CHAPTER V

THE SYNTHESIS OF HAEMOGLOBIN IN ROOT NODULES AND CHLOROPHYLL IN LEAVES IN RELATION TO NODULE FORMATION.

Porphyrin and chlorophyll biosynthesis in plants and micro-organisms has been actively studied with the general conclusion that chlorophyll biosynthesis follows the same pathway upto protoporphyrin as does haem in animal tissues (Rimington, 1957). From the fact that the two pigments haem and chlorophyll are built upon the same basic tetrapyrrole plan, certain ideas with reference to their metabolic utilisation suggest themselves. In view of the occurence of high concentrations of haematin pigments in the leaves of legumes (Hill and Scarisbrick, 1951) the changes in the haemoglobin content of nodules might be related to the other haematin pigments of the plant as for instance the chlorophyll of leaves. Such a proposition derives strength from the fact that in chlorotic leaves the total haematin and chlorophyll concentrations are proportionately reduced (Davenport, 1953).

The studies of Granick (1951) have shown that the biosynthetic chain in the formation of the two haem pigments -- chlorophyll and haemoglobin -- has its origin from glycine and acetate in the basic metabolic mileu. Glycine and acetate are also utilised by green plants in the synthesis of chlorophyll (Della Rosa et al., 1953). The specific

utilization of glycine for porphyrin synthesis has also been demonstrated in the formation of the haem protein in the root nodules of leguminous plants (Richmond and Saloman, 1958).

In view of these facts it is possible to envisage a physiological relationship between the metabolisms of haemoglobin and chlorophyll in nodulated legumes.

Virtanen (1955) has shown that the haemoglobin content of root nodules and their capacity for nitrogen fixation are directly related. Bonnier and Sironval (1956) have observed the formation of haemoglobin in nodules of plants grown in 16-hour day but not in those of an 8-hour day. Subsequently, Bonnier, Sironval and Verlinden (1957) have suggested that a physiological relationship between the metabolism of haemoglobin and chlorophyll could exist.

Presently in studies on Arachis hypogaea, the photoperiodic control in nitrogen fixation and haemoglobin synthesis in root nodules has emphasised the regulatory influence of foliar factors. Thus, a reduction in day length from 10 hours to 4 hours caused a pronounced reduction in root nodule haemoglobin content of nodules. Besides, a reduction in haemoglobin in relation to

soil reaction was associated with pronounced chlorosis under conditions of extreme alkalinity.

The present field work was, therefore, done to study the synthesis of haemoglobin in root nodules and chlorophyll in leaves in relation to nodule development so that principles might emerge which would enable a better understanding of nodule growth and function.

Since Jordan and Garrard (1951), in their field studies on soybean, have shown that varieties of host plants differed both in regard to the maximum concentrations of root nodule haemoglobin produced and the rate at which the peak production was reached, it was thought deisrable to choose from different varieties of Arachis hypogaea in the present work. These are: Varieties TMV 2 (Bunch), TMV 3 (Spreading), TMV 5 (Spreading), and MG 1 (Spreading) varieties.

Plant of field work:

The four varieties of groundnuts were grown in the field soil and were not artifically inoculated. Nodulation was a result of infection by the rhizobial populations of the soil. The plants were provided with a plentiful supply of water. All the plants grew well

and had a dark green foliage. Nodules began to form on the root systems at 18 days from the time of sowing of seeds.

Nodules were harvested from random selection of plants from among different varieties, at intervals of 7-10 days and especially at the time of maximum flushes of flowering as obtain under field conditions of growth. The root nodules were excised from the root systems and their number, weight and volume along with haemoglobin concentration were determined (vide: Materials and Methods) as described earlier. Chlorophyll estimations were also carried out (vide: Materials and Methods) with leaves chosen at random in each variety of host plant. All the varieties grown showed excellent plant growth and were of a healthy dark green foliage. The experiment lasted for 80 days from the time of sowing the seeds.

The relationship between haemoglobin synthesis in nodules and of chlorophyll in leaves also illustrated in Figs. 12 and 13. The increase in average nodule number and weight per plant calculated on an analysis of 5 plants are represented in Figs. 14 and 15.

A. The number and weight of nodules show a steady increase from two weeks following nodule initiation until 65 to 70 days following the germination of the host plants.

B. A varietal variation existed in the host plants in regard to the fluctuations of haemoglobin in the root nodules.

C. ~~Since~~ haemoglobin concentrations in root nodules were estimated alternately with flowering as well as in between flowering flushes, it appears reasonable to state that haemoglobin variations in root nodules have been influenced greatly by the successive production of crops of flowers in all the 4 host varieties. A pattern of flowering distinguished by alternation of high and low frequencies throughout the flowering period was observed in the present investigation. This specific character of the groundnut plant (Smith, 1954) in its pattern of flowering, influences the levels of haemoglobin in root nodules. This period of flowering under the conditions of the experiment ranged from 25 to 60 days.

D. The maximum concentration of haemoglobin in root nodules of field grown plants.

The maximum concentration of haemoglobin in the root nodules of varieties TMV 2 and 3 was attained in between 45-50 days and as the results show on the 48th day following sowing of seeds. In varieties TMV 5 and HG 1 these maxima were attained in 60 days following sowing.

The maximum concentrations of haemoglobin attained in the root nodules of the four varieties were far in excess of 200 µg/g. fresh weight of nodule tissue. This threshold concentration was chosen initially as a criterion for defining effectiveness in terms of root nodule haemoglobin concentration in a preceding experiment.

TABLE 5

THE MAXIMUM CONCENTRATION OF HAEMOGLOBIN IN THE ROOT NODULES OF ARACHIS HYPOGAEA VARIETIES.

Variety	Days from sowing	Root Nodule haemoglobin µg/g fresh weight of nodules
TMV 2	48	295
TMV 3	48	340
TMV 5	60	340
HG 1	60	330

(For comparison: The haemoglobin concentration of root nodules in var. TMV 2 of *Arachis hypogea* in sand cultures, on inoculation with various strains, ranged from 145-245 µg/g fresh weight of nodule tissue).

E. From the preceding table it is clear that the rhizobial strains in the field soil were effective in nitrogen fixation in terms of root nodule haemoglobin concentration.

F. The parallel developmental pattern of haemoglobin in nodules and chlorophyll in leaves.

In terms of the developmental pattern of haemoglobin in nodules and chlorophyll synthesis in leaves of the 4 varieties, one interesting observation bears reference to the parallel development of the two porphyrin pigments in the host plant. Whilst there was a parallel in the gradual development of chlorophyll in the leaves and the formation of haemoglobin in the root nodules, the maximum concentrations of haemoglobin in the root nodules of all the four varieties were attained before the foliar levels of chlorophyll began to decline.

Whilst the decline in foliar chlorophyll content marked a period of transition from the reproductive stage to seed formation in the host, it was distinguished by a gradual change of the red into the green pigment. This confirms the pigment transformations observed in an earlier experiment.

Concomitantly, the transformation of the red into the green pigment during a period of 70-80 days from sowing estimations of root nodule haemoglobin were discontinued owing to the interference of the developing green pigment in nodules.

2. THE SYNTHESIS OF HAEMOGLOBIN IN ROOT NODULES AND CHLOROPHYLL IN LEAVES IN RELATION TO SPECIFIC RHIZOBIUM STRAINS IN ARACHIS HYPOGAEA.

In the course of strain testing in nitrogen fixation with local (R_4, R_5) and American (3G4B9, 3G4B10) strains of Rhizobia from Arachis hypogaea, a marked pattern of chlorosis developed in plants when the latter two strains were used as inoculants in sand culture work.

While this showed the probable adaptation of local strains of nodule bacteria to local plant hosts (Nutman et al., 1952) it was decided to study this phenomenon further since recent work on soyabean (Erdman et al., 1956, 1957; Johnson et al., 1958, 1959 a, 1959 b) has shown varying degrees of upper leaf chlorosis consistently in plants given certain inoculants. Chlorosis, however, was absent when a highly effective Rhizobium strain was used as an inoculant (Ura Mae Mears et al., 1960).

In the light of these observations, it appeared worthwhile to study the influence of Rhizobium strains R_4 and R_5 (local) and 3G4B9, 3G4B10 (U.S.A) on the synthesis of haemoglobin in nodules and chlorophyll in leaves in Arachis hypogaea (All the four strains of rhizobia were isolates obtained from Arachis hypogaea).

Plants of the bunch variety (TMV 2) of groundnuts (Arachis hypogaea) were grown in sterile sand - nutrient solution (vide Materials and Methods) substrate, in glazed containers. The process of seed and sand sterilization, inoculation with rhizobia, watering, supply of nutrient solution and care of plant assemblies are described earlier. Four series of five pots, each containing three plants, were inoculated with various strains, with five pots serving for uninoculated controls. The plants were maintained in the green house and were harvested at 45 days from sowing. Determinations were made of chlorophyll (vide Materials and Methods) and haemoglobin. For chlorophyll determinations, the leaves at the 2nd, 3rd, 4th and 5th nodes from plants grown in association with each strain were collected, weighed and chlorophyll estimated from a fresh weight of 5.0 grams of leaf material.

Observations of the root systems inoculated with the four strains did not show any perceptible difference in terms of nodulation, although in association with strains 3G4B9 and 3G4B10 the plants showed a distinct chlorosis. Leaf chlorosis ranging from light green to nearby white leaves was observed uniformly in plants grown in association with the two strains.

The foliar chlorophyll content and root nodule haemoglobin with reference to the four strains in symbiotic association with Arachis hypogaea are represented in Table 6.

TABLE 6.

The haemoglobin content of root nodules and of chlorophyll in leaves in relation to specific rhizobial strains in Arachis hypogaea var. TMV 2.*

Strain.	Origin.	Chlorophyll a. mg/l.	Chlorophyll b. mg/l.	Total chlorophyll mg/l.	Root Nodule Haemoglobin. µg/g. fresh weight of Nodules.
R_4	Local	213.6	168.7	382.4	300
R_5	Local	204.2	162.5	366.7	295
3G4B9	U.S.A.	58.1	42.4	100.6	84
3G4B10	U.S.A.	61.0	54.6	115.7	75

* Plants were grown in sterile sand inoculated with the various strains for 45 days. The plant nutrient solution was free from combined nitrogen.

The chlorophyll analyses show that the chlorophyll content of leaves from plants inoculated with the local strains of bacteria (R_4 and R_5) were far in excess of and thrice the values obtained with the strains 3G4B9 and 3G4B10. There was a correlation between the chlorophyll content of leaves and haemoglobin content of root nodules. Reduction in haemoglobin content of nodules was associated with chlorosis in host plants grown in association with the strains 3G4B9 and 3G4B10.

In preceding studies on the synthesis of haemoglobin in root nodules and chlorophyll in leaves a certain correlated development of the two haem pigments in relation to nodule development was observed. The present results show that the interaction between haemoglobin and chlorophyll formation in the host involve a third complementary factor viz., the influence of the bacteria in the symbiotic state.