CHAPTER III

REVIEW OF LITERATURE

Account of work done on leaf protein abroad
The word "protein", introduced by Mulder in 1839, is derived from the Greek "proteious", which means "first". It reflects Mulder's belief that this class of substance is of primary importance in the chemistry of living organisms.

Proteins in green vegetation began to get attention only around 1920 with the realization of their importance in the nutrition of farm animals (Osborne, 1924), although the occurrence of glutinous substances in leaves was reported by Rouillé in 1773 even before the term "protein" was coined. Osborne and Wakeman (1920) and Chibnall and Schoyerer (1921) separated crude protein preparations from extracts of spinach and cabbage leaves respectively. Around 1925, a suggestion was put forth to make from fodder, a protein concentrate suitable for non-ruminants (Ereky, 1926). Later, Slade (1937) argued that the use of proteins from grass was more economical than meat obtained after feeding the grass to animals. By 1939, mostly as a result of the pioneering work of Chibnall and his colleagues, some information on the quantity of protein in leaves and their properties became available (Chibnall, 1939). But, an interest in the use of extracted leaf protein as human food did not arise until later, when during the World War II a search for improved methods of
food production began in Great Britain. It was during this period, Pirie (1942) advocated the large-scale production of leaf protein for food and began his systematic studies which opened the vast area of the potentialities of leaf protein in human nutrition. After twenty years of systematic work Pirie (1962) described how progress in biochemical engineering may make possible the extraction of leaf protein into concentrated forms suitable for human consumption and could greatly broaden our choice of crop plants.

There were two possible approaches for getting low-fibre or fibre-free protein food material from vegetation: (a) mechanical disintegration of the cell wall to release the protoplasmic fluid followed by coagulation of protein and (b) enzymatic breakdown of cell wall to extract the protein or to use the whole fermented/hydrolysed mass as a composite food. Pirie adopted the former approach. In his laboratory, many types of screw expellers, designed for pressing oil out of fish or seeds were found unsatisfactory but the domestic meat mincer did an admirable job with soft leaves for getting protein on a small scale. For large-scale extraction, various disintegrating devices
were tested. Ball mills, rod mills, edge and end-runner mills and dough breakers were tried and found usable but unsatisfactory. Davys and Pirie then made a series of versatile pulpers able to handle one to two tons of crop per hour when driven by 25 HP motor (Davys and Pirie, 1960), a 2 HP press for separating the juice from this pulp (Davys and Pirie, 1965) and a smaller extraction unit that gets out the juice from 100 to 200 kg batches of leaf in one operation 'village unit' or batch extractor (Davys and Pirie, 1963). These machines have undergone constant improvements.

Other extraction machinery which, like those used by Davys and Pirie is based on mechanical disruption of the tissues by cutting, shearing or pressing have been described by Crook (1946), Tilley, Barnes and Raymond (1954) and Raymond and Tilley (1956).

Pirie (1961) also described an apparatus for the disintegration of soft tissues, including leaf tissues, in the absence of air and Festenstein (1961) reported that the extraction of protein from small quantities of young tobacco leaves by this press was comparable with high speed maceration. In the same year, Chayen et al. (1961) described the disintegration of leaf material by
the impulse rendering process and its use in the isolation of leaf proteins. Since 1964, this impulse rendering unit has been operating under license in Haifa, Israel, for making alfalfa leaf lipid protein (Smith, 1967).

With the help of a grant from British National Committee of the IBP an equipment to measure the extractability of leaf protein from 2 - 3 kg of leaf in different climatic zones, in a systematic and reproducible manner was designed at Rothamsted (Davys and Pirie, 1969; Davys et al., 1969). This equipment is now in use at many centres including this laboratory.

The IBP pulper (Davys and Pirie, 1969) is a hammer mill with 58 fixed beaters in six rows and a drum, which is 44 cm long, 27 cm in diameter at the feed end and 32 cm at the discharge end; the difference in diameter ensures an air flow in the direction of movement of the pulp. In the laboratory, the rotor is driven by a 5 HP motor; the speed and direction of rotation are so arranged, that the pulper can also be mounted on a 'Landrover' and driven from the power-take-off. The pulper can be fed uniformly at rates up to 1.5 kg per minute. The pulper is supplied with several pulleys so
that it can be run at different speeds. Although designed as a small agronomic tool, when fed mechanically at a uniform rate with chaff-cut leaf, it can take as much as 6 kg per minute. The ability to handle 360 kg of crop per hour makes this pulper a useful small-scale production unit.

In the IBP press (Davys et al., 1969), 900 g of pulp is spread evenly on a cloth on a square grooved platen 23 cm each way, the cloth is folded so as to make the area of pulp 450 cm$^2$ and another grooved platen is placed on top. The assembly is mounted with the grooves vertical and subjected to a pressure of 1 ton applied by means of a bell-crank with 40 to 1 ratio. These conditions closely resemble those in the large press and the dimensions are chosen so that the unit can be carried and, like the IBP pulper, can be put in a 'Landrover' and used in the field. But this press, is totally unsuited for production. Davys and Pirie (unpublished) have therefore, made a small belt-press, a scaled-down version of the larger belt-press suited to the size and output of the IBP pulper.

Koegel et al. (1971) feel that maceration and/or fractionation of green forages may be carried out by means
of rollers or rotary presses. Important characteristics of the rolling process such as peak pressure, dwell time, and most important: pressure gradient may be varied to fit needs by adjusting different variables.

Large-scale production process as outlined by Morrison and Pirie (1961) is extremely simple. Juice from fresh pulped leaves is freed from the starch grains and fibre, then coagulated with steam at $80^\circ$. The protein coagulum is filtered off, washed with water at pH 4, and pressed into blocks or dried while frozen.

Leaf protein concentrate (LPC) thus obtained is a dark green cake containing about 60% water; on dry basis it contains 60 - 70% protein, 25 - 30% lipid, some starch and mineral matter, and very little fibre. This wet cake could be directly used in cooking or preserved by drying, salting, pickling or canning. The texture, colour and nutritive value of the product is influenced by the condition of drying and Arkcoll (1969) described how a product can be made with as good an appearance, texture, keeping quality and digestibility as made by freeze-drying. This work is of great value in countries where freeze dryers are expensive.
A gray-brown powder could be obtained by solvent extraction of lipids from the LPC (Pirie, 1957). This is the basis of a process described by Huang et al. (1971). Leaf lipids are highly unsaturated (Lima et al., 1965; Buchanan, 1969). Because of this degree of unsaturation, the lipids associated with leaf protein readily oxidise when exposed to air, especially when the material is kept warm after incomplete drying. Pirie (1969) considers that it would be advantageous to avoid the solvent extraction as some lipids are useful components of diet and these highly unsaturated may be especially valuable. Furthermore, unless some use is proposed for the separated lipid, its removal would be an extra process that would increase the cost of the product and make it difficult, if not impossible, to consider leaf protein production as a process that could be organized on a local or village basis.

The solvent extraction also removes the carotene. Arkcoll and Holden (1971) have demonstrated that \( \beta \)-carotene is remarkably stable in the extracts used to make leaf protein and about a third is extracted and precipitated with the protein. LPC contain between 1.4 and 1.7 mg/g. According to W.H.O. a young child needs 1.8 mg/day. Hence only 2 g leaf protein per day
would meet the requirement (Pirie, 1971). Moreover, conversion of $\beta$-carotene to Vitamin A in the serum is more efficient if given as a vegetable than as $\beta$-carotene in oil.

The amino acid composition of unfractionated, or whole, leaf protein from different species is similar and it is not affected by the physiological age or state of the plant, or by fertilizer treatment (Bryant and Powden, 1959; Pleshkov and Powden, 1959; Chibnall et al., 1963; Gerloff et al., 1965; Wilson and Tilley, 1965; Byers, 1971). The similarity in amino acid composition might indicate that the major proteins in the leaf concentrate which are the proteins involved in photosynthesis and metabolism of leaf cells, are similar in many plant cells (Stahmann, 1963).

The average percentages of amino acids that are deficient in many other proteins—lysine, methionine and tryptophan are 6.3, 2.1 and 1.6. Leaf protein is not therefore as good nutritionally as the animal proteins such as milk and egg, but it is better than the cereal and legume seed proteins.
When first tested, the nutritive values of leaf protein concentrates were found to be low (Carpenter et al., 1954; Ellinger, 1954). Later it was shown, however, that heating during the processing above 82° could damage the proteins and that, when high temperatures were avoided, the products made from different species, could have as high or higher nutritive values than soyabean proteins when tested on chicken and rats (Duckworth and Woodham, 1961; Henry and Ford, 1965; Woodham, 1965), or with enzymatic methods (Akeson and Stahmann, 1965). Duckworth et al. (1961) carried out a trial on pigs and showed that with about 7% of LPC the rate of growth and efficiency of feed utilization were as good as for diets containing 3% of fish meal.

Waterlow (1962) used leaf protein in mixture with milk on Jamaican infants recovering from malnutrition. He found that a mixture of equal parts of milk and leaf protein gave the same nitrogen retention as milk alone. Some variations in biological values between species were reported (Henry, 1963; Woodham, 1965). Lexander et al. (1970) also found very great differences in nutritive values of the proteins from different species, the highest value (Amaranthus caudatus) was twice the lowest one (Medicago sativa).
Before coagulation, a leaf extract can be separated into 'chloroplast' and 'cytoplasmic' fractions by differential heat treatment of the extract.

Nutritionally, the chloroplastic material was found inferior (Subba Rau et al., 1969; Lexander et al., 1970) and the cytoplasmic one superior to the unfractionated leaf protein. Pirie (1971) considers that the quality of the 'chloroplast' fraction depends to a great extent on the technical skill of those making the original extract; quinones, polyphenols and other tanning agents are more abundant in the chloroplasts than the cytoplasm of many species and may combine with protein in the presence of air. To varying extents this impairs its nutritive value.

In terms of absolute amount, methionine has been found to be limiting essential amino acid in all leaf protein preparations and its addition improves diets containing unfractionated protein (Henry and Ford, 1965; Shurpalekar et al., 1969). Adding lysine (Subba Rau et al., 1969; Shurpalekar et al., 1969) or iso-leucine (Henry and Ford, 1965) to the same diet had no effect.

Byers (1971) has reported that less leucine and more histidine and lysine are found in cytoplasmic protein than
in the chloroplastic one. She has shown that the methionine, is 'available' in sufficient quantity in cytoplasmic and unfractionated protein, but it may be marginal in some chloroplastic preparations. She considers that it would be economical to use all the protein that is extractable from the leaf, and supplement it, if necessary, with methionine, to improve its nutritive value.

Synge et al. (1970) applied van Slyke's nitrous determination procedure (which removes any ε-amino groups of lysine not bound to quinones and polyphenols) to various proteins. The results on some leaf protein agreed well with those obtained by other chemical methods (Woodham, 1965). But, the amount of lysine in chloroplastic fractions was not much above the recommended FAO minimum (4.2%).

Food habits and culinary techniques vary in different regions and hence, presentation of leaf protein on table and its use in diets is a matter of research in each region. Morrison and Pirie (1960), Byers et al. (1965) outlined some of the principles underlying presentation and described the English dishes in which leaf protein could be incorporated. Oke (1966) prepared dishes suitable
for use in Nigeria. In a recent paper Oke (1971) has given the responses of various groups to whom the dishes containing leaf protein were offered.

There are two by-products of this process; the fibre, which contains 1 to 3% of N (on dry matter) and the liquor or the whey which contains water soluble components of the leaf. The former could be used as feed for ruminants (Pirie, 1966b; Stahmann, 1968). Oelshlegel et al. (1969) observed that silage made from the residue, from several species, was readily eaten and chemical analysis suggested that lucerne silage made from the residue was preferable to that made from the fresh crop.

The whey may cause local pollution. Hartman et al. (1967) spray-dry the whole extract. This procedure is harmful as shown by Subba Rau et al. (1969) and is also condemned by Pirie (1969a) on theoretical grounds. Hollo and Koch (1971) coagulate and filter the extract and concentrate the filtrate in a multiple effect evaporator before adding it back to protein. Kohler and Bickoff (1971) concentrate it and consider it valuable because of certain 'unidentified growth factors'. At Uppsala, Jönsson (1962) compared the whey from pea-vines and
other leafy material with various other substances using seven microorganisms and proved particularly satisfactory for growing *Rhizobium meliloti* and adequate for *Penicillium chrysogenum* and *Aspergillus niger*. Shah et al. (1970) used extracts from three species of plants and found that nine strains of yeast were able to make use of the organic matter in the fluids.

Hollo and Koch (1971) consider a basic change in human eating habits unlikely. They are of the opinion, that crops harvested at a time when they are at the peak of their vegetative growth should be processed and fed to animals. Such a scheme would avoid protein losses. The procedure begins in Hungary towards the middle of April, with processing rape either alone or in mixture and ends in December or January with field kale. The raw material in the appropriate stage of development is mechanically disintegrated without the addition of water. The grist so obtained is pressed. The juice passes through certain operations of pre-treatment to vacuum evaporator. The concentrate obtained in the concentrator is processed together with the fractions derived in the pre-treatment of the juice to a main product by atomizing in a drier. This product as well as the pressed residue are produced in pelletized form. The individual drying operations are
carried out at low temperatures and the type of technology is more or less the same as in the production of cane sugar.

While Pirie is of the opinion that a serious effort should be made to gain the green product in developing countries, Americans consider that, for a wide usage in most markets, the lipids will have to be extracted and greyish-white products produced. Kohler and Bickoff (1971) consider that an economically sound process must yield high value feed products in addition to LPC. Since they are not so much interested in maximum yields of protein but in the pressed cake the choice is made of sugar-cane roll press (Knuckles et al., 1970) to replace both grinding and pressing operations of Davys and Pirie (1960, 1965). Kohler and Bickoff approach is divided into two phases: Phase I -- to develop a basic wet separation process to produce, (a) standard grade dehydrated alfalfa meal; (b) 50 % protein, high-xanthophyll concentrate for poultry; and (c) forage solubles concentrate for use as a UGF supplement for livestock. Phase II -- a research programme to develop further economically sound steps to yield pigment-free, palatable LPC plus xanthophyll concentrate from the intermediate or end products. Based on Phase I, a full scale plant that processes 50 to 70 tons of alfalfa per
hour has gone into production. The addition of ammonia in the process has been found to have many beneficial effects (Spencer et al., 1971).

Very little is known about the cost of leaf protein. Pannenbaum (1969) feels that Pirie’s estimate may not hold true in tropical rural villages due to severe shortages of potable water and cheap electricity (or fuel), two commodities readily available in Britain. Uniroyal, Canada have released estimates of 10 - 20 ⁵ per lb of crude product (70% protein) not including profit or marketing cost. A solvent-extracted product would cost 2 - 3 times as much. On this basis, Uniroyal feels that LPC would be competitive with Single Cell Protein or Fish Protein but not the Soy Protein although the cost may lower with improvements in the agricultural or processing techniques (personal communication from J.H.Wilson to Mr. Joshi). The cost of leaf protein is being assessed by the Tropical Products Institute, U.K. and the results are awaited.

At Rothamsted, the amount of extractable protein from more than a hundred different species has so far been determined on the laboratory scale using domestic mincer and later, the IBP unit (Crook and Holden, 1948; Rep. Rothamsted exp. Sta. since 1957). It was observed that
the extractability of protein from leaves varies between different species and also between different ages of the same species (Crook and Holden, 1943; Singh, 1961); and the composition of protein made from extracts also varies with the age and species of leaf used (Morrison and Pirie, 1961).

The choice of species for large-scale extraction at Rothamsted was restricted to those crops for which there was already an established technique of husbandry; many of these are cultivated for the production of seed rather than leaf (Pirie, 1969a).

Intensive agronomic research work was also undertaken. The object of these agronomic studies was to determine the system of cropping which would yield the maximum amount of extracted protein per hectare per year in Great Britain. Byers and Sturrock (1965) assembled the results of yield measurements during 6 year and concluded that, with a suitable succession of crops, the total yield could be 1000 kg/ha in a year. Byers and Sturrock (1965) observed that the yield of protein depends on species, variety, season, age of the plant, the amount of fertilizer given and the ability to regrow after cutting. The average amount of protein N
extracted was greater from cereals than from the legumes. The knowledge gained was used to maximise the annual yields and yields have now reached 1200 kg protein/ha with legumes and 2000 kg/ha with crops given 530 kg N/ha (Arkoell and Festenstein, 1971).

The essential amino acid production for ten crops harvested for forage and fifteen crops harvested for seed was calculated from average crop yields in the United States for the ten year period of 1953 to 1962 (Akeson and Stahmann, 1966). Highest yields per acre of essential amino acids were calculated from forages which could be processed into leaf protein concentrates. Alfalfa produced the highest yield per acre. From these results the authors concluded that leaf protein would give a greater return of edible protein in U.S.A. than any other form of husbandry. Stahmann (1968) emphasized that a leaf crop can synthesize considerable more protein than a conventional seed crop, and by direct human consumption the great turnover losses associated with meat production could be avoided.

Little (1968) reprinted most of the papers published before 1967 on the use of water weeds. Some other points on protein extraction and on the other uses to which water weeds could be put were made by Pirie (1970). In
U.S.A. Boyd (1968, 1969, 1971) extracted protein from a
number of aquatic plants in quantity sufficient enough to
justify their being considered as potential sources of a
protein-rich supplementary food. Water hyacinth, water
lettuce and Hydrilla sps are most serious weeds in almost
all tropical and sub-tropical nations. Chemical analyses
showed that all these three species contain large amounts
of crude protein and had satisfactory level of amino
acids.

In New Zealand, Vartha and Jones (1970) observed that
the substantial improvement in herbage yield of lucerne
with irrigation was paralleled by the yield of extracted
protein. Protein yield was at a maximum at 5 weeks
frequency of cutting and Wairau strain yielded 2000 kg/ha.
The extractability of protein nitrogen increased at

In Sweden, the amount of protein produced per $m^2$
by plants cultivated in soil in a greenhouse at three
levels of fertilizer application, for about 10 weeks was
estimated by Kjeldahl analysis of the TCA - insoluble
fraction of freeze-dried material (Lexander et al., 1970).
The quantity of extractable protein was determined on
non-dried but frozen material after disintegration.
Twenty-nine species and varieties were investigated. Large differences between species were found. Protein extractability varied between 5 and 80 %, while the extractable protein produced per m² ranged between 1 and 140 g. *Amaranthus caudatus* and the Chenopodiaceae investigated were considered as the most suitable species for large-scale production. The authors emphasize, that a search for suitable species should not be limited to typical crop species because those that have hitherto not been used as crops may be the most advantageous ones for the production of an unconventional food as leaf protein concentrates.

Growth regulators affect both the shape of a treated plant and also the amount of protein in it. Byers and Jenkins (1961) observed that Gibberellic acid did not significantly affect the yields of dry matter or crude protein from spring vetches.

Singh et al. (1970) reported significant increases on the levels of protein and soluble amino acids in the leaves of bush beans, peas and sweet corn by spraying s-triazines.
It must be borne in mind, at this stage, that most of the work done on the choice of crops and yields of leaf protein relate to crops grown in the temperate zone. The immediate problem, however, is not the discovery of more ways for producing protein in this already well-fed region. It is in the tropical and sub-tropical countries where diets are deficient in protein that leaf protein as a supplementary food would be of use (Pirie, 1966a). Intensive research should therefore be done in these countries.

Byers (1961) studied the extraction of protein from the fresh leaves of 60 tropical species growing in Ghana. The legumes were the best source of good quality leaf proteins, but good results were also obtained with a few of the more common weeds.

Akinrele (1962) extracted protein from a few species in Nigeria and got very low protein yields. He, therefore, concluded that leaf protein had no future in Nigeria. Oke's work (1964, 1965) showed otherwise. Using the IBP unit Oke (1971) could extract from 35 to 50 % N from cereals; 60 to 80 % from legumes and 40 to 90 % from green vegetables. There was always an optimum for each species at which the extractable nitrogen was highest.
CHAPTER III

REVIEW OF LITERATURE (CONT.)

Account of work done on leaf protein in India
In India interest in leaf protein was evident as early as 1943, when attempts were made to use leaf protein by late Professor B.C. Guha during the Bengal famine (Guha, 1960). Jayprakash Narayan, Sarvodaya leader, and Professor K.N. Kaul met Professor Pirie and obtained his views on organizing work on leaf protein in India (Pirie, 1969b). Later, Singh (1964) investigated 38 species of cultivated and wild plants available around Lucknow with a view to make a choice of plants for leaf protein production in bulk.

Special attention was paid to the investigation of water-hyacinth in the Department of Biochemistry, University of Calcutta and Datta et al. (1966a; 1966b) described a method for extraction of protein concentrates from this noxious weed, under different conditions. A blade-type hammer mill was used and Na₂CO₃ was found to extract the protein material most efficiently. It was observed that protein extracted without carbonate was more digestible *in vivo* and had a greater biological value than protein made by carbonate extraction. Later, Ghosh (1967) observed some improvements by supplementing water-hyacinth leaf protein with methionine. Pirie (1967) estimates that about 6% of Na₂CO₃ and equivalent amount of HCl is required to obtain 1% of dry protein
from about 100 kg of weed from water-hyacinth by the method outlined by Datta et al. It would raise the cost of protein considerably. Pirie considers that 250-500 tons (wet weight) of leaves can be harvested from a hectare of water-hyacinth in a year and even if the extraction is without carbonate and as low as 12%, the plant would yield about 500 to 1000 kg of protein/ha/yr. This yield is as large as any edible legume seed. Work has recently started at the Indian Statistical Institute, Calcutta, and preliminary studies using IBP unit (Matai et al., 1971) have shown that a number of plants growing in marshy land, wild plants could be used for protein production. Encouraging results were obtained from Solanum nigrum, Alocasia indica, Cestrum diurnum, Oldenlandia corymbosa and Croton sparsiflorus.

Although Dr. V. Subrahmanyan and his colleagues did some work on leaf protein in mid-fifties (Sur and Subrahmanyan, 1955; Anandaswamy and Date, 1956), systematic investigations started at the Central Food Technological Research Institute (CFTRI), Mysore, only in late 1964. Singh used a batch extractor (Davys and Pirie, 1963) and the procedure adopted for coagulation etc. was according to Morrison and Pirie (1961). The main features of the work and findings at the CFTRI are
briefly summarized here.

The vegetation of 24 plant species was studied. This included a number of cover crops, green manures, fodders and by-product leaves. From well cultivated lucerne the yields of leaf protein (N x 6.25) were as high as about 1500 kg/ha/year and from not so well reared lucerne between 500-700 kg/ha/year. The yield from the by-product vegetation ranged between 66 to 160 kg/ha when the main economic crop was harvested within 70 days from sowing (Singh, 1967).

Protein coagulation under different conditions was studied. The acid coagulation was found to yield less nitrogen than heat coagulation. Fractional coagulation by differential heat treatment of lucerne yielded a cyto-fraction with 77% protein and a chloro-fraction with 45% when ordinary unfractionated leaf protein had 60% protein (Singh, 1969).

Various drying and preservation techniques were tried. The problem of preserving wet leaf protein cake was satisfactorily solved by treatment of the slurry with acetic acid/vinegar leaving a residual 2% acidity (Subba Rao et al., 1967).
Nutritional studies showed that even under a state of vitamin and mineral deprivation, leaf protein led to significant improvements when added to protein deficient rice diets. In diets adequate with respect to vitamins and minerals, the responses to leaf protein were comparable to skim milk powder at 5% protein levels (Singh, 1967).

The leaf protein, coagulated by acid pH adjustment of the extracts, was found to be significantly inferior to that coagulated by steam injection. The cytoplasmic protein was found to be superior to chloroplast (Subba Rau et al., 1969). The spray dried whole extract was unsuitable as food, failing to support growth and being toxic (Subba Rau et al., 1969).

Singh (1969) observed great variations in the nutritive value of leaf proteins prepared from different vegetations. Some, like that from carrot, taooma failed to support any growth, while that from beet root, dhaincha, and horsegram were inferior and those from crucifers (knolkkhol, radish, cabbage, turnip, cauliflower) superior to lucerne leaf protein. There was no toxic effect observed in leaf protein from any source.
In a 6 month long feeding trial with children, it was observed that leaf protein from lucerne, besides being an efficient lysine supplement was also a satisfactory food protein supplement. There was a remarkable improvement in the quality of low protein ragi-diet by addition of leaf protein to give 10 g of extra protein per child per day (Doraiswamy et al., 1969).

A device, based on steam jet ejector principle, was described for treating large volumes of leaf extract to coagulate protein for use in small or industrial-scale production of leaf protein (Ahmed and Singh, 1969).

An industrial pulper and press (Davys and Pirie, 1960, 1965) has been installed and some information on protein yields is now available from the Coimbatore Agricultural College (Samuel, personal communication). In addition to species recommended by Singh (1967), Sesbania grandiflora, Clitoria ternata, Acalypha indica and by-product leaves of groundnut, cucurbits and cotton are considered suitable. Sweet potato grown for fodder purposes yielded 700 kg/ha; Glyricidia maculata grown as green manure gave 600 kg/ha and Sesbania speciosa yielded 500 kg/ha. Russian Cotton var. 84/4, yielded 505 kg of
protein whereas Russian Cotton var. 72/2 yielded 515 \( \text{g} \) of protein when harvested after two pickings of main product. D.L.Avarai 692 harvested three times in five months yielded 440 \( \text{g} \) of protein/ha.

The effect of fertilizer treatments on four popular varieties of grasses cut five times showed that Centchromus glaucus was the best as it yielded 357 to 511 \( \text{g/ha} \), Panicum maximum was next best with 224 to 330 \( \text{g/ha} \); Brachiaria mutica gave 180 to 275 \( \text{g/ha} \) and Centchromus ciliaris 150 to 253 \( \text{g/ha} \).

An experiment was also conducted to study the effect of periodicity of cutting of grasses on the yield of leaf protein. Panicum maximum gave highest yield of protein (1400 \( \text{g/ha} \)) among the three grasses investigated in 18 months when cut at the interval of 30 days. Harvesting every 60 days was suited for Centchromus glaucus and Brachiaria mutica.

At Sri Aminashilingam Home Science College, Coimbatore, studies on acceptability of food preparations containing leaf protein concentrates have been conducted since 1967 and the results have recently been summarized
by Kamalanathan et al. (1969) and Kamalanathan and Devdas (1971). At 5 g level, the scores of the dhal balls, leaves chutney, chutney powder and ragi addai were close to the standard. At a higher level of 15 g all the samples were reported to have leafy flavour, saw-dust texture and bitter taste. LPG was more acceptable when introduced at the completion of cooking, and when the strong flavours were masked by spices or fully ripe bananas.

The work on leaf protein in this Department was initiated by Joshi in 1968. More than 60 species have so far been screened through hand mincer and results published (Deshmukh and Joshi, 1969; Dev and Joshi, 1969a, 1969b).

The programme of work on the production of protein from green vegetation was approved as a National Project by the Indian Committee of the IBP and is being carried out in collaboration with the U.K. IBP (IBP News 20, 1969). Systematic work on the agronomic aspects of leaf protein production began in 1969 and the results obtained have been summarized (Joshi, 1971). Using the IBP unit some other results obtained in this laboratory are briefly described here.
It was possible to extract protein from most of the tropical weeds which grow exuberantly during the monsoon and compete with the cultivated crops (Gore and Joshi, 1971). It is felt that some of these weeds could be regarded as potential crop plants, or plants with a positive value rather than undesirable nuisances. The by-products of the process were profitably used to grow useful fungi (Deshpande and Joshi, 1969, 1971).

Dev (1972) selected lucerne for intensive agronomic trials and found that it is an outstandingly productive crop of consistent performance. The crop harvested at three weeks frequency of cutting gave more than 125 tons forage, 6 tons crude protein and more than 3.2 tons extracted protein per year. Species of *Amaranthus*, *Lablab* and *Dolichos* yielded protein at a rate of 6 g/ha/day.

Gore (1972) has shown that *Sesbania sesban*, a cover crop of the region is capable of giving 2 tons and Hybrid Napier 2.5 tons/ha/year. By-product leaves from cole crops gave more than 120 g/ha in 75 days. Although tetraploid berseem produced crude protein at a rate of 20 g/ha/day it was possible to extract only about 5 g protein/ha/day (Mungikar, 1972). Mustard, *Tithonia* and fodder maize have also been found promising.