STUDIES ON THE CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF PLANT TISSUE FLUIDS. PART I.

Effects of age and environment on the tissue fluids of French beans (*Phaseolus vulgaris*).

By C. Narasimha Acharya and B. N. Sastri.

In recent years, several attempts have been made to correlate the physiological conditions of plants with the physico-chemical constants of their tissue fluids. Particular attention has been paid to the effects of environment on osmotic pressure (Dixon and Atkins, *Sci. Proc. Roy. Dublin Soc.*, 1912, **13**, 219; 1915, **14**, 374; **15**, 51) and hydrogen-ion concentration (Hurd, *J. Agr. Res.*, 1924, **27**, 725; Rea and Small, *Protoplasma*, 1926–27, **1**, 324; **2**, 428). The study of tissue fluids has also been extended to investigation of plant disease (Contributions to the study of the spike-disease of sandal, *J. Indian Inst. Sci.*, 1928, **11A**, 23, *et. seq.*). No systematic attempt has, however, been so far made to study (*a*) the relation between the chemical composition and the physical constants of tissue fluids derived from different parts of the plant, and (*b*) changes in the composition and properties of tissue fluids with age, nutrition, environment and physiological condition respectively.

Studies of changes in the chemical composition and physical properties of blood under different conditions of health and disease have led to important findings in animal physiology (Myers, *Physiol. Rev.*, 1924, **4**, 274; MacLeod, Physiology and Biochemistry in Modern Medicine, 1926). It may, in like manner, be expected that detailed study of changes in the kinetic tissue fluids of plants which are responsible for the distribution of nutrients, translocation of metabolism products and the various biochemical changes leading to synthesis and storage of reserve products, or degradation and disposal of waste materials, will lead to useful findings in vegetable physiology.

In the present investigation, an attempt has been made to study the changes in the composition and properties of the tissue fluids from the different parts of *Phaseolus vulgaris*. A few preliminary trials have also been carried out to determine the relation of (*a*) conditions of light and shade, and (*b*) height and general development, on the composition of tissue fluids expressed from the plants.

EXPERIMENTAL.

The seeds were obtained from a local nursery, and sown on 28th April, 1928, in unglazed clay-pots containing a suitable make-up of soil and farmyard manure and provided with bottom drainage. The pots were watered freely from day to day, as is usual with garden crops.

Extraction of tissue fluids.—The plants were removed at intervals of 6 to 7 days in sufficient numbers to provide the required material for each set of determinations. After removal of adhering soil by washing in running water, the plants were divided into leaves, stems and roots. About 150 g. of each tissue were weighed into stoppered bottles, and after addition of a little toluene the bottles were cooled in a freezing mixture of ice and salt and left overnight, when the fluids from the different tissues were expressed by means of a hydraulic press at one ton to the square inch, since preliminary experiments showed that the fluids obtained at pressures of 1, 1·5 and 2 tons were nearly the same in quantity as well as total-solid content; higher pressure than 1 ton was not applied. The fluids thus obtained were centrifuged to remove suspended impurities and preserved in the ice-chest. The following determinations were carried out:—(1) Specific gravity, (2) refractive index, (3) osmotic pressure by calculating measurement of depression in the freezing-point using a micro-Beckmann apparatus, (4) hydrogen-ion concentration by the electrometric method, (5) total solids, (6) loss on ignition at 600°, (7) calcium, potassium and phosphorus in the ash by the A.O.A.C. (1925) methods, (8) total nitrogen by Gunning's modification of the Kjeldahl method, (9) total as well as reducing sugars by the method of Davis and Daish (*J. Agric. Sci.*, 1913, **5**, 437) and (10) diastatic activity as described by Sastri and Sreenivasaya (*J. Indian Inst. Sci.*, 1928, **11A**, 23).

Specimens of plants were collected at the following stages:—(1) Emergence of the first leaves (4th May), (2) formation of petioles (8th May), (3) appearance of the nodules on most of the roots (13th May), (4) formation of flower buds (21st May), (5) opening of the first flower (26th May), (6) formation of the pod (1st June), (7) first appearance of the seeds (6th June), (8) maximum development of the first pod (10th June), (9) ripening of seed and (10) harvest of beans (18th June).

Meteorological observations (Table I) during the period of investigation show that although the average minimum temperature remained about the same throughout, the corresponding figures for maximum temperature and hours of sunshine tended to decrease, and the relative humidity to increase towards the later stages. The periods of flowering and seeding were marked by somewhat dull weather which should have led to the slackening of those two processes. It may be inferred that with a hot and dry weather as prevailed in the beginning, the changes corresponding to the formation and the maturation of the bean would have proceeded more rapidly than observed in the present investigation, although no doubt the chemical mechanism of the processes would have been the same.

TABLE I.

Meteorological observations from 23rd April to 21st June.

Period Week ending on	Max. temp.	Min. temp.	Hours bright sunshine	Rainfall, inches	Relative humidity, percentage
28th April	97·4	71·9	9·3	...	41
4th May	98·4	72·5	9·5	0·002	47
10th ,,	94·9	70·6	7·8	0·015	56
16th ,,	95·2	69·6	10·1	0·230	49
22nd ,,	96·6	71·9	9·1	0·048	52
28th ,,	94·3	69·3	10·2	0·062	50
3rd June	94·1	69·8	10·0	0·202	51
9th ,,	84·0	67·6	3·6	0·015	65
15th ,,	85·2	67·9	4·2	0·087	68
21st ,,	87·3	66·6	6·3	0·012	58

General observations.—With the growth of the plant, the shoot lengthened from 6 cm. on the 7th day after sowing to 30 cm. at the stage of maximum development of the pod; there was no increase during the ripening of the seed (10th to 18th June). A similar observation was made with regard to the root-length measured from time to time. The average total weight of the plant increased from 1·65 g. when the first leaves emerged to 19·4 g. when the pod was fully formed (10th June) but fell to 17·6 g. when the seed was fully ripe (18th June). Changes in the weight of leaf and stem respectively were similar to those of total weight, but the weight of the root increased right up to the end. The yield of tissue fluid from the leaf was more or less constant (85—89 per cent.) up to the stage of seed development but showed a slight decline (76 per cent.) as the seed ripened: the yields from stem and root—decreased to a greater extent with the age of the plant, and fell from 84 to 57 per cent. in the case of the stem and from 83 to 66 per cent. in that of the root.

Specific Gravity, Refractive index, Osmotic pressure, Total solids and Mineral ash.

The figures (I—IV) show that tissue fluids from different parts exhibit progressive but similar variations at various stages during the growth of the plant. The curves are generally double topped ones: and show either two maxima or two minima corresponding to the stages of development of flower buds and formation of seeds respectively.

As the plant grows the tissue fluids, thin and dilute in the early stages, become richer in both mineral and organic matter, showing a progressive increase in specific gravity, osmotic pressure, total solids and mineral ash content, till the period of flowering. When flower buds are formed however, there is a drop in the concentration of the sap, the plant foods being transported to the centre of reproduction. The curves show a minimum immediately after the flowers open, and maintain a low level till the seeds are ripe. Such a stage may be expected to represent a period of low vitality in the plant. It is probably on this account that a plant is particularly susceptible to disease at the flowering stage.

The leaf-sap has generally a higher specific gravity and contains more of total solids than the stem. The fluid from the root gives invariably lower values than those from the leaf and stem for all attributes mentioned above, thereby suggesting that it is more dilute than the others.

The following were the ranges of the refractive indices of the tissue fluids from the different parts of the plant, measured at 26° :—Leaf, 1·3375—1·3402; stem, 1·3369—1·3408; and root, 1·3356—1·3368. There was generally a close correlation between the refractive indices and the corresponding total solid contents.

Reaction (Fig. V).—The hydrogen-ion concentration of the tissue fluids changes progressively during growth. Thus the P_H values for the fluid of the leaf ranges from 5·66 to 6·21, that of the stem from 5·6 to 5·9 and that of the root from 5·85 to 6·29. In all cases maximum acidity is reached at the time of flowering after which there is a steady return to the initial reactions.

Nitrogen (Fig. VI).—In the early stages, when vegetative growth is rapid, the sap is comparatively rich in nitrogen. With the approach of the flowering stage, the nitrogen content decreases rapidly and reaches a minimum when the first flower buds appear. The low level of nitrogen continues during the seeding stage, but tends to rise again as the plant ages. This observation is in accordance with those of

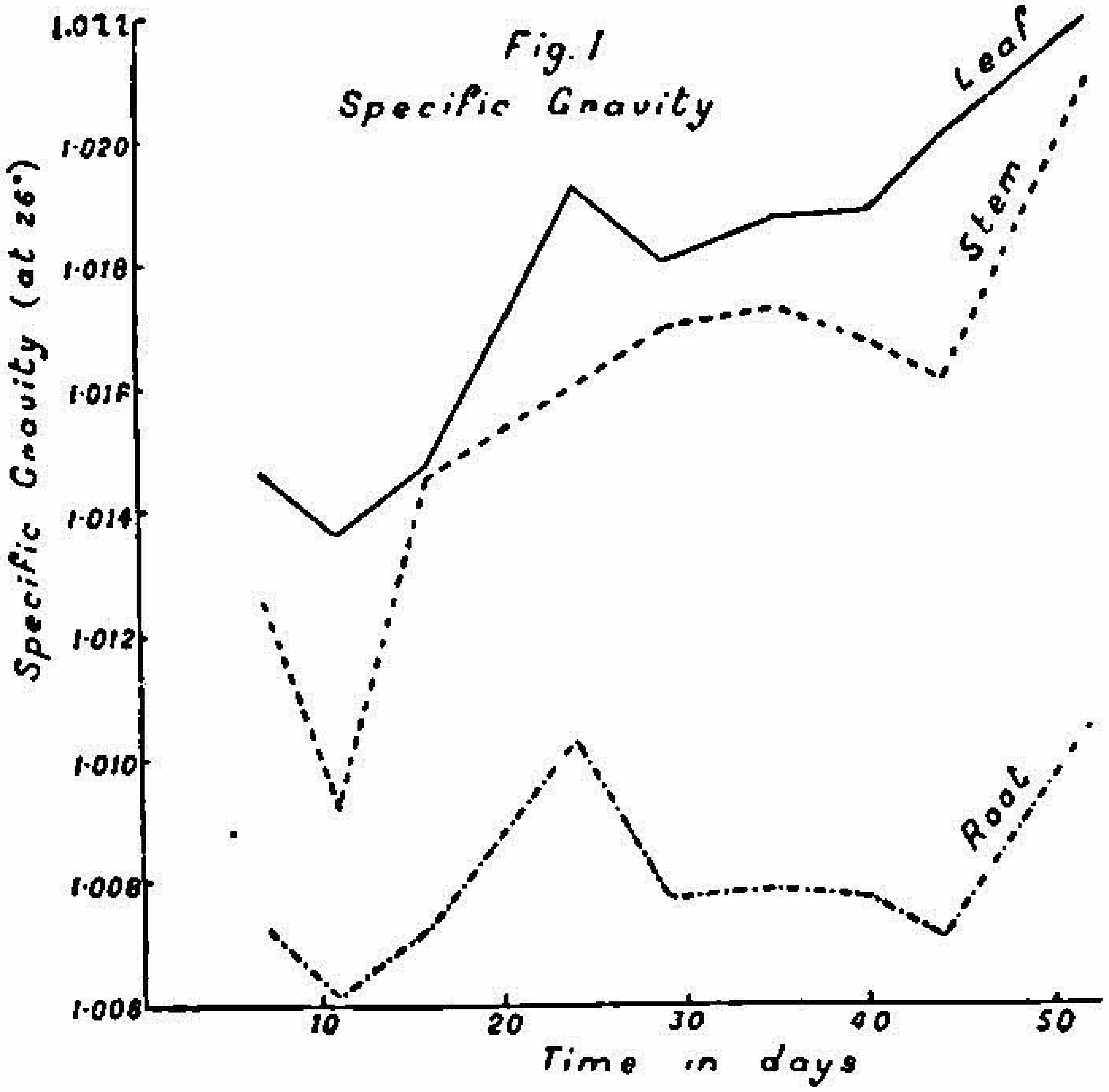

Fig. I
Specific Gravity

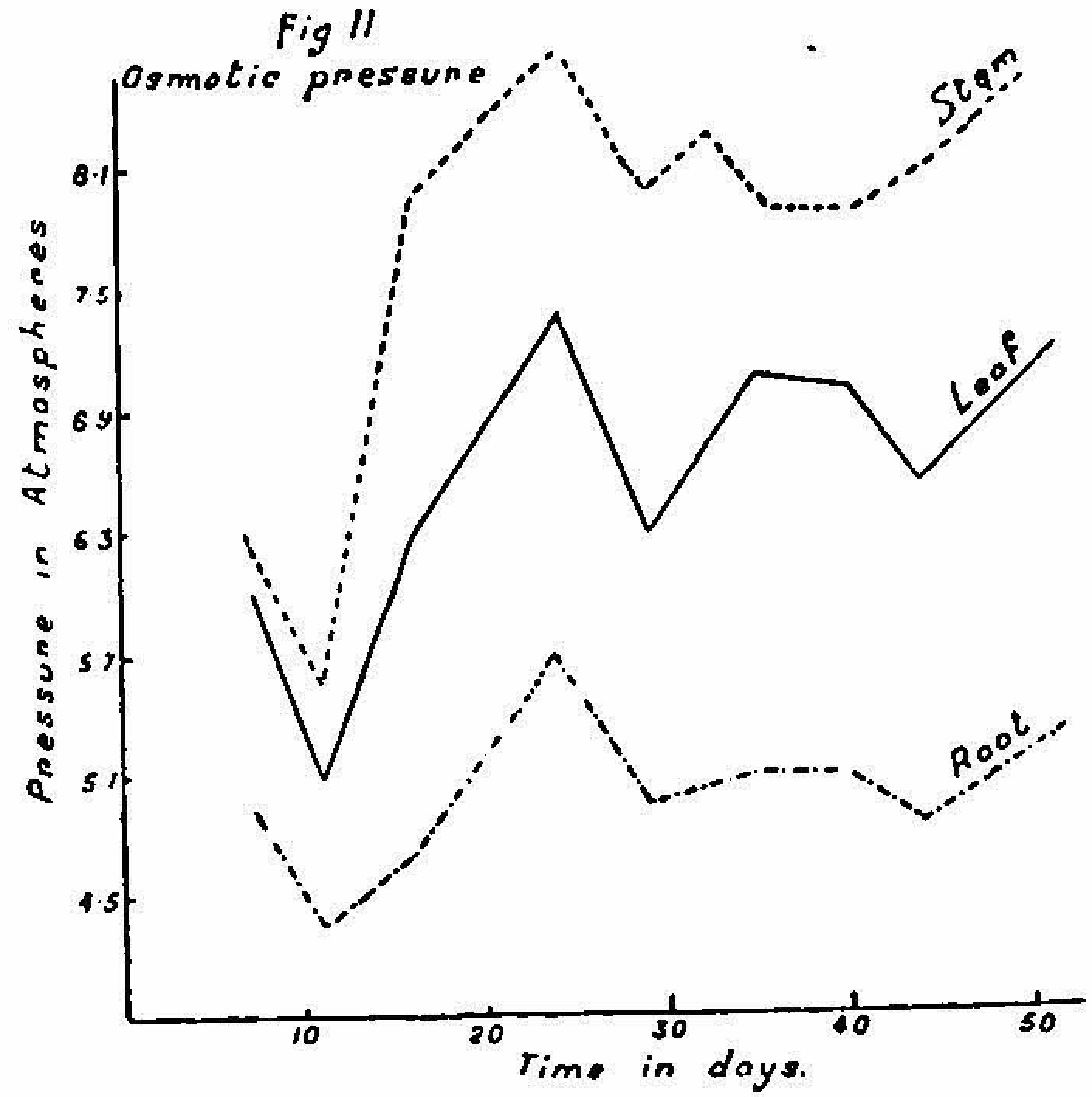

Fig II
Osmotic pressure

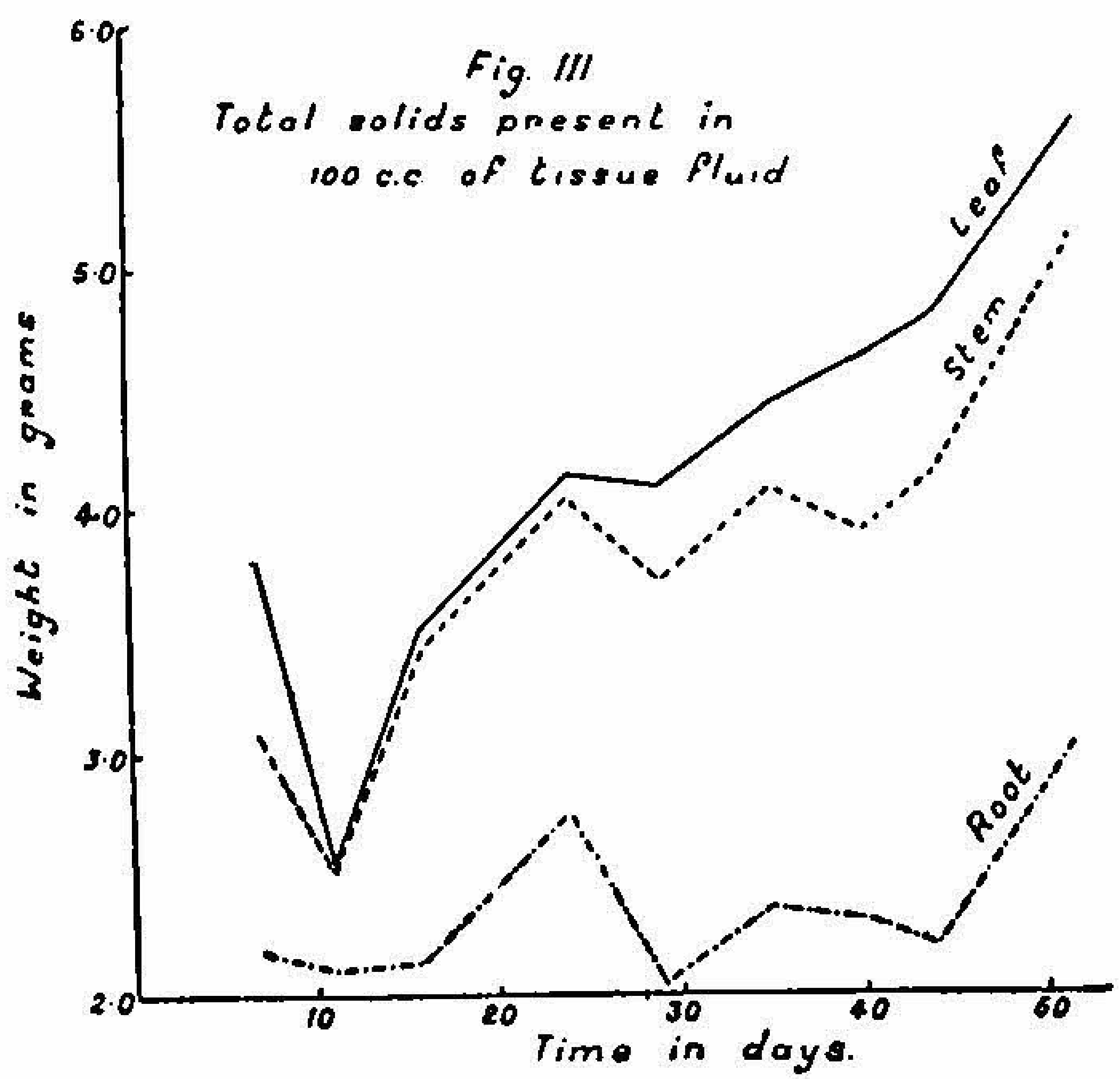

Fig. III
Total solids present in
100 c.c of tissue fluid

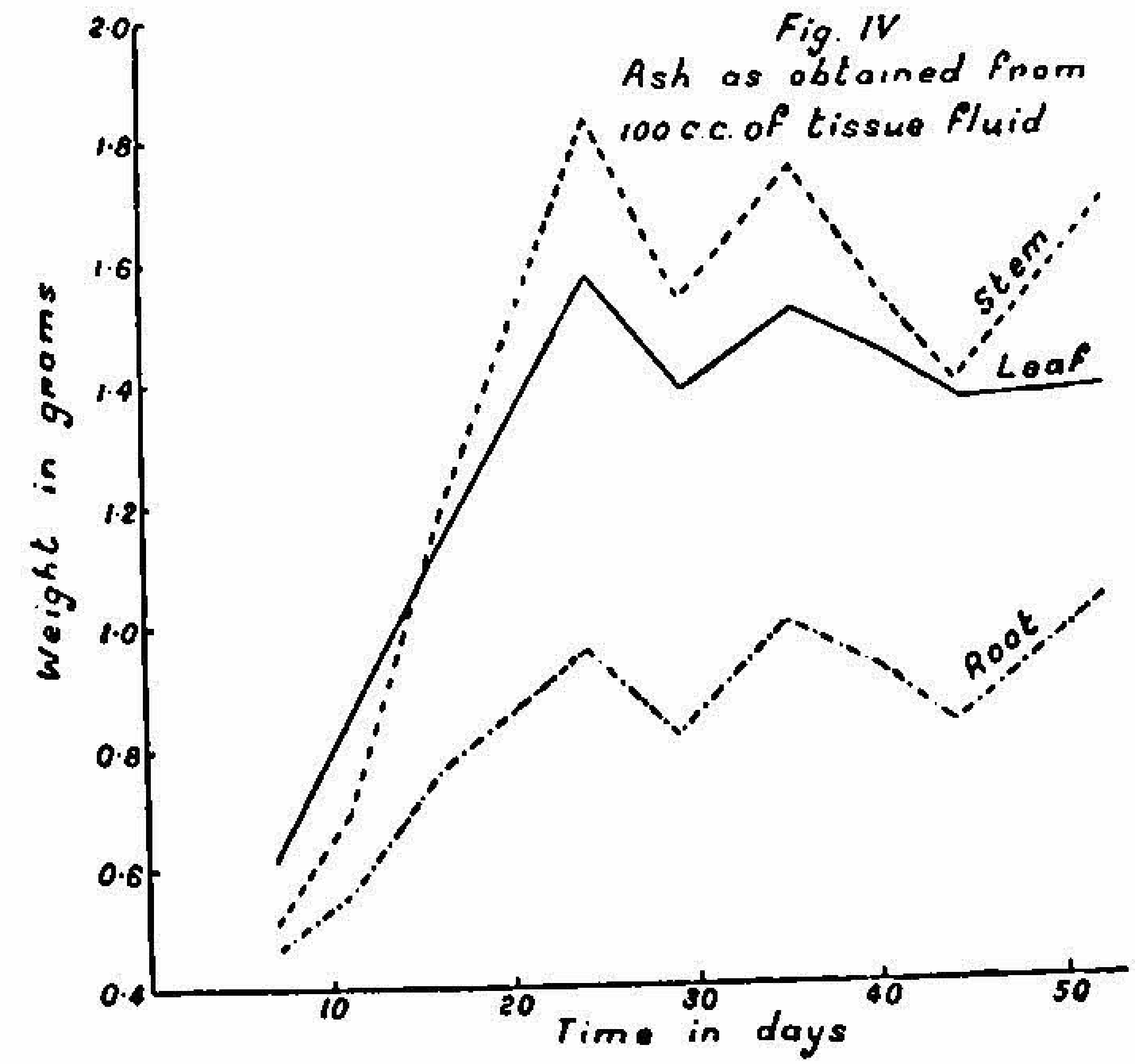

Fig. IV
Ash as obtained from
100 c.c. of tissue fluid

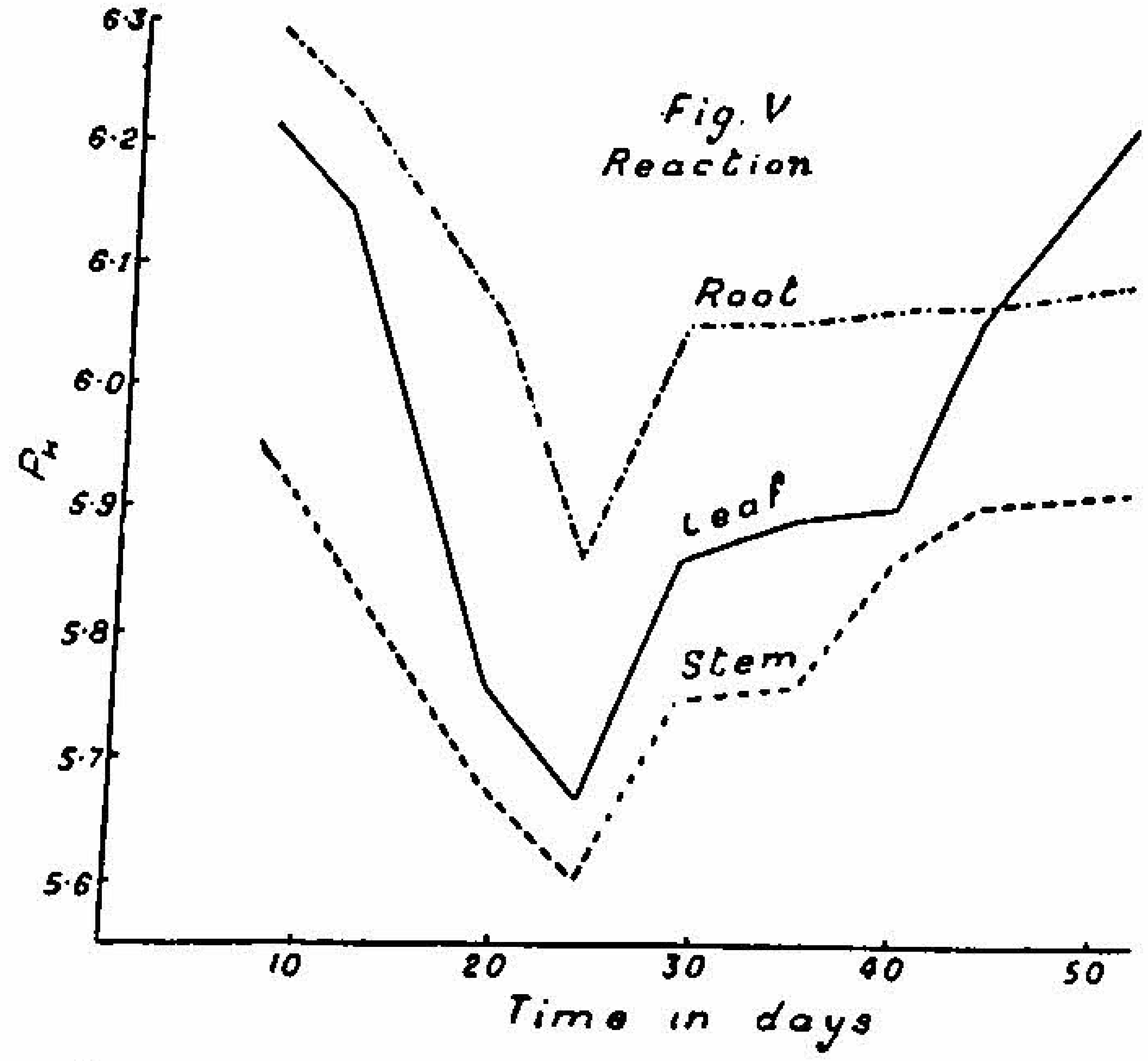

Fig. V
Reaction

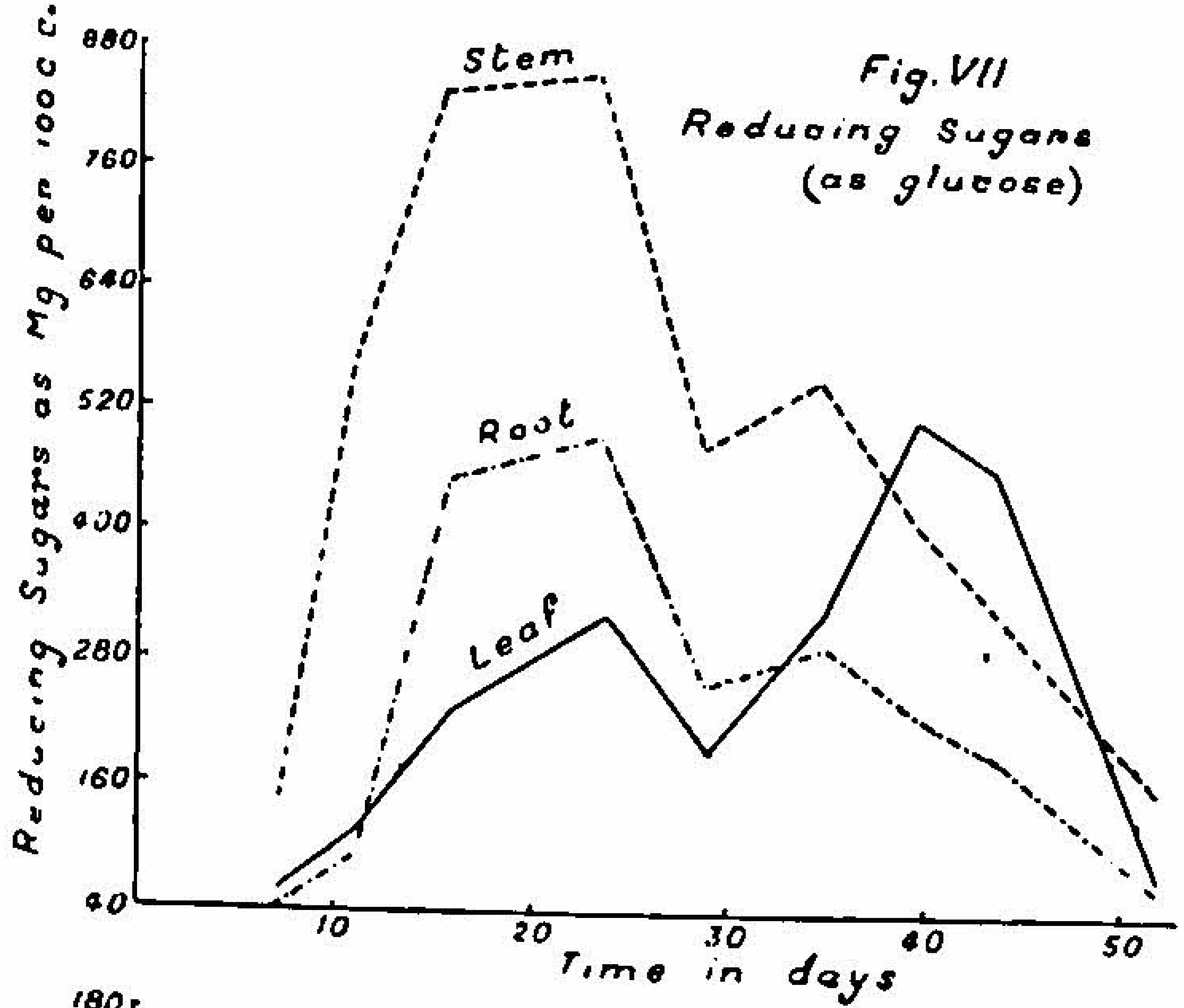

Fig. VII
Reducing Sugars
(as glucose)

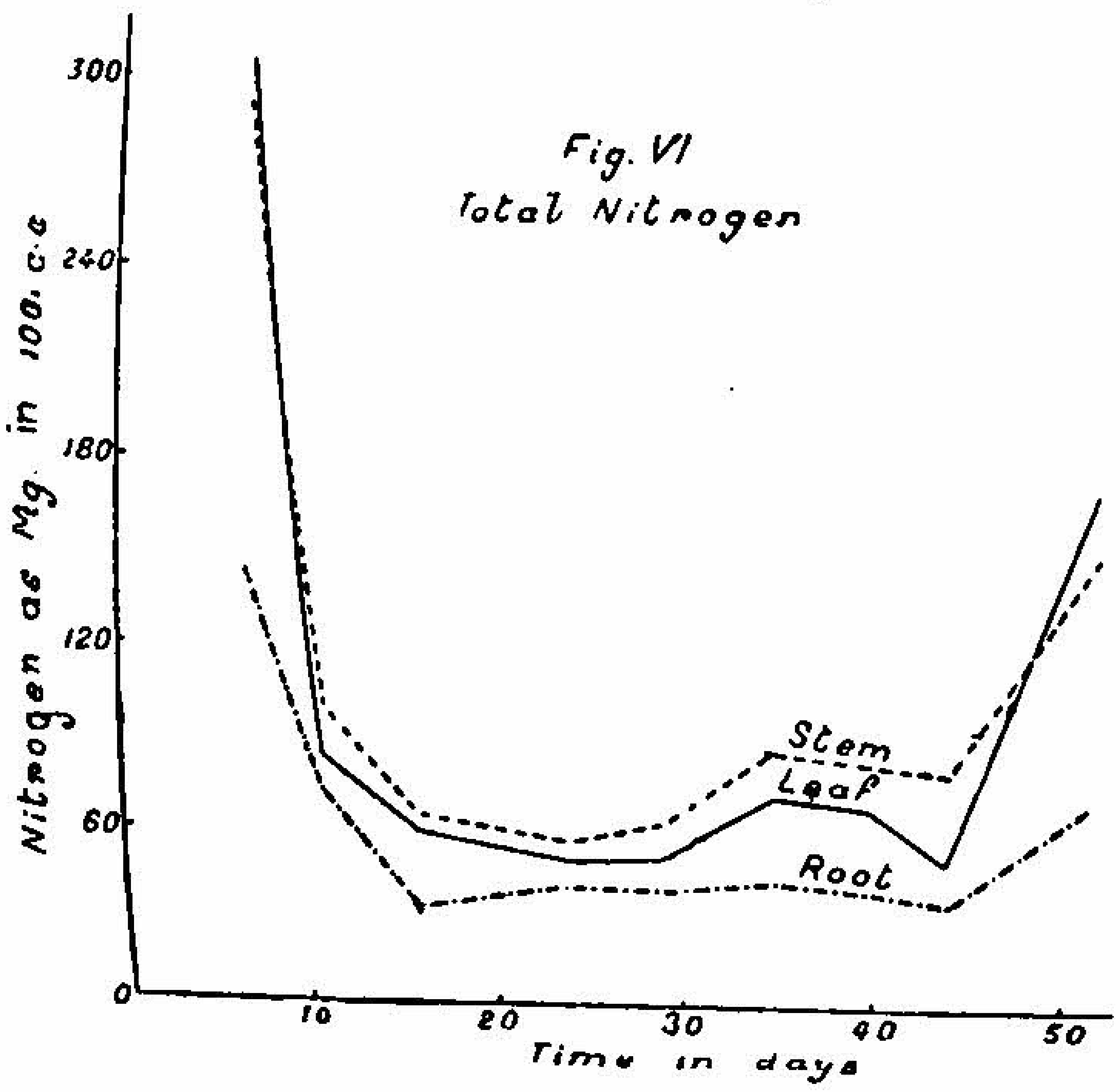

Fig. VI
Total Nitrogen

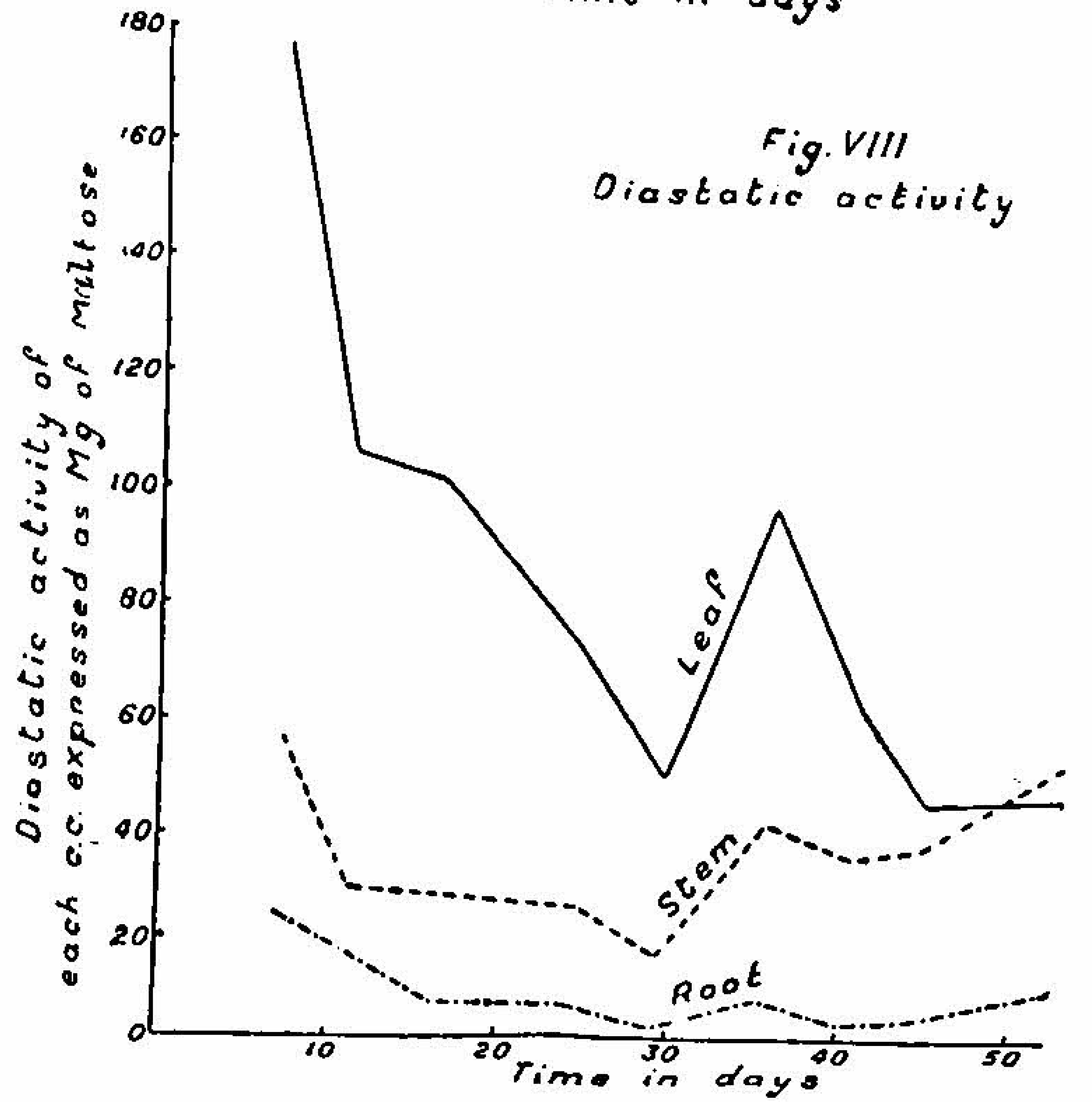

Fig. VIII
Diastatic activity

Hicks (*New Phyt.*, 1928, **27**, 108), Gericke (*Soil Sci.*, 1922, **13**, 135) and others.

Sugars.—Reducing sugars account for nearly all the total sugar present in the different tissue fluids, so these only are presented in Fig. VII. As contrasted with nitrogen, the sugars increase steadily in the fluids from the different parts of the plant until the flowering stage is reached. Decrease then sets in, and excepting a slight tendency to increase at the time of seed development, the sugars diminish until they become almost negligible at the time of harvesting.

Diastatic activity (Fig. VIII).—Diastatic activity is prominent in the fluid from the leaves particularly in the early stages, but declines rapidly: a brief pause is noticed at the time of flowering, but soon the decline is resumed until the activity at harvesting becomes less than one-third of the original. The activity of the fluid from the stem is generally lower than that from the leaf, and is not so much affected by the age and condition of the plant. The diastatic activity of the fluid from the root is generally very much less than that of the other tissues, and is almost negligible at the time of flowering and seeding.

Tall and stunted plants.—Although all the plants belonged to the same variety and were raised under identical conditions, it was noticed that some plants were taller and better developed than others. In view of the possibility of chemical and physico-chemical attributes of such plants differing from each other, the determinations described above were carried out with them.

It was observed that rapid vegetative growth was accompanied by lower specific gravity, refractive index and total solids in the fluids from the stem and the root, while in the case of that from the leaf the reverse was true. The data for osmotic pressure, nitrogen, ash content and diastatic activity of the fluid from all parts of the tall plants are also lower than the corresponding figures for the stunted ones.

Effect of darkness.—To study the effect of darkness and consequent arrest of photosynthesis, representative plants of different ages were stored in the dark for 24 hours and their tissue fluids then examined in the manner described already.

It was observed that the fluid from the leaf was the first to suffer. Specific gravity, refractive index and total solids, originally high, became very much less as the result of storage in darkness. The reaction was also affected. Thus, whereas the reactions of the sap from leaf, stem and root were originally P_H 5·88, P_H 5·75 and P_H 6·04 respectively, they became P_H 6·71, P_H 6·44 and P_H 6·2 in darkness. This observation is in accordance with the findings of

Garner, Bacon and Allard (*J. Agric. Res.*, 1924, **27**, 119), who noted that decrease in the length of daily illumination is marked by corresponding reduction in the active acidity of the sap.

It was also noted that decrease in the carbohydrate content of plants kept in darkness was accompanied by accumulation of nitrogen in the leaves.

Composition of ash (Fig. IV).—There is considerable variation in the mineral ash contents of the saps expressed at various stages in the life of the plant, ranging from about 0·46 g. per 100 c.c. of the fluid from the root to about 1·71 g. in that from the stem. The composition of the mineral matter is fairly uniform. Potassium oxide forms the major constituent in all cases and accounts for 30 to 50 per cent. of the total mineral matter. The highest percentage is found in the stem, which is also the richest in carbohydrates.

The figures for calcium (as CaO) show more variation. There is but little calcium in the root sap, at no time more than 6 per cent. of the total ash. The percentages in tissue fluid from the stem range from 6 to 12 per cent. while those for the fluid from the leaf increase from about 7 per cent. in the earlier stages to about 20 per cent. at the time of harvest.

The amounts of phosphorus present in the fluids from the different parts are invariably low, being at no time more than 3 per cent. of the total ash. It was also noted that the phosphorus and potash contents of fluids from plants kept in darkness were higher than those from illuminated plants.

DISCUSSION.

The foregoing observations show clearly that the tissue fluids of *Phaseolus vulgaris* undergo changes in chemical composition and physical properties which correspond to different stages in the life of the plant.

As the plant develops from the seedling stage, its roots collect minerals from the soil and the leaves build up sugars and other photosynthetic products, which are subsequently translocated to the other parts of the plant. The total solids, the sugars, and the minerals in the tissue fluids from the different parts of the plant increase in consequence and continue to do so until the flowering period is reached. After that stage, however, though the assimilation of nutrients through the roots and photosynthetic activity still continue, translocation of carbohydrates and minerals into the flowers proceeds so rapidly that the quantities of those substances in the tissue fluids from different parts of the plant are considerably lowered. The decrease in the different constituents continues throughout the flowering and the seeding stages, after which there is a tendency for the original composition to be restored.

By showing a continuous decrease throughout the life of the plant, nitrogen of the tissue fluids appears to be an exception to the observations described above: but considering that the total nitrogen in the whole plant increased almost to the day of harvest, it may be inferred that the apparent diminution in the nitrogen percentage of the tissue fluids is due to the proportionately greater photosynthetic activity. Nitrogen is being assimilated from the soil as well as fixed from the atmosphere throughout the life of the plant. The quantities of nitrogen thus passing into the tissue fluids are, however, proportionately less than the carbohydrates and other products of photosynthetic activity in the fluids at the same time. The decreased proportion of nitrogen is therefore due not to diminished intake, but to disproportionately greater photosynthetic activity, throughout the life of the plant.

The change in reaction at the time of flowering may be attributed to rapid translocation of the different sugars and mineral salts from the various parts of the plant to the flower buds. A poorly buffered condition results and a somewhat acid reaction is observed. After the flowers are formed, there is less translocation from the different parts with the result that increasing amounts of the different buffering materials collect in the tissue fluids and, in consequence, the original reaction is rapidly attained.

The diminution observed in diastatic activity, particularly in the leaf tissue fluid, suggests that as the plant ages there is less and less hydrolysis of starch taking place. Since, however, the translocation of carbohydrate materials to the different parts, particularly to the flowers and the seeds takes place throughout the life of the plant, it may be inferred that such changes are probably not preceded by hydrolysis of starchy materials. The rapidity of the changes, particularly at the flowering stage, suggests that the photosynthesised materials are carried by the tissue fluids from the leaf and, to a smaller extent, from the other parts directly to the flower buds.

The study of the composition of the tissue fluids from the tall and the stunted plants shows that there is greater photosynthetic activity in the former than in the latter. The occurrence of considerably greater percentages of minerals and nitrogen in the tissue fluids from the stunted plants is obviously due to diminishing amounts of water and photosynthesised materials passing into them. The observation also suggests that the stunted condition is due to some of the plants having received more nitrogen and minerals in the earlier stages than was really needed and in consequence, 'lodging', a phenomenon commonly observed in the field crops whenever soluble plant nutrients are supplied in excess.

The composition of the fluids from plants kept in darkness has been affected considerably. The respiration of the plant proceeds throughout the period of storage in darkness resulting in the utilisation of the mobile carbohydrates in the tissue fluids. There is no photosynthetic activity to make up for the loss, and hence follows the diminution in the various constituents of all tissue fluids. The hydrogen-ion concentration is also affected by steady disappearance of the different materials, and probably also by development of certain organic acids. The mineral and nitrogen contents also decrease as the result of storage in darkness, showing that in absence of photosynthesis, supply of the nutrients essential to the active functioning of the roots in the absorption of minerals, and of the root-nodule bacteria in the fixation of atmospheric nitrogen, has been retarded.

SUMMARY.

The more important physical properties and chemical constituents of the tissue fluids from different parts of *Phaseolus vulgaris* at various life-stages were determined and their relation to age and environment studied.

The observations show that the tissue fluids become enriched with minerals and the different products of photosynthesis up to the stage of flowering, when rapid translocation of the different constituents to the flower buds take place. After that period, although the assimilation of minerals from the soil, the fixation of nitrogen from the air and the photosynthetic processes continue at the same rate, yet the translocation of the various substances into the flowers and seeds proceeds so rapidly that the tissue fluids in the other parts are rendered comparatively weaker in the various constituents almost up to the time of harvest.

The tissue fluids from tall and stunted plants of the same age were examined. The 'lodging' observed in the case of the latter was found to be associated with accumulation of nitrogen in the leaf and low water content of the tissue fluids from the different parts of the plant.

Examination of plants stored in darkness showed that owing to prolonged respiration of carbohydrate materials without compensation

by photosynthesis, the tissue fluids from the different parts are rendered poor in carbohydrates and solid matter in general. Owing to inadequacy of carbohydrate nutrition, nitrogen fixation in the nodules and assimilation of minerals by the roots are distinctly affected.

In conclusion, we beg to acknowledge our grateful thanks to Dr. V. Subrahmanyan for his kind interest in the progress of the work and suggestive criticism.

Department of Biochemistry,
Indian Institute of Science,
Bangalore.

[Accepted, 11–3–31.]

Printed and published at the Diocesan Press, Post Box 455, Madras—1931. C1606.