestine, similar to cases involving tapeworms or other intestinal parasites. Rarely, congenital transport-protein deficiencies, including transcobalamin II deficiency, can also lead to vitamin B12 deficiency [7, 11, 14].

SYMPTOMS OF VITAMIN B12 DEFICIENCY

Severe Symptoms

- a. Haematological disorders.
 - i. Pancytopenia (leukopenia, thrombocytopenia).
 - ii. Pernicious anaemia.
- b. Neurological disorders.
 - i. Sensory and motor deficiencies.
 - ii. Subacute degeneration of spinal cord.
 - iii. Dementia.

- c. Reduced growth and healing.
- d. CVS problems.
 - i. Coronary disease.
 - ii. Myocardial infraction [15].

Common Symptoms

Common symptoms include pale skin, loss of appetite, tinnitus, weakness and fatigue, numbness, blurred vision, sore tongue or mouth ulcers, breathlessness, depression. , decline in memory, judgment, and understanding [15, 16].

INDUCTION OF VITAMIN B12 DEFICIENCY

Vitamin B12 deficiency has become a significant health concern today. Therefore, it is necessary to study vitamin B12 deficiency and treatment associated with it. Mammals (human, rats, etc.) rely entirely on dietary sources for vitamin B12 as they cannot synthesis vitamin B12. Induction of vitamin B12 deficiency and deficiency related complications in mammals is difficult as very few animal models are available for induction. The normal range of Vitamin B12 in rats is 500-840 µg/ml. Following are the experimental models for vitamin B12 deficiency and its complications.

- a. The Rat Model of Nitrous Oxide-Induced Vitamin B12 Deficiency
- b. Restricted Diet Model
- c. Modified vitamin B12 free diet Model

The Rat Model of Nitrous Oxide-Induced Vitamin B12 Deficiency

Since animals cannot synthesize vitamin B12 on their own and must get it through diet, it can be difficult to induce vitamin B12 insufficiency and the complications that come with it in animals. Since rat's store vitamin B12 in their kidneys and livers and release it when needed, it is challenging to cause a real vitamin B12 deficiency in animals by dietary limitations. Therefore, in order to assess the effectiveness of vitamin B12 supplements, it is crucial to convert the stored vitamin B12 into an inactive form.

- Principle: It is observed that continuous exposure to nitrous oxide gas can induce vitamin B12 deficiency in animals and humans. As nitrous oxide acts as oxidizing agent and converts cobalamin -I (active form) to cobalamin -II (inactive form). cobalamin -I (active form) promotes transferring of methyl group from methylcobalamine to homocysteine for the formation of tetrahydrofolate and methionine causing alteration in DNA synthesis, protein synthesis. Therefore, nitrous oxide gas in combination with oxygen can act as vitamin B12 deficiency inducer.
- Materials and method
 - o Animal selection: Preferably Healthy adult Female Wistar Albino Rats aging 8-10 weeks, in the range of 200-250g are selected.

- o Housing condition: Feeding Regime: Vitamin B12 Deficient pellet feed and pure drinking water will be supplied ad libitum.
- o Procedure: Animals will be kept in gas mixture of 20% N2O and 80% O2 for 48 hours in a sealed chamber. After 48 hours the animals are removed from gas chamber and blood is collected and test for vitamin B12 deficiency immediately after removal from chamber [17].
- Advantages:
 - o Induction time is less.
 - o Can induce complication like- numbness, peripheral neuropathy, megaloblastic anaemia like complication associated with Vitamin B12 Deficiency.
- Limitations:
 - o Brain and nerve damage
 - o Hypoxia
 - o Memory and cognitive impairment
 - o Disorientation, Dissociation
 - o Dizziness
 - o Peripheral neuropathy
 - o Diet restriction of Vitamin B12 is also required [17, 18]

Restricted Diet Model

Mammals such as human, rodents required Vitamin B12 in its food as they cannot synthesis Vitamin B12 on their own. Therefore, the food used to feed laboratory animals naturally contains vitamin B12, making it a direct source of this nutrient for them. As a result, assessing the effects of newly developed drugs becomes challenging. To accurately study these effects, it is essential to remove vitamin B12 from the diet. However, inducing vitamin B12 deficiency by diet restriction is a long-term process that takes several months to develop.

- Principle: The primary source of vitamin B12 for mammals that cannot produce it themselves comes from animal-derived food products. Research has shown that strict vegetarians, vegans, and elderly individuals are more susceptible to vitamin B12 deficiency due to insufficient dietary intake of this nutrient. Consequently, to assess vitamin B12 levels in animals, it is necessary to eliminate vitamin B12 from their diet and evaluate the resulting levels.
- Materials and Method:
 - o Animal selection: Preferably Healthy adult Female Wistar Albino Rats aging 8-10 weeks, in the range of 200-250g are selected

- o Housing condition: Feeding Regime: Vitamin B12 Deficient pellet feed and pure drinking water will be supplied ad libitum.

- Procedure:

Vitamin B12 deficient food is prepared by removal of vitamin B12 supplement

from diet. Normal diet of animals is replaced by vitamin B12 free diet and then the animal is regularly observed for induction of vitamin B12 deficiency. Development of vitamin B12 deficiency is evaluated by blood test. The ingredients for preparation used are as presented in Table 3.

Table 3: vitamin B12 free diet

Sr.no.	Ingredients	Quantity (g)
1	Defatted soyabean	400
2	Glucose anhydrous	443
3	DL- Methionine	5
4	Soyabean oil	100
5	Salt mixture	50
6	Vitamin B12 free vitamin mixture	5
7	Choline chloride	2
8	Cellulose powder	10
Total Feed		1015 g

- Advantages:

- o Use of anaesthetic gas such as nitrous oxide is not required
- o Safe in comparison with Nitrous oxide model

- Limitations:

- o Time consuming require a month for induction.
- o Difficult to induce vitamin B12 related problems in short duration [19].

Modified Vitamin B12 Free Diet Model

Many studies have indicated crucial role of vitamin B12 in humans. Therefore, it is necessary to study about vitamin B12 deficiency. The main purpose of inducing vitamin B12 deficiency in experimental animals is to study the symptoms or problems induced by vitamin B12 deficiency.

- Principle: This study aims induce vitamin B12 deficiency by modifying the diet of animals. This modified diet is prepared by using 50g pectin/ kg.

Pectin binds to intrinsic factor in stomach and does not allow binding of vitamin B12 to intrinsic factor and makes vitamin B12 less bioavailable.

- **Materials and Method:**
 - o **Animal selection:** Preferably Healthy adult Female Sprague Dawley Rats aging 8-10 weeks, in the range of 200-250g are selected
 - o **Housing condition: Feeding Regime:** Vitamin B12 modified diet pellet feed and pure drinking water will be supplied ad libitum.
- **Procedure:** Vitamin B12 modified food is prepared by addition of 50g pectin/kg in normal diet and then it is replaced by normal diet. Food intake is recorded daily to establish direct correlation between the amount of food intake and extent of vitamin B12 deficiency induction. Blood is collected after 8 weeks and 16 weeks to evaluate the extent of vitamin B12 deficiency
- **Advantages:**
 - o Safe in comparison with Nitrous oxide model
- **Limitations:**
 - o Lengthy process.
 - o Difficult to induce vitamin B12 related problems in short duration.

- o After induction of vitamin B12 deficiency with pectin we need to stop the administration of pectin as it can damage the vitamin B12 content from the formulation as well. Furthermore, for evaluation of the efficacy of the formulation or sources we need to feed animals with Vitamin B12 free diet [20].

HAEMATOLOGICAL EVALUATION OF VITAMIN B12 DEFICIENCY

Defining vitamin B12 deficiency is challenging due to its low but critical requirement. While scientific literature uses pmol/L, clinical labs measure it in ng/L. There is ongoing debate about whether serum holotranscobalamin levels reliably indicate B12 absorption, malabsorption, or deficiency. To better assess deficiency, additional markers like methylmalonyl-CoA (MMA) and homocysteine, which are part of cobalamin-dependent pathways, are evaluated. Elevated levels of these markers suggest “tissue deficiency” even when serum B12 levels appear normal. Following are the tests done for evaluation of vitamin B12 levels from blood serum

- Serum vitamin B12 level
- Methylmalonic acid (MMA) test
- Homocysteine test
- Holotranscobalamin (holo-TC) test [21, 22, 23, 24].

CONCLUSION

In summary, the development and refinement of animal models for inducing vitamin B12 deficiency are fundamental to advancing our understanding of its complex pathophysiology and evaluating potential therapeutic strategies. The three primary methods—nitrous oxide-induced functional deficiency, restricted diet, and modified diet incorporating pectin—each offer distinct advantages and limitations. While the nitrous oxide model allows rapid induction and replication of both neurological and hematologic symptoms, concerns remain regarding its safety and potential confounding effects such as hypoxia and neurotoxicity. The restricted diet approach provides a safer, more gradual onset of deficiency but at the expense of time, whereas the modified diet with pectin offers a novel way to address issues concerning B12 bioavailability. Careful selection of the most appropriate model, tailored to specific research objectives, is crucial to ensuring clinically relevant and reproducible results. A thorough understanding of B12 absorption mechanisms and the appropriate use of diagnostic tools—such as measurements of serum vitamin B12, methylmalonic acid, homocysteine, and holotranscobalamin - further empowers researchers to accurately characterize deficiency states. By providing a comprehensive overview of available methodologies, this review serves as a valuable resource to guide future investigations, ultimately contributing to improved prevention and

treatment strategies for vitamin B12 deficiency.

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