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ROLE OF POLYPHOSPHOINOSITIDES IN BRAIN

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IT is well known that the membranes of brain cells contain a high percentage of lipids which play a role in their structural and functional development. Among the brain lipids, polyphosphoinositides have the highest turnover and seem to play a leading role in signal transmission.

Studies carried out on the rat brain suggest that polyphosphoinositides exist as two different pools—one easily acted upon by hydrolases and the other not so easily hydrolyzed. For want of better nomenclature they are referred to as metabolically 'labile' and 'inert' pools[1-3]. The 'inert' pool may influence the structural integrity of the membrane whereas the 'labile' pool may be involved in cell proliferation and membrane function.

Recent studies show that the extracellular signals such as hormones, neurotransmitters, growth factors etc, combine with receptors in the brain cell membrane and activate enzymes which breakdown polyphosphoinositides. Originally, it was thought that phosphatidylinositol, 4,5-bisphosphate (PIP_2) gets converted to phosphatidylinositol-4-phosphate (PIP), phosphatidylinositol (PI), 1,2-diacylglycerol and a mixture of inositol-1-phosphate (IP) and inositol 1,2-cyclic phosphate by sequential reactions. But recent studies have shown that extracellular signals activate phospholipase C which hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacyl glycerol (DG) which act as secondary messengers. Phosphatidyl inositol appears to be a precursor of PIP_2 and not a product of hydrolysis of PIP_2[4] (figure 1).

IP_3 stimulates the release of calcium from endoplasmic reticulum and prevents its reuptake[5]. At the same time, extrusion of calcium is prevented by the regulation of the calcium pump by membrane phosphoinositides. The possibility also exists that inositol 1,4-bisphosphate (IP_2), a product of hydrolysis of phosphatidylinositol-4-phosphate (PIP), increases the permeability of plasma membrane to external calcium. The resulting increase in calcium facilitates events such as the release of neurotransmitters from the synaptic vesicles, activation of calcium/calmodulin-dependent protein kinase and activation of appropriate target enzymes[6].

Two isomers of IP_3, namely, 1,4,5-IP_3, with a very short half-life, and 1,3,4-IP_3, with a half-life of 30 min have been detected. The former is believed to be involved in releasing calcium from endoplasmic reticulum whereas the latter may help in transferring signals to the nucleus for cell division[7]. 1,3,4-IP_3 is not formed from 1,4-5-IP_3 and its mode of formation remains unknown.

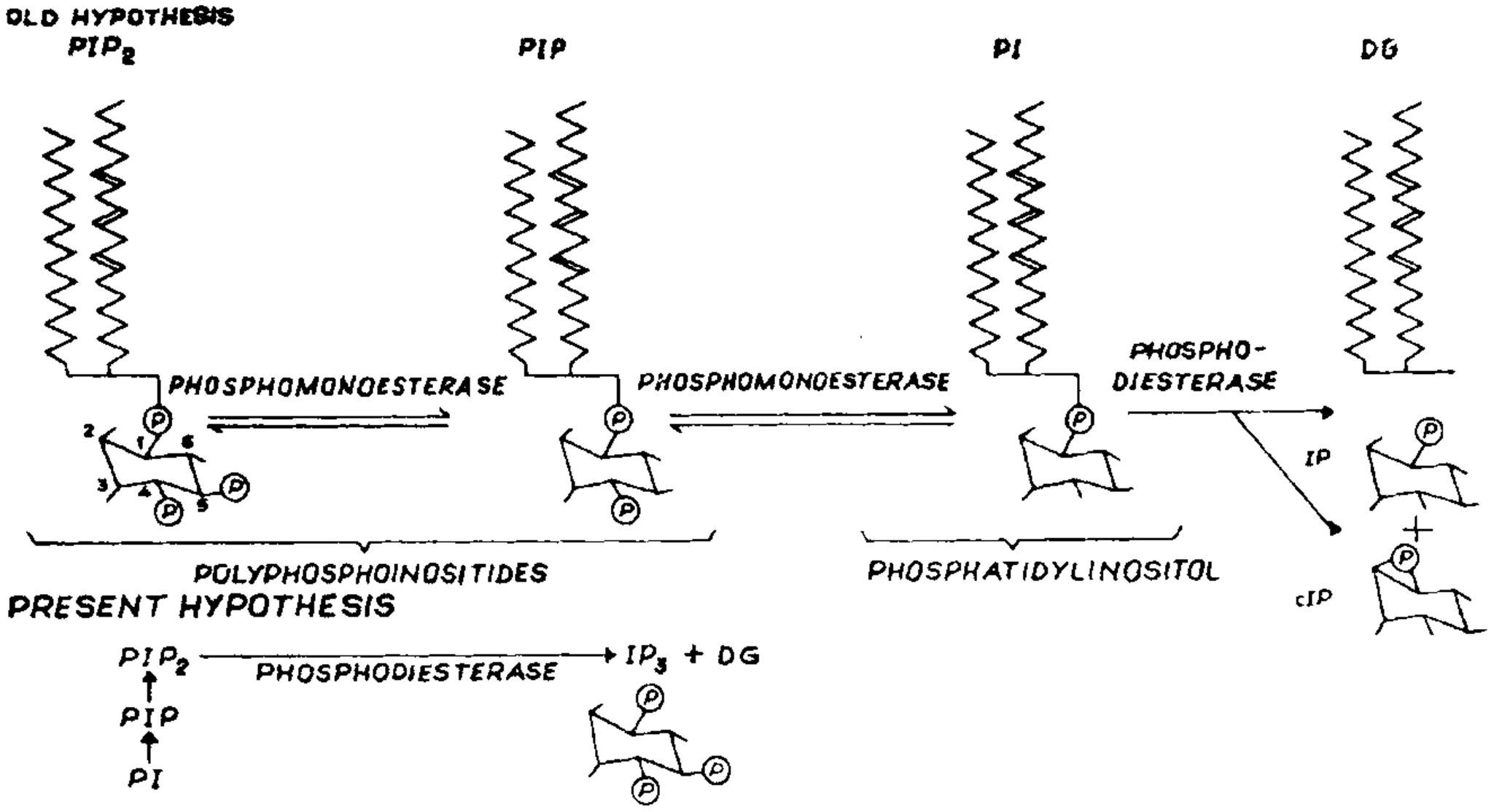
In this connection it is tempting to suggest that two molecular species of PIP_2—$PIP_2{}^1$ containing diacyl glycerol moiety with no arachidonic acid and 1,4,5-IP_3 moiety and $PIP_2{}^2$ containing diacylglycerol moiety with arachidonic acid and 1,3,4, IP_3 moiety—may be formed from PIP. The latter may be predominant during cell proliferation whereas the former may be predominant in the mature cell. Either the same phospholipase C or different isoenzymes may act on $PIP_2{}^1$ and $PIP_2{}^2$ (figure 2).

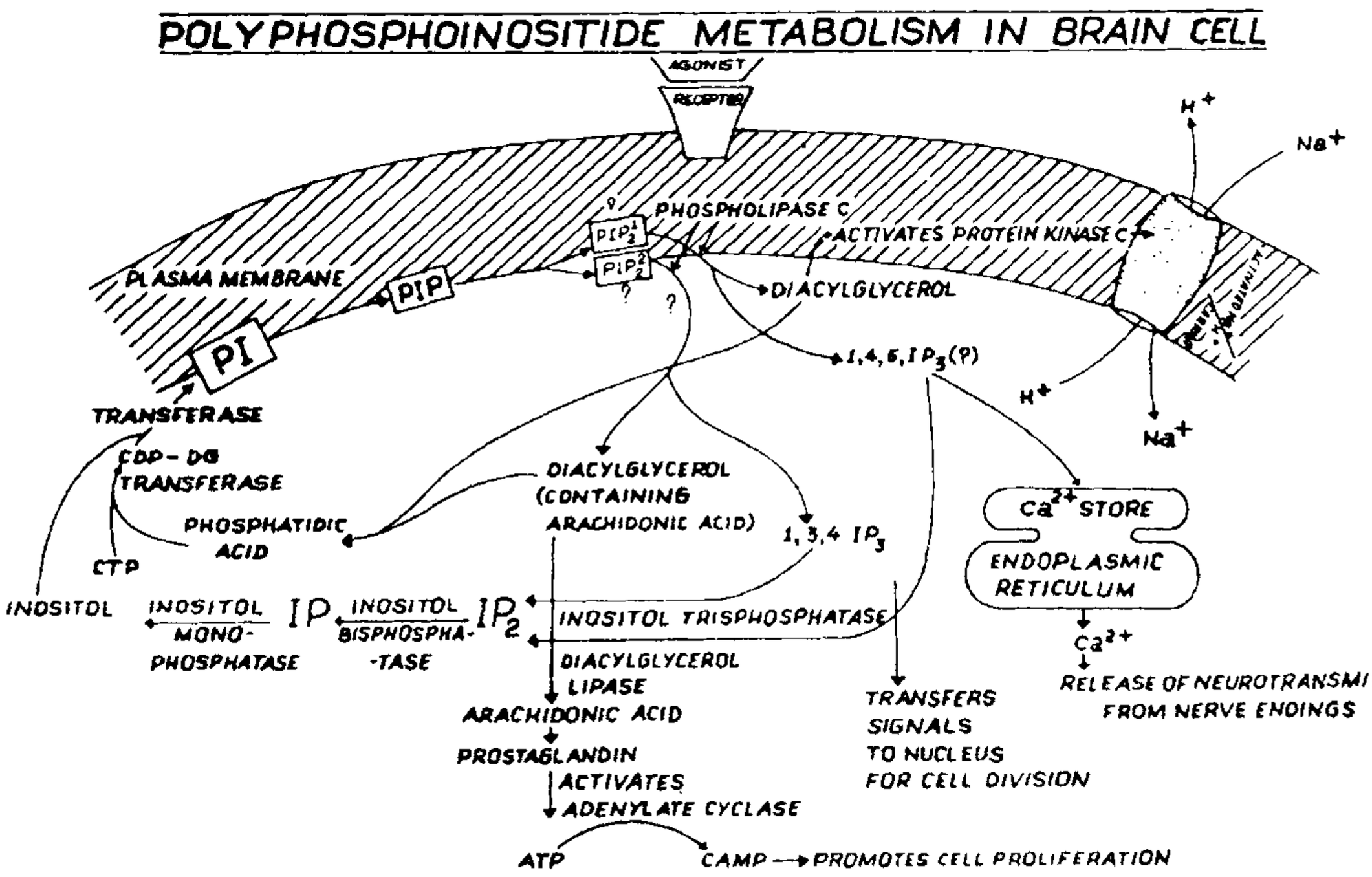
It is now suspected that the labile pool of polyphosphoinositides may turn out to be a mixture of different molecular species such as $PIP_2{}^1$, $PIP_2{}^2$ etc.

1,4,5-IP_3 formed from $PIP_2{}^1$ may be the messenger needed for the release of calcium from endoplasmic reticulum. 1,3,4-IP_3 formed from PIP_2^2 may be involved in transferring signals to the nucleus for cell division during cell proliferation.

Diacylglycerol formed from $PIP_2{}^1$ may not contain arachidonic acid and may activate protein kinase C responsible for activating Na^+/H^+ carrier involved in the influx of sodium into neurons during depolarization[8]. This may also activate calcium/phospholipid (phosphatidylserine) dependent protein kinase. But the physiological substrates for this kinase have not yet been identified.

Diacylglycerol formed from $PIP_2{}^2$ may contain





Figures 1,2. PI, Phosphatidylinositol. PIP, Phosphatidylinositol 4-phosphate. $PIP_2{}^1$, Phosphatidylinositol 4,5-bisphosphate containing diacylglycerol moiety with no arachidonic acid and 1,4,5-IP_3 moiety. $PIP_2{}^2$, Phosphatidylinositol 4,5-bisphosphate containing diacylglycerol moiety with arachidonic acid and 1,3,4-IP_3 moiety. 1,4,5-IP_3, Inositol 1,4,5-trisphosphate. 1,3,4-IP_3, Inositol 1,3,4-trisphosphate. IP_2, Inositol 1,4-bisphosphate. IP, Inositol 1-phosphate. CTP, Cytidine 5′-triphosphate. DG, 1,2-diacylglycerol. CDP-DG, Cytidine 5′-diphosphodiacylglycerol. cAMP, Cyclic adenosine 3′,5′-monophosphate. C/P, Cyclic inositol 1,2-monophosphate. IP_3, inositol 1,4,5-trisphosphate.

arachidonic acid moiety and act as substrate as well as activator of diacylglycerol lipase for the production of arachidonic acid[9], which in turn, acts as substrate for prostaglandin synthesis[10]. Prostaglandin endoperoxide activates adenylate cyclase involved in the synthesis of cyclic AMP[11] which promotes cell proliferation.

The polyphosphoinositide cycle operates for controlling the turnover of inositol phosphates and diacylglycerol. Figure 2 summarizes polyphosphoinositide metabolism in brain cell.

Since the enzymes of polyphosphoinositide cycle play a considerable role in regulating the turnover of polyphosphoinositides, several laboratories including ours are trying to isolate the enzymes of the cycle and study the regulation of these enzymes. It is suspected that distinct inositol phosphatases acting on IP_3, IP_2 and IP may be present as there are reports of the presence of inositol triphosphatase in red blood cells[12], inositol diphosphatase in insect salivary gland[13] and inositol monophosphatase in bovine brain[14].

Studies carried out in this laboratory on the developmental pattern of polyphosphoinositides in the rat brain[3] show that both 'inert' and 'labile' pools increase during pre-weaning and post-weaning periods implying their involvement in the development of neurons and glia. It would be of interest to study different molecular species of the 'labile' pool as different molecular species may be involved in cell proliferation and cell maturation.

Studies in progress in this laboratory on the effects of nutritional deficiency and alcohol administration suggest that the synthesis of the 'structural' pool (inert pool) of PIP_2 may be affected both by pre-weaning undernutrition and post-weaning protein deficiency but not by alcohol administration. The synthesis of the labile pool of PIP_2 seems to get affected only by post-weaning protein deficiency. In the case of alcohol administration in both the pre-weaning and post-weaning periods, no change is observed in the value at 1 min postmortem from which the labile pool is estimated suggesting that the enzymes hydrolysing 'labile' pool might have been inactivated. As far as PIP (whose functions apart from being a precursor for PIP_2 formation are not yet known) is concerned, 'structural' and 'labile' pools decrease only in pre-weaning undernutrition. The effect of alcohol on PIP is exactly the same as in PIP_2 (table 1).

Studies on different molecular species of 'labile' pools of polyphosphoinositide, their hydrolyzed products and different enzymes involved in polyphosphoinositide cycle at different stages of development of the brain in normal and stressed rats would enable us to understand their roles during cell proliferation and maturation and the differential effects of stresses on polyphosphoinositide metabolism in rat brain.

Table 1 *Effect of nurtitional deficiency and alcohol feeding on rat brain polyphosphoinositides*.*

	Preweaning period		Postweaning period	
	Under-nourished	Alcohol fed	Protein deficiency	Alcohol fed
	% of control			
PIP_2				
Inert pool	41	100	86	100
Labile pool	100	‡	80	‡
PIP				
Inert pool	63	100	100	100
Labile pool	77	‡	100	‡

‡ No change in 1 min post-mortem value, which is required to calculate labile pool, suggesting that enzymes hydrolysing labile pool might have been inactivated.
PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP, phosphatidylinositol-4-phosphate.
* Summarized from the data of Uma and Ramakrishnan[15], Shah *et al*[16] and Kurien and Ramakrishnan (unpublished).

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EFFECT OF SESBANIA MOSAIC VIRUS INFECTION ON THE NITRITE REDUCTASE ACTIVITY IN LEAF TISSUES OF DHAINCHA (*SESBANIA SESBAN* (L.) Merr.)

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DHAINCHA has gained great popularity as a green manure crop for rice, potato, sugarcane and cotton crops. In 1978 a viral disease was observed on it and around Gorakhpur. The virus was identified tentatively as sesbania mosaic virus (SeMV) and its effect on the host metabolism was investigated[1]. The effect of SeMV infection on the nitrite reductase (NiR) activity in leaf tissues of dhaincha is reported in this paper.

NiR, a flavoprotein, is utilised in the biosynthesis of amino acids in plants. It enables the reduction of nitrite ions by using reduced ferredoxin, which is an important direct source of electrons for nitrite reduction in leaves[2]. Virus infection is known to alter the course of nitrogen metabolism of the host[3] but the NiR activity of virus infected plants has not been investigated so far.

Dhaincha [*Sesbania sesban* (L.) Merr. var. picta (Prain)] Cv Shevari was used as the host and SeMV as the pathogen for systemic multiplication. Nine-day old dhaincha seedling plants were arranged in two groups of 120 each. The first group of seedlings was left as healthy control, while the second group was inoculated with SeMV by leaf rubbing method. NiR activity in dhaincha leaves was estimated from fresh samples at 10-day interval[4]. The experiment was performed in triplicate and the average recorded as a function of time (post inoculation).

The data in figure 1 revealed that virus infection caused a proportionate decrease in the NiR activity of test plants. An increase in the enzyme activity was however observed on the 20th day of inoculation in both the healthy and diseased leaves followed by steady decrease.

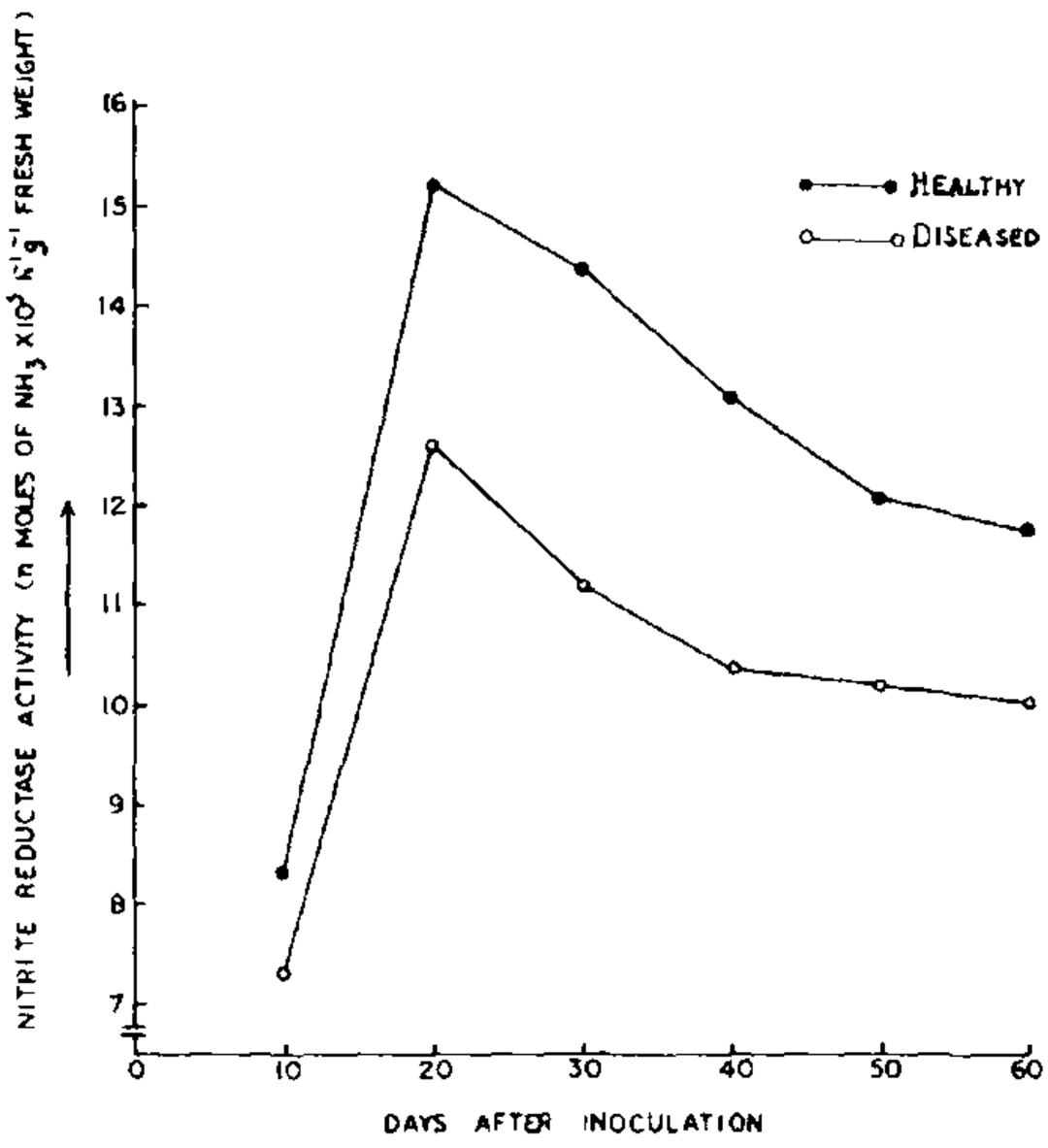

Figure 1. NiR activity of test plants.

The enzyme (NiR) has been reported to occur in chloroplasts[5-7]. Any damage to this pigment can be taken as reflection of the activity of the enzyme. The virus infection caused symptoms of mosaic, chlorosis and also reduction in leaf size[1]. The intracellular manifestation is either the loss of chlorophyll or the breakdown of the chloroplast or both[8]. Thus, the lowered NiR activity in the present case is associated with loss of chloroplast/chlorophyll in virus-infected leaves. Loss of chloroplast pigments due to virus infection has been reported in virus infected host leaves by earlier workers[8-11].

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