Ph.D. THESIS

by

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"GASEOUS EXCHANGE IN PLANTS"

A. "The Effect of Salts and Other Substances on the Respiration of Elodea Canadensis Michx."

B. "The Effect of Potassium and Phosphorus Starvation on the Carbon Assimilation Rate of Barley."

with an additional paper:

"The Percy Sladen Expedition to Lake Huleh, Palestine. 1935.

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A.

The Effect of Salts and Other Substances on the Respiration of Elodea canadensis Michx.

Table of Contents

Introduction

The process of respiration, in its more common form, is one in which an oxidation takes place; this may involve simple compounds, as, for instance, in the Sulphur or Nitrifying Bacteria, or, more characteristically, the oxidation and degradation of complex organic substances such as fats, carbohydrates and proteins.

Blackman (3) has formulated a scheme which indicates the course of respiration of carbohydrates. As a result of more recent work, however, by Bennet-Clark (2) and others, and of studies in the relationship of nitrogen metabolism to respiration by Spoehr and McGee (28), Schwabe (26) and others, it became increasingly apparent that a schema involving protein metabolism as well as carbohydrate metabolism was necessary to give a more complete picture of the process. Such a schema has been formulated by Gregory and Sen (8), in which the various sources of carbon dioxide (production of which is often taken as a measurement of the intensity of respiration) are indicated.

As would be expected from the nature of the process, such factors as temperature, etc., may affect the rate of respiration. A large number of workers have also demonstrated, by means of culture experiments, the relationship between certain ions and the respiration or other physiological phenomena of plants. The more recent publications of Gregory and Richards (7) and their collaborators/

collaborators deal very fully with the effects of Potassium, Nitrogen and Phosphorus on the physiology of the barley plant.

Jacobi (12), working with aquatic plants, found that various salts, when dissolved in water, had the effect of increasing the rate of respiration to varying degrees, according to the salt used. Lyon (18) also found a similar stimulation when using phosphate solutions. Stiles and Leach (29) state that "weak solutions of mineral salts and inorganic acids appear to cause an increase in respiration intensity." Further, Wehner (33) found that a mixture of nitric acid and sodium nitrate caused Fontinalis antipyretica to respire more actively. The present work is an attempt to follow the course of respiration over long periods in the dark when inorganic salts are used, and is also an attempt to discover the underlying causes of the stimulation.

METHODS AND MATERIAL

The estimation of oxygen dissolved in water

In such experiments as the following it is convenient to measure the rate of respiration by the amount of oxygen consumed. The more usual methods of estimating oxygen dissolved in water are derived from the method described by Winkler. The principle involved depends on the instability of manganous hydroxide in the presence of free oxygen. In this estimation the water to be analysed is first rendered alkaline with a solution of alkaline potassium iodide; a solution of a salt of manganese ($-SO_4$ or $-Cl_2$) is then/

then added to the water and the following reaction takes place:-

$$Mn\,SO_4 + 2\,KOH \rightarrow Na_2SO_4 + Mn(OH)_2 \quad \ldots\ldots\ldots\ldots (1)$$

In the presence of dissolved oxygen,

$$2\,Mn(OH)_2 + {}^1/_2\,O_2 + H_2O \rightarrow 2\,Mn(OH)_3 \quad \ldots\ldots\ldots (2)$$

On subsequently acidifying the medium, the manganese hydrate acts as an oxidising agent and liberates free iodine from the potassium iodide supplied with the alkali in the first step. The manganous hydrate is regenerated and this forms the salt of the acid

$$2\,Mn(OH)_3 + 2KI + 6\,HCl \rightarrow 2\,MnCl_2 + 2\,KCl + 6\,H_2O + I_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The manganic hydrate acts as a "carrier" of oxygen.

A drawback with the original method is in the fact that a large quantity of water is required - one hundred ml. or more. Estimation on a smaller scale is limited by the difficulty of eliminating the short periods in which the water to be analysed comes into contact with atmospheric air. More recently, micro-methods have been described which can be used with a high degree of efficiency, as, for example, those of Nicloux (21) and Ellis (6). The method employed in the present work is similar to that described by van Dam (5). In his method, one ml. of water can be used, and the oxygen content accurately measured. The present author has applied this method/

method to a study of photosynthesis in aquatic plants [Jones (14)] where a current of water was passed over the plants. In that work, owing to the small amount of water used in the estimation and to the method of sampling direct from the water stream, very careful consideration had to be given to the accuracy of the rate of flow of water over the plants. The oxygen estimation apparatus consisted of a syringe which had an accurately ground piston, and was so made that a very precise quantity of water could be taken in. The titration was done by means of a modified Rehberg burette [Rehberg (24)].

This apparatus is of a rather expensive nature, and when the present work on respiration in this University was begun, it was decided that some cheaper form of apparatus be used. A ten ml. syringe pipette embodying the same principles as the van Dam model is described by Krogh (15), and this is also of a high degree of accuracy. With this larger syringe pipette a less expensive form of micro-burette could be used, viz: 2 ml. divided into 1/100ths. of a ml. so that accurate readings could be made to the nearest 0.005 ml.

The syringe itself is completely made of glass, and consists of (D) a solid glass piston which is very accurately ground into the substantial glass barrel (E). The fit is so perfect that if the barrel (E) is pressed with the fingers the piston can be moved only with difficulty; otherwise the manipulation of the piston is quite/

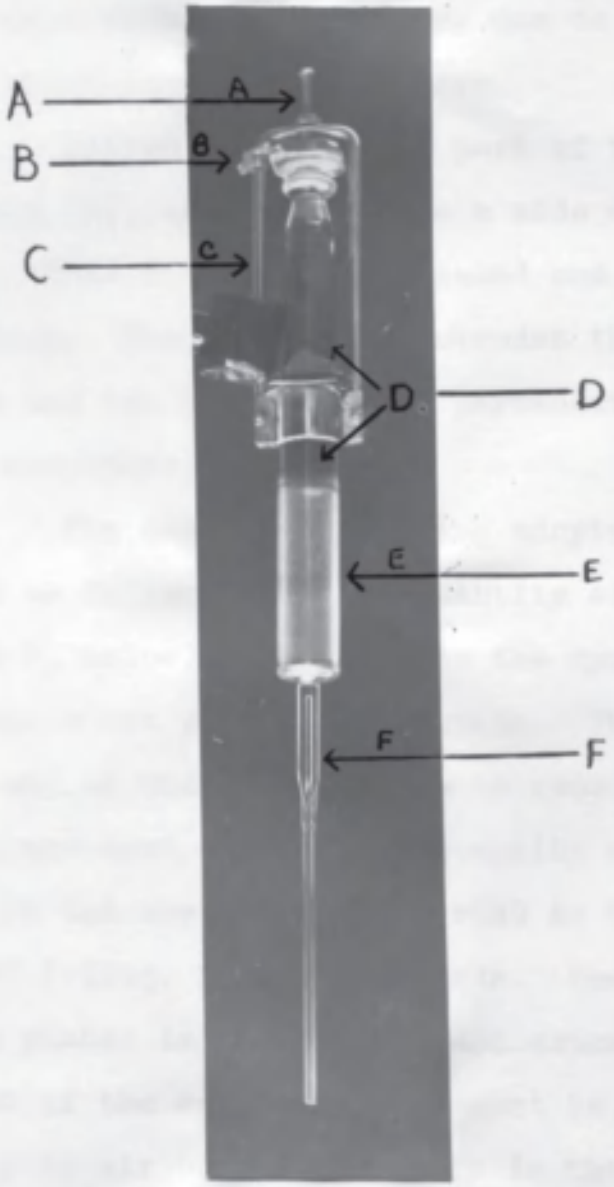

Figure 1 Photograph of syringe pipette. For description, see text.

quite easy. Extending from the barrel is a capillary tube (F) about twelve centimetres long. The narrow cannula can be clearly seen in the photograph, the small meniscus/

meniscus next to the barrel being due to a small droplet of water which has collected there.

Fitted to the glass part of the syringe is a metal guard (C), and attached to a side of this is a stop (B). This stop can be loosened and set firmly in any position. The screw (A) protrudes through the top of the guard and can be fixed in a permanent position by means of stop nuts.

The method of sampling adopted by the present author is as follows: a small quantity of alkaline KI (Solution 1, below) is taken into the syringe and the inside rinsed out with this solution. This is then expelled and as much as possible is removed by moving the piston up and down. A further quantity of the solution is taken in and the syringe inverted so that the capillary opening of F (Fig. 1) faces upwards. The piston is then carefully pushed in so that air and excess solution are forced out of the cannula. Care must be taken that absolutely no air bubbles are left in the syringe. As a matter of routine it is also necessary to turn the top of the piston "square on" so that slight variations in the volume of the dead space (due to a certain unevenness of the flat base of the piston) are avoided. The end of the syringe is now plunged into the water to be analysed, and the piston drawn up slowly. By turning the square top of the piston it comes into contact with stop B. It is advisable to turn the top of the piston to its fullest extent/

extent, as, in this way, it can be drawn out to exactly the same degree every time; thus a very precise volume of water is taken in at every estimation. Water is then quickly rubbed off the end of the syringe, and the end placed in a solution of manganese chloride (Solution 2, below). The piston is now turned, so that, being free from stop B, the manganese solution can be drawn in. The screw A (Fig. 1) is fixed at a definite point above stop B in order that, by the last movement, a definite volume of Solution 2 is drawn into the syringe. A small piece of rubber pressure tubing, filled with water and plugged at one end with a solid glass rod, is quickly fitted over the end of the capillary.

The movements performed thus ensure (1) the precise intake of solutions, and (2) comparatively little contact of the water with air. The length of the capillary, together with the fact that it is filled with Solution 2, obviates any influence of the oxygen in the water of the water stopper on the estimation.

As is usual in micro-chemical analysis, the movements and times for each comparative estimation are always the same.

The precipitate formed inside the syringe is now dispersed by a simple circular movement of the wrist. Too violent shaking is apt to cause movement either of the piston or of the water stopper, with consequent risk of the appearance of bubbles of gas; this must be carefully guarded against. A small bubble of air or gas in the syringe during an estimation shows a very definite difference in the/

the final titration value.

After the dispersal of the precipitate, the syringe is clamped upright and left for a definite period, half an hour at the most, to allow the precipitate to settle on the piston. After the required period, the water plug is carefully removed - the glass rod plug first, then the rubber, as in this way very little negative pressure is caused inside the syringe; such would tend to cause the formation of gas bubbles, or the inclusion of these, with a consequent reduction in the volume of liquid to be titrated. The titrating vessel is a clear glass specimen tube of dimensions 3 cm. diameter and 9 cm. height, and containing 1.5 ml. of HCl solution (one part concentrated acid to four parts water). With the removal of the water stopper the end of the syringe is placed under the acid, and a small quantity of clear liquid can be expelled before any of the precipitate gravitates out. Some of the liquid mixed with acid is drawn back in again by bringing the piston back to screw A. This acidification brings about the reaction in equation 3, above. A slight oscillation of the piston (with the end of the cannula under the acid) mixes the acid and water together, so that this part of the procedure is quickly finished. The solution of liberated Iodine is now gently and evenly expelled into the titrating vessel. The Iodine is very volatile, so the titration must be commenced at once and finished as quickly as possible. N/200 sodium thiosulphate

is/

is used for the titration. The greater part of the thiosulphate necessary (known approximately from previous trials) is added without shaking the titrating vessel. The Iodine left in solution is now in such dilute concentration that there is very little fear of loss by volatilisation. When the colour is almost disappeared three drops of starch solution (Solution 4, below) are added and the titration finished with moderate care. The titrated liquid is then sucked back into the syringe pipette (which contains some Iodine solution in the "dead space") and expelled three times, and with the recovery of the blue colour the titration is very carefully finished. If the titration movements are completed within a short time, there is comparatively little liberation of Iodine by the action of the acid on KI.

Solutions used

(1) NaOH ..... 33 gms.
KI ..... 10 gms.
Water to ..... 100 ml.

(2) Manganous chloride ... 40 gms.
Water to ..... 100 ml.

(3) HCl conc. ..... 20 ml.
Water to ..... 100 ml.

(4) Soluble Starch ..... 1 gm.
Conc. NaCl soln. to.. 100 ml.

The substances used in the above solutions are of "Analar" purity. In the experiments described below, where substances of "Analar" standard could not be obtained/

obtained, only those of highest purity were used.

The proportions of Solutions 1 and 2 used are such that the quantity of Solution 2 is half that of Solution 1. These quantities are, of course, determined by the amount of Solution 1 required to fill the dead space of the cannula, etc. This volume is calculated by filling the dead space with a solution of known normality, washing this out with distilled water, and titrating with another solution of known normality. The screw A (Fig. 1) is adjusted so that on pulling out the piston from B to A, half the volume of the dead space is drawn in. The syringe used for these experiments has a dead space of 0.301 ml., found by filling the dead space with N NaOH and titrating with N/20 acid and averaging over several readings. The position of stop B is fixed so that by drawing up the piston from its lowest limit ten ml. of water are taken in. This is determined by filling the dead space with water, attaching the syringe to a burette containing water by means of a water-filled piece of rubber tubing, and observing the movement of the meniscus in the burette on withdrawal of the piston to stop B. In point of fact, this volume (i.e. in addition to the volume of the dead space) is made to be ten ml. for ease of calculation. The volume of liquid drawn in on moving the piston from B to A is also determined by the same method. Krogh has found that Solutions 1 and 2 can vary in their proportion within wide limits, but, of course, the proportion decided upon must be strictly adhered to in every/

every comparative estimation. By fixing screw A in the position determined by the use of the burette (as described above), the volume of Solution 2 used is always half that of Solution 1, viz: 0.15 ml. of Solution 2 is used.

The Experimental Error of the Technique

In order to test the technique it is, of course, necessary to use water with a constant oxygen content. Water of low oxygen tension, obtained by bubbling nitrogen overnight through a winchester of water standing in a thermostat, may be used as (a) this would probably produce a more constant or only slowly-changing oxygen content, and (b) variation in technique due to differential contact with atmospheric oxygen would become obvious. The following table shows the results of three successive trials.

TABLE 1

Results as ml. of N/200 Sodium thiosulphate	
Trial number	Ml. thio. (N/200)
1	0.207+
2	0.210
3	0.210

The slight discrepancy in the first reading, although comparatively small, is known to have been caused by too hasty removal of the water stopper, so that some bubbles of air replaced a small quantity of liquid in the cannula just before acidification. In such a case the reading would be discarded as being unreliable.

A similar degree of accuracy can be obtained with/

with water in equilibrium with air if the micro-technique described by Krogh (15) is carefully followed. It is very often possible to obtain identical readings for consecutive estimations. For example:

Ml. thio. required as in Table 1 above

(1) ..... 2.290 ml.
(2) ..... 2.290 ml.

Occasionally, however, a slight difference up to 0.0375 ml. oxygen / litre of water, as maximum divergence, occurs. This may be due to small fluctuations in the oxygen tension, but the maximum divergencies occur, generally, when a fault in technique takes place, on observance of which the titration would be discarded. Table 2, below, presents two series of estimations on two different lots of water.

TABLE 2

Water lot number	Ml. N/200 thio. used for 10 ml. sample
1	2.250 2.250 2.260 2.250+
2	2.290 2.295

Table 2 shows that comparative estimates are possible within 0.7%, even with faulty technique, but this must be considered as maximum. In general, the results can, with care, show a variation of only 0.2%. There is/

is no doubt that in these trial experiments, successive estimations on the same water would be closer if care were taken to ensure the absolute constancy of the oxygen tension in the water.

The Plant Chamber, etc.

It was decided to use 'still' water in these experiments, as a current method involved the use of rather more expensive apparatus. Although there are certain objections to the use of still water, as, for instance, the formation of local diffusion gradients of oxygen around the plants so that the oxygen tension at the leaf surface is not absolutely known, it was nevertheless hoped that, in spite of the objections, a satisfactory comparative series of results would be obtained.

A first essential aimed at in devising the apparatus was (1) ease of manipulation of the plant chamber, and (2) easy replacement of the solutions when required. Several types of plant chambers were tried. Some were of simple design and had the opening closed either with liquid paraffin or by a wax float as recommended by James (13) for his 'stock' solutions. In no case were satisfactory results obtained.

Another type, where the water from the stock solutions on a shelf gravitated through a glass spiral immersed in the thermostat and entered a thin tubular plant chamber through the bottom, was also unsatisfactory. This/

This plant chamber was closed by a rubber stopper through which passed a length of capillary tube which was bent out to the edge of the thermostat. This capillary tube could be closed by a water stopper such as previously described for the syringe pipette. When the oxygen tension of the water in the plant chamber was to be measured, the water was slowly expelled by running in new water through the bottom of the chamber. A sample was taken direct from the stream of the out-going water. Although this type might have been made satisfactory, it was discarded as there was too much manipulation of taps and rubber connections when the chamber was lifted out to be shaken (q.v.). It was also considered to be more satisfactory if the stock solutions were not outside the thermostat but actually in it, so that the solutions could be brought into equilibrium with air at the required temperature. Otherwise, it was essential to remove some of the air or dissolved gases from the stock solutions before using the solutions in the experiments, as gas bubbles tended to be formed in the chamber.

The apparatus finally adopted is that shown in Fig. 2, below. The plant chamber is seen to be the size and shape of an ordinary boiling tube except that a narrow tube has been fused on to the opening.

A description of the plant chamber is continued overleaf.

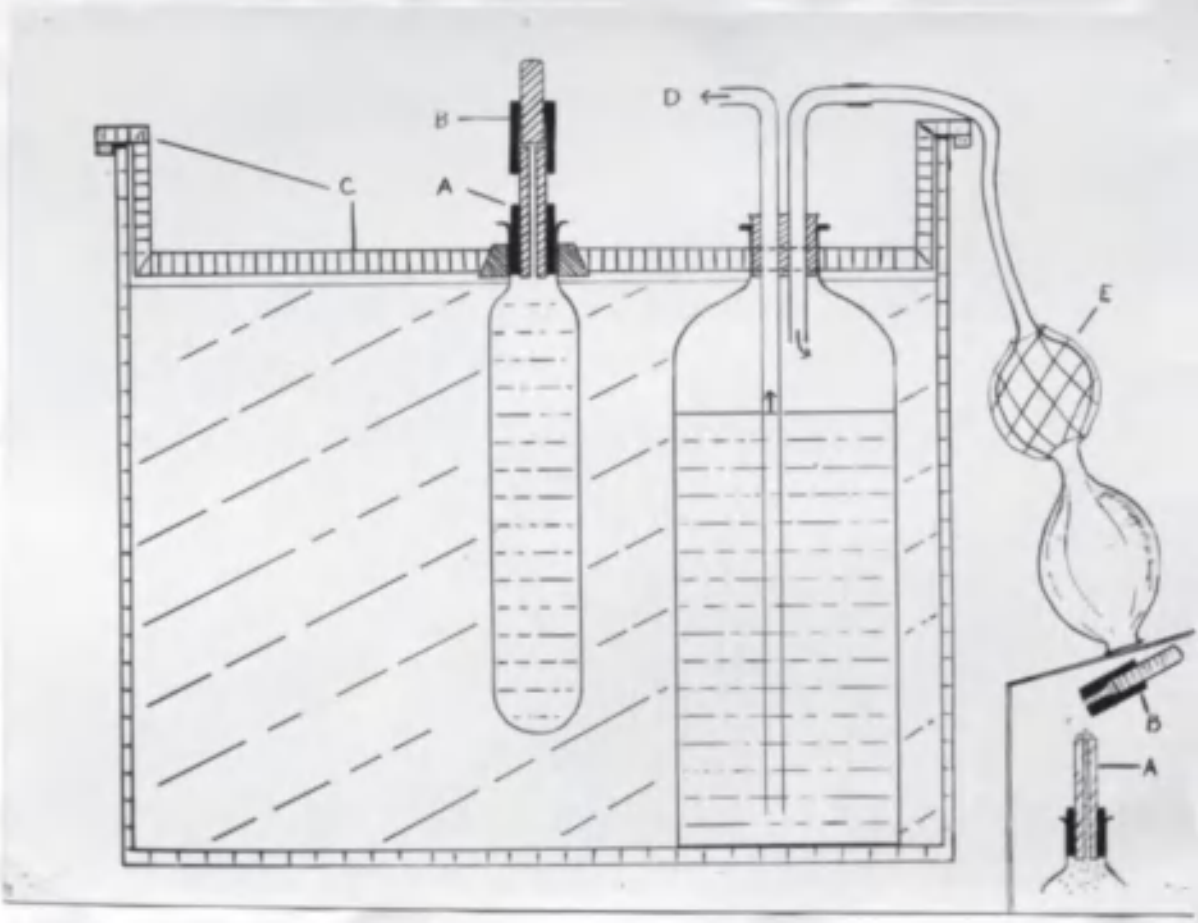

Figure 2 Diagrammatic representation of apparatus:

A .... first part of the chamber stopper

B .... second " " " " "

C .... wooden holder for the plant chamber

D .... exit for solutions

E .... bulb of scent spray type.

Lettering in inset as above.

The experimental solutions used are brought to equilibrium with air by having them in winchesters in the thermostat and by bubbling air through them overnight. The method of filling the chamber with the solutions is based on the principle of the scent spray. A length of glass tubing reaches to the bottom of the winchester and is bent at right angles above the stopper as shown in Figure 2, D. This is the exit which leads to the chamber. A piece of glass tubing, bent at right angles, is attached to the opening D by means of a length of rubber tubing. One arm of the glass tubing is long enough to reach to the bottom of the plant chamber. In Figure 3 a plant chamber, fixed into a wooden holder, is on the bench in the foreground, and the glass tubing, attached to the length of rubber, is clearly seen passing into the chamber.

Another bent glass tube passing through the stopper of the winchester is attached to the bulb E (Fig. 2) which is of the scent spray variety. By pumping this, a positive pressure is caused in the atmosphere above the solution in the winchester and this forces the solution out along D and into the bottom of the plant chamber. Figure 2 shows only one winchester, but where more are necessary they can be attached to bulb E by three-way taps. Figure 3 shows the bulb attached to a three-way tap fixed to the shelf, and used for two winchesters.

The method of closing the plant chamber is as follows: the chamber is filled with the required solution until it very thoroughly overflows. The first part of the/

the stopper (Fig. 2, A. Also inset), consisting of a capillary tube pushed through a piece of rubber pressure tubing, is then inserted, care being taken that no air bubbles are included. This is easily done by (a) having the capillary tube protruding slightly beyond the rubber tubing and (b) commencing the operation with the 'stopper' inclined to the mouth of the chamber. The stopper, on being pushed in, replaces some of the water and this comes out through the capillary tube. The second part of the stopper (Fig. 2, B. Also inset) consists of a piece of rubber pressure tubing filled with water and plugged at one end with a piece of solid glass rod. This is pushed over the capillary tube of A until the two pieces of glass meet. Excess water of the water stopper is simply forced out. If the whole operation is carried out quickly, there is little fear of change in the oxygen tension of the solution in the chamber (vide "Blank Experiments with the Apparatus", below).

To remove the stopper the reverse process is carried out except that the glass plug of B is removed first. If this is done too hastily there is always the possibility of the negative pressure so formed bringing gas bubbles out of solution and also of upsetting the hydrostatic pressures of the plant material. It has been found more desirable to hold the capillary tube of A and to push the rubber tubing of B downwards so that the glass plug of B is forced out without any fear of causing a negative/

negative pressure inside the chamber. The stopper A is now removed and the water sample taken.

The rubber parts of the stopper must be waxed, and the most satisfactory results have been obtained by placing the rubber in molten wax under reduced pressure as described by Pregl (23). Another reason for having the stopper in two parts is that there is difficulty in closing the chamber with the usual type of stopper as this always tends to be pushed back out by the positive pressure created inside.

The plant chamber is held in position by means of the teak wood part C (Fig. 2). This is about two inches broad and about half an inch in thickness. It is shaped as in Figure 2 so that the chamber can be brought down to the water level of the thermostat. The wooden holder has a central hole through which a split cork can be fitted, and this is bored so that it can hold the chamber firmly in position as shown in Figure 2. In practice, the actual neck of the chamber does not protrude above this cork as much as is shown in Figure 2, but is flush with the cork so that all the water inside the chamber is beneath the surface of the water in the thermostat.

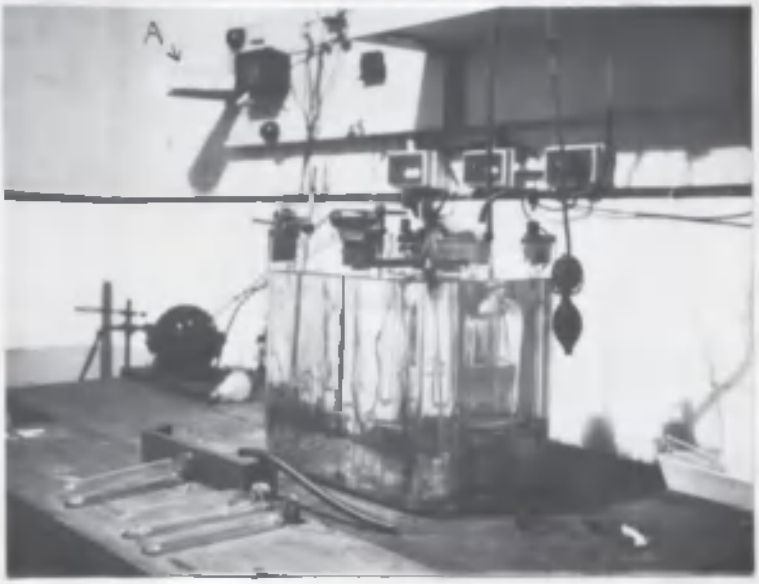

Figure 3 Conditions of Experiment.

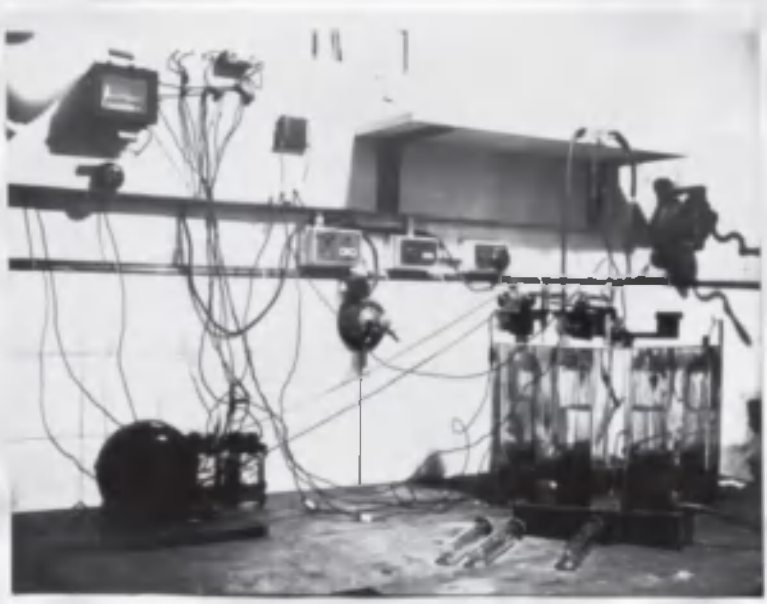

Figure 4 Conditions of Experiment.

The Thermostat

This consists of (1) a glass tank (since, with this, assimilation experiments could be carried out if desired without much alteration in the apparatus); (2) a thermo-regulating apparatus, electrically controlled, and (3) a stirrer of the propeller type.

The thermo-regulating apparatus consists of (a) a grid-type mercury-toluene thermo-regulator as seen in the tank in Figures 3 and 4, and (b) a mercury switch relay working from a four-volt battery. The battery is kept charged through a carbon filament lamp. A certain amount of trouble was caused by an insufficiency of mercury in the relay switch; this was remedied by the makers and by inserting a 0.1 mf. fixed condenser across the switch terminals. Sparking in the thermo-regulator is controlled by a 2.0 mf. condenser across the points, and by a 25,000 ohms fixed, wire-wound resistance across the condenser terminals.

The stirrer is motivated by the electric motor seen in Figures 3 and 4. The water of the tank is earthed, as are also the other danger points, and a safety fuse is inserted in the mains circuit.

The thermostat is maintained at 23'C ± 0.02'C, the small variation in such an unlagged tank being in part due to the room temperature which is kept approximately the same as that of the thermostat. However, the important temperature to be considered is that of the solution in the plant chamber, and here no appreciable variation could

be /

be detected with a Beckman Thermometer. The temperature control is therefore considered to be highly satisfactory.

Plant Material

Owing to the paucity of aquatic material in the district a stock of plants had to be grown in the greenhouse. In the first place a number of different species were cultivated:

(1) Vallisneria spiralis L. ♀.

(2) the Sagittaria natans of aquatic plant dealers,

(3) Callitriche autumnalis L.,

(4) Myriophyllum spicatum L.,

and (5) Elodea canadensis Michx.

A certain difficulty was met in initially starting the aquaria, probably owing to the time of transplanting being after the normal growing period. The S. natans presented no difficulty, but the V. spiralis showed a great tendency to shed most of the older leaves. These latter plants recovered later, however, if the temperature was maintained at 23°C and an extra boost of light given from a 300 watt lamp. The Elodea was most difficult to start, except during the normal growing season when transplanting the plants complete with 'roots' was completely successful. Elodea material for the initial trial experiments had to be collected fresh.

In every case a soil compost was most successful as compared with sand cultures (see also the work/

work of Snell (27)). The compost used was three parts of riddled loam to one part of sharp sand, the whole mixed together with the addition of a little leaf mould. The compost was placed on the bottom of the aquarium to a depth of two inches, and, on top of this, half an inch of washed sharp sand was spread. This top layer of sand prevented contamination of the water with soil during the filling of the aquaria (running the water on to a sheet of paper in this operation prevented the sand being washed away), and in planting the material.

The experiments recorded below were all done on E. canadensis as the V. spiralis and the S. natans with their strap-shaped leaves were not too suitable for the type of chamber described above; such leaves floated out through the opening and it was difficult to fix the stopper without damaging them.

The water in the aquaria, since this has become 'matured', has remained clear, in some cases for more than a year. It is always advisable to change the soil each year although the 'matured' water may be used again. The Elodea shoots used are not so robust as may sometimes be found with outdoor material, but it nevertheless compares very favourably with outdoor material in greenness and in the lack of etiolation. By using tap water the plants became accustomed to the medium in which the salts and substances under consideration are dissolved. The water of Glasgow is of exceptional purity and is often used in place of distilled for many purposes.

EXPERIMENTAL PROCEDURE

The shoots of Elodea canadensis used were two inches long, cut clean with a razor, and were always terminal. The shoots employed were usually eight to ten in number. They were washed in running water in order to remove any epiphytes, and were then treated according to the needs of the experiment.

The method of measuring the respiration rate of the shoots is as follows: the shoots, after the required preliminary treatment, are transferred to darkness and placed in the chamber in the thermostat. As a matter of routine the growing points are always facing upwards in their normal orientation to gravity. The time of placing the plants in darkness is taken, and from this time is calculated the "hours in darkness". At the commencement of an experiment the solution in the plant chamber is rapidly poured off and the exit tube (attached to D of Fig. 2) from the winchester containing the required solution is placed into the chamber. The solution is now pumped from the winchester into the plant chamber so that this is filled until it overflows. While the solution is still running, (the pressure in the winchester being maintained for a short time), the end of the syringe pipette, the outside of which has been wiped of the solution 1 which fills the dead space, is plunged into the chamber and a sample of water withdrawn. At this moment the stop watch is started. The piston must not be drawn/

drawn up too quickly as the resistance of the capillary tube may cause the formation of gas bubbles inside the syringe. Simultaneous with the finish of the sampling, a tap in circuit E (Fig. 2) is closed to prevent any 'suck-back' of the solution from the chamber into the winchester on the reduction of pressure in this latter. The syringe is then withdrawn and kept facing downwards. The end is quickly though gently wiped, and placed into Solution 2 which is then drawn in as described elsewhere. The syringe may now be inverted and the water stopper, previously prepared, fitted over the end. The syringe is gently rotated a few times to mix the solutions inside and to cause the formation of a finer precipitate, and is then clamped upright. The process of sampling can be completed in about a half to three-quarters of a minute after a little experience. The inlet tube from D is now lifted out almost to within an inch of the top of the plant chamber and clear of the respiring shoots. The tap in circuit D is opened and a few pumps given to the bulb E. (Fig. 2). While the solution is still running the inlet tube is completely taken out of the chamber. The chamber, together with its wooden holder C is removed from the tank and the capillary stopper A fitted, the water stopper B fixed on this, and the chamber replaced in the thermostat. The whole operation from commencement to finish generally occupies less than a minute. As the solution in the winchester is in equilibrium with air, the/

the short contact of the experimental solution with air during the sampling process will not affect results. Any affected solution would, in any case, be removed by the process of overflowing carried out as described above. In addition, the superficial layers of solution in the neck of the chamber are also removed through the capillary of the stopper A when this is put on. Any diffusion of oxygen from the water stopper must traverse the length of the capillary of the stopper before affecting results; it is considered, however, that such a possible occurrence would not significantly alter the determination of respiration rate.

The formation of air bubbles in the chamber is not common since the solutions used are in equilibrium at the experimental temperature. There is also little chance of bubbles being held by the leaves if the solutions are forced in steadily; if any bubbles are caught they can easily be removed by the inlet tube. It must be mentioned that the complete operation of sampling is performed in the diffuse light of the 16 c.p. carbon filament lamp covered over with a cylinder of asbestos as seen in Figure 3 A. The exposure to this light is very small and this must have only a negligible effect on the shoots in the thermostat. The shoots are never exposed to this light except during those short times when the actual water sampling is being done.

The shoots are now left to respire for a period

of/

of one hour as measured by a stop-watch. With everything prepared beforehand, the second sample can be taken in a very short time. About ten to fifteen seconds before the completion of the hour, the chamber attached to the holder is lifted out and inverted and reverted a few times so that a few glass beads inside the chamber can gravitate through the solution of the chamber. This is done to remove any local differential distribution of the oxygen inside the chamber so that in every case the samples are taken from solutions of uniform oxygen tension.

Each sample, before and after the experimental period, is titrated as described above, and the difference between the two readings gives the amount of oxygen consumed in respiration per 10 ml. of solution. A short calculation gives the total oxygen consumed from the whole volume of solution in the chamber. (This latter is obtained from burette measurement and is corrected for the volume taken up by the glass beads and by the stopper when this is fixed in position. The volume taken up by the plant material is not considered as this must be relatively small, the greater part of the volume of fresh material being in itself water). After the end of the experiment the shoots are dried at 100°C for 15 hours at least, and the dry weights taken. From this the ml. of oxygen consumed / gm. dry weight / hour can easily be obtained.

During the first lot of experiments only one plant/

plant chamber was in use so that where two lots of shoots were being considered these had to be transferred to and from the plant chamber, and kept, at other times, in boiling tubes hanging in the thermostat and containing the requisite solution. These latter were protected from air contamination by cotton wool plugs. With only one chamber three hours are necessary for complete determinations on two lots of shoots. In the later experiments more plant chambers were made, having relatively equal volumes (e.g. one pair was 117 and 119 ml., and another 121 and 125 ml.). Any slight difference in the actual volumes was not considered to be serious as the oxygen tension at the end of an experimental period at high respiration rate was still fairly high - viz: rarely less than five-sixths of the saturation obtained with air. With two plant chambers, containing one lot of shoots each, readings for both could be completed within two hours. Each chamber would have its own holder, and one lot of shoots would be placed in the dark half an hour before the other as comparative readings are taken half an hour after one another. Each reading is made and the syringe etc. prepared for the next sample within half an hour, if the precipitate is left to settle for exactly 18 minutes. Thus all the samples are taken within one and a half hours and the apparatus ready for a new series of estimations within two hours. During these experiments only one syringe pipette was in use; for comparative determinations/

determinations on a larger number of lots of shoots, more syringes would be required. The author tried to obtain further supplies but on each occasion the syringes arrived broken. As they come from abroad (Krogh, Laboratory of Zoöphysiol., University of Copenhagen, Denmark), the breakage appears to take place at the customs.

Blank Experiments with the Apparatus

Some trial experiments have been made to determine the suitability of the apparatus. The results in Table 3, below, show that water of normal oxygen tension can be left in the plant chamber for an experimental hour without much variation occurring in the oxygen content of the water.

TABLE 3

Oxygen content of the water before and after the experimental period. Values in terms of ml. N/200 Thio. per 10 ml. of water.

Experiment number	Initial Oxygen	Final Oxygen	Difference
1	2.285	2.285	0.00
2	2.215	2.2225	0.0075
3	2.230	2.230	0.00

The method of sampling must therefore be considered to be highly satisfactory, as there is only occasionally a sequence of readings which show a divergence of about 0.3% of one another.

EXPERIMENTAL RESULTS

Ammonium chloride

In the following experiments the effect of ammonium chloride on the respiration of Elodea is compared with parallel experiments in tap water.

TABLE 4

Plant : *Elodea canadensis*

Treatment: Collected from aquarium and placed immediately in the solutions contained in the thermostat in darkroom.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In water
1 - 2	1.432	1.538
4 - 5	2.439	1.664

TABLE 5

Plant : *Elodea canadensis*

Treatment: The plants had been lying free-floating in water for nearly a month and showed slight etiolation. Experimental shoots were cut and placed immediately in the solutions in the thermostat in darkroom.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In water
1.25 - 2.25	2.364	1.302
21 - 22	1.524	2.279

Table 4 shows the stimulating effect of

0.05/

0.05 M ammonium chloride within four to five hours as compared with parallel experiments in water.

In Table 5 the shoots were more attenuated and seem to have responded to the solution much quicker. This experiment also shows that whereas there is an increased respiration due to the presence of the solution during the first few hours, the difference is later in the reverse direction: the respiration of the plants in water has increased while that in the salt solution has decreased after 21 hours.

Table 6, below, is a similar experiment to the above, although in this case the increase in the respiration in water seems to occur within a shorter period than the last (Table 4).

TABLE 6

Plant : Elodea canadensis
Treatment: Collected from aquarium and placed immediately in solutions contained in the thermostat in darkroom.
Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In water
1 - 2	1.682	1.247
4 - 5	2.033	2.30
23 - 24	1.319	2.179

However, Table 6 again shows that a depression is felt in the respiration rate of the ammonium chloride series after 23 - 24 hours, as opposed to/

to the increased rate of the water series.

Experiments were also tried with a weaker solution of ammonium chloride, viz: 0.02 M. In the two following experiments (Tables 7 and 8) the shoots were placed in darkness and the respiration rates taken in water, after which one lot of shoots was placed in the salt solution.

TABLE 7

Plant : Elodea canadensis

Treatment: Material collected fresh from a stream. Two lots of shoots cut in evening and left in water all night under a 60 watt lamp. First readings taken in water.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In water	
1 - 2	1.045	1.119
3	In 0.02M NH_4Cl.	In water
4.25 - 5.25	1.473	1.457
8.25 - 9.25	1.736	1.615
24.25 - 25.25	0.6965	2.169
28 - 29	0.8035	0.9448

In the last experiment there appears to be no relative stimulation due to the salt, but the usual depressent effect of the salt occurs after 24 hours. A corresponding depression in the respiration rate of the shoots which have been kept in water appears only after 28 hours.

TABLE 8

Plant : Elodea canadensis

Treatment: Shoots cut from aquarium plants and left in water overnight under a 60 watt lamp; then transferred to thermostat where the first readings were taken in water.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In water	
0.75 - 1.75	0.7731	0.8794
1.75	In 0.02 M NH_4Cl.	In water
4.5 - 5.5	1.345	0.8989
24.75 - 25.75	2.213	2.423
28 - 29	0.9317	1.508

The depressent effect of the salt on the respiration rate only becomes evident after 28 hours in this experiment. There is also a definite stimulating effect within a few hours of placing the shoots in the salt solution. Although there is a difference in the general course of the respiration in Tables 7 and 8, nevertheless the deleterious effect of the salt used is definitely outstanding.

Some attempt was also made to isolate the affect, if any, of the NH_4 - ion. This part of the work was hindered by a lack of syringe pipettes, as serial determinations in moderately close sequence could not be made on a sufficiently large number of series of shoots. In addition, there appears to be a stimulation of the respiration rate by salts in general, so that any

variation/

variation in the reaction of the plant material to, say, NaCl, would tend to obscure any positive effect of the NH_4 - ion in NH_4Cl. Other factors, such as the relative permeability of the protoplasm to various ions, introduces another complex source of error, as also might the previous history of the plant material. However, some experiments were tried, using ammonium chloride and sodium chloride solutions.

TABLE 9

Plant : Elodea canadensis

Treatment: Previously attenuated shoots were cut and placed under a 60 watt lamp for 46 hours; then placed in darkness into the solutions contained in the thermostat.

Temp. : 23.5°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In 0.05 M NaCl
3 - 4	1.208	1.396

There does not appear to be much significant difference in degrees of stimulation in the last experiment. Longer term experiments did not help in clearing the issue. Examples of longer term experiments are given in Tables 10 and 11.

TABLE 10

Plant : Elodea canadensis

Treatment: Shoots collected from aquarium plants and each lot left in one of the solutions all night, under a 60 watt lamp. Solutions used are indicated below. In the morning the shoots were placed in the darkroom, into the appropriate solution in the thermostat.

Temp. : 23.5'C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In 0.05 M NaCl
2.25 - 3.25	1.83	2.346
24 - 25	1.505	1.248

TABLE 11

Plant and treatment as above, except that the shoots were left in water all night. Treatment with solutions commenced when the shoots were placed in the dark.

Temp. : 23.5'C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05 M NH_4Cl	In 0.05 M NaCl
2.5 - 3.5	2.685	2.236
24 - 25	1.625	1.769

The reaction of the plants does not seem to be precise enough in this type of experiment. Perhaps more than the usual 8 to 10 shoots used in the experiments might produce better results, but the apparatus is not quite able to deal with a larger number in its present form. It must be admitted, therefore, that no specific effect of the NH_4 - ion, as opposed to an effect of the Na - ion, could be discriminated from the above respiration experiments.

Where/

Where the plants have been pre-treated in the light with the salts there seems to be no tendency for the plants to respire during the initial period in darkness at a comparatively low rate, followed within a varying space of time by an increase to a maximum. In such cases of pre-treatment in the light, the respiration rate starts at a high level and shows an immediate tendency for a progressive fall.

TABLE 12

Plant : *Elodea canadensis*

Treatment: Shoots cut from aquarium plants; one lot then placed in 0.02 M NH_4Cl, and the other in water; both left under a 60 watt lamp all night, then placed in the thermostat in appropriate solutions.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.02 M NH_4Cl	In water
1 - 2	1.116	0.889
4 - 5	1.064	

Table 12 above demonstrates the reduction in respiration rate, after 4 to 5 hours in darkness, of plants pre-treated in the light. This is also the case in pre-treatment of the plants with other salts, as, for example, Na_2SO_4 or NH_4NO_3.

TABLE 13

Plant : Elodea canadensis

Treatment: Shoots cut from aquarium plants and left in 0.01 M Na_2SO_4 all night under a 60 watt lamp; then transferred to thermostat.

Temp. : 23.5'C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	Lot A	Lot B
1.5 - 2.5	2.529	2.294
5.5 - 6.5	2.262	2.284
29 - 30	0.5763	0.6473

The above experiment demonstrates the similarity of the reaction of analagous lots of shoots under parallel identical conditions. Experiments such as those shown in Tables 8 and 9 show the similarity of the initial respiration rate of analagous lots of plants left in water overnight. In Table 14 are given the respiration rates of shoots similar to and collected under the same conditions as the shoots used in Table 13, except that those of Table 14 were left longer, viz: 44 hours in the solutions (0.01 M Na_2SO_4 and 0.01 M NH_4NO_3).

TABLE 14

Plant : Elodea canadensis

Treatment: Shoots cut and left in solutions for 44 hours, in light, before transferring to darkness. The solutions used are indicated below. The lamp used was 60 watt, as formerly.

Temp. : 23.5°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.01 M NH_4NO_3	In 0.01 M Na_2SO_4
1.75 - 2.75	1.795	1.547
5 - 6	1.396	1.016
25.5 - 26.5	1.517	0.836
Further treatment	The shoots in the above experiment were transferred to nitrate and sulphate solutions to which sucrose had been added, after 27 hours in darkness.	
27	Transferred to M/200 (NH_4NO_3 + sucrose)	Transferred to M/200 (Na_2SO_4 + sucrose)
29.75 - 30.75	1.163	0.836
50 - 51	1.03	

From the respiration rates obtained in such experiments as Table 14, there would appear to be an accumulative effect of leaving the shoots for a longer period in the salts. The respiration rate of the shoots treated with sodium sulphate in the last experiment are definitely lower than those pre-treated for a shorter time as in Table 13. The general course in prolonged darkness is similar, however.

Further, Table 14 shows that the addition of sucrose to the solutions does not prevent the lowering of the rate of/

of respiration. The material was of a particularly hardy nature and the apparent differential effect of the NH_4NO_3 will not be stressed until further critical data have been obtained from shoots with different previous histories.

Sodium Nitrate

The following experiments are on the same lines as the previous, but the salt sodium nitrate is studied. The similarity of the reaction of the plants to this salt may be seen in Table 15, i.e. there is an increase to a maximum but the salt would appear to depress the rate slightly, compared to a similar series in water. It is generally the case, however, that the salt causes a stimulation with a later relative depression in the respiration rate as compared with parallel experiments in water.

TABLE 15

Plant : Elodea canadensis

Treatment: Shoots cut from aquarium plants and left in water all night under 100 watt lamp; then placed in darkness - one lot in water, and the other in 0.02M $NaNO_3$

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.02 M $NaNO_3$	In water
0.5 - 1.5	1.809	1.633
3.5 - 4.5	2.424	3.232
24.25 - 25.25	1.119	1.48
27.25 - 28.25	0.8206	0.919

Pre-treatment with such dilute solutions as 0.001 M sodium nitrate produces the same type of course as the pre-treatment experiments described for other salts.

Such/

Such a pre-treatment with dilute solutions is given in Table 16 below. The results are also graphically represented in Figure 5.

TABLE 16

Plant :*Elodea canadensis*

Treatment: Shoots cut and left overnight in (a) 0.001 M $NaNO_3$ and (b) water, both under 100 watt lamp; then transferred to thermostat with appropriate solutions.

Temp. : 23°C

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.001 M $NaNO_3$	In water
0.5 - 1.5	1.245	2.981
4.25 - 5.25	1.111	1.525
24.25 - 25.25	0.934	1.317

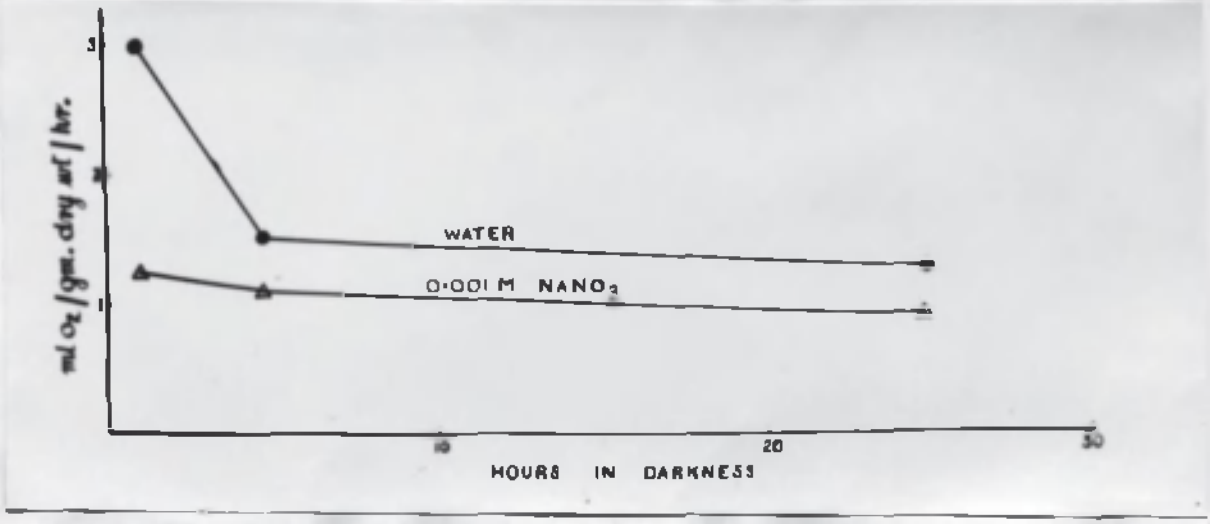

Figure 5 Graph showing the effect of pre-treatment with 0.001 M $NaNO_3$, with a parallel experiment in water. Data from Table 16 above.

The effect of pre-treatment, even with such dilute solutions, appears to be a depression in the respiration rate. Certainly, the course of respiration in water, given in Table 16 above, does not appear to be characteristic, but, from the present author's experience, is a type sometimes found in winter material. Nevertheless, the effect of the nitrate is evident.

In general, the course of respiration in prolonged darkness in water is one in which the rate increases in varying degree to a maximum, with a subsequent fall after about 24 hours. Whatever is the cause of this sudden collapse of the respiration, it would not appear to be connected with the carbohydrate reserve, as the following experiments show.

TABLE 17

Plant : *Elodea canadensis*

Treatment: One lot of shoots left overnight in 0.001 M sucrose and another in water, both under a 100 watt lamp; then transferred to thermostat with appropriate solutions.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.001 M sucrose	In water
0.75 - 1.75	1.844	1.924
4.25 - 5.25	1.496	2.219
24.0 - 25.0	1.597	2.685
28.0 - 29.0	1.060	0.621

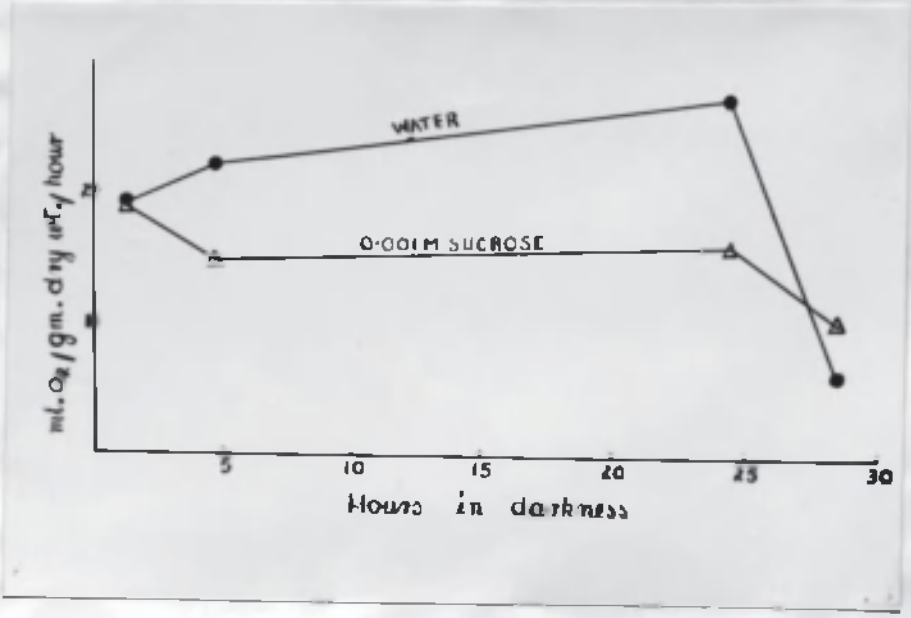

Figure 6 A graphical representation of the data from Table 17, showing the effect of pre-treatment with 0.001 M sucrose.

TABLE 18

Plant : Elodea canadensis

Treatment: Shoots left overnight in water under 100 watt lamp, then placed, in morning, in thermostat - one lot in 0.001 M glucose, and the other in water.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.001 M glucose	In water
0.75 - 1.75	1.892	1.588
3.75 - 4.75	2.830	2.018
23.75 - 24.75	2.001	2.968
27.0 - 28.0	0.907	0.979

Apart from any small variation from type, the figures obtained from the use of sucrose or glucose as sources of easily available carbohydrate suggest that these substances cannot of themselves prevent the onset of a low respiration rate when the plants are subjected to prolonged darkness.

When plants are treated with solutions of salts the course of respiration seems to be similar to that in water only, except that the salts seem to hasten the onset of the low respiration rate. Experiments with sodium nitrate together with glucose indicate that here again the effect can not be altered, even to the slower deterioration in the absence of salts. Where the salts are used in low concentrations the effect is still rather detrimental.

Table 19 shows a comparative effect of pre-treatment with dilute solutions, which in later experiments are used together.

TABLE 19

Plant : *Elodea canadensis*

Treatment: Shoots left for three days under ordinary conditions of temperature and light, etc. One lot kept in 0.001 M $NaNO_3$ and the other in 0.0001 M glucose.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.001 M $NaNO_3$	In 0.0001 M glucose
1 - 2	0.774	1.15
4 - 5	--	0.716

It would appear that such a solution as 0.0001 M glucose can hasten the onset of the low respiration rate if/

if the shoots are pre-treated with this solution for three days. The two following experiments show the effects of pre-treating analagous shoots, collected on the same day under the same conditions but with one series having two days and the other six days pre-treatment with nitrate-glucose solution.

TABLE 20.

Plant : Elodea canadensis

Treatment: Shoots collected from aquarium and left for two days under natural conditions of temperature, light, etc. The solution was changed daily. Solution used was (0.001 M $NaNO_3$ + 0.0001 M glucose) i.e. $NaNO_3$ present in concentration 0.001 M and glucose in concentration 0.0001 M.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour In 0.001 M $NaNO_3$ + 0.0001 M glucose
0.5 - 1.5	1.322
2.0 - 3.0	1.454
4.0 - 5.0	1.256
5.5 - 6.5	1.14
24.75 - 25.75	0.9253

After the experiments recorded in Table 20, above, the plant material was transferred to water and left in the greenhouse under the normal conditions of light and low temperature. After a further four days in these conditions the plants were again transferred to the thermostat and the respiration rate measured.

TABLE 20 (continued)

Treatment: As described above.
Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour
	In water
0.5 - 1.5	0.826
3.0 - 4.0	0.925
4.5 - 5.5	0.760

The data from this experiment are shown graphically in Figure 7, below.

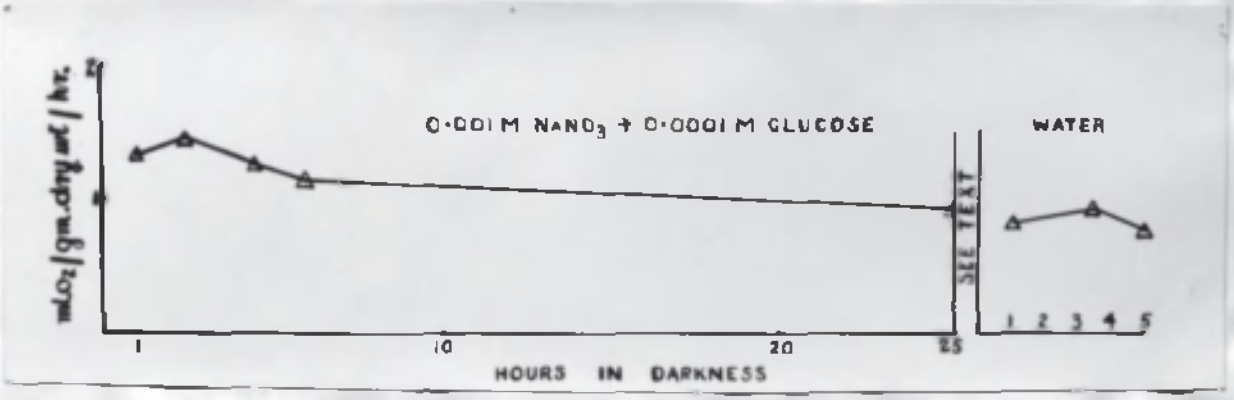

Figure 7 Showing the course of respiration after pre-treatment with the solution designated.

From the results of the experiment given in Table 20 and shown graphically in Figure 7 it would appear that the effect of the treatment is to cause some permanent injury to the plant so that the normal rate of respiration cannot be recovered. This permanently low rate seems to be caused by the additional exposure to prolonged darkness and not totally due to the solution, as the next experiment shows. In this, analagous material to that used in the last experiment has been/

been left in the solution for a longer period under natural conditions; it will be seen that the course of the respiration in the dark is similar, but here again the presence of the glucose has not prevented the onset of the low rate.

TABLE 21

Plant : *Elodea canadensis*

Treatment: As in the last experiment, using shoots gathered at the same time under identical conditions, except that the period of pre-treatment has been extended to six days.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm dry wt./hour
	In 0.001 M $NaNO_3$ + 0.0001 M glucose
0.5 - 1.5	1.300
4.25 - 5.25	1.387
24.0 - 25.0	1.206
28.5 - 29.5	0.901

The effect of the longer exposure to the solution does not seem to affect the initial rate of respiration on transferring the plants to darkness, but this experiment, together with the previous, shows that the low rate of respiration cannot be obviated by the addition of an easily available form of carbohydrate to the medium. A composite experiment is given below in Table 22, where parallel experiments have been done with a nitrate-glucose solution and with water. The effects of changing the concentration or nature of the solution, after a period in darkness, are also given.

TABLE 22

Plant : Elodea canadensis

Treatment: Shoots cut from aquarium plants and left overnight in water under 100 watt lamp; in morning one lot transferred to the glucose-nitrate solution and the other left in water. Left a further four days under natural conditions of light, temperature, etc., before transferring to thermostat and darkness. Solutions changed daily.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.001 M $NaNO_3$ + 0.0001 M glucose	In water
0.5 - 1.5	1.908	1.748
4.25 - 5.25	0.996	2.204
24.0 - 25.0	0.979	2.53
27.5	Transferred to 0.02 M $NaNO_3$ + 0.0001 M glucose	
28.0 - 29.0	0.829	0.718
32.0 - 33.0	0.708	
48.0 - 49.0	1.128	0.668
51.0		Transferred to 0.02 M $NaNO_3$ + 0.0001 M glucose
52.0 - 53.0	0.896	0.967

From Table 22 it can be observed that the effect of pre-treatment becomes evident after four hours in the dark, when the series in the nitrate-glucose solution has attained the low respiration rate. When this low rate was established the shoots were transferred to a (0.02 M $NaNO_3$ + 0.0001 M glucose) solution. In the series pre-treated with dilute solution this transference was made after 27.5 hours in the/

the dark; but in the series in water it was not made until after 51.0 hours in the dark. The results show an apparent stimulation with the stronger solution, but in the series originally in the dilute solution this only becomes evident 24 hours after the transference and is possibly due to some pre-death influence. This may also account for the very slight stimulation of the series originally in water. It would appear that the experimental treatment causes the shoots to lose their power of reaction to 'stimulating' solutions to a very great degree. The data of Table 22 are given graphically in Figure 8 below.

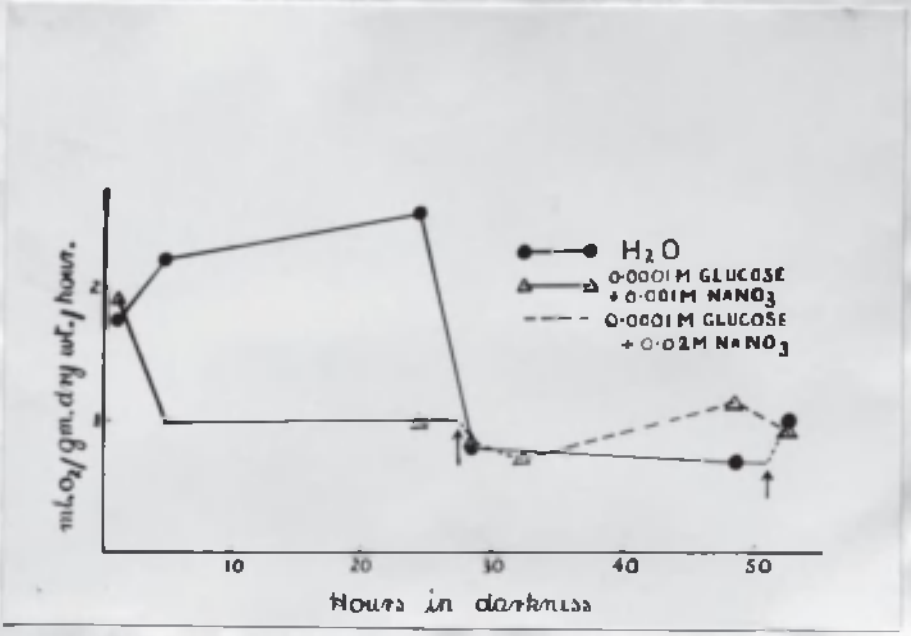

Figure 8 A graphical representation of the data of Table 22. The arrows indicate the time of changing the solutions, and the dotted lines represent the subsequent respiration.

This experiment again seems to show that the onset of the low respiration rate is not directly connected with the presence or absence of carbohydrates.

A number of trial experiments have been done, using Asparagine as a source of organic Nitrogen. The following long-term experiments have been designed to demonstrate the main results of the Asparagine trials.

The Asparagine was always supplied as a 0.05% solution, a concentration successfully used by Schwabe (26). Like that author, the present experiments show that the rate of respiration of Elodea is increased by Asparagine. Although the increase obtained by Schwabe was greater than in the present instance, the ultimate effect of a higher rate of respiration is nevertheless very evident in the present experiments when Asparagine is present in the solution.

One type of Asparagine experiment is given in Table 23 where, in the first place, parallel experiments were done in (a) water and (b) 0.05% Asparagine. After a period of 53.25 hours in the dark the series in water were transferred to the Asparagine solution. Similarly, after 75 hours in the dark, the series in Asparagine were transferred to a (0.05% Asparagine + 0.001 M glucose) solution. The various effects of treatment are given in Table 23, and are also given in graphical form in Figure 9.

(Overleaf)

TABLE 23

Plant : Elodea canadensis

Treatment: Shoots collected from aquarium plants and left in water under natural conditions for two days; then transferred to thermostat - one lot placed in 0.05% Asparagine and the other in water.

Temp. : 23'C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05% Asparagine	In water
0.5 - 1.5	1.687	2.441
4.0 - 5.0	1.265	2.379
24.0 - 25.0	1.047	1.764
28.0 - 29.0	1.279	0.943
48.0 - 49.0	1.076	0.759
52.0 - 53.0	1.134	0.677
53.25		Transferred to 0.05% Asparagine
72.25 - 73.25	1.265	1.477
75.0	Transferred to 0.05% Asparagine + 0.001 M glucose	
76.0 - 77.0	1.541	
77.5 - 78.5	1.454	
96.0 - 97.0	1.919	
97.5 - 98.5	1.832	
101.25 - 102.25	1.352	
120.25 - 121.25	1.803	
173.0 - 174.0	2.646	

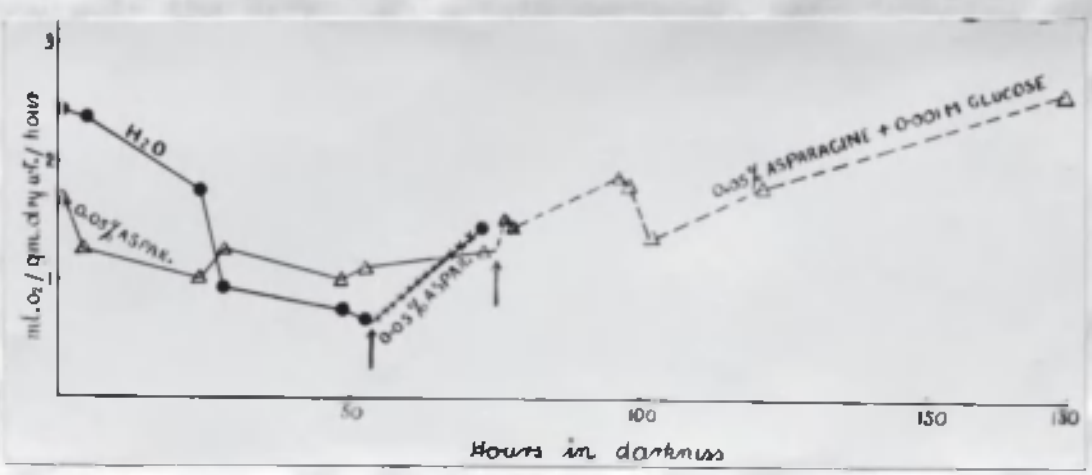

Figure 9 A graphical representation of the data of the experiment shown in Table 23. The arrows show the times of changing the solutions, and the dotted lines indicate the course of respiration subsequent to this. The solutions used are indicated in the figure.

In this experiment the respiration rate in water is seen to start at a high level and to subsequently fall to the characteristic low rate after about 24 hours. After 53.25 hours in the dark, this series is able to respire at a higher rate on transference to a 0.05% solution of Asparagine. The new rate is not dissimilar to that of the plants treated with Asparagine in the first place. On the other hand, the Asparagine series shows/

shows a more or less constant respiration even up to 75 hours in the dark. When this series is transferred at this point to a (0.05% Asparagine + 0.001 M glucose) solution the respiration is observed to increase gradually, showing that the mechanism for drawing carbohydrate into the respiratory cycle is still able to function. The high reading after 173 hours in the dark may conceivably be due to some pre-death influences, although this is not certain as the plants 'feel' healthy even after this long period in darkness. The visible changes in the appearance of the plants under such treatment are discussed elsewhere.

In view of the 'beneficial' effect of the Asparagine some experiments were tried using this substance together with ammonium chloride and/or glucose. Such an experiment is given in Table 24 where one series was treated with a solution of 0.05% Asparagine to which NH_4Cl had been added so that the concentration of this salt was 0.05 M. This series was compared with a parallel experiment in which the shoots were treated with 0.05 M NH_4Cl only. After 73 hours, glucose was added to the Asparagine--Ammonium chloride solution so that the glucose was present in a concentration of 0.001 M. This experiment is also represented in Figure 10.

TABLE 24

Plant : Elodea canadensis

Treatment: Shoots cut from aquarium plants and left overnight - one lot in 0.05% Asparagine and the other in water, both under 100 watt lamp; both lots then placed in thermostat in darkness - one series in (0.05M NH_4Cl + 0.05% Asparagine) and the series originally in water placed in 0.05M NH_4Cl.

Temp. : 23°C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	In 0.05% Asparagine + 0.05M NH_4Cl	In 0.05M NH_4Cl
0.5 - 1.5	2.613	2.013
3.5 - 4.5	2.157	1.437
24.0 - 25.0	4.335	1.222
28.0 - 29.0	1.701	1.096
48.0 - 49.0	1.494	1.114
52.0 - 53.0	1.617	1.007
72.0 - 73.0	0.954	0.8626
73.0	As above with 0.001 M glucose added	
75.75 - 76.75	1.286	
77.25 - 78.25	1.203	

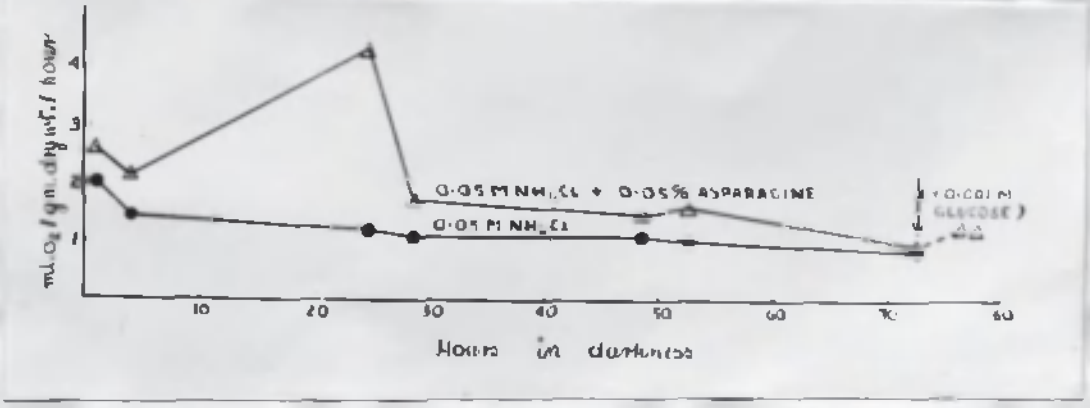

Figure 10 Graphs showing course of respiration after treatment with solutions indicated. Time of addition of glucose (→ 0.001M concn.) to Asparagine-ammonium solution shown by arrow.

In this last experiment measurements on the ammonium chloride series had to be stopped after 73 hours in the dark as the plants showed obvious signs of deterioration. When the 0.05% Asparagine solution contains also NH_4Cl in a concentration of 0.05 M it may be observed that the course of respiration is of the normal form except that the rate is higher than in the case without the presence of Asparagine. Nevertheless, the concentration of ammonium chloride used obviously causes a deterioration of the plant tissue, and after 73 hours in the dark the plants seem to have lost the ability to benefit by the presence of the Asparagine and a low rate of respiration has set in. However, even at this point there is an almost immediate reaction to the addition of glucose, showing that the mechanism of carbohydrate respiration has not been destroyed. There is an abnormally high reading for this series after 24 hours in the dark, but this point will not be emphasised as further critical work on this high rate has yet to be done.

It would appear, therefore, that the presence of 0.05 M ammonium chloride has a deleterious effect on the plant with or without the presence of Asparagine. Another experiment, designed to show the effect of a more dilute solution of this salt, is given below. In this, the solutions used were (a) 0.05% Asparagine + 0.01 M NH_4Cl and (b) 0.05% Asparagine. After 54.25 hours in the dark, glucose was added to each solution so that it was in a concentration of 0.001 M.

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The data of Table 25 are also graphically represented in Figure 11.

TABLE 25

Plant : *Elodea canadensis*

Treatment: Shoots left overnight in 0.05% Asparagine under natural conditions; placed, in morning, in thermostat - one lot in (0.05% Asparagine + 0.01 M NH_4Cl) and the other in 0.05% Asparagine only.

Temp. : 23'C.

Hours in darkness	Ml. oxygen/gm dry wt./hour	
	In 0.05% Asparagine + 0.01 M NH_4Cl	In 0.05% Asparagine
0.5 - 1.5	1.845	1.629
4.0 - 5.0	1.845	1.393
24.0 - 25.0	2.967	1.196
26.0 - 27.0	1.937	1.55
28.25 - 29.25	1.661	1.34
48.0 - 49.0	1.507	1.235
52.0 - 53.0	1.691	1.235
54.25	glucose → 0.001M concentration added to above	glucose → 0.001M added to above
72.0 - 73.0	2.306	2.063
76.5 - 77.5	2.091	1.563

The data of Table 25 above are given graphically in Figure 11, overleaf.

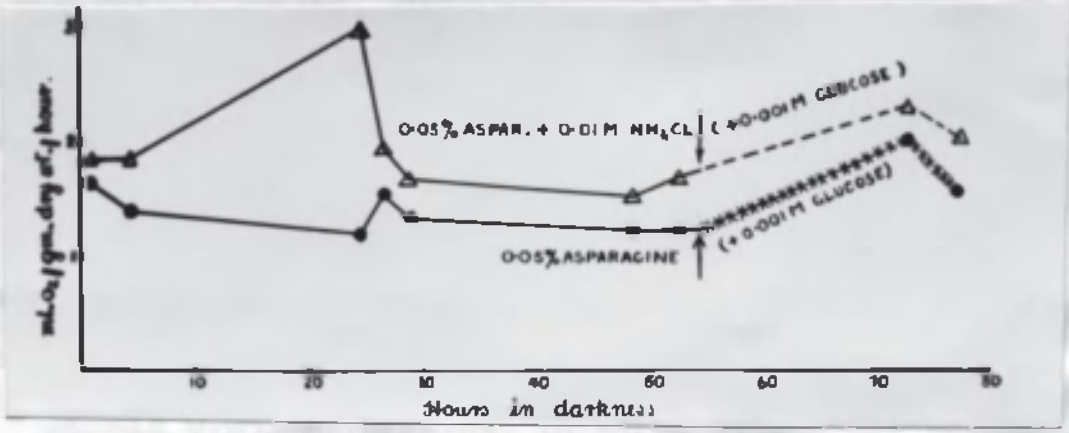

Figure 11 Graphs of parallel experiments using the solutions indicated in the figure. The arrows indicate the time of adding glucose (giving 0.001M concentration) to the solutions.

In this type of experiment the more usual course of respiration is again found in the presence of the salt, with a maximum after about 24 hours in the dark. After 53 hours the respiration rate in the presence of the salt is still higher than in the corresponding Asparagine series. In both series an addition of 0.001 M glucose causes an immediate rise in the respiration rate. In addition, the general condition of the plants in both series was good, and the ammonium chloride in 0.01 M concentration in the presence/

presence of Asparagine did not have the detrimental effect found with 0.05 M NH_4Cl as in the previous experiment (Table 24).

The experiment described below (Table 26) is on similar lines to the last but with 0.001 M glucose being present in both solutions in the first place.

TABLE 26

Plant : Elodea canadensis

Treatment: Shoots left overnight, under 100 watt lamp, in 0.05% Asparagine; in morning, one lot placed in (0.05% Asparagine + 0.01 M NH_4Cl + 0.001M glucose) and the other lot in (0.05% Asparagine + 0.001M glucose).

Temp. : 23'C.

Hours in darkness	Ml. oxygen/gm. dry wt./hour	
	0.05% Asparagine + 0.01 M NH_4Cl + 0.001 M glucose	0.05% Asparagine + 0.001M glucose
0.5 - 1.5	1.754	1.693
5.5 - 6.5		1.738
23.75 - 24.75	2.806	1.932
28.25 - 29.25		2.080
48.75 - 49.75	2.054	2.332
52.25 - 53.25	2.021	1.798
72.5 - 73.5	2.105	2.556
76.25 - 77.25		2.347

The data given above are shown in graphical form in Figure 12, overleaf.

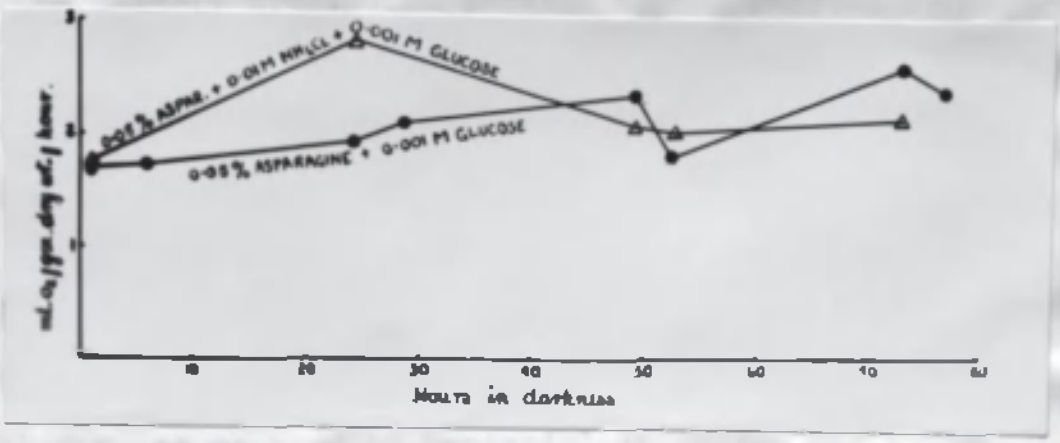

Figure 12 A graphical representation of the data from Table 26. The solutions used are indicated in the figure.

A striking feature of this type of experiment is that, in both cases, a superior rate of respiration is maintained with relatively little deterioration in the plant tissues. The usual 'stimulation' due to the presence of the salt is still observed within the first 24 hours. This tendency for a higher rate due to the salt is lost after about 50 hours of darkness when the rate is inclined to be relatively lower than the corresponding series without the salt.

Bacterial Infection

An obvious criticism in work such as has been described, is that of bacterial infection. The presence of bacteria in such cases would assist in oxygen consumption,

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so that the reading for this characteristic of respiration would be falsely attributed totally to the respiration of the plant material. In short, in the presence of a number of bacteria, the respiration rate calculated for the plant material would be of too high an order.

In the experiments described above, the present author is convinced that the respiration figures given are true for the plant material used under the experimental conditions, as no sign of bacterial infection was found during the period of the experiments recorded. Although tap water has been used, there is never any development of bacteria in this (nor, incidentally, of algae) if it is kept free of air contamination. The author has a bottle of a solution of (0.0001 M glucose + 0.001 M $NaNO_3$) which has been lying in the laboratory, in a south window, for nine months; the solution is still perfectly clear, and neither a bacterial scum has been formed nor have any algae developed on the sides of the bottle. There have also been occasions when Asparagine solutions, left after an experiment conducted at 23'C, and kept thereafter in the darkroom at ordinary temperatures for a fortnight or more, have similarly remained absolutely clear.

In any case, the results of the experiments themselves do not indicate bacterial infection of any significant degree. In the presence of sugars at 23'C, when oxygen consumption would increase if bacteria were present (since the bacteria would undoubtedly multiply under/

under such conditions), the facts show that the oxygen consumption falls after the plants have been in darkness for a certain period. Trial experiments on the solutions themselves, to determine if there is any variation in the oxygen content during an experimental period, would not successfully answer the criticism as infection might take place from the plant material itself when this is used.

The water in the aquaria remains remarkably clear, being protected from the air by means of glass covers. In only one aquarium has there recently developed any suggestion of algae and, in this, their presence has only been detected by a small translucent area on the glass sides of about two square inches. The quite "sterile" nature of the aquaria would therefore obviate much of the opportunity for infection from the plant material. Further, most, if not all, of the epiphytes would almost certainly be removed when the shoots are washed in running tap water before being submitted to the experimental treatment. From the point of view of unwanted epiphytes, the cultivated material is probably more desirable than the freshly gathered.

In the cases where glucose has been added to the experimental solutions after a certain period has elapsed, there is a definite rise in the rate of respiration when Asparagine is present; on the other hand, the rise, if any, is insignificant when Asparagine is absent. In the Asparagine-glucose type of experiment, however, the maintained clarity of the solutions and a microscopical examination/

examination of the material after the experiment both serve to proclude any attribution of a superior oxygen consumption to the presence of bacteria.

DISCUSSION.

It will be convenient to divide this discussion into different parts, viz:

(1) The Onset of the Depressed Rate of Respiration in Water during Prolonged Darkness.

(2) The Onset of the Depressed Rate of Respiration in the Presence of Salts during Prolonged Darkness.

(3) The Effect of Salts on the Metabolism of the Plant.

(4) Protein Synthesis from Inorganic-N Salts.

(5) The Mechanism of Salt Effect.

(6) Degradation of Chlorophyll.

(7) The Gases of the Lacunae in Water Plants.

(1) The Onset of the Depressed Rate of Respiration in Water during Prolonged Darkness.

A study of the results presented elsewhere indicates that the effect of prolonged darkness, when the plants are in water only, is to induce, ultimately, a low respiration rate. While the mere presence of solutions of easily available carbohydrate may in themselves hasten it and certainly do not prevent it (Tables 17, 18, 19), the conclusion/

conclusion is that the onset of the low rate is not directly dependent on the presence or absence of carbohydrate Moreover, shoots left in water for more than 24 hours have been shown to have such a reserve of starch as would appear to be inconsistent with the appearance of the depressed respiration rate. On the other hand, addition of Asparagine to the water (Tables 23, 25) produces an equable rate which is superior to that finally obtaining in water only. It appears, therefore, that the onset of the low respiration rate, when Elodea shoots are kept in water only and in darkness, is closely connected primarily with the nitrogen metabolism and probably not directly with the carbohydrate metabolism.

Unfortunately, it has not yet been possible to make the necessary chemical analyses with a view to understanding the precise nature of this connection with the nitrogen metabolism. Nevertheless, a study of the current views on protein synthesis and degradation, together with the measurements of oxygen consumption here recorded under the various conditions, permit a speculation of the processes at work. The following attempt at an explanation of the results is therefore only tentatively made from the circumstantial and direct evidence available, although it is realised that the solution of the problem will probably lie in biochemical analysis.

The view has gained ground that protein syntheses and degradation/

degradation proceed along separate paths and that these processes are catalysed by independent catalytic systems. This conclusion has been reached by Gregory and Sen (8), Mothes (20) and other workers as a result of their experimental work. If both processes are proceeding concurrently then the velocities of the independent reactions will determine the protein content at any particular moment. In addition, it seems probable that the amino acids, produced during synthesis and proteolysis, are different in either case. On this view, also, proteolysis may proceed irrespective of the concentration of the precursors of protein synthesis.

Growth due to meristematic activity is generally associated with protein metabolism. It is known that protein synthesis takes place in the light although it is not yet certain that this always happens in darkness other than by a re-synthesis from pre-existing organic nitrogen compounds. When Elodea shoots are placed in the dark and in water only, there is only a slight indication of significant meristematic growth and this may conceivably be partly due to a low rate of protein synthesis (relative to degradation in the dark). The presence of Asparagine, however, causes an extension of the shoots which has every appearance of normal, meristematic growth, although, as would be expected, the young leaves are more or less devoid of chlorophyll. Moreover, the plants in water only finally present visible signs of deterioration and this

is associated with the onset of the low respiration rate, whereas the shoots in Asparagine remain as "crisp" and turgid as freshly-gathered material, and the stems remain bright and shiny in contrast to the dull, water-logged appearance of shoots otherwise treated. Asparagine is considered to be a precursor (though not necessarily an essential, integral step) of protein, so that the inference would be that the augmentation of protein synthesis by Asparagine is the process which prevents the onset of the low respiration rate. As protein degradation is almost certainly taking place in the dark, the supposition would be that, in water only, proteolysis is proceeding in excess of synthesis. Such a loss of protein has been known, in other cases, to precede deterioration of plant tissue. Further, if, as it would appear from the circumstantial evidence of growth in darkness, an amidated substance like Asparagine produces an increase in the rate of protein synthesis, then it might be inferred that synthesis in water only is limited by a stage preceding the formation of aminated compounds.

Until further experimental data have been obtained, the following suggestion is put forward as a probable explanation of the processes at work under the experimental conditions in prolonged darkness:

The amino acids of proteolysis are not directly used in a rapid re-synthesis of protein. The probability is that the amino-acids so formed are removed in a great measure/

measure by oxidative deamination. The accumulation of NH_3 will in turn depend on the factors regulating amination (q.v.). Thus, in water only, proteolysis exceeds synthesis and the amino acids, as such, are to some extent removed from the metabolic cycle by oxidative deamination. The resultant depletion of the protein reserve induces an incipient death condition with which the low respiration rate may be found to be correlated. The rise in respiration during the first 24 hours or so could be the result of a pre-death stimulation, sometimes found to be a characteristic of dying tissues. Suitable analyses will indicate if this increasing oxygen consumption during the first 24 hours is in fact due to an increase in the rate of oxidative deamination as a result of a greater concentration of amino acids from a progressively increasing rate of proteolysis. The sudden collapse of the respiration after 24 hours or so might therefore be found to be correlated with the depletion of protein reserves as this would impose a limit on the rate of proteolysis and, pre-:sumably, of oxidative deamination as a corollary.

The question of the part played by carbohydrate in the respiratory cycle must not be lost sight of. It would appear, however, that carbohydrate does not play a significant part in the respiratory cycle unless a certain level of nitrogen metabolism obtains. A recapitulation of the results in prolonged darkness will serve to demonstrate this:

(1) In water ..... pre-death (?) stimulation of the respiration with, subsequently, a fall to a low rate.

(2) In carbohydrate ... as in (1) above.

(3) In Asparagine ..... a more or less steady respiration rate which is higher than the final low rate in (1) and (2); no abnormal "stimulation".

(4) In Asparagine + glucose ... a respiration rate at a level higher than (3) above.

A schema in which is shown the relation of carbohydrate metabolism to nitrogen metabolism has been given by Gregory and Sen (8).

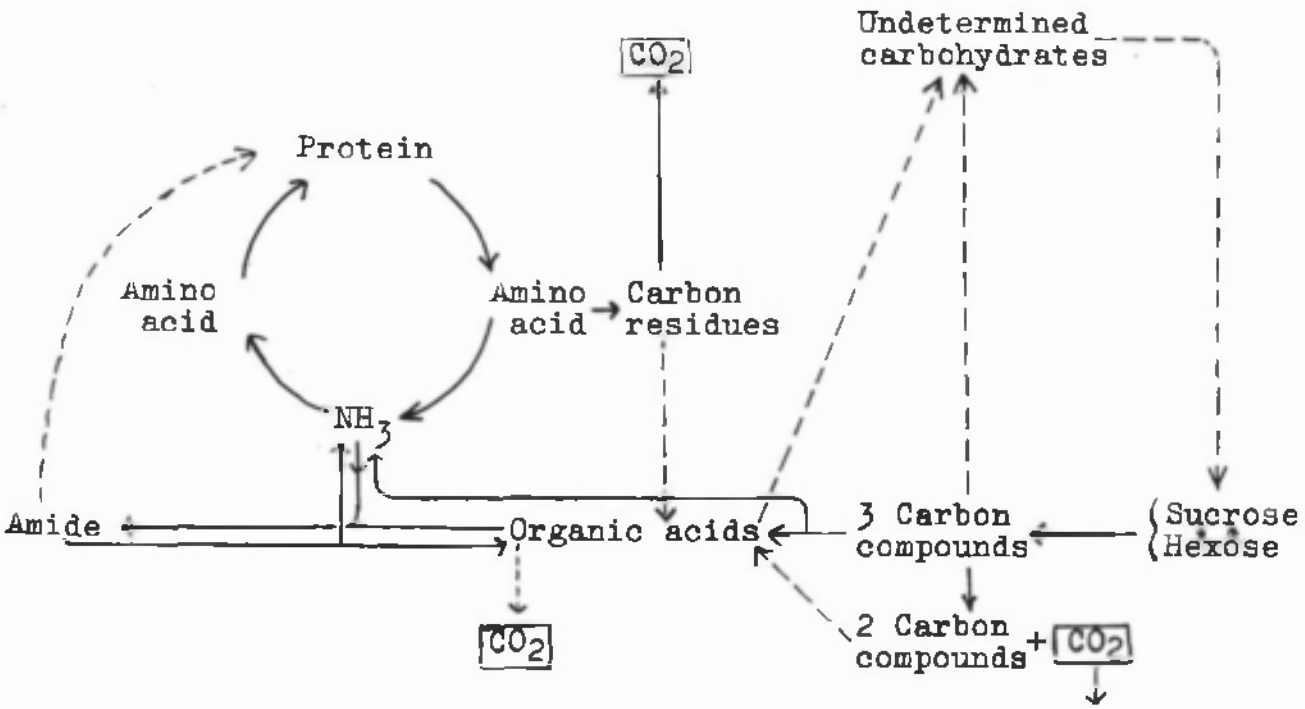

Figure 10 of Gregory and Sen (8) A schema of the relations of the carbohydrate and protein metabolic cycles indicating the possible origin of CO_2.

Oxidation of the 3 carbon compounds from glycolysis results in the formation of 2 carbon compounds and these may be/

be resynthesised to organic acids. The carbohydrate metabolism is linked to the nitrogen metabolism through organic acids. The authors suggest that the carbohydrate metabolism is in some obscure manner "controlled" or "regulated" by the rate of protein synthesis.

The present results would seem to find their explanation in the schema given above, It has been suggested that, in prolonged darkness, where the medium is water only, there are pre-death influences at work, and it would certainly appear that these in some way inhibit a primary synthesis of protein. If this inhibition is on some such stage as amination then the carbohydrate could not be drawn into the respiratory cycle and addition of glucose would not affect the oxygen consumption. This has in fact been found to be the case (Tables 17, 18, 19). When the pre-death effect is obviated by the use of Asparagine then it might be expected that primary protein synthesis would proceed. If this potentially increased synthesis was limited by carbohydrates, then addition of glucose to the Asparagine solution would ensure that carbohydrate was not limiting while, in addition, the primary protein synthesis would proceed at a higher rate. This, on the above hypothesis, would be reflected in the rate of oxidation of the 3 carbon compounds derived from the glycolytic process. Such an increase in oxygen consumption has in fact been found on addition of glucose to the Asparagine solution (Tables 23, 25, 26).

As a point of interest, the experiments presented in Table/

Table 23 indicates that the mechanism for utilising carbohydrate in the respiratory cycle is still present after 174 hours in darkness (Figure 9).

(2) The Onset of the Depressed Rate of Respiration in the Presence of Salts.

$NaNO_3$

From the results of Lovell (16), KNO_3 increases the respiration of Elodea by 50% over that of shoots respiring in water only. This stimulation can be reproduced both in freshly-gathered material and in material which has been kept under laboratory conditions for a period of nine days. Lovell does not state, however, how long the shoots were in darkness or how long they had been pre-treated with the salt before comparative determinations were made, but it seems very probable that the apparent "stimulation" is that which may be observed during the initial period in darkness, as, on the present findings, the respiration in water alone later reaches a high rate. The degree of stimulation due to the presence of the salts and the relation of this to concurrent respiration in water would therefore depend on a time factor and would vary according to the length of pre-treatment and to the length of the period in darkness before measurement. In addition, there is no doubt that the previous history of the plant material has a reflection on work of this nature as very hardy plants seem more able to overcome the effect of salts.

In/

In view of the stimulation of respiration by other salts such as NaCl and KCl etc. [Jacobi (12)] it might be suspected that the stimulation by $NaNO_3$ in the present work (or by KNO_3 in Lovell's experiments) is not altogether due to an integral participation of the inorganic nitrogen in the metabolic processes. If nitrate did assist in an increase of, say, protein metabolism and reserve, one would expect the respiration to proceed more equably with such a reserve of nitrogen assisting in the maintenance of protoplasmic vitality (cf. previous discussion). In the present experiments attempts were made to determine if exposure of Elodea to nitrate (in dilute solution) and under the influence of light, would increase the stability of the plant in its reaction to prolonged darkness. The effects were invariably similar to those in Tables 20, 21 and 22. Although in these latter experiments the course of respiration in water is not of the usual type (occasionally found by the present author) the ultimately greater depression in the respiration rate due to the nitrate is very evident.

Treboux (31), Benecke (1) and others have shown that mineral salts generally reduce the photosynthetic activity and this would be reflected in the carbohydrate reserve. The experiment presented in Table 19, where the plants were pre-treated for three days in (a) 0.001 M $NaNO_3$ and (b) 0.0001 M glucose, indicates the ultimate depressent effect on respiration by the nitrate; while the experiments presented/

presented in Tables 20, 21, and 22 prove that even with an artificially supplied carbohydrate the depressent effect of the salt still occurs. The effect of the nitrate in relation to the depressed rate of respiration is thus not connected with the presence or absence of carbohydrate. The ultimate deterioration of the tissues would again suggest that the nitrogen metabolism is involved.

NH4Cl

In the experiments with NH4Cl it is evident that the usual initial stimulation of the respiration occurs and that there is a post-maximal low rate. The maxima in water and in the salt solutions are more or less of the same order so far as these results show, but determination of this with certainty would necessitate a series of readings at closer intervals over a 24-hour or longer period. It has not been possible to do this.

(3) The Effect of Salts on the Metabolism of the Plant

The question which naturally arises is whether the increasing and ultimately depressed rates of respiration during a period of prolonged darkness, as found in salt solutions and in water alone, are due to the same fundamental causes.

Evidence for a specific effect of the salts on the metabolism of the plant is demonstrated in the experiments where the shoots are pre-treated in the light with a salt solution (Tables 12, 13). In these experiments, on transference to darkness, the respiration may commence at

a/

a high level but invariably shows a progressive fall to a low level; in contrast to this, respiration in water alone increases to a maximum followed by a rapid decrease to a low minimum. Moreover, some of the experiments suggest that the time taken to reach the low respiration rate requires a less period of exposure of the shoots to salt solution when there is a preliminary pre-treatment in the light. It would be necessary, however, to arrange a critical series of experiments before such a conclusion could be definitely reached. In this connection, it may be mentioned that Ingold (11), in his work on Elodea, found a more rapid ion absorption in the light than in the dark although Steward (30) states that this is a result of the effect of light on the carbohydrate content and of factors related to oxygenation. Nevertheless, it may definitely be stated that in addition to an effect of prolonged darkness there is a superimposed effect produced in the presence of salts. It has been shown that this effect cannot be obviated by the use of carbohydrate (glucose) in the case of $NaNO_3$ at least. Evidence of Rosenfels (25), in his work on K Br absorption, indicates that the low respiration rate with Elodea also occurs in his sucrose-K Br solution. Unfortunately, experiments have not been tried with NH_4Cl-glucose solution, but there seems no reason to doubt that the ultimately depressed rate in NH_4Cl solution only will also be shown to be practically independent of the carbohydrate supply. It is realised, however, that ammonium salts affect the carbohydrate/

carbohydrate metabolism of Elodea. e.g. Benecke (1) found that starch is not easily formed in Elodea in the presence of ammonium salts, although this author does not find the exosmosis of sugars demonstrated by Wächter (32) in the case of the bulb scales of onion. The present work suggests, however, that the effect of mineral salts is primarily on the nitrogen metabolism, in so far as the respiration rate in prolonged darkness is concerned. Evidence for this is found in the NH_4CL-Asparagine experiments.

NH_4Cl as a 0.01 M solution causes a comparatively early deterioration of the tissues in prolonged darkness except when supplied in the presence of Asparagine; in this latter case the shoots retain their (relatively) healthy appearance (see also "Chlorophyll Degradation" below). This is not so for 0.05 M NH_4Cl in the presence of Asparagine, where an ultimate deterioration and yellowing of the visible aspects of the plant occurs. In addition, the respiration rate in (0.01 M NH_4Cl + Asparagine) shows the usual increase to a maximum, but the post-maximal rate tends to be higher than the corresponding rate in Asparagine only (Table 25). On the other hand, a (0.05 M NH_4Cl-Asparagine) solution causes a similar rise to a maximum (apparently higher than in the last) but the ultimately depressed rate is similar to that found in 0.05 M NH_4Cl only (Table 24). Apparently the effect of NH_4Cl is a dual one, viz: (1) a tendency to maintain a higher rate of respiration and (2) the usual detrimental effect/

effect with the ultimate causation of a low respiration rate. The experiments would indicate that (2) predominates if a certain balance in the nitrogen metabolism is not maintained by an organic N substance such as Asparagine; such, apparently, would be the case when the concentration of the salt is too high. It is suggested that it is the balance of the protein metabolism which is upset directly or indirectly by the presence of the salt under such conditions.

The dual effect of the salt as indicated above may therefore be primarily due to the same cause, viz: in the balance of the relative rates of protein degradation and of synthesis. Thus, in the presence of a salt only, proteolysis is in excess of synthesis, the rates of oxygen consumption reflecting the rates of the oxidative deamination process. This condition also holds for the same experimental conditions in water alone, but the rate of proteolysis is greater in the presence of the salt; the critical concentration of protein would therefore be reached within a shorter period in the presence of the salt. A consideration of some of the previous experiments shows that the rate of the post-maximal decrease is, in fact, greater, and may occur earlier in the presence of the salts as compared with that in water. This is believed to be the case with salts in general, irrespective of the ionic constitution, and is true also of inorganic-N salts.

(4) Protein Synthesis from Inorganic-N Salts

However, the possibility of an ammonium salt playing an/

an orthodox part in primary protein synthesis must also be considered. If this primary synthesis were not inhibited (e.g. in the presence of Asparagine) and if the ammonium salt assisted in this process, then a higher respiration rate would be the result of (a) an increased rate of oxidative deamination due to the mere presence of the salt (cf. above) and (b) a higher rate of carbohydrate respiration, since the greater rate of primary protein synthesis in the presence of the ammonium salt would cause a greater rate of carbohydrate respiration (cf. previous discussion). Thus, the respiration rates in an NH_4Cl-Asparagine solution and in an Asparagine-glucose solution would tend to be lower than that in an NH_4Cl-Asparagine-glucose solution. This might partly explain the early stages of the experiment represented graphically in Figure 12 where, incidentally, the maximum found in the NH_4Cl-Asparagine-glucose solution is definitely higher than that found for any of the solutions used throughout the work with the exception, possibly, of the 0.05 M NH_4Cl-Asparagine solution in such an experiment as that given in Table 24 (Figure 10). Nevertheless, after 29 hours in darkness, the respiration rates in the Asparagine-glucose solution, with or without the addition of 0.01 M NH_4Cl (Figure 12) tend to be approximately equal, and this would suggest that the balance of the metabolism in the two solutions is reached in different ways. The possibility is (Figure 12), (1) in an NH_4Cl-Asparagine-glucose solution the/

the high oxygen consumption might to a greater extent be due to the oxidative deamination process, so that, while the Asparagine prevents the onset of a critical protein concentration, the possible primary protein synthesis, with its reflection in carbohydrate respiration in the presence of the salt, is for some reason not so great as in (2) an Asparagine-glucose solution in which the ultimately high rate is probably mainly due to an increased carbohydrate respiration, consequent upon an increasing rate of the primary synthesis from the products of glycolysis and the NH_3 from the deamination process. If this were true, then there should be a higher concentration of protein in the plants in the Asparagine-glucose solution than in the NH_4Cl-Asparagine-glucose solution. It is indeed significant that in the Asparagine-glucose solution apparent meristematic growth in the dark is much more obvious than in the NH_4Cl-Asparagine-glucose solution, and this in itself, together with the respiration data, might suggest that there is a high rate of protein degradation and a low primary synthesis in the presence of the salt, as compared with a low protein degradation and a high primary synthesis in the Asparagine-glucose solution. Thus, the equally high rates of respiration finally obtaining in both solutions (Figure 12) are possible, although the carbohydrate/amino acid respirations need not be in the same proportions in either case.

It must be concluded, therefore, that although

primary/

primary protein synthesis is possible under the experimental conditions, this may only take place to a significant degree in the dark in the presence of such a substance as Asparagine, the primary synthesis resulting from the products of the plant's own metabolism. The presence of a salt such as NH_4Cl introduces complexities related to an increased rate of proteolysis due to the presence of the salt, but primary synthesis involving the ammonium salt may nevertheless proceed. In regard to this latter, the present author has obtained slow growth of Sagittaria natans and Vallisneria spiralis in an 0.01 M NH4Cl solution, while the plants died in an 0.01 M NaCl solution. The plants were in light, however. In addition, the conclusions arrived at with these plants need not necessarily be true for Elodea.

While ammonium salts may assist in a primary protein synthesis in Elodea when certain conditions hold, there does not seem to be any evidence that nitrate can take part in this process. Unlike NH_4- the NO_3- ion does not inhibit chlorophyll degradation (q.v.). Other workers have found a deleterious effect of nitrate on water plants, as, for instance, the inhibition of root growth in Ranunculus fluitans found by Snell (27).

Since it appears that Asparagine actively increases protein synthesis, there is no reason to suppose that Elodea in nature would not utilise organic nitrogen compounds for its metabolic processes. There is a large amount/

amount of organic decay, generally, in the sub-aqueous substratum so that these compounds could be liberated in soluble form and could be absorbed by the leaves of the plant. Nevertheless, the function of the "roots" of Elodea is not always realised, as they are not merely a means of anchorage. Snell (27) has shown that Elodea plants "rooted" in soil show a greater growth rate than "unrooted" plants; in addition, these rates are both greater than in similar experiments with sand. The present author has also observed that growing axillary buds of Elodea are often associated with these "roots". It is significant that when a "root" reaches and penetrates the soil, the associated axillary bud commences to grow very quickly. It has been suggested that the function of the "roots" is to tap the high concentration of CO_2 in the substratum, but it is unlikely that this is the whole reason for the "root" system. In an actively growing season, the absorption of organic nitrogen for protein synthesis could take place through the "roots" from the soil, and this would be of very valuable assistance for the growth of the plants. Since there would be a limit to the amount of organic nitrogen which could be made available for the plant, the loss of this, after some years of supporting a dense aquatic flora, could very well account for the sudden disappearance of Elodea from many localities - a curious phenomenon, the reason for which has not yet been discovered.

(5) The Mechanism of Salt Effect

It is known that salts affect the nature of the protoplasm and also the relative concentrations of the nitrogen fractions of the cell. Steward (30), in reference to the work of one of his collaborators (Preston), states that the respiration of potato discs in solutions of salts varies with the amino N and protein N fractions, rather than with the concentration of sugars. Also, the relative concentrations of the nitrogen fractions are affected.

Mention may also be made of the work of Lundegärdh. This author states (17) that if wheat roots are placed in a solution of a neutral salt, the PH. of the protoplasmic membrane increases; this is believed to be caused by the absorption of Cations in exchange for H- ions, so that the membrane becomes negatively charged relative to the solution. This negative charge permits easy passage of the Cations into the protoplasm, but energy is required for the uptake of anions and this energy is supplied by an oxidation. Pantanelli (22) has demonstrated a differential absorption of cations over anions for the cells of Elodea. In such a case the presence of the cations may affect the PH. of the protoplasm with a subsequent effect on the nitrogen metabolism. In so far as these results would indicate, this effect is apparently on protein degradation.

The increased respiration caused by the salts and the resultant depletion of the protein reserves could in itself accentuate the deterioration along the lines suggested by/

by Briggs and Petrie (4) for carrot tissue immersed in salt solutions. These authors suggest that respiration in itself affects the PH. of the tissue and this would reflect on the ionisation of indiffusible substances such as proteins. It is apparent, therefore, that the total effect of the salts on the protoplasm is a complex one, but the accumulative effect is a final degradation of protein.

(6) Degradation of Chlorophyll

When the plants are kept under prolonged darkness a certain amount of chlorophyll degradation takes place. With addition of Asparagine to the medium this would appear to be somewhat curtailed in the older leaves, though in the younger leaves, which appear on the upper part of the shoot where extended growth has taken place, chlorophyll is nearly absent. These yellow, young leaves very clearly show the extent of growth in the Asparagine solutions. Ultimately, however, in Asparagine all the leaves finally become yellowish although they still retain their healthy crispness.

In general, salts, including nitrate, do not prevent the degradation of chlorophyll. It is noteworthy, however, that the ammonium salts used seem very definitely to inhibit loss of chlorophyll. In the 30-hour experiments at least, after the experimental period, the shoots in the more dilute ammonium salt solutions are definitely more green than those in water or in other salt solutions. In stronger ammonium salt solutions (0.05 M) the ultimate deterioration of the plant tissues, after two to four days in darkness, is accompanied/

accompanied by a great loss of chlorophyll, even in the presence of Asparagine. The conclusions that the effect of the experimental conditions is primarily on the protein metabolism have their reflection in this question of chlorophyll, which is also an organic N substance. Michael (19) finds that feeding his plants with Asparagine and sugar prevented yellowing of the leaves and caused protein synthesis, indicating a close connection between protein metabolism and chlorophyll production.

Degradation of chlorophyll is probably along the lines of oxidative deamination, but some trials, where the medium was in equilibrium with (a) gaseous nitrogen and (b) air, produced rather inconclusive results, as one might have expected chlorophyll degradation to be curtailed in the absence of oxygen.

(7) The Gases of the Lacunae in Water Plants

The gases in the lacunar regions of many water plants contain a certain proportion of oxygen. Gorski (9) finds that the gas escaping from assimilating Elodea is composed of: oxygen 21 - 50%; nitrogen 50 - 80%; CO_2 2%. The percentage of oxygen varied with the light intensity. There is thus a potential source of oxygen for the plant in the lacunae, and this may possibly be used in respiration. During the night, in the growing season, when the temperature of the water and of the substratum is relatively high, the respiration must be proceeding at a higher rate as compared with/

with the winter respiration at low temperatures. Where there is a competition with other plants for oxygen a 'reserve' of this gas in the lacunae would naturally be very valuable. In the present experiments, the shoots were cut so that there was always the possibility of an outward diffusion of the oxygen into the medium (or vice versa) and this could affect results somewhat. This direct connection between lacunae and medium could be avoided by pricking off the shoots some days before the experiment so as to give time for the plant to heal the cut. A brown formation always occurs at the cut end and this may be a brown excretion into the lacunar cavities [similar to the excretion into older lacunae of other water plants as found by Hochreutiner (10)], or some wound reaction similar to that on cut potato. In the experiment shown in Table 23, the shoots were cut two days before the experiment and left in water. The subsequent reaction in darkness was similar to that of a previous day's experiment where the shoots had been cut the evening before and left overnight in water under a 100 watt light. The absence of any initial rise to a maximum in both these experiments seems somehow to be related to a winter condition of the material.

On the other hand, some experiments were tried with complete plants of Sagittaria natans. The lacunae in this plant are very large and must contain a correspondingly large 'reserve' of gases. A curious feature of these experiments was that very little respiration could be detected even after two days in darkness, as if the oxygen in/

in the lacunae was more readily used than the oxygen of the medium. This interesting point might be developed in a later work.

A feature of the experiments with Elodea, both with and without salts, was that the stem lacunae seemed to become waterlogged. In addition to the rigidity of the stem being caused by cell turgidity, it is possible that the pressure in the lacunae acts in the same way as a football bladder. When this pressure is lost by waterlogging, the rigidity of the stem would also disappear. A noteworthy feature of the Asparagine experiments is that, where the solutions do not in themselves detrimentally affect the cells, the stems in every case are of the characteristic 'sparkling' appearance due to the air spaces inside. It would appear that the Asparagine assists in maintaining a gaseous pressure inside the lacunae, a probable corollary to the maintenance of protoplasmic vitality.

SUMMARY

1) An apparatus, which can be used for the measurement of rates of respiration of Elodea shoots, is described. In this apparatus, "still" tap water is used during an experimental period of respiration.

2) Measurements of respiration were based on oxygen consumption. The micro-technique of the oxygen estimation is indicated. It is found that the method of water sampling yields results which are highly satisfactory.

3) The experimental results show that mineral salts generally increase the respiration rate of Elodea canadensis, but that this stimulation is of a temporary nature.

4) The use of carbohydrates in easily available forms does not prevent the onset of the depressed respiration rates in water or in salt solutions during a period of prolonged darkness. In such conditions deterioration of the plant tissues may still occur.

5) Asparagine, as a source of organic nitrogen, causes Elodea to respire at a more or less equable rate for at least 73 hours in darkness. The plant tissues also remain turgid and relatively healthy. It is suggested that Asparagine actively augments protein synthesis; thereby a critical concentration of protein reserve is avoided and the actual occurrence of death is delayed for a period in excess of 174 hours of darkness.

6) It is suggested that, unless the vitality of the cell processes is maintained by such a substance as Asparagine, proteolysis, during a period of prolonged darkness in water or in salt solution, is relatively in excess of protein synthesis; the increasing respiration during the initial 24 hours or so is possibly due to an increasing rate of oxidative deamination of the amino acids of proteolysis. The resultant depletion in protein reserve might be found to be correlated with the onset of the depressed rate of respiration.

7) It is suggested that carbohydrate respiration is dependent on the rate of primary synthesis of protein. It would appear that this primary synthesis in darkness (and hence the occurrence of carbohydrate respiration) is inhibited as a result of pre-death influences under the experimental conditions, unless the vitality of the cell is maintained by a substance such as Asparagine.

8) The possibility of ammonium salts playing a part in primary syntheses is discussed. It seems apparent, however, that this primary synthesis is masked by the stimulation of proteolysis which occurs in the mere presence of the salts.

9) The mechanism of salt effect is discussed. It is probable that the ultimate result of a differential absorption of cations would alter the PH. of the protoplasm, and that this might alter the nature of indiffusible substances such as proteins in such a way that the rate of proteolysis is relatively increased. The increased respiration, due to the oxidative deamination of the amino acids of proteolysis, could intensify the effect on proteins along the lines suggested by Briggs and Petrie (4).

10) The effect of the experimental conditions on the process of chlorophyll degradation are discussed. It is found that ammonium salts have an inhibiting influence on chlorophyll degradation.

11) A certain level of nitrogen metabolism would also appear to be a necessary concomitant of the maintenance of gaseous pressures in the lacunae.

This work was done during the tenure of a Carnegie Scholarship, and the writer wishes to record his sincere thanks to the Carnegie Trust for the Universities of Scotland for financial assistance. The writer is also indebted to Dr. G. Bond, Lecturer on Plant Physiology, University of Glasgow, for his continued interest and advice throughout the period of the work.

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B.

The Effect of Potassium and Phosphorus Starvation on the Carbon Assimilation Rate of Barley.

Table of Contents

Introduction

The process of carbon assimilation by plants has for many years engaged the attention of a large number of investigators. The comparatively recent monographs on photosynthesis by Stiles (31) and Spoehr (29) constitute a comprehensive review of the experimental results of a very wide range of workers.

Carbon assimilation is, however, a process of great complexity, and much remains to be done before complete elucidation of the problem shall have been accomplished. Briefly, the process is controlled by two sets of factors and these have been referred to as external and internal factors. Blackman (2) first directed attention to the principle of Limiting Factors and this has stimulated research along lines which have led to the understanding of the nature and control by the external factors (such as light, carbon dioxide concentration, etc.) and their relation to carbon assimilation. The principles formulated by Blackman have been discussed by Benecke (1), while, further, Maskell (21 and 22) has considered the interaction of the factors of light and CO_2 in terms of their effects on diffusive resistance. He postulates a series of such resistances at different stages of the diffusion path, constituting variable resistances in series, the contribution of each to the total diffusive resistance depending on variation in CO_2 supply, stomatal aperture and other internal factors./

factors. A detailed presentation of the chemical processes involved in carbon assimilation has been given by Warburg (35) and by Willstatter and Stoll (36). The more recent work has been reviewed by Briggs (6) and Emerson (10).

With regard to the nature of the internal factors and their control on carbon assimilation, however, our experimental knowledge has not progressed to the same degree. It is accepted that the seat of the photosynthetic process is at the surface of the chloroplast. Further than this, the most generally accepted schema of the process is that conceived by Willstatter and Stoll (36) who believe that at least five stages are involved. These may be summarised as follows:

1) The passage of CO_2 from the cell surface to the chloroplast. (Hydro--diffusion stage).

2) Combination of CO_2 with some component of the chloroplast system, and finally with the chlorophyll molecule.

3) Activation of compound molecules by light, chlorophyll possibly acting as a photocatalyst, forming some intermediate compound. (Light reaction).

4) Chemical reaction (catalysed by enzyme) among the activated molecules leading to the splitting-off of oxygen and formation of the first product of photosynthesis. (Dark reaction).

5) Polymerisation of the first product of assimilation. (Sugar or starch).

A study of the effect and control of the internal factors presents many difficulties. Such factors as chlorophyll content have received a certain amount of attention, but the nature and effect of various other protoplasmic factors have not yet been established. Briggs (5) has contended that there is 'a reactive chloroplast surface' which determines the proportionality factor between carbon dioxide supply and photosynthetic activity. This surface possibly corresponds to the surface between the aqueous and lipoid phases of the chloroplast as postulated by Stern (30). Briggs, furthermore, has shown that this 'reactive chloroplast surface' may be modified when plants are starved of certain mineral elements.

The present work is an extension of previous work performed by Gregory and his collaborators on the effects of manurial deficiency on the growth of barley. Gregory and Richards (16) have shown that some important physiological processes may be seriously affected when barley plants are grown under conditions of deficiency in potassium, phosphorus or nitrogen. Deficiency in these elements further affects such factors as chlorophyll content, rate of leaf production, tiller formation and other general characteristics of growth. These effects must be accompanied by a change in the internal organisation of the plant and hence it was thought that a direct study of the effect of such deficiencies on the rate of carbon assimilation might profitably be pursued.

The leaves used for measurement of the assimilation rates/

rates were those produced successively on the main stem of the barley plant. Each leaf was taken at the time at which it reached maturity, and when the auricles had expanded. In this manner comparable leaves from plants of different treatments could be measured at comparable stages in their development. It was further endeavoured to maintain the plants under natural conditions in so far as this was possible and desirable. Use was therefore made of atmospheric air as a source of carbon dioxide, sunlight as a source of light, and the leaves remained attached to the plant during the experiment. A gasometric method was employed for estimating assimilation rates. It was also considered desirable to ascertain the maximum possible assimilation rates of the barley leaf, by this method, in order to compare this with the results obtained from growth analysis and dry weight increase.

Method and Material

A pure strain of barley (Plumage Archer) was used in these investigations, which were carried out at the field laboratory of the Imperial College of Science and Technology at Rothamsted Experimental Station. The seeds were grown in glazed pots which held 30 lbs. of washed sand. The pots were about 10 inches high and 10 inches in diameter. Near the base of each pot there was an opening in the side which could be closed by means of a rubber bung. Through this bung there passed the short arm of a right angle glass tube.

A/

A 'pad' of glass wool, placed behind the bung, prevented the sand from escaping into the tube. The long arm of the glass tube was kept vertical outside the pot and reached almost to the top of this. Equal saturation of the sand could be ensured by adding water until the levels of the water in the glass tubes were the same (approximately one inch) in all cases. In the event of water-logging by heavy rains the tubes could be turned down so that surplus water from the pots could be drained into receiving bottles. This drainage water would contain nutrient solution and was therefore returned to the pots as occasion required. Normal routine watering of the pots was done by pouring the water into earthenware pots inserted in the sand at the surface. In this way, disturbance of the surface of the sand was avoided. The total number of pots used in this work was 136.

Selection of seeds for uniformity of size and colour was made by eye. Before sowing they were sterilised in 0.2% formalin for four hours. The seeds were then sown in the pots at the rate of nine seeds per pot. They were spaced equidistant from one another in a circle with a radius more than half that of the pot. Sowing was carried out at a uniform depth of 1.25 inches. The first crop was sown on May 1st., 1934, and the first signs of germination were noted on May 11th. By 14/5/34 germination was complete and was practically uniform at 100% germination. When the plants were at the second leaf stage, selection

was made so that only three uniform, vigorous, equally spaced plants were left in each pot. The nutrient solutions were applied in three doses, the first on 15/5/34, the second on 29/5/34 and the third on 12/6/34.

Three nutrient levels of potassium and phosphorus were employed. These will be referred to as:

F.M. (Fully Manured)

K_3 (one-ninth standard level of potassium ion)

K_5 (one eighty-first do. do. do. do.)

P_3 (one-ninth standard level of phosphate ion)

P_5 (one eighty-first do. do. do. do.)

In Table I are given the weights of salts as supplied to each pot of the F.M. series.

TABLE I

Salts used	Weight of salts in grams per pot
Na_2HPO_4, $12H_2O$	2.52
$NaNO_3$	9.10
K_2SO_4	1.85
$CaCl_2$ $6H_2O$	0.37
$MgSO_4$	0.61

A trace of Manganese and of iron was added to each pot.

In the potassium deficient series no compensation was made for the simultaneous reduction in the quantity of $-SO_4$ ion present as this was known to be in adequate amounts [Gregory and Crowther (15)]. In the phosphorus deficient series, however, there was a simultaneous reduction of the/

the sodium ion, due to the variation of the sodium phosphate added, and this was compensated by adding the requisite amount of sodium sulphate. The variation in $-SO_4$ was not considered to be important.

When the plants had reached the second or third leaf stage the assimilation experiments were begun. As growth proceeded experiments were carried out almost continuously, as daily estimations were necessary during the period of maximum leaf production rate. The aim of the experiments was to measure the assimilation rates of the leaves of the mineral deficient series and to compare these with the assimilation rates of the corresponding leaves of the F.M. series. As far as possible, the assimilation rates in the three levels of deficiency in a series were compared on any one day. At the same time a series of control values gave a reliable estimate of the amount of CO_2 in the volume of air used. The average amounts of carbon dioxide removed from the air by the assimilating leaves could thus be estimated and the value for assimilation expressed in terms of mg. CO_2/unit area of leaf surface/hour.

As the area of the leaves was comparatively small it was necessary to run the experiments for a sufficiently long time to give an easily measured amount of CO_2 assimilation. This time was generally taken as seven hours. In addition, as previously stated, leaves were used which were at the same stage of development, namely, at the time of complete expansion of the leaf and also of the auricles. In/

In the phosphate deficient series, however, the considerable reduction in rate of leaf production made it impossible to compare either the P_3 and P_5 directly, or these with the corresponding leaf in the F.M. series, and thus a compromise was necessary. This difficulty did not arise in the potassium series as here leaf production was almost identical throughout the series. As stated, only leaves of the main shoot were used and identification was made possible by marking the shoot with indian ink before tillering was advanced. Every third leaf, as it emerged on the main shoot, was marked in the same way.

Apparatus

The principle involved in the apparatus was that of the usual gasometric method. Since the apparatus was to be used in the field it was made easily transportable by mounting it on a wheeled trolley; in addition, it could be housed in a hut when no experiments were in progress.

In order to facilitate the description of the apparatus it will be convenient to refer to each component as it occurs along the path of a gas stream. There are six such gas streams and each circuit consists of (1) a leaf chamber, (2) a flow regulator consisting of (a) the capillary resistance and (b) the manometer, (3) the absorbing apparatus and (4) the pump, together with devices for controlling the gas flow.

(1) The Leaf Chamber

The/

The area of too large a leaf chamber introduces certain difficulties. The supply of CO_2 to the leaf depends on diffusion, so that the deeper the chamber the greater is the diffusive resistance. Indeed, with a large chamber much of the CO_2 contained in the air may not be available to the leaf during the passage of the air through the chamber. Under these conditions it becomes impossible to estimate the "effective" concentration of CO_2, although the composition of the emerging gas may be accurately knwon.

To meet these very important requirements in work of this kind, a special leaf chamber was designed by Doctor, now Professor F. G. Gregory of the Royal College of Science, London.

The leaf chamber is entirely made of aluminium, except for a glass screen. It comprises three parts, (1) a lower flat plate on which the inlet and outlet tubes for air flow are fitted; (2) a central water trough which has a longitudinal groove along its lower side, this being closed by a glass plate above. This longitudinal groove constitutes the actual leaf chamber. The water trough is thus separated from the leaf chamber by the glass plate, which admits light to the leaf. The water trough is also covered above by (3) a second glass plate fitted into a metal holder (Fig. 1, C). Attached to the lower flat aluminium plate is a steel rod by means of which the chamber can be fixed and clamped in any desired position. The overall dimensions of the chamber are 15 cm. x 10 cm. x 5 cm.

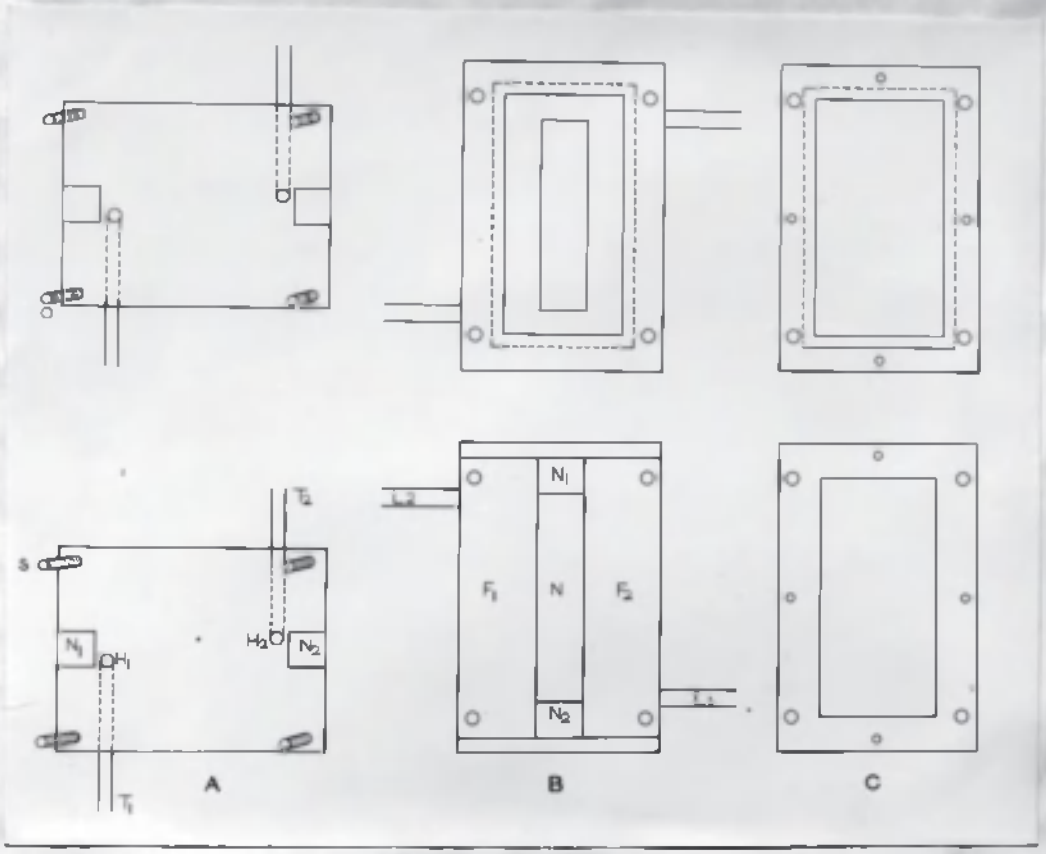

Figure 1 A diagrammatic representation of the different aspects of the leaf chamber.

The lower metal plate is one centimetre in thickness. The inlet and outlet tubes T1 and T2 (Fig. 1, A) are fixed into the body of the plate and connect with H1 and H2 (Fig. 1, A) which are small holes bored from the uppermost surface to the level of T1 and T2. N1 and N2 are two depressions scooped out from the metal. When the chamber is in use, these depressions hold washers of gelatine upon which/

which the leaf rests as it lies along the line N1 - N2. At the four corners of this lower plate are four large screws (S) which fit through corresponding holes in the upper parts of the chamber, and by means of wing nuts the whole can be securely assembled. When the upper parts of the chamber are thus screwed on to the lower metal plate, the depressions N1 and N2 of 1B (Fig. 1) fit above those of 1A, and the shallow groove N of 1B forms, with the lower flat plate, the actual leaf chamber. The leaf, held between the gelatine cushions in N1 and N2, lies along this groove N and rests on the lower plate. F1 and F2 in 1B (Fig. 1) are flat flanges which, when lightly vaselined, form a perfect airtight fit with the lower flat plate.

In 1B (Fig. 1) L1 and L2 are the outlet and inlet for water which is made to flow through the water trough. The water screen is approximately 1.5 cm. deep. By maintaining a flow of cool water through the screen, any deleterious effects due to the heat rays of the sun are obviated. When the apparatus has been in use for some time the glass plates may require cleaning; except for this contingency the two upper components illustrated remain screwed together and are not separated.

The gelatine cushions are made by pouring molten gelatine of suitable consistency into N1 and N2 (upper and lower parts), these being temporarily closed at the ends by means of cork strips so as to form moulds. The

correct/

correct consistency of gelatine is determined by experiment and should be such that the temperature conditions do not appreciably affect the gel.

Photographs of the different aspects of the parts of the leaf chamber are shown in Figures 2 and 3. Figure 4 indicates the method of assembling the parts together.

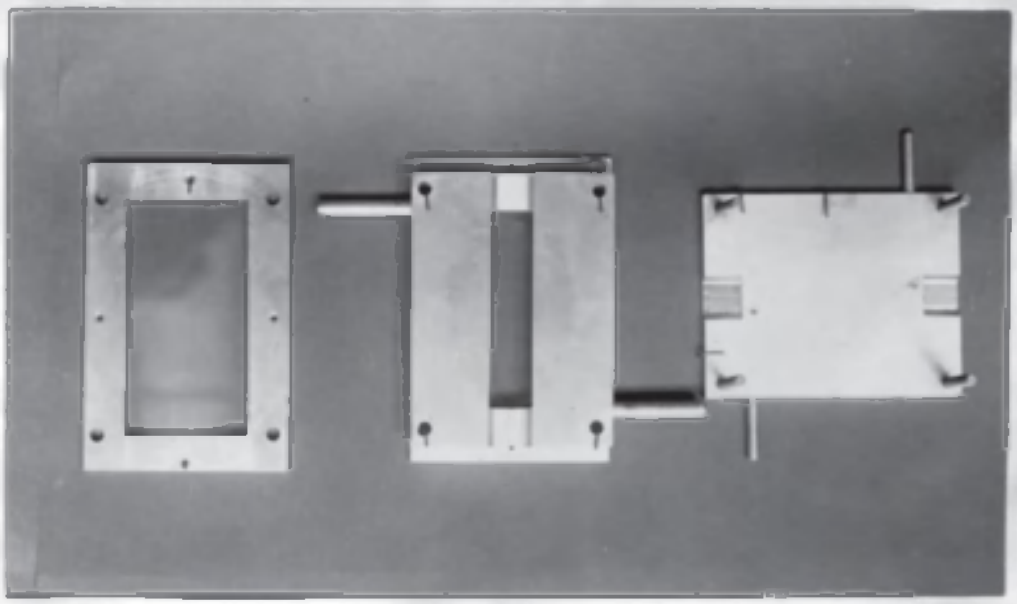

Figure 2 Showing the longitudinal groove on the under side of the central piece; the lower plate with the air inlet and outlet; the upper metal frame and glass plate.

(Figures 3 and 4 overleaf)

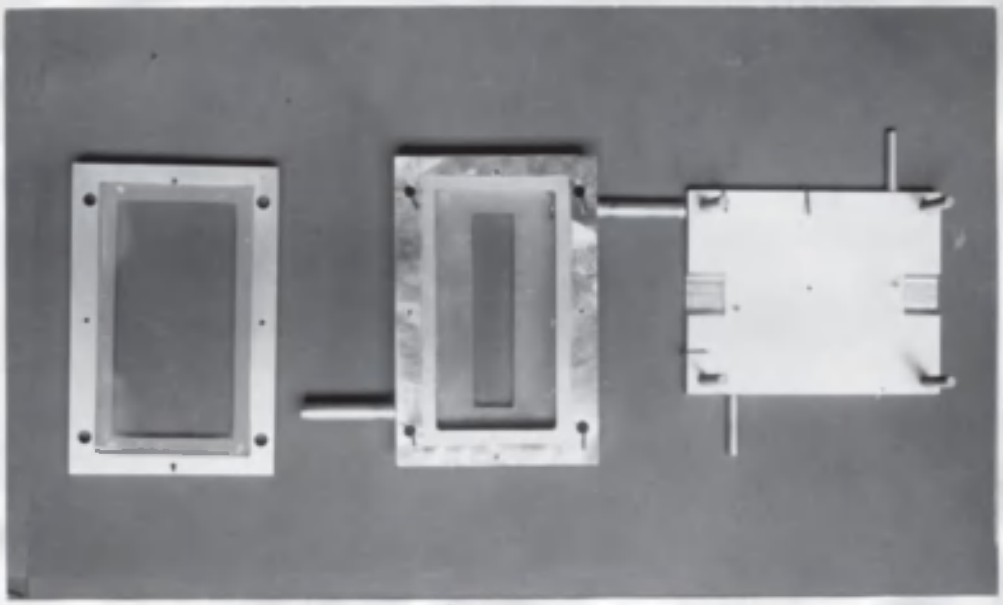

Figure 3 Actual photograph of the water screen with the top piece removed.

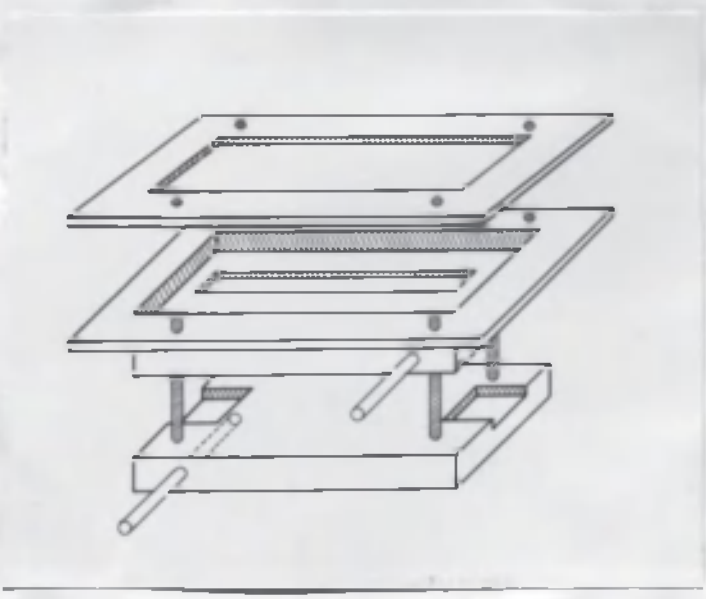

Figure 4 Showing the method of assembling the parts.

After leaving the leaf chamber the air stream passes through a capillary resistance into the absorbing towers. The relations of the various parts of the apparatus may be observed in Figure 5.

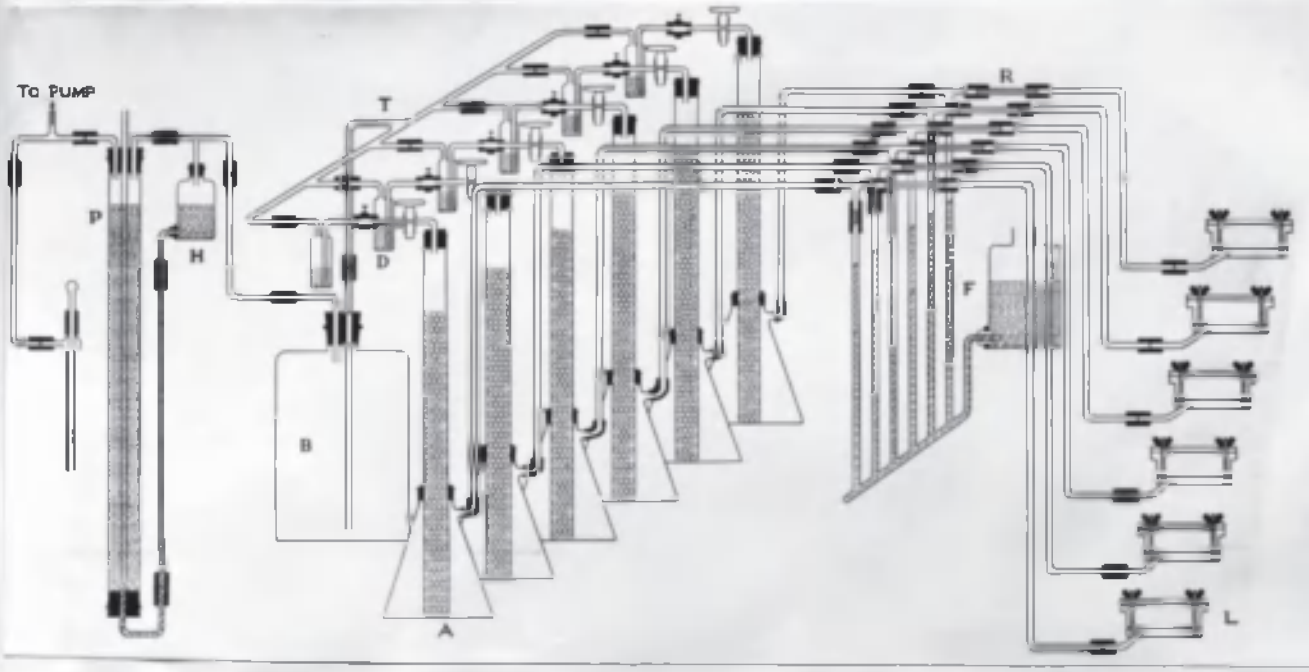

Figure 5 A diagrammatic representation of the whole apparatus, showing the relation of the various parts to one another.

(2) The Flow Regulator

It is essential that the flow of air through each of the six circuits be equal, in order to obtain a reasonable degree of accuracy in the results. To obtain this, the following simple method is adopted. Each air stream passes through a capillary resistance R (Fig. 5). As the accuracy of the rate of air flow of each stream depends, in a great measure/

measure, on the equality of the resistance of these capillaries, it is necessary to have these of equal value. By using the arrangement described below, capillaries of equal resistance can be obtained.

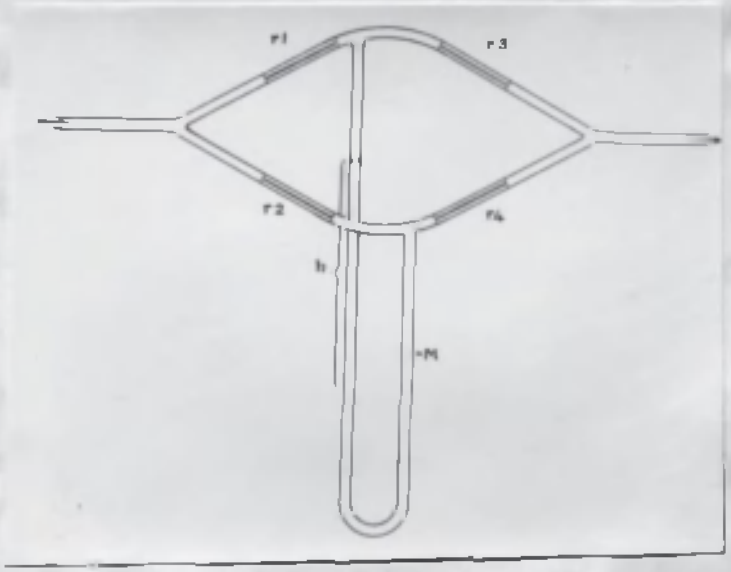

Figure 6 Simple device for obtaining capillaries of equal resistance.

The principle employed in the device illustrated in Figure 6 is that of the Wheatstone's Bridge. r1, r2 and r3 are capillaries of fixed resistance and of convenient length; r4 is the capillary to be standardised. A manometer M is inserted across the bridge and this registers any differential pressures in this part of the circuit. In order that the height 'h' of the liquid in each arm of the manometer be equal, it is necessary that the following condition/

condition be the case:

$$\frac{r1}{r2} = \frac{r3}{r4}$$

This ratio is independent of the rate of flow, although the sensitivity increases as the rate of flow increases. Each of the capillaries to be standardised are in turn placed in the position of r4. It is advisable to have these somewhat longer than necessary so that they can be gradually ground down until they cause equal heights 'h' in the manometer when they are placed in the position of r4. By this method it is easy to obtain a complete set of resistances R which are of equal value.

When the assimilation apparatus (Fig. 5) is working, the resistance of these capillaries causes a negative pressure on the pump side of the circuit and this is registered by the rise of the liquid in the common manometer F (Fig. 5). This manometer is comprised of six vertical tubes joined along their lower ends by a horizontal tube, one end of which is closed; the other end is connected to a bottle containing the manometer liquid, the surface of which is exposed to the atmosphere. The six vertical arms are each connected through a T-piece to its circuit by means of rubber pressure tubing; into this rubber tubing is pressed a small length of thermometer tubing, and this helps to damp out pressure fluctuations which might cause an oscillation of the liquid in the manometer arms.

(3) The CO_2 Absorbing Apparatus

This part of the apparatus consists of a series of ordinary filter flasks A, 20 cm. high. Through the rubber stopper of each flask is fixed a wide glass tube, 75 cms. high and 2.5 cms. in diameter. This wide tube is so placed as to be almost touching the bottom of the flask. The tube is filled to within a few inches of the top with glass beads of 2 mm. diameter.

At the beginning of an experiment the flasks each contain 100 ml. of N/5 (approx.) baryta. The suction caused by the pump draws the baryta out of the flask and holds it in the tower, so that the air stream is drawn into the flask and then passes up the absorbing tower. This absorbing system is found to be a very successful scrubber and complete absorption of CO_2 is obtained at a rate of flow much higher than that experimentally used. The baryta traps D (Fig. 5) act as indicators of incomplete CO_2 absorption by the system. Glass taps join the top of the absorption towers and the baryta traps, and these are fixed by means of rubber tubing over which screw clips are fitted. These screw clips serve for making minor adjustments in the rate of flow in each circuit.

A noteworthy feature of the absorption apparatus is its inexpensiveness as compared with other absorption systems. There is also easy accessibility of the parts, ensuring the possibility of thorough cleaning.

(4) The Pump and the Controlling Devices for Air Flow

After passing through the absorbing apparatus, the gas streams pass into a common circuit T (Fig. 5) leading to the pump.

An electrically-driven oil pump was used as there was no suitable water supply to obtain suction by the usual means. The principle of the pump is the same as that of the filter pump used on water taps. Oil is raised from a resevoir and forced through a tube with a constriction as in the filter pump. The gas is drawn from the apparatus through a hole in this constriction and the oil is returned to the resevoir.

Working at a speed of highest efficiency, the pump causes too great a flow of air through the apparatus, so this is reduced by inserting in the circuit of the apparatus a valve of the pattern described by Gregory (13). This consists of a solid glass rod, carefully ground into a close-fitting tube (vide. Fig. 5). Part of the suction force of the pump is dissipated by drawing air from the atmosphere through this valve so that the air flow through the apparatus is decreased. The amount of air passing through the valve from the atmosphere can be regulated by varying the resistance to its passage with the sliding glass rod.

Fluctuations in the suction force occur, but these are practically eliminated by the use of (a) the carboy B (Fig. 5) which is of large volume, and (b) the piece of apparatus P (Fig. 5) which is based on the principle of the Mariotte Bottle. This piece of apparatus (P) consists of a long, wide/

wide tube closed at both ends by rubber stoppers. Through the lower stopper the interior of the wide tube is connected with a bottle H (Fig. 5). Inlet and outlet tubes for the passage of air are fixed in the rubber stopper, as also is a tube leading from the atmosphere to the bottom of the wide tube. This wide tube is filled with water as shown in the diagram. The height of the water is regulated by the height of the bottle H. This is so arranged that when the pump is working, the suction causes air to bubble constantly from the submerged end of the inner tube leading from the atmosphere. Thus, if any variations occur in the pressure of the system, a greater or lesser amount of air escapes through this inner tube.

With such devices, the rate of flow of air through the plant chambers is kept constant and the volume of air passing through the system can also be regulated.

Experimental Procedure

At the commencement of the work the bottle attached to the manometer F (Fig. 5) had its lowest position determined, i.e. so that the liquid in the manometer arms rested at a convenient height. This height was marked off on each arm. The levels obtained with the required rate of air flow were also determined and these were similarly marked. Uniformity in level was obtained by simply raising the bottle containing the manometer liquid to the required level. The levels were marked by black thread so that it was found desirable/

desirable to check these before each experiment.

At the commencement of an experiment only the lower flat plate of each leaf chamber, with its gelatine cushions, was in position. The gelatine was lightly smeared with vaseline to prevent absorption of water from the leaf. The plant pots were placed at the correct height and position, so that, while still attached to the plant, the leaves could lie across the flat plates without stress. The tip of each leaf rested on the extreme gelatine cushion and passed across the nearer one. The flanges F1 and F2 (Fig. 1, B) of the lower and upper parts of the chamber were vaselined lightly, after which the upper part of the chamber, with its corresponding gelatine cushions, was lowered on gently and screwed into position.

Generally, corresponding leaves from two plants of the same manurial level and series occupied two leaf chambers. When three pairs of leaves were being used, each pair of leaf chambers was attached to a single gas circuit and the three circuits without plant chambers were used as controls. The water screens were then joined in series, outlet to inlet, and water from a tank allowed to gravitate through. The tank, built on the gasometer principle, may be seen in the left-hand corner of Figure 7. Thermometers at either end of the water stream were read every hour and the readings averaged over the duration of the experiment.

(Figure 7 overleaf)

Figure 7 Photograph showing the conditions of experiment.

When the apparatus was ready for the measurement of assimilation, the pump motor was switched on and the sliding rod of the variable resistance fully drawn out. The rod was then gradually pushed in until the air had commenced to bubble from the inner tube of the valve P (Fig. 5), and also until the highest marked levels in the arms of the common manometer had been reached approximately. A final adjustment for each separate circuit was done by means of the screw clips mentioned elsewhere. Minor adjustments found necessary throughout/

throughout the day were also done by means of the screw clips.

At the end of the experimental period, the glass taps at the top of the absorbers (Fig. 5) were turned off simultaneously. This prevented any 'suck-back' from the baryta traps.

The Titration

The baryta of each absorbing tower was titrated in turn. The rubber stopper of each flask, together with the absorbing tower, was gently lifted out so that the baryta and glass beads remained in the flask. The tower was then washed down with a definite quantity of CO_2-free distilled water. From previous trials the maximum safe volume of $N/5$ acid which could be added was known; this was quickly added, after which the titration was finished with care. The indicator used was phenol-pthalein.

The $N/5$ acid was made by diluting the required amount of concentrated HCl to 10 litres and titrating this against the standardised NaOH of the Chemistry Department. The acid was made up to $N/5$ strength by suitable adjustment and re-standardisation.

The standard $Ba(OH)_2$ was made by dissolving the solid crystals in water in the usual manner and filtering the solution quickly into a 10 litre container through a Buchner's funnel. In addition, 63 gms. of $BaCl_2$ were added to reduce the solubility of the $BaCO_3$ and the mixture shaken up. This solution was standardised against the standard acid.

Blank Experiments for Experimental Error

Unfortunately, in this part of the work on mineral nutrition, time did not permit of a long series of blank experiments with a view to determining the experimental error statistically. Further, in order to make obvious any possible fault inherent to any particular gas circuit, the circuit numbers used for controls and for assimilating leaves were varied regularly. Because of this, it has not been possible to construct a balanced table for an Analysis of Variance on the blanks throughout the course of the work. Chinoy (8), however, used the same circuit numbers for his four controls when using the same apparatus. An Analysis of Variance, performed by Professor F. G. Gregory on Chinoy's results, shows that the error due to the inequality of the capillaries amounts to 1.06% for a single deter-:mination. For the mean of four estimations the standard error is therefore 0.5%. Since the assimilation rate is estimated from the difference between a single determination of the CO_2 in the stream led over the leaf, the S.E. of the determination would be $\sqrt{1.06^2 \times 5/4} = 1.18\%$. The method is thus seen to be capable of high accuracy. Professor Gregory has informed the present writer that the experimental error in this work would appear to be of the same order.

EXPERIMENTAL RESULTS

Before considering the results obtained from the measurement/

measurement of rates of carbon assimilation it might be of interest to describe some of the other effects of manurial deficiency.

The Visible Aspects of Manurial Deficiency

When barley is grown under conditions of potassium deficiency there is a definite reduction in the chlorophyll content of the leaves, which are light green in colour. The rates of leaf production and of tillering seem to be normal; the leaves, however, undergo early death, and the tillers die off at the time of elongation of the stem. Later, however, a fresh lot of tillers appear. In addition, there is a reduction in the length of the stem. These characteristics become more marked as the level of potassium is decreased.

Figure 8

Photograph of a pot of three F.M. plants.

Figure 9 Photograph of a pot of three plants from the K_3 series.

Figure 10 Photograph of a pot of three plants from the K_5 series.

The photographs shown in Figures 8, 9 and 10 were all taken after the time of elongation of the stem, and ear emergence is about to commence. The progressive difference in size and growth is clearly seen. The lettering used in the figures are those of another part of the work of the Institute on plant nutrition. The only variable ion, however, is the potassium ion. The greatest growth is seen in the Fully Manured series (Fig. 8, PIKI), with a progressive decrease through the K_3 series (Fig. 9, PIK3) to the K_5 series (Fig. 10, PIK5).

In contrast to the above, deficiency of phosphorus causes a reduction in the rate of leaf production and in the number of tillers, indicating thus a reduced meristematic activity. The chlorophyll content of the leaves is not reduced; in fact, the leaves are a darker green than those of the fully manured series. There is, however, a reduction in leaf size, and early death of these occurs, though this does not happen so rapidly or so spectacularly as in the potassium deficient series. There is also characteristic development of anthocyanin. These characteristics of phosphorus starvation also become more obvious with increasing deficiency.

Also to compare with the F.M. plants shown in Figure 8 are photographs of plants of the P_3 and P_5 series in Figures 11 and 12.

(Figures 11 and 12 overleaf)

Figure 11 Photograph of a pot of three plants from the P_3 series.

Figure 12 Showing the subnormal growth at the P_5 level of deficiency.

Figures 8, 11 and 12 demonstrate the progressive reduction in height of stem, in leaf size and in number of tillers, as P-deficiency is intensified.

Affected also by the level of potassium and phosphorus deficiency are the water relations of the leaf. Unfortunately, dry weights were not taken during the earlier stages of the work, so that calculations involving dry weights only refer to the fourth or fifth and later leaves; nevertheless, the results obtained demonstrate very clearly the various effects of the manurial deficiences on the water relations. The data collected refer to leaves used in the assimilation experiments, together with some measurements on the first few leaves from plants of the second F.M. crop. The data include measurements of (1) leaf area, (2) fresh weight and (3) dry weight. All the measurements on leaves of the same number on the stem and in the same manurial series have been averaged.

The following water relations are considered:

(1) Ratio of water content to leaf area,

(2) Ratio of water content to dry weight, and

(3) Ratio of dry weight to leaf area.

The data of the above water relations are presented in Table 2 for the F.M., K_3, K_5, P_3 and P_5 series, and are graphically represented in Figures 13 to 18.

TABLE 2

$\frac{\text{Weight of water}}{\text{Leaf area}}$ = centigrams water per dm^2 Leaf Surface

$\frac{\text{Dry weight}}{\text{Leaf area}}$ = weight in centigrams per dm^2 Leaf Surface

Leaf No.	Fully Manured			K_3 series			K_5 series		
	Wt. of water. Leaf area	Wt. of water. Dry weight	Dry weight Leaf area	Wt. of water. Leaf area	Wt. of water. Dry weight	Dry weight Leaf area	Wt. of water. Leaf area	Wt. of water. Dry weight	Dry weight Leaf area
3	195.2	5.04	38.74						
4	200.5	5.11	39.27						
5	200.9	5.22	38.01	226.2	5.80	39.02	223.5	7.16	31.23
6	234.0	6.16	37.97	236.4	6.32	37.98	223.2	7.40	30.16
7	221.3	6.47	34.23	220.9	6.13	36.10	177.4	6.30	28.34
8	192.6	5.99	32.15	195.3	6.13	31.86	179.3	7.39	24.26
9	180.2	5.21	34.60				164.8	5.99	27.43
10	141.4	3.66	38.62	136.0	3.52	38.77	156.2	5.57	28.65
11	133.8	3.01	44.44	161.5	4.89	33.29	124.1	4.04	30.70

Leaf No.	P_3 series			P_5 series		
	Wt. of water. Leaf area	Wt. of water. Dry weight	Dry weight Leaf area	Wt. of water. Leaf area	Wt. of water. Dry weight	Dry weight Leaf area
3	191.7	6.08	31.54			
4						29.47
5			39.56	192.3	5.14	37.45
6	211.2	5.54	38.15	167.3	5.34	31.39
7	174.9	4.44	39.41	151.3	4.53	32.72
8	170.4	4.32	39.68	182.1	4.50	40.59
9	142.5	3.55	40.24	174.8	4.69	37.37
10	124.3	2.63	47.61	176.7	4.03	43.88

(1) $H_2O/_{L.A.}$ Weight of water per unit area of leaf surface.

(a) K-deficient series

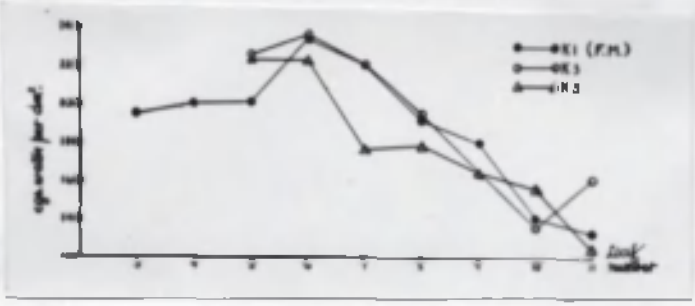

Figure 13 Graph showing the weight of water per dm^2 of leaf surface in the F.M., K_3 and K_5 series.

In Figure 13 it is seen that the curve of the F.M. series (K_1) rises until the sixth leaf after which there is a steady fall until the eleventh leaf. A similar course is followed by the curve of the K_3 series except for a diversion at the eleventh leaf where the value is higher than that of the F.M. The same effect of age is shown by the K_5 series, although in this case the values are generally slightly lower than the corresponding values for the F.M. series.

(b) P-deficient series

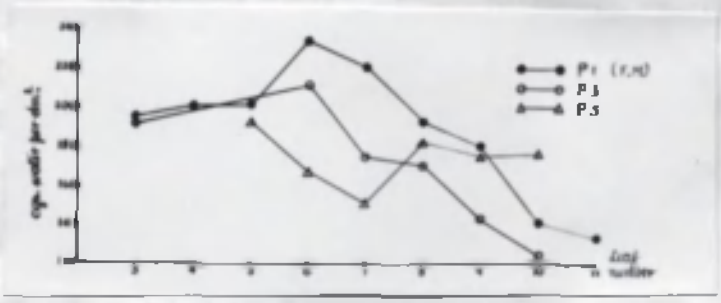

Figure 14 Graph showing the water content per dm^2 in the F.M., P_3 and P_5 series.

The effect of age in the P_3 series is also observed as

a/

a progressive reduction in the value of water content per unit area, although these values tend to remain definitely lower than those of the F.M. series. The P_5 curve is of uncertain position, being lower than the F.M. curve until the eighth leaf, after which it is relatively higher.

(2) $^{H_2O}/_{D.W.}$ X 100. Water content as percentage dry weight.

(a) K-deficient series

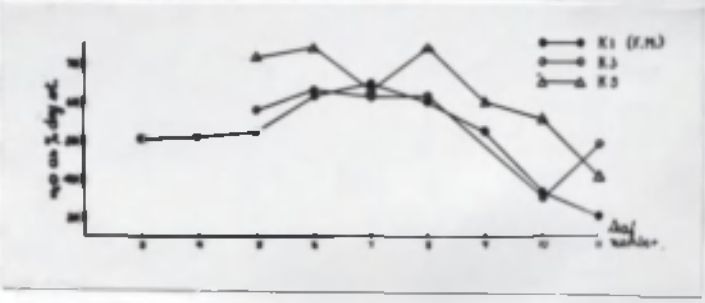

Figure 15 Graphical representation of the water content as percentage dry weight in the F.M., K_3 and K_5 series.

The values for this ratio in the F.M. series rises until the seventh leaf, after which there is a continuous, rapid fall until the eleventh leaf. A similar variation occurs in the K_3 series except for the value at the eleventh leaf. Although the K_5 curve presents the same general characteristics as those of the K_3 and F.M. series (except that the fall with age does not commence until the eighth leaf), it is nevertheless at a higher level than the other two.

(b) P-deficient series

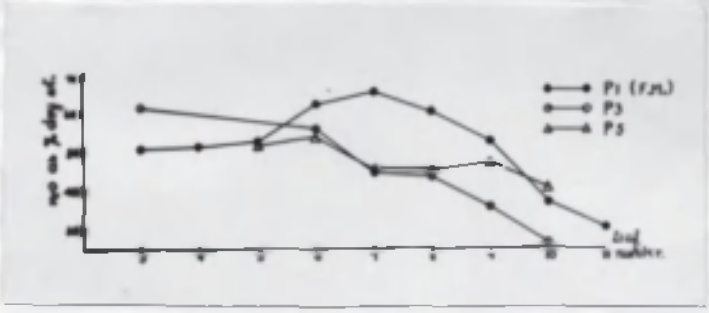

Figure 16 Graphical representation of the water content as %age dry weight of the successive leaves of the F.M., P_3 and P_5 series.

It would appear that, in general, phosphorus starvation causes a decreased succulence as measured on a dry weight basis, and that this further decreases with age in the same manner as the F.M. series. The lowest level of phosphorus manuring (P_5) seems to produce what would appear to be anomolous results in that the values are between those of the F.M. and P_3 series until the ninth leaf, after which the succulence seems to be relatively greater than that in the corresponding leaves of the F.M. series.

(3) $^{D.W.}/_{L.A.}$ Dry weight per unit area of leaf surface.

(a) K-deficient series

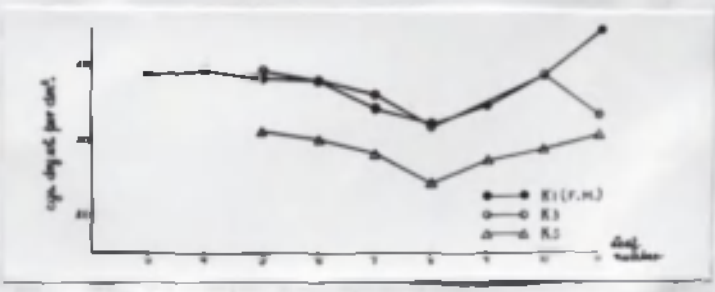

Figure 17 Graph showing the dry weight per unit area of leaf surface in the F.M., K_3 and K_5 series.

The dry weight per unit area of leaf surface in the F.M. series shows a progressive, slight decrease in value until the eighth leaf, after which there is a definite rise to a highest value in the eleventh leaf. The same is also true of the corresponding measurements in the K_3 series. The corresponding values in the F.M. and K_3 series are always approximately equal with the exception of the values at the eleventh leaf. The K_5 curve presents the same general characteristics as the F.M. curve except that the former is at a lower level than that of the F.M.

(b) P-deficient series

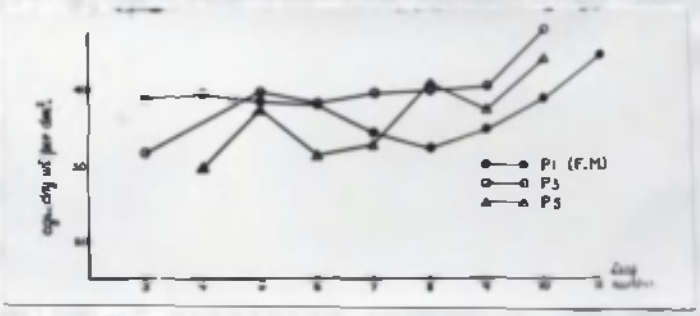

Figure 18 Graph showing the relation of the F.M., P_3 and P_5 series in regard to the dry weight per unit area of leaf surface.

A consideration of Figure 18 indicates that, in general, phosphorus starvation of a P_3 level causes an increase in the D.W./L.A. ratio and that this value seems finally to increase with age. The same is true of the P_5 series although the value for the ratio is lowest in order until the seventh leaf, after which the value remains between that of the P_3 and F.M. series.

General Characteristics of the Water Relations

From the foregoing experimental data it will be seen that the percentage fall in water content due to age is greater on a dry weight basis as compared with water content calculated on a leaf area basis. In all cases, potassium deficiency of a K_3 level seems to have little effect on the water content of the leaf. In the K_5 series, however, there is much less succulence, compared to the F.M. series, on a leaf area basis and more on a dry weight basis. These variations seem to be connected with the variations in the D.W./L.A. ratio. This latter is probably also true for the phosphorus deficient series although certain anomalies occur. It is also generally concluded that phosphorus deficiency causes a decrease in succulence both on the leaf area and dry weight basis. The anomalies in the P_5 series may be associated with the difficulty of obtaining leaves of the same status at approximately the same time as analagous leaves in the other series.

The water relations described above are in close agreement with those previously found by Gregory and Richards (16) for the same plant.

The Rate of Carbon Assimilation

In the following tables are presented the assimilation rates of successive leaves on the main shoot of the F.M. series, the K_3 and K_5 series, and the P_3 and P_5 series. The ratios are given in the first place as

mg.CO_2/

mg.CO_2 assimilated per hour per square decimetre of leaf surface, and in the second place as mg.CO_2 assimilated per gm. dry weight per hour.

Reference has already been made to the difficulty in regard to the phosphorus deficient series and to the compromise necessary under the existing conditions. In the P_3 series, leaves of the usual status of maturity could only be compared with leaves in the F.M. series which were of the same leaf number but only of an approximately equal status. The discrepancy in the P_5 series was more serious, and although the leaves of the P_5 and F.M. series which were compared were at the same stage of emergence (i.e. at complete expansion of the auricles), these were definitely not of the same number on the stem. The leaf numbers of the P_5 series with which successive leaves of the F.M. series were compared are thus specifically indicated in the tables. Since the assimilation rate in the leaves of F.M. plants falls with age, any significant reduction in this rate due to phosphorus manuring, as found in the present experiments, would therefore have been still greater if the P_5 leaves had been compared with F.M. leaves of precisely the same status as regards number on the stem, etc.

(Tables overleaf)

TABLE 3

Assimilation Rates of Barley
Series F.M., K_3 and K_5.

Results in (A) mg. CO_2 / dm^2 / hour
and (B) mg. CO_2 / gm. dry wt. / hour

Plants sown on 3rd. May, 1934

Date	Leaf No.	Av. Temp. Leaf Chamb °C	F.M. A	F.M. B	K_3 A	K_3 B	K_5 A	K_5 B
June 5	2		20.		21.			
6	2	13.			12.4		9.28	
7	3	16.	20.1		22.6			
8	3	19.2			11.9		9.57	
9	3	19.8	13.6		9.6		14.1	
13	4	22.2	32.8		18.5		8.2	
15	5	20.5	23.43		21.8		2.8	
18	6	28.7	30.02	80.4	19.9	59.5	12.7	33.3
19	5	18.4	22.62	66.0	14.7	37.8	2.2	7.1
22	7	21.8	19.95	54.98	12.7	34.9	1.18 (½ LEAF)	4.2
23	6	20.2	20.2	54.3	15.95	39.0		
24	7	18.8	14.02	43.8	5.98	16.1	2.5	9.6
26	7	19.6	16.6	46.3	9.07	25.4	0.4	1.4
28	8	18.1	14.52	48.4	6.7	22.9	1.5	6.4
July 1	8	28.7	18.95	60.2			7.0	24.2
2	8	26.6	16.6	49.4	10.17	31.3	4.41	19.3
6	9	28.5	14.6	45.8	11.07		1.7	5.98
12	10	18.7	11.1	30.4	8.3	22.3		
13	11	20.6	19.6	44.2	12.3	37.1	0.5 (¼ LEAF)	2.2
14	10	18.4	20.2	49.3	12.8	33.6	1.5	4.6
19	11	22.1					-ve result	

TABLE 4

Assimilation Rates of Barley.
Series F.M., P_3 and P_5.

Results in (A) mg. CO_2 / dm^2 / hour
and (B) mg. CO_2 / gm. dry wt. / hour

Plants sown on 3rd. May, 1934.

Date	Leaf No.	Av. Temp. Leaf Chamb. °C	F.M. A	F.M. B	P_3 A	P_3 B	P_5 A	P_5 B
June 11	3	25.95	22.68		18.6		14.81	
12	3	22.1	32.5		17.9		8.05	
14	4	22.9	23.1		(18.86) (18.2)			
17	5	28.2	37.31	83.8	19.3	49.2	4.9 (Fourth Leaf)	23.6
20	6	20.6	(23.2 (23.6	59.5) 59.8)	7.15	17.9		
23	6	20.2	20.2	54.3			9.06 (Fourth Leaf)	24.2
27	7	16.1	17.03	52.4	11.6	29.6	10.61 "	29.0
29	8	20.3	15.71		12.17	29.2	9.8 (Sixth Leaf)	
July 1	8	28.7	18.95	60.2			7.4 "	15.7
3	8	25.5	17.76	52.2	5.36	14.03	2.98 "	9.4
7	9	30.5	23.4	60.9	3.23	8.8	1.06 (Seventh Leaf)	3.9
11	9	29.7	15.67	41.5	4.4	10.6	3.9 "	11.3
12	10	18.7	11.1	30.4	9.4	20.5		
16	10	20.9	14.08	31.4	13.5	26.9	5.2 (8th Leaf)	13.8
19		22.1					(4.68 " (9.00 "	11.5) 20.9)
30	5	25.3	26.3	66.0			5.3 (9th Leaf)	14.1
Aug. 15		23.6					4.5 (10th Leaf)	11.2

In order to complete measurements on the last leaf of the P_5 series, another crop of F.M. plants was sown on June 30th., 1934. The same procedure was followed in this as in the sowing of the previous crop. The seeds had completed germination on July 4th. and the manuring was done in three stages, viz: on July 7th., July 20th. and August 2nd. The leaves of this new F.M. series were used in obtaining comparative measurements of assimilation in leaves of the same status and manuring; they were also used for completing the data for the water relations of the earlier leaves and for comparison with the last of the P_5 leaves.

A consideration of Table 3 indicates that there is a large reduction in the assimilation rate in the K-deficient series. The results are represented graphically below in Figure 19; in this are also shown the average temperatures of the water, as taken on the date of each comparative set of measurements.

(Figure 19 overleaf)

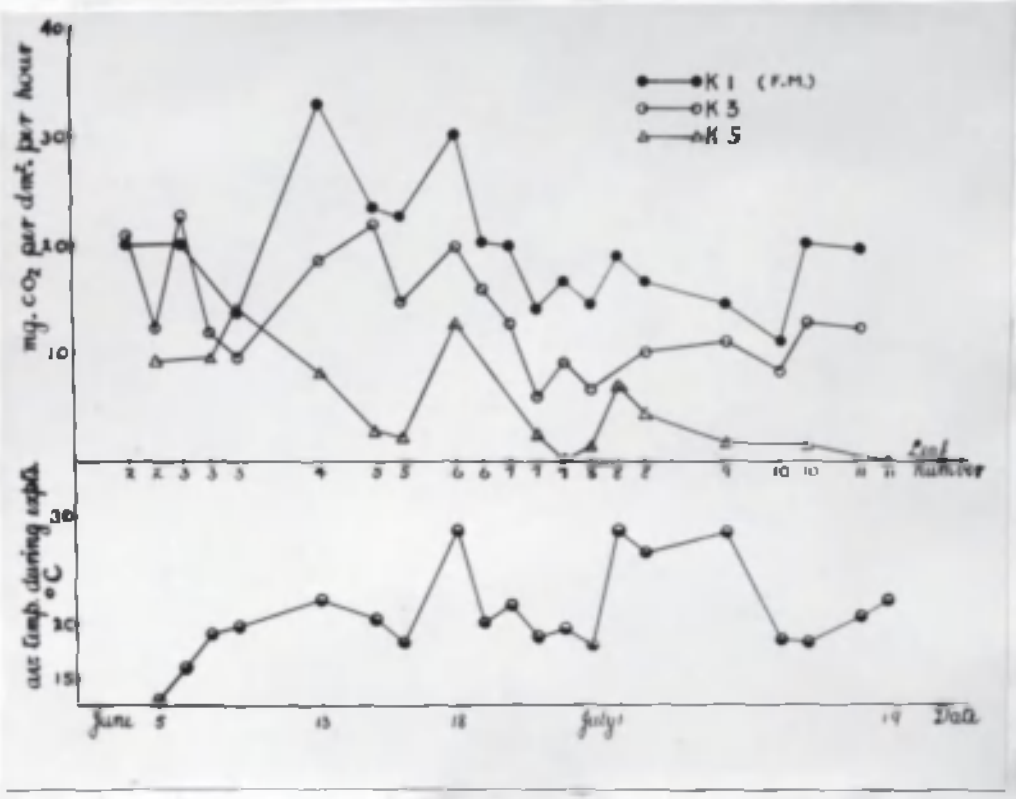

Figure 19 Assimilation rates of successive leaves on the main shoot of F.M., K_3 and K_5 series in mg. CO_2 / dm^2 / hour. The lower graph represents the average temperature during each of the experiments.

In Figure 19 each point on any ordinate represents the figures obtained during the experiment on any particular date indicated. All the measurements on all the leaves are also plotted in relation to the dates of each experiment.

It would appear from Figure 19 that the effect of manuring does not become uniformly evident until after the third/

third leaf stage, after which the K-deficient series have an assimilation rate which is consistently lower than the F.M. series. In addition, the reduction progressively follows the level of potassium manuring; the K_3 curve takes up a position between the F.M. and the K_5, which latter series has a very much reduced assimilation rate.

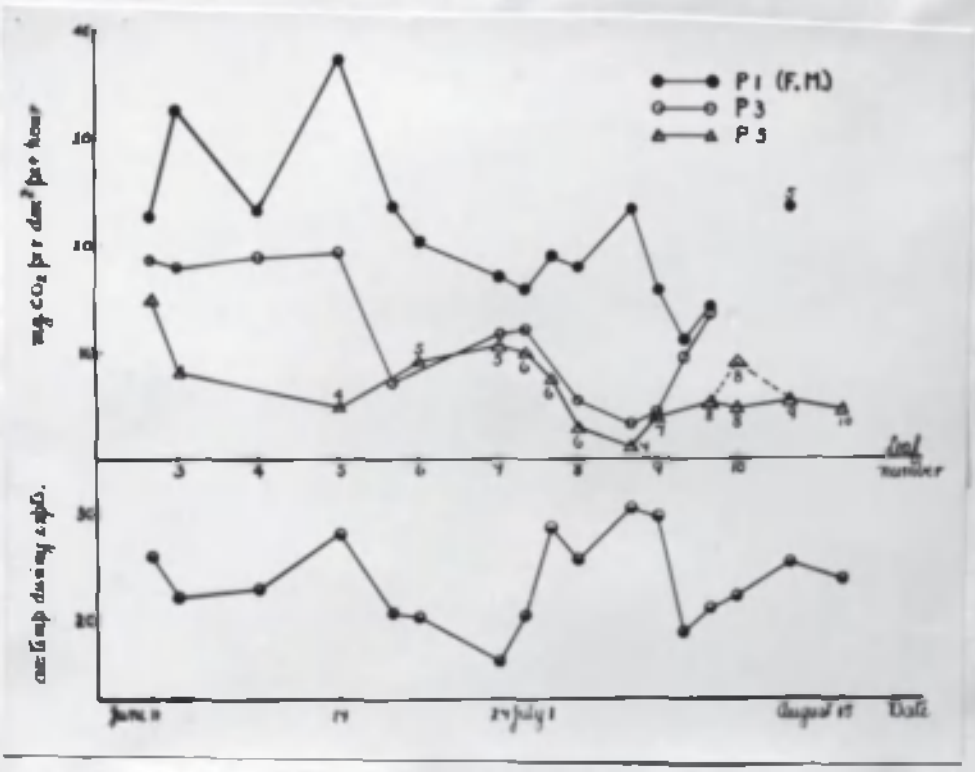

Figure 20 Graph of the assimilation rates of successive leaves on the main shoot of the F.M., P_3 and P_5 series in mg. CO_2 / dm^2 / hour. The average daily temperatures of the experiments are shown in the lower graph.

The assimilation rates of all the leaves used are also shown in Figure 20 for the F.M., P_3 and P_5 series. The values have been plotted according to the dates of the experiments. In order to facilitate interpretation, the leaf numbers of the P_5 series are indicated in the graph and are thus compared with the leaves of the other series which were used in the comparative experiments of any particular date. The fifth leaf of the second crop of the F.M. series is also separately shown in the appropriate position in regard to the date of the experiment and to the P_5 leaf used on that date.

In the phosphorus deficient series, therefore, the effect of manuring is apparent at the third leaf stage. Until the fifth leaf of the F.M. and P_3 series the progressive reduction in the assimilation rate follows the level of phosphorus manuring when these are compared with the values found for P_5 leaves on the same day. The reduced values for the later leaves of the P_3 series are, however, of a similar order to those of the P_5 series found on the same day. There is a remarkable recovery of the assimilation rate of the 10th. P_3 leaf to a value near that of the corresponding F.M. leaf.

The correlation between the temperature and the assimilation rates of the F.M. leaves, as represented in Figures 19 and 20, only appears positive to a significant degree during the first half of the life cycle (until approximately the 7th. leaf stage). Later there are

some/

some disconcerting variations in assimilation rate with simultaneous opposite variations in the temperature, viz: high temperatures in the latter part of the work do not always induce higher assimilation rates. The opposite was the case in the work of Chinoy (8) where high assimilation rates in the latter part of the life cycle were associated with lower temperatures. No explanation is given for this phenomenon.

In order to eliminate the effect of variations in external factors, such as temperature, light etc., the following calculations have been made showing the assimilation rates of the leaves of the various deficiency series as a percentage of the rates of the F.M. series. All the percentage assimilation rates for all the experiments on any particular leaf number of a series have been averaged, and this figure is plotted as an average value for the leaf. Although this is not a strictly legitimate procedure, it serves to crystallise some of the results of the work. Table 5 gives these results as calculated on a leaf area basis, while Table 9 presents the data calculated on a dry weight basis.

(Table 5 overleaf)

TABLE 5

Relative assimilation rates, on a leaf area basis, of the K_3, K_5, P_3 and P_5 series. F.M. = 100

Leaf number	K_3	K_5	P_3	P_5
2	105.0			
3	73.8	67.3	68.7	45.2
4	56.4	25.0	80.3	13.1
5	79.1	10.8	51.7	52.1
6	72.8	25.9	31.0	39.4
7	55.0	10.1	68.2	14.5
8	53.8	24.5	54.0	36.8
9	76.0	5.8	18.7	20.1
10	69.2	5.2	90.2	
11	62.7	-ve result		

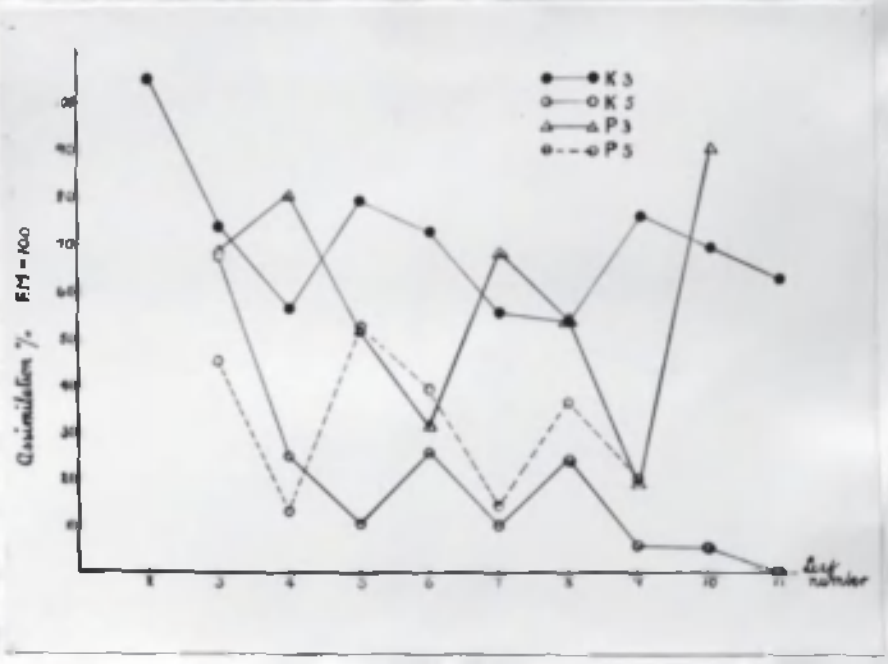

Figure 21 Graph showing the assimilation rates, on a leaf area basis, of the K_3, K_5, P_3 and P_5 series. Assimilation rate of the F.M. series = 100.

On a leaf area basis, the K_3 series shows an average assimilation which varies between approximately 50 - 80% that of the F.M. series, while the K_5 series, after a higher rate at the second leaf, maintains an assimilation relative to the F.M. of approximately 10 - 20%. At the eleventh leaf stage, however, the rate in the K_5 series becomes zero or less/

less (in these graphs "net" assimilation rates are being considered).

Compared with the last, the oscillations in the phosphorus deficient series are of much greater magnitude and these are probably due to the difficulty described in obtaining leaves of equal status in regard to age, etc. The variations in the P_3 series are between 20 - 80% of the corresponding assimilation rates in the F.M. series, the mean value being circa 50%. The highest value is found in the 10th. P_3 leaf. On the other hand, the P_5 series has a mean assimilation rate of approximately 30%, the variations throughout the life cycle being 10 - 50% of the rates in the F.M. series.

There is, therefore, an obvious reduction in the assimilation rate due to manuring, and this reduction is intensified with increase in deficiency. On the basis of the calculations presented in Table 5, and on a consideration of the graphical representation of these in Figure 21, the progressive reduction in the assimilation rate due to manuring is given as follows: K_3, P_3, P_5 and K_5, the last-named being the lowest in order.

Unfortunately, dry weights were not taken until the fourth or fifth leaf stage so that the data presented in Table 6, below, represent the assimilation rates for the latter part of the life cycle only.

(Table 6 overleaf)

TABLE 6

Relative assimilation rates on a dry weight basis of K_3, K_5, P_3 and P_5 series. F.M. = 100

Leaf number	K_3	K_5	P_3	P_5
4				28.2
5	57.3	10.8	58.7	49.0
6	72.9	24.5	30.0	22.1
7	51.3	12.5	56.5	16.6
8	53.4	30.8	28.1	44.0
9		6.6	20.1	21.4
10	70.8	7.2	76.6	
11	84.0			

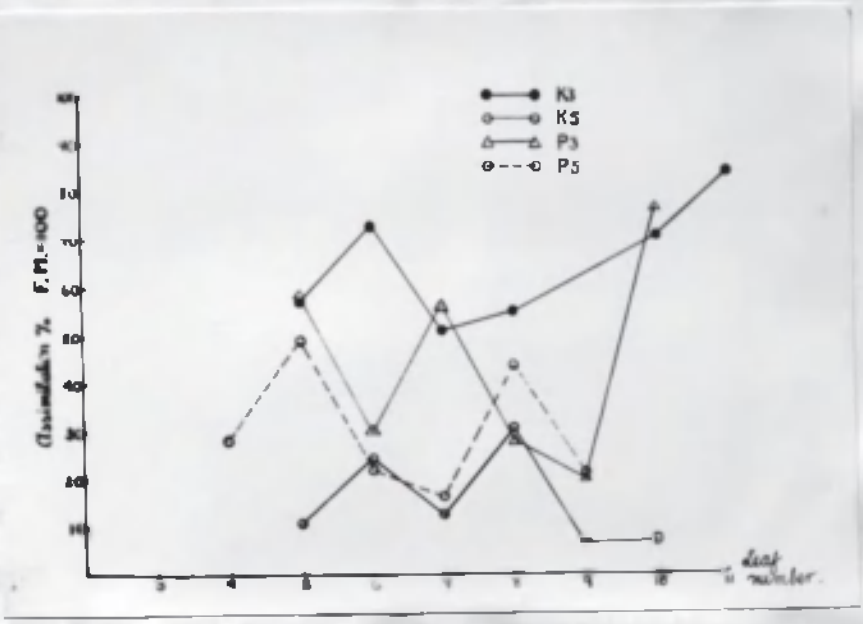

Figure 22 Graphical representation of the data of Table 6, showing the assimilation rates, on a dry weight basis, of the K_3, K_5, P_3 and P_5 series. Assimilation rate of the F.M. series = 100.

It is apparent, therefore, that the percentage assimilation rates on a dry weight basis are not fundamentally unlike those on a leaf area basis although certain minor differencies occur. The confusing rates of the P_5 series relative to the P_3 series may be observed at the eighth and ninth leaves.

On/

On a basis of leaf areas or of dry weights, the progressive reduction of the assimilation rates in the deficiency series are the same, relative to one another, in both cases, viz: K_3, P_3, P_5 and K_5, the last-named having the lowest values.

Discussion

In order to facilitate the discussion, the features of interest in the present work and other relevant data are reviewed under separate headings, viz:

(a) Increase in the dry weight of the plant.

(b) Comparative study of the rates of assimilation obtained by other workers.

(c) Translocation in attached leaves.

(d) Potassium and Phosphorus starvation and their effects on the assimilation process.

(e) The Maximum value for the assimilation rate under natural conditions of CO_2 supply.

(a) Increase in the Dry Weight of the Plant.

It is of interest to compare the figures for the production of organic matter as calculated from the present data (obtained by the gasometric method described above) with those obtained by growth analysis data from experimental work by other workers.

If the assimilation rates of the F.M. series (including eight determinations not given in the preceeding tables) be averaged over the whole season, the figure is found to be 21.2 mg. CO_2 / dm^2 / hour. With this assimilation rate, the calculated/

calculated average increase in carbohydrate (as $C_6H_{12}O_6$) would be at the rate of 14.45 mg. / dm^2 / hour. From Table 2 the average for all the data available for the ratio $\frac{D.W.(gms.)}{dm^2}$ = 0.3755 gms. On this calculation the increase in carbohydrate would therefore be 37.9 mg. / gm. dry weight of leaf / hour. For the purposes of this discussion the average "assimilating day" may be taken as being of 16 hours duration, so that, from the previous figure, there would be a daily production of carbohydrate of 0.606 gm. / gm. dry weight of leaf. This value is calculated on the data of "apparent" assimilation rates and consequently takes into account the loss of weight due to respiration during the period of the experiment. There is, however, a loss during the eight hours night respiration and a value for this must be subtracted from the above figure for daily carbohydrate production.

The average night temperature can be taken as 15 - 17° C. and from data by Gregory and Sen (17), respiration at that value, for the barley plant, is about 5 mg. carbohydrate / gm. dry weight / hour, or 40 mg. / gm. dry weight for an eight-hour period. Thus the "net" gain per day would be 0.566 gm. carbohydrate / gm. dry weight of leaf. There would thus be an increase of 56.6% of the dry weight of the leaf per day or about four times the original dry weight of the leaf per week.

On a basis of Gregory's experiments of 1922 (12) by a method of growth analysis, the "net" assimilation rate has/

has been found to be 1 gm. dry matter / dm^2 / week, and this, by Gregory's method of calculating percentage increment of organic material, is 39.7% per day. In more recent growth analyses in the same Institute, Mathur (23) for 1932 finds a percentage production of dry matter per day, during the first two weeks of growth, to be 29.5%, while Verma (34) for 1933 and 1934, finds a value of 39.1%, this latter being strikingly near the figure obtained by Gregory eleven years previously (1922). In both these years the weather was exceptionally warm and no doubt contributed to the higher rates obtained in both cases.

Nevertheless, the figures obtained by growth analysis are consistently lower than the figure obtained by calculation from the data of the present investigation. There are several factors which would tend to accentuate the discrepancy between the results by the two methods, e.g. the present experiments took place between 10 or 11 a.m. and 5 or 6 p.m., during which the assimilation would be at a maximum with the result that a calculated assimilation for a 16-hour day would be considerably exaggerated. Further, the leaves were in a strong current of air, whereas the results from growth data were from plants normally in a relatively still atmosphere where local CO_2 diffusion gradients etc. would tend to limit carbon dioxide assimilation. In addition, the area of leaf employed was very probably one which would give maximum assimilation per unit area.

(b) Comparative Study of the Results obtained by Other Workers

The present discussion will be confined to results which, in general, have been obtained under more or less natural conditions. The assimilation rates have been obtained by very different methods and the plants employed varied widely in their structural organisation.

Mention must first be made of the work of Sachs (28), who made the first real attempt to measure rates of assimilation. Sach's "Half-leaf method" was based on the increase in dry weight of selected parts of the leaves after definite periods of insolation. He found an assimilation rate for Helianthus annuus of approximately 26 mg. CO_2 / dm^2 / hour, using natural sunshine and air. In contrast to this, Brown and Escombe (7) found the assimilation rate of Helianthus to be 6.8 mg. CO_2 / dm^2 / hour, using diffuse natural light and air. This value was obtained by a gas-current method. These authors also determined the assimilation rates of Catalpa bignonioides, using concurrently dry weight increase and gasometric methods. Calculations on the increase in dry matter showed considerable discrepancies according to the method used, viz: an increase in dry weight of 6.69 mg. / dm^2 / hour by the dry weight method, and 2.35 mg. / dm^2 / hour as calculated from the CO_2 assimilation by the gasometric method. On the basis of these experiments they criticised the Half-leaf method of Sachs as giving an inordinately/

inordinately high value for assimilation. Thoday (32), however, in a critical survey of Sach's method, has improved and refined this. He shows that the improved method continues to give an assimilation rate for Helianthus annuus which agrees closely with that of Sachs, namely 26 mg. CO_2 / dm^2 / hour. Using Catalpa bignonioides Thoday (33) also obtained slightly higher results though these were concordant with those obtained by Brown and Escombe (7) using the dry weight method, namely 8 mg. / dm^2 / hour. Thoday preferred to criticise the results obtained by Brown and Escombe by the gas current method, pointing out that the leaves in their plant chamber were abnormally situated and were wilted by the influence of strong sunlight.

Other workers, using gasometric methods with air as the source of CO_2, have also obtained low values for assimilation rates which do not compare favourably with results obtained by dry weight methods. For instance, Giltay (11), using a gas-current method, found the assimilation rate of Helianthus to be 5.8 mg. CO_2 / dm^2 / hour, a result not dissimilar to that obtained by Brown and Escombe. Giltay's estimation for Nicotiana was 4.4 mg. CO_2 / dm^2 / hour by the gasometric method, while Müller's was 20 mg. CO_2 / dm^2 / hour by the Half-leaf method. Blackman and Matthaei (3) employed a gas-current method and obtained results for Helianthus which were comparable with those of Sachs, but the former authors employed CO_2 in a concentration of 6.37%; also, the relatively high temperature (30° C) and strong sunlight together assisted/

assisted in the production of a high rate. Low values for assimilation rate in the sun leaves of Sambucus nigra were found by Boysen Jensen, viz: 4.6 mg. CO_2 / dm^2 / hour using CO_2 in a concentration of 0.55 mg. CO_2 / litre. A very low rate of assimilation for the leaf of sugar cane was found by McLean (24) under field conditions, viz: 5.14 mg. CO_2 / dm^2 / hour.

An obvious fault in many of these gasometric experiments is that the gas has been passed over the plant at a low rate, but where this has been increased higher assimilation results have been obtained, e.g. Dastur and Chinoy (9), employing a gasometric method, estimated that the maximum apparent assimilation under the experimental conditions was 17.58 mg. CO_2 / dm^2 / hour, whereas on a dry weight basis a maximum of 8.92 mg. CO_2 / dm^2 / hour was found. This latter result was not corrected for loss of weight by translocation and must necessarily be somewhat lower than it should.

A number of the results of other workers have been collected into the following table, and these have all been given in terms of mg. CO_2 / dm^2 / hour for ease of comparison. The workers obtaining the results, the methods employed (gasometric or otherwise), and the conditions of illumination and CO_2 concentration have been indicated.

(Table 7 overleaf)

TABLE 7

Assimilation rates obtained by various workers by the dry weight method and the gas-current method. All readings in mg.CO_2/dm^2/hour.

Investigator	Method	Plant	CO_2 concn.	Temp °C	Source of Light	CO_2/dm^2/hour
Sachs	Half Leaf Method	Helianthus	Air	25	Sun	26.4
"		Cucurbita	"	24	"	22.0
Muller	"	Nymphaea	"	-	Illumination	36.0
"	"	Rumex	"	-	Changing (natural)	34.0
"	"	Petasites	"	-	"	30.0
"	"	Helianthus	"	-	"	28.0
"	"	Nicotiana	"	-	"	20.0
"	"	Tulipa	"	-	"	20.0
"	"	Arum	"	-	"	16.0
"	"	Colchicum	"	-	"	18.0
"	"	Allium	"	-	"	18.0
Thoday	"	Helianthus	"	27-29	Sun	26.0
"	"	Catalpa	"	-	"	8.0
Weber	Estimation from production of dry matter	Tropaeolium majus	"	-	Illumination Changing	6.6
"		Phaseol. multipl.	"	-		4.8
"		Ricinus communis	"	-	"	7.8
"		H. annuus	"	-	"	8.2
Giltay	Gas-current method	Helianthus	"	13-27	"	5.8
"		Nicotiana	"	12-29	"	4.4
"	"	Helianthus	"	28-36	"	7.6 to 8.0
"	"	Cassia	"	28-35	"	12.2
"	"	Cadrela	"	28-34	"	9.0
"	"	Nicotiana	"	31-35	"	9.0
"	"	Acalyphon	"	33-35	"	7.0

Table 7 continued.

Investigator	Method	Plant	CO_2 concn	Temp. °C.	Source of Light	CO_2/dm²/hour
Brown and Escombe	Gas-current method	Helianthus	air	20	Diffuse light	6.8
"	"	Tropaeolium	"	20-25	Sunlight with a canvas screen	3.4
"	"	Catalpa	"	20	Intermittent sunshine	6.0
"	"	Polygonum weyrichii	"	20	Sunlight with screen	7.6
Boysen Jensen	"	Sinapis alba	0.55 mg. CO_2 per litre	20	Excess light (natural)	12.0
"	"	Senecio sylv.		20		9.0
"	"	Rumex acitos.		20	"	4.6
"	"	Sambucus nigra (sun leaves)		20	"	4.6
Dastur and Chinoy	"	Rice	air	28-30	Artif. illum.	17.58
Blackman and Matthaei	"	H. annuus	6.37%	30	Strong sunlight	39.0
Willstatter and Stoll	"	"	5%	25	48000 lux	55.
Miller	Estimation from production of dry matter	Dwarf yellow Milo.	air	-	Sunshine	26.7
"		Pumpkin	"	-	Under field conditions & during growing season	26.4
"		Cowpeas	"	-		12.5
"	"	Soybeans	"	-		11.75
"	"	Corn	"	-		28.0
"	"	Kafir	"	-		22.0
"	"	Milo	"	-	"	28.0
Dastur and Chinoy	Diff. in total carbohydrate	Rice	"	28-30	Under field conditions	15.6

Table 7 continued.

Investigator	Method	Plant	CO_2 concn.	Temp. °C.	Source of Light	$CO_2/dm^2/hour$
Gregory	'Net' Assim^n rate from growth data	Barley	air	Sand culture		7.1
Gregory and Mathur	"	Barley	"	Sand culture		6.77
Dastur and Desai	Gas-current method	Ricinus communis	5%	25	Artif. illum.	19.5
"	"	Abutilon asiaticum	5%	25	"	11.6
"	"	H. annuus	5%	31	"	59.7
"	"	Phaseolus vulgaris	5%	28	"	95.0
Present Investigation	"	Barley	air	28	Sunshine	37.3

The evidence shows, therefore, that in most instances there is a divergence between the results obtained by (1) gas-current analyses and (2) dry weight methods, the values found by the latter methods being generally higher than those found by the gasometric methods. The reasons for this discrepancy will be fully dealt with elsewhere but it may be indicated here that the low readings obtained by gas-current methods are mainly explicable on the grounds that (1) the rate of the current of air has not been great enough and (2) the plant chambers have been too large. The maximum value obtained by the present investigation (37.3 mg. CO_2 / dm^2 / hour) under natural conditions of light, temperature, air etc., is certainly comparable with the maxima obtained by Sachs. It is thus demonstrated that the gas-current method can be profitably used in the estimation of maximal assimilation rates and can now be favourably compared with methods of dry weight increase. In view of the special considerations given to the flow of air over the assimilating leaf it is not surprising that the maximum rate found in the present experiments is probably of the highest order yet obtained for assimilation rate of plants in air and natural light, etc.

(c) Translocation in attached leaves

In the calculation of some of the results obtained by the dry weight method, it is obvious that allowances must be made for translocation in order to obtain a more accurate value for the total increase in weight due to assimilation.

Thus/

Thus, Miller (25), in his work on the leaves of Sorghum found that during the day from 5 a.m. - 5 p.m. the leaf showed an increase of 11.2 gms. of dry matter / square metre of leaf surface. This represents the "net" gain and in no way indicates the loss by translocation and respiration. Miller also determined that 9.4. gms. dry matter / square metre of leaf surface disappeared from the leaf from 5 p.m. - 5 a.m. For the purposes of this discussion, if we assume that the loss is the same by day as by night, then the gross assimilation from 5 a.m. - 5 p.m. should be represented as 20.6 gms. dry matter / sq. metre. This value is therefore 17.1 mg. dry matter / dm^2 / hour, or an assimilation rate of 26.7 mg. CO_2 / dm^2 / hour, a figure which compares favourably with the findings of the present work conducted under still more favourable conditions.

However, such calculations for translocation are only necessary in attached leaves, as obviously there would be no translocation in detached leaves. Sachs (28) did in fact find a greater increase in dry weight of leaves of Helianthus annuus detached from the plant as compared with attached leaves, a difference which he attributes to translocation. Sachs also determined that Helianthus leaves lost 0.1 gm. dry weight / dm^2 throughout the night. Other authors have confirmed the actuality of translocation proceeding by day as well as by night.

Translocation can therefore account for much of the discrepancies of rates of assimilation by dry weight methods between/

between attached and detached leaves. Subsidiary effects due to detaching the leaves may further tend to increase the assimilation rate in these leaves. Detachment may to some extent increase the supply of water to the leaf, the increased water content permitting a wider opening of the stomata and thus a greater possibility for CO_2 diffusion. There would thus be a greater potential assimilation capacity. Brown and Escombe (7) have, in fact, shown an increased diffusion of CO_2 into detached leaves of Catalpa as compared with attached leaves. This result finds support in Thoday's work (33) on the stomata of Catalpa bignonioides although he points out that there was not any evident change in the stomata on detaching leaves of Helianthus.

It is apparent that a method such as that employed in the present work is highly desirable, as, by the method, "net" assimilation rate is not affected by translocation to any extent, and disturbances due to detaching leaves are avoided by using attached leaves.

(d) Potassium and Phosphorus Starvation and the Effect on the Assimilation Process.

K-Starvation : The reduction in the assimilation rates of barley under conditions of potassium deficiency in the present investigation is quite in accordance with the findings from previous work in the same Institute. Gregory and Richards (16) found that (a) the respiration is supernormal and (b) the "real" assimilation rate subnormal in/

in K-deficient leaves. The accumulative effect of increased respiration and of decreased assimilation would result in a still more subnormal "net" assimilation. This reduced "net" assimilation would be expected to cause a reduction in the carbohydrate of the leaf, a fact substantiated by Gregory and Baptiste (14) for the same variety of barley as used in the present experiments.

While Richards (26) finds no direct correlation between the level of potassium and respiration, Gregory and Richards (16) suggest a positive correlation between the level of this ion and the assimilation rate. The hypothesis of these authors is that the potassium ion is in some way connected with an early stage in the assimilation process, viz: the hydro-diffusion stage. By means of a K-bicarbonate $\rightleftharpoons$ K-carbonate dynamic equilibrium there would be a rapid mode of transference of CO_2 to the chloroplast surface. Potassium would be more valuable in such a reaction than, for instance, sodium or calcium, owing to the extreme motility of the potassium ion and the ready permeability of the cytoplasm to it. This latter feature might be concluded from the work of Mann and Wallace (20) who have shown that rain can leach this element from leaves. It is probable that this would happen only when the potassium is present in greater concentration than is essential for the assimilation process.

P-Starvation : The ultimate effect of phosphorus starvation/

starvation also results in a reduction of the assimilation rate of barley. While it is intelligible that phosphorus may play a direct part in respiration, the exact effect of this element on the assimilation process is not quite clear. Certainly, Gregory and Richards (16) found that there was a highly significant correlation between phosphorus content and assimilation. There may be some necessity for phosphorus, in some manner not yet understood, to maintain the efficiency and activity of the seat of the assimilation process, viz: the chloroplast surface.

The reduced rate of tillering and of leaf production, together with the general tendency for reduction in leaf area, is presumably a result of the reduced rate of protein synthesis caused by phosphorus deficiency (Richards and Templeman - 27).

(e) The Maximum Value for the Assimilation Rate under Natural Conditions of CO_2-supply.

It has been shown that rates of assimilation, obtained by dry weight methods, are generally higher than those which investigators have found when using gas-current methods in which air and natural light are utilised. This discrepancy may be attributed to three causes:

(1) In many cases, the leaf area used has been too large so that the air has undergone too great a depletion of CO_2. Kostychev (18) has criticised the work of Boysen Jensen and points out that the air leaving the leaf chamber was/

was almost devoid of CO_2. This certainly is not the case in the work described in this thesis as the titrations show that only one third of the CO_2 has been removed by the assimilating leaf. The actual rate of flow of air was about 30 litres / hour, a rate in excess of that suggested by Kostychev et al (19) as being the minimum necessary for maximum assimilation, viz: 1 litre of air / sq. cm. of leaf surface / hour. Previous work by Chincy (8), using the apparatus described elsewhere, had slightly less than this minimum flow recommended by Kostychev. Consequently, though Chincy's maximum assimilation rate was very high, by virtue of the special plant chamber, it was definitely lower than the maximum rate found in the present work, viz: 37.3 mg. CO_2 / dm^2 / hour.

(2) Apparently, much of the work done by other investigators has been done with plant chambers which were so large and deep that much of the air passing through the chamber never came into contact with the assimilating leaf. The special leaf chamber designed by Professor F. G. Gregory, which was used in this work, has overcome this fault.

(3) Previous work by Gregory and his collaborators have stressed the importance of using leaves of identical morphological status, and also of growing the plants under standard conditions. These precautions have been carefully observed in the present investigation, (except where the compromise was necessary in the case of the slow-growing P_5 series). As many investigators in this field have failed to/

to realise these essentials, their findings are somewhat invalidated.

This investigation was carried out under the direction of Dr. F. G. Gregory, now Professor, of the Royal College of Science, London, and my thanks are due to him for suggesting the problem and permitting me to use the apparatus which he designed for the purpose. I was further given free access to the previous data available at the Imperial College of Science and wish to record my thanks for this privilege, as well as for the ready help and advice I received throughout My thanks are also due to Mr. Tooley, of the same Institute, for the photographs used in Figures 1 - 12.

SUMMARY

(1) An apparatus, employing the principles of gasometric analyses, and used in the determination of the assimilation rates of leaves under field conditions, is described. A special feature of the apparatus is the leaf chamber, in which the dead space is reduced to a minimum so that all the air passing through the chamber comes in contact with the leaf surface.

(2) The effect of potassium and of phosphorus starvation on the visible characteristics of the plant body have been/

been described. Further, the water relationships of the successive leaves of barley under two deficiencies of potassium and two of phosphorus have been studied and compared with those of the F.M. plants.

(3) The percentage increase in dry weight per week has been calculated from the data of the present investigation, and the results compared with those found by the method of dry weight production from growth data collected in the same Institute by Gregory and his collaborators.

(4) These calculations, utilising the dry weights of the leaves employed, show that the plant would increase its dry weight by four times during a week's growth. This result compares favourably with those obtained by actual growth analysis.

(5) The assimilation rates for the successive leaves on the main shoot of barley grown under conditions of potassium and phosphorus starvation at (a) K_3 and K_5 levels, and (b) P_3 and P_5 levels, are given. These are compared with the corresponding rates found for the leaves of the F.M. series.

(6) It is found that there is a reduced assimilation rate under potassium deficiency, and that the rate varies with the level of potassium. Phosphorus deficiency also reduces the rate of assimilation. The results show that the reduction in assimilation due to manuring/

manuring are in the following order: K_3, P_3, P_5 and K_5, the last-named being the lowest in order.

(7) Assimilation rates obtained by other investigators using dry weight and gas-current methods have been discussed.

(8) The mechanisms of the effects of potassium and phosphorus on the assimilation process are tentatively indicated.

(9) It is found that the maximum assimilation rate obtained by the present method, viz: 37.3 mg. CO_2 / dm^2 / hour is the highest yet obtained by a gas-current method using atmospheric air as a source of CO_2.

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The Percy Sladen Expedition to Lake Huleh, Palestine.

1935

Table of Contents

INTRODUCTION.

It is only within comparatively recent years that there has been an intensive study of the biology of Palestine. A comprehensive survey of the flora and fauna was made by Tristram (9) but more recent reviews are given in Bodenheimer's "Animal Life in Palestine" (3) and in Post's "Flora of Syria, Palestine and Sinai, (7). There are also publications in Hebrew from the Hebrew University at Jerusalem, although these are not easily available at the present time. Reference may also be made to the work of Dr. Eig of that University whose studies in the botanical field will ultimately serve to elucidate the various floristic problems of Palestine.

In regard to the Lake Hulah district however, investigation has been curtailed and fragmentary. Previously this was due to the inaccessibility of the district and also to interest being diverted to more apparently interesting regions such as the Dead Sea valley etc. In more recent years also, when scientific work in Palestine has been greatly stimulated, the tension which has for a decade existed between the Arab and Jewish peoples, has rendered it inadvisable to visit/

visit exposed regions such as the Huleh district unless in the short times of civil peace.

Thus, while the hydrophytic flora of other parts of Palestine have been more carefully studied, that of Lake Huleh has received rather less attention. Associated as this area is with the almost unique valley of the Jordan, it was felt that a floristic and ecological investigation might prove to be of great interest. Again, in view of the fact that a Jewish Land Development organisation had acquired the rights of the lake and swamp and were, in 1935, preparing plans for the drainage and canalisation of the whole area, the need was more urgent for a record of the plants of this interesting district before complete loss of the aquatic vegetation took place. Consequently, in July 21st. 1935, an expedition with a personnel of two (Mr. R. Washbourn, B.A., as Zoologist, and the present writer as Botanist) was sent out by the Trustees of the Percy Sladen Memorial Fund. The Expedition stayed throughout the hot summer months and until January 3rd. 1936, when the heavy winter rains made further work impossible.

Among the earlier references to Lake Huleh, those/

those to the Waters of Merom in the Old Testament are of historical interest only. Josephus, the ancient historian, lived for a time at Tiberias on the Sea of Galilee and he also refers occasionally to that part of the Jordan Valley which is the Huleh district. References of biological interest are, however, made by early travellers such as Cotivus (4), Adrichomus (1) and de la Roque (8). All three travellers maintained that there were considerable fluctuations in the level of the lake and that it dried up in summer. Adrichomus describes how, instead of crossing the lake, he had to cross a plain of blackish earth "dry and without a drop of water". A more popular account which also contains many interesting observations on the reed swamp adjacent to the lake is that of MacGregor (6) in "The Rob Roy on the Jordan".

More recently some general accounts of the flora and fauna of Lake Huleh have been given by Washbourn and Jones (10 and 11).

AIMS AND METHODS OF THE EXPEDITION.

The Expedition was stationed at the Malaria Research Laboratory, of the Hebrew University of Jerusalem, in Rosh Pinna, a village situated on the hills/

hills bounding the west side of the Huleh valley. Journeys to the lake and swamp were made every day except when the necessary sorting and preserving of collected specimens was to be done. Work on the lake was operated from Yesud ham Ma'ala, since at this village boats were available from those employed in the local fishing industry.

The vegetation of the lake and swamp was studied in detail where this was possible (q.v.). The whole area of the lake and swamp was about 18 square miles and this was somewhat too extensive to be completely studied during the time of stay of the Expedition, with such a limited personnel. Especially was this so in the large swamp where the means of travel was chiefly by reed rafts, an extremely slow and tedious procedure (Figures 6 and 7). Some navigable channels had been cut through the Papyrus by the local fellahin at Mallaha, a village on the malaria-infested edge of the swamp. This village was a convenient base from which to make excursions into the swamp. While species were collected from as many different parts of the swamp as possible, some areas, notably the N.E. area of the swamp have been left unexplored. This area/

area was difficult to reach, owing to its distance from the base, during one day's journey; furthermore, the District Commissioner warned the Expedition against visiting the Eastern side of the District owing to some local trouble with the Bedouin (Figures 8 and 9). Ecological notes of the swamp were therefore taken from its more accessible parts viz: the S.E. area, the W. and N.W. areas and in the vicinity of the River Jordan and the lake.

The plants collected were identified provisionally, where this was possible. Algae were preserved in formalin as also were the various plankton hauls. It was impossible to make quantitative plankton hauls as the density of the aquatic vegetation and the shallow depth of the lake made it necessary to draw the net at irregular levels and directions. The plankton nets were of fine-mesh bolting silk suitable for phytoplankton. Some hand-net plankton hauls were made in the open water of the swamp and in some of the springs.

In addition, temperatures of the lake were taken by the Expedition at various temperature stations on the lake and swamp. A complete record of the air and/

and water temperatures at a small meteorological station at Yesud ham Ma'ala has also been kindly given to us by the recorder of the station. Oxygen estimations were made of the water of the swamp and lake with a view to completing a chemical study of these two habitats. The estimations were done by Alsterberg's (2) modification of the Winkler method. A B.D.H. Comparator was employed for measuring the PH. values of the water and some winchester water samples were brought back for chemical analysis.

THE HULEH DISTRICT.

Lake Huleh (Figure 1) is situated on the River Jordan in the region of Upper Galilee, and is the most northern of the series of three lakes* in the Jordan Valley system. The lake and its adjacent swamp (Figures 2 and 3) together form a lateral extension of the River Jordan and lie in the broad Huleh plain. This plain is bounded along the East side by a range of Syrian hills; on the North by Mount Hermon and its foothills; and on the West side by the mountains of Upper Galilee, which separate the valley from the Mediterranean Sea. The geological formation of these latter is like most of the mountains of Palestine in that/

* Lake Huleh, Lake Tiberias (Sea of Galilee), and the Dead Sea, the latter being the most southern of the series.

that there is an overlayering of limestone; the hills bounding the East of the plain, however, have an additional, superficial stratum of basalt.

The three main sources of the River Jordan are in Mount Hermon and the other Syrian mountains to the North and the resulting wadis* and streams from these flow down into the Huleh plain where they unite to form the River Jordan proper. The river then continues to flow southwards and soon enters the Huleh swamp, where its identity becomes somewhat obscured owing to the main stream splitting into various channels and spreading through the swamp vegetation. There is, however, a well-defined river through the swamp at present (Figure 4) but local tradition has it that the shallow banks sometimes give way so that a new route is taken through the dense vegetation.

After passing through the swamp, the river and its derivatives flow directly into Lake Huleh, which, in fact, is contiguous with the swamp. This northern extremity of the lake is the broad end of a pear-shaped stretch of water, the narrow end being in the extreme/

* A Wadi is generally the designation of the channel of a watercourse which is dry except in the rainy season. This definition is not always adhered to in Palestine, e.g. Wadi el Barid, which carries a large volume of water all the year round.

extreme south. To the south of the lake the Huleh valley ends in a low range of hills lying East-West and it is through a narrow gorge in this range that the Jordan, after leaving the lake from its southern extremity, flows southwards on its swift, steep way to Lake Tiberias (Figure 5). Lake Huleh is approximately at the level of the Mediterranean Sea while Lake Tiberias, some twelve miles to the South, is 670 feet below the Mediterranean Sea Level.

The level of Lake Huleh is maintained by a large deposition of boulders and gravel (brought down from the surrounding hills) at this southern exit of the Jordan so that a "hump" has developed in the river bed, forming thus a lip over which the excess water of the lake constantly escapes.

PHYSIOGRAPHICAL FEATURES OF THE DISTRICT.

There are, broadly speaking, only two seasons in the Huleh plain, - the dry season which is also the hot season, and the rainy season, which is also the cold season. The dry season extends from June - October during which period there is generally no rainfall whatsoever. The temperature during this period often reaches a maximum in August when temperatures of 90°F to/

to 100°F in the shade may be maintained and sometimes exceeded. After September or October, periodic showers of rain come with more and more frequency and intensity until about January or February, after which there is a great rainfall until March or April. January is the coldest month but temperatures below freezing are not common. There is very little precipitation in May and with the advent of high temperatures the hot dry summer begins.

Heavy rain is not continuous during the winter, when, in fact, long dry spells may intervene. Thus, although the average annual precipitation on the western hills of the valley may be low, the amount of rain which may fall, in a storm of only a few hours duration, is tremendous. Wadis which have been dry all summer, become rushing torrents, and from the whole hillside boulders and loose earth are poured into the valley beneath. The Jordan becomes swollen and overflows the shallow banks (Figure 10), and the springs, with an augmented supply of water, well up with greater force.

During the winter, therefore, the lake and swamp, which occupy the shallow basin of the valley, receive/

receive an immense volume of water. The Jordan river remains in flood practically throughout the winter, due no doubt, to the snow and rain storms on Mount Hermon etc.; the rainwater from the hills surrounding the valley does not have time to permeate the soil to any comparatively large degree and runs straight into the lake and swamp; and since only a restricted volume of water can find exit through the narrow gorge in the south, the level of the water of the lake and swamp rises. During the summer, when only the Jordan (which even then continues to bring down a very large volume of water), a few wadis (which run all the year round), and the springs, are the only means of maintaining a water supply to the lake and swamp, the water level falls. This fall is accentuated by the great loss in evaporation from the lake and in transpiration by the swamp plants. It was learned from quite an authoritative source that the difference between this summer level of the lake and the maximum winter level, is about 3 feet. In the region of the lake and part of the swamp, the shore line may recede 10-20 yards, but the present writer has observed recessions of 50-500 yards of the water's edge in the region of the swamp.

Thus/

Thus the area of the lake and swamp is subject to an annual "tidal" effect and this naturally has a very definite effect on the shore limits of the aquatic and swamp flora. The majority of subaerial plants in Palestine develop during the rainy season when water is plentiful and when periods of quite high temperatures occur (Figure 11). Consequently, only those perennials which can withstand a period of immersion and can flower when the water level falls, and those annuals whose seeds can germinate in these lower damp areas and are able to survive the summer heat, are to be found in the lower littoral zone in the summer season.

With the aquatic and swamp flora on the other hand, growth takes place when the temperature of the water and of the soil becomes propitious. These optimum conditions in the Huleh basin occur in the summer when the water level has receded nearly to its minimum, and this level would therefore determine the limits of the aquatic and swamp vegetation.

THE LAKE.

The lake occupies a shallow basin in the southern/

southern part of the plain, at the base of the E. range of hills and about a half to one mile from the W. range. At its widest it is about 2½ miles broad E-W. and about five miles long N-S.

The average depth of the water is about 5 feet but a few areas near the centre reach 8 or 10 feet. The slope of the bottom is very gradual towards the centre and it is possible to wade out considerable distances. In fact, at some points, one can wade 50 or 100 yards with the water reaching only waist high.

The lake bottom is generally of a characteristic brownish or greyish mud formed by the deposition of silt from the Jordan and from the wadis but nevertheless some striking differences may be observed. For instance, along the N. side of the lake, parallel to the S. boundary of the swamp and stretching from the W. to within half a mile or so of the E. shore, there is a mud bar. Near to the swamp, the mud bar is usually within 2 feet of the surface of the water but in some parts it is only a few inches beneath the surface (e.g. at the mouth of the Jordan). It would appear that this deposit is brought down mainly by the Jordan and the Wadi el Barid since it is most noticeable at/

at the points at which these enter the lake. A certain amount may also be brought down by a flow of water through the breadth of the swamp; especially might this be the case in the winter when the floods occur and when the springs, which no doubt abound in the swamp, are in full force. Assisted by the off-shoots of the Jordan, this broad stream would carry down with it a quantity of the organic debris which has collected under the swamp vegetation (q.v.).

Another notable difference in the character of the lake bottom occurs in the N.E. Corner of the lake where the Wadi Darbashiya, a summer-and-winter-running wadi, enters the lake. This wadi arises in the basaltic hills of the east and runs along the S.E. side of the swamp, to enter the lake in its N.E. corner. In its course it collects a quantity of rough basaltic gravel and deposits it on its delta in the lake. This delta is composed of old mollusc shells and shell gravel, together with the basaltic gravel and a little partially decomposed organic matter. Very little mud is to be found on this delta but the rough gravel gradually changes to the characteristic mud as the delta extends southwards into the silted areas of the lake/

lake.

A similar condition exists practically right down the E. shore of the lake where it is almost entirely composed of boulders and gravel of basalt mixed with old mollusc shells, although this appearance of the bottom does not extend far into the lake. Within a very short distance it becomes the typical mud characteristic of the greater part of the lake. Deltas formed by the other wadis of the east are very small and the force of water is not sufficient to clear any considerable area from the heavy silt deposit.

The western shore line, on the other hand, reflects the geological nature of the western range of hills. The level of the western plain, with its alluvial soil and limestone boulders, gradually slopes from the base of the hills to the lake, but at the lake side there is a more or less sudden drop of several feet to the water level. This drop forms the western shore, the main feature of which is the large quantity of limestone pebbles mixed with shell gravel. It is probable that these pebbles have been brought down from the hills in some past era when the whole of the west/

west side might have been, more or less, one vast wadi. Even at the present day, the large Wadi el Hindej which is only a winter-running wadi, bringing down in the rainy season large quantities of pebbles and silt, assists in the formation of this steep-sided, pebbly beach. Where the wadi enters the lake, it has produced a large steep-sided spit of pebbles and sand which juts out into the lake and at the end of this a very definite delta, exposed in the summer, has been formed. It may be imagined that if another similar wadi were present, say, a short distance south of the Wadi el Hindej, a similar spit would be formed and a little bay between the mouths of the wadis would result. Such a condition would, in fact, give an appearance similar to that found right up the west shore as far north as El Almaniya; little bays enclosed between old steep spits occur right up this region and the shore line has a dentate appearance. The limestone pebbles of this west shore do not extend very far into the lake before they become covered by mud.

The water of the lake, even in the summer, is brownish-green in colour and rather opaque. At first it was thought that this colour was due, to a large extent/

extent, to fine brown silt suspended in the water, but the clarity of the Jordan water and of the wadis showed showed that very little silt was being brought down in the summer season. When a plankton haul was made, however, an enormous amount of phytoplankton was brought up so that the opaque nature of the water and its colour, must have been due almost entirely to this phytoplankton flora.

The day temperatures of the lake, at any one time, varied, of course, on the location of the temperature station, e.g. in shallow or deep water, but where the conditions of depth etc., were similar, it was found that very little variation occurred. Although temperatures in the shallow regions near the shore, may reach 90°F or even more, the average temperature of the lake must be considered to be lower than that. The following are some of the temperatures recorded:-

TABLE I.

24/9/35. Temperatures of lake at stations.

Station number.	Readings on compass	Surface	Middle	Bottom	Depth.
H1(a)	G44; F244	76.6°F	75.7°F.	73.2°F	4ft
H1(b)	G41.5;F247	77.9°F	76.1°F.	74.3°F	6.5ft.
H1(c)	G59;F256.5	77°F	74.3°F.	73.2°F	7 ft.
H1(d)	G48.5;F267	77°F	75.2°F.	74.7°F	7.5ft.

All measurements of bearing from water guage (G) at mouth of Wadi Darbashiya and from point at end of Eucalyptus trees, el Almaniya, (F).

Temperatures taken in bottle water samples between 11 a.m. and 12 noon, local time.

TABLE 2.

2/10/35. Temperatures at lake stations.

Station number.	Readings on compass	Surface	Middle	Bottom	Depth.
H2(e)	P165;F261.5	74.5°F	74'F;73°F	72.5°F	8ft.
H2(f)	P169;F241	76°F	75°F;74°F	72°F	7ft.
H2 (g)	P169;F221	76°F	74°F;71°F	69°F	7ft.

All measurements from F (see previous table) and from Pumping house, Yesud ham Ma'ala (P).

Temperatures taken between 9 a.m. and 11 a.m. local time, by means of maximum and minimum thermometer.

It is seen from the above tables that, in general, the temperature of the lake bottom is only a few degrees below that of the superficial layers and that this difference is gradual so that there is no sharp distinction between a warm epilimnion and a cool hypolimnion/

hypolimnion as is sometimes found in some deeper lakes e.g. Lake Windermere.

In special locations one may find in Lake Huleh a more definite disparagement between the upper and lower layers but these are presumably due to the presence of submerged springs. Similarly, where the cooler waters of the Jordan river enters the lake differences of 10°F may be found between the superficial layers and the bottom. e.g. 8/10/35 Temperature of surface in Nuphar 75°F. Temperature of bottom 65°F.

As would be expected, in the colder seasons the day temperature of the lake becomes much lower and in November or December this may be in the region of 60°F. Presumably, still lower temperatures occur in the coldest months of January and February. The vast volume of cold water being brought down by the river Jordan and by the wadis will greatly accentuate this lowering of the temperature in the winter.

The water of the lake was of medium hardness and normal alkalinity. The following is an analysis of the lake water:-

TABLE 3.

Sample taken 15th October, 1935.

Results expressed in pp/100.000

Suspended solids	Minute trace.
Dissolved solids	23.4
Chloride (as Cl_2)	1.5
Free Ammonia	0.006
Albuminoid Ammonia	0.018
Nitrate (as N_2)	Nil.
Nitrite	Very faint trace.
O_2 consumed by permanganate (4 hrs. at 80°F).	0.528
PH value	7.9
Temporary hardness	11.09
Permanent hardness	3.77.

It will be observed that the organic matter is rather high but this is consistent with the presence of the large amount of phytoplankton and with the circumstance of the main supply of water to the lake passing through the swamp vegetation.

Some estimations of the oxygen content of the water of the lake were made and these were shown to be, on the whole, normal. The estimations were made/

made, primarily, to provide relative data in a study of the habitats of the lake and swamp. It will be noted from these tables (5, 6 and 7) that the oxygen content of the water of the swamp is, on an average, only about one-sixth that of the lake. This would naturally be expected in view of the large amount of decaying organic matter present in the swamp water (q.v.). The estimations were made by the macro-Winkler method for the estimation of oxygen in water using a potassium permanganate adaptation in the presence of organic material. (Alsterberg (2)).

VEGETATION OF THE SHORE AND LAKE.

The Shore.

A description of the shore vegetation must, in view of the "tidal" effect referred to, include that area between the low summer level of the lake and (approximately) the high winter level.

A most obvious feature in this area is the belt of the grass Cynodon dactylon (L) Pers. which practically extends all along the shore on both the east/

east and west sides of the lake. The upper limit of the zone seems to mark, approximately, the height to which the water level of the lake rises during the heavy rains of January and February. Associated with C. dactylon is Cyperus longus L. and (in the vicinity of wadis and marshy places), Panicum repens L., Echinocloa crusgalli (L) Beauv., Eclipta alba L., Lippia nodiflora (L). Rich. and a few other swamp plants.

The zone is more obvious on the east side where it forms an almost continuous fringe some 8-20 yards wide, (Fig.12). It is succeeded by the vegetation of the hillside which during the summer months consists of the dried, scorched stalks of the springtime vegetation. The relative positions of the consociations in this dried vegetation are apt to be confused on this east side due to cultivation, and rather better data has been collected on this matter at Yesud ham Ma'ala (q.v.).

Where, as is characteristic on this eastern side, the wadis split fanwise far up the hillside, into several smaller streams which flow directly down into the lake, the grass zone is succeeded, between/

between the arms of the wadis, by a dense, summer-growing, herb-shrub type of vegetation which is dependent on this proximity to water (Fig.13). The slash of green formed presents a very pleasing relief to the eye, from the monotonous, reddish-brown, barren appearance of the rest of the hillside. Typical plants of this dense undergrowth associated with these wadis are (Figures 14 and 15):-

Anemone coronaria L.

Arundo Donax L.

Dianthus strictus Banks and Soland

Erythraea spicata (L).

Ficus sp.

Lotus sp.

Lysimachia dubia Ait.

Nerium Oleander L.

Panicum repens L.

Plantago major L.

Polygonum aviculare L.

Polygonum senegalense Meism .

Pulicaria vulgaris Gaertn.

Quercus Aegilops L.

Rapistrum sp.

Ricinus/

Ricinus communis L.

Rubus ulmifolius Schott

Sorghum halepense (L) Pers.

Stachys longespicata Boiss and Kotschy.

Trifolium fragiferum L.

Typha angustata Bory and Chaub.

Verbascum sinuatum L.

Verbena officinalis L.

Vitex Agnus-Castus L. etc.

As the arms of the wadis pass across the narrow breadth of the grass zone the shallow banks support a flora to be found invariably in such locations in this area e.g.

Glycyrrhiza echinata L.

Lysimachia dubia Ait.

Melilotus alba Desr.

Ononis spinosa L.

Scrophularia alata Gilib.

Xanthium Strumarium L.

Growing in the water or on the little deltas in the lake one finds:-

Mentha longifolia (L). Huds.

Nasturtium officinale R.Br.

Polypogon monspieliensis (L.) Desf.

On/

On the west side of the lake, as opposed to the east, the grass zone may be either non-existent, or be present as a narrow fringe about a yard wide, or, in some parts only, it may be several yards wide. The extent of the zone is certainly correlated with the nature and slope of the shore. The higher limits of the zone (when this has not been confused by cultivation or some such) generally yields dominance to Alhagi maurorum Boiss. or Centaurea calcitrapoides L.

Near Yesud ham Ma'ala, for instance, there is a very definite vertical succession from the C. dactylon consociation (Figure 16). Succeeding this latter is an Alhagi maurorum consociation where the species Centaurea carduiformis DC., Daucus (Carota ?), Scolymus hispanicus L.. Sinapis incana L.. Torilis neglecta R. and S. are to be found. The Alhagi maurorum is generally infested with Cuscuta monogyna Vahl. and Cuscuta pedicellata Ledeb.

Within a few yards this latter consociation is replaced by a Centaurea calcitrapoides consociation and associated with this are Lactuca scariola L. Plumbago europaea L., Rubus ulmifolius Schott.

Near the Wadi el Hindej the species Vitex Agnus-Castus/

Agnus-Castus L. occupies a position between the C. dactylon and A. maurorum consociations. The V. Angus-Castus provides shade or support for species such as:-

Bryum argenteum Hedw.

Hordeum murinum L.

Linaria Elatine (L.) Mill.

Polygonum aviculare L. etc.

The steep sides of the spit formed by the Wadi el Hindej are occupied by a narrow fringe of C. dactylon close to the lake edge, followed by V. Agnus-Castus; while on the broad top of the spit Prosopis farcata (Banks and Soland) Eig. is locally dominant or co-dominant with A. maurorum. On the open stoney ground of this area Heliotropium villosum (Willd.), Plantago Lagopus L. and Ammi Visnaga (L.) Lam. are frequent while Chenopodium album L and Hedypnois Cretica (L.) Dum. - Cours are occasional. The actual banks of the wadi running through the middle of the spit are a dense growth of V. Agnus-Castus, a species which is found only in such habitats and in the near vicinity of wadis.

In the dry beds of the wadis which cease to run in the summer, a different flora is found. A good/

good example of such a dry wadi is the Wadi el Hindej above. It is noteworthy that some of the species here are confined to this wadi while only a few occur in another similar situation. Actually in the bed of the wadi, within the last 50 yards or so of its course, before it enters the lake, there occur the species,

Amaranthus chlorostachys Willd.

Chrozophora obliqua (Vahl) A. Juss.

Cyperus pygmaeus Rottb.

Euphorbia Chamaesyce L.

Glinus lotoides L.

Heleochloa schoenoides (L.) Host.

Heliotropium villosum Willd.

Hemarthria altissima (Poir). Stapf. and Hubbard.

Portulaca oleracea L.

Tribulus terrestris L.

At the exit of this wadi are found:

Amaranthus sylvestris var. graecizans (L.) Boiss.

Cyperus glaber L.

Polygonum lapathifolium L. etc.

while on the delta (Figure 17) grows,

Alternanthera sessilis (L.) R.Br.

Amaranthus sylvestris var graecizans (L.) Boiss.

Ammi/

Ammi Visnaga (L.) Lam.

Chenopodium urbicum L.

Digitaria sanguinalis (L.) Scop.

Erodium malacoides (L.) Willd.)

Euphorbia petiolata (Banks and Soland).

Portulaca oleracea L.

Scorpiurus subvillosus L.

Solanum nigrum L.

Sonchus oleraceus L.

Sorghum halepense L. etc.

THE LITTORAL ZONE

By the littoral zone is meant that damp fringe along the water's edge when the lake is at its summer low level.

This is very sparsely populated and no very striking difference is found between the vegetation of the east and that of the west, despite the very different natures of these two shores. The vegetation is growing in rather open formation on the patches of moist or wet mud which have settled between the boulders and stones (Figure 18). The following is a list of the species typical of this zone:-

Alternanthera/

Alternanthera sessilis (L.) R.Br.

Cyperus flavescens L.

Cyperus longus L.

Cyperus fuscus L.

Echinochloa crusgalli (L.) Beauv.

Fimbristylis bis-umbellata (Forsk). Bub.

Jussiaea repens L.

Portulaca oleracea L.

Spirodela polyrhiza (L.) Schleid

Trifolium resupinatum L. etc.

THE SPRINGS.

Mention may here be made to those springs occurring near the lake and swamp. The two most notable on the west side are (a) Ain el Mallaha (b) the spring at Jahula and (c) the "Sulphur" springs at El Almaniya. (a) Ain el Mallaha (Figure 19) which is near the village of Mallaha, merely gushes out at several points beneath huge boulders - the actual sources are hidden. This spring - or series of springs - yields a vast amount of water and, in fact, is the main source of the Wadi el Barid. An important feature at the springs is that in the shaded, damp environment of the overhanging boulders/

boulders, the fern Adiantum Capillus-Veneris L. is to be found (Fig. 20 and 21).

(b) The spring at Jahula arises in a large cave (Figure 22). The Adiantum Capillus-Veneris is also found in the cave together with mosses such as Eurhynchium tenellum Milde and Weisia verticillata Brid. This is one of the few places near the lake and swamp where mosses are to be found in the summer. At the mouth of the cave, growing on the overhanging rock was a specimen of Parieteria officinalis L.

(c) Another series of springs near El Almaniya are very curious. They are known as the "Sulphur" springs and arise simply in little pools (Figure 23). The 'sulphur' nature is caused by the occasional bubbling of sulphurous gases which would seem to be the product of decay in some subterranean region. It is possible that the gases have been formed under the swamp or lake and a part is forced out or comes out of a solution saturated under pressure, through these springs at El Almaniya. The flora consists of algae and these were, of course, collected.

AQUATIC VEGETATION OF THE LAKE.

The/

The distribution of the flora of the lake does not seem to bear any relation to depth of water except in so far as this may determine the shore limits of any of the consociations. Apart from the algae on stones etc., and a few isolated patches of Vallisneria spiralis L., there is little submerged vegetation within several yards of the shore. Generally this distance is about ten yards but where the slope inwards of the lake bottom is gradual, no vegetation may be found for 50 yards or so.

The factors which might possibly determine the position of any one consociation would seem to be (a) rate of silting and/or (b) nature of silt. Unfortunately, very little data has been obtained towards elucidating these possibilities. In some cases, the mud supporting one consociation may appear similar to that supporting another; but this much is certain - striking differences in the physical nature and composition of the substratum can be related with differences in distribution of the flora.

The whole basin forming the lake is practically completely covered with a mass of vegetation. These plants/

plants grow to such an extent that in many cases, the uppermost branches are floating on the surface of the water.

In order to determine the distribution of the consociations to be found in the lake and to discover the mutual limits of these it was necessary to perform a series of dragging operations over the area. This was accomplished by slowly rowing in a boat approximately along a pre-determined line between two fixed known points. By continually casting out a three-pronged drag and hauling this up one could obtain an idea of the aquatic flora of the area over which the boat was travelling. Where it was necessary to fix any particular point (e.g. at a transition point between two consociations or some such,) this could be done by taking compass readings on two or more fixed points on the shore. It could then be possible later to plot the point on a map from these compass readings.

By travelling along a series of radiating lines from Yesud ham Ma'ala base, a quantity of comprehensive data was obtained and from these a vegetation map was plotted. (Figure 48). The various consociations and their relative positions are shown but it must not be assumed that the lines of demarkation between the consociations are as clear as indicated. Rather was there/

there a gradual transition (to a greater or lesser degree) from one to the other. Also it has not always been indicated in the map where isolated specimens of a species have been found away from the main locus of the consociation as this would rather tend to obscure the otherwise very definite distribution of the consociations in the lake.

The main consociations to be found in the lake are those dominated by (1) Myriophyllum spicatum (L) (2) M. spicatum and Potamogeton lucens L. (3) Nuphar luteum (L) Sibth and Sm. (4) Vallisneria spiralis L. and Naias marina L. (5) Potamogeton pectinatus L. (6) Potamogeton nodosus Poir. (the P. natans and P. fluitans of Palestine authors). (7) Ceratophyllum demersum L. and (8) Phragmites communis Trin.

(1) Myriophyllum spicatum consociation.

This consociation occupies the greater area of the southern half of the lake, where it is pure for the species. The plants are growing in a typically light brown-grey mud. At the limits of its distribution it gradually becomes co-dominant with other species or, in some cases, the dominance is completely usurped. Thus/

Thus, in the north-west limit of this consociation, it becomes co-dominant with P. lucens forming the

(2) M. spicatum and P. lucens consociation. This consociation stretches northwards to the limits of the broad Nuphar luteum consociation where its identity becomes completely lost.

(3) N. luteum consociation.

This is a very striking population occupying a very prominant position in the general flora of the lake. While small colonies of this plant are to be found in many sheltered corners, its most extensive development is reached in the region of the mud bar, which has been noted as stretching along the north side of the lake. It is an unusual sight to see, this band of yellow water lilies, about two miles or more long, and anything up to 300 yards broad. The consociation commences about one-third of the way down the west shore, at El Almaniya (Figure 24) and continues north to the vicinity of the swamp where it curves round (Figure 25) and follows an easterly direction (Figure 26) to within a half mile or so of the east shore. The N. luteum is, of course, dominant here, but C. demersum is of quite frequent occurrence, while occasionally there is a plant/

plant of N. marina. Little colonies of Ranunculus aquatilis L. occur locally near the mouth of the Jordan. Near the mouths of the Jordan and of the Wadi el Barid, where the mud bar is very near the surface of the water, the Nuphar is interrupted by "islands" of Phragmites communis, Cyperus Papyrus (q.v.) and Typha angustata Bory and Chaub.

(4). V. spiralis and N. marina consociation.

This consociation is very conservative and is practically confined to the north-east corner of the lake where the Wadi Darbashiya has its exit. The substratum here is different from that of the rest of the lake and is principally composed of shells and shell and basaltic gravel mixed with partially decomposed organic debris and some mud. The V. spiralis only is found in other parts of the lake where coarse gravel has been deposited e.g. at the mouths of some of the wadis. In such cases, the V. spiralis is small-growing and in shallow water, but in the main area of the consociation growth is much more extensive. This may be due to the nature of the substratum and the depth of the water (4 feet or so).

(5) Potamogeton pectinatus consociation.

Except/

Except for occasional plants in other parts of the lake, this species is absolutely confined to the east side as a consociation some 30-40 yards from the shore and about two miles long. It approaches the shore to where the water is 1½ feet deep and maintains dominance to a depth of three to four feet of water where it is gradually replaced by the M. spicatum consociation.

(6) P. nodosus consociation.

This species occurs in its customary habitat in the shallower waters and is found at various points around the lake, but notably in the silted areas at the mouths of some of the wadis (Figure 27).

(7) C. demersum consociation.

While C. demersum occurs frequently, especially along the north side of the lake, among the Nuphar, it only becomes the dominant species in one area at El Almaniya on the west side of the lake. Here the consociation occupies an area of about 1000-2000 square yards and is in a position between the N. luteum and a Phragmites communis consociation (q.v.) near this shore. The Ceratophyllum occurs in such quantity in this area that an attempt at walking through it is attended/

attended with the utmost laboriousness and discomfort. Indications seem to point to the fact that this species has succeeded a part of the Nuphar as an investigation of the substratum shows the presence of old and decaying Nuphar rhizomes under the silt. In the shallower parts nearer the shore, where C. demersum is less dense, occasional plants of Chara vulgaris L. are found - a species found nowhere else in the lake.

(8) Phragmites communis consociation.

As would be expected, the P. communis occurs along the shore in the silted areas; such is the case at various points along the east and south sides of the lake and more especially on the west side where there is a continuous stretch of P. communis from El Almaniya to as far north as the swamp. Indeed, this zone, with little interruption, continues on this west side right up to the north of the swamp as a continuous consociation. In the lake the consociation is about 50 yards broad and extends from the water's edge in the summer, to about 3 feet of water. There are very few species associated with the P. communis; the species found are Sparganium erectum L. and Lycopus europaeus L., these generally occurring where the black mud/

mud is incompletely covered with water.

Incidentally, at El Almaniya, cultivation is carried on right up to the edge of the Phragmites zone, and in a low-lying field adjacent to the Phragmites. a few very small mounds have been left due to the typical Arab habit of ploughing around an obstruction rather than removing it. This is fortunate as it leaves the only few specimens of Datura Stramonium L. that are to be found in the Huleh district.

THE SWAMP. (ARD el HULEH).

The large swamp to the north of the lake covers an area of approximately 12 square miles. This is almost completely a dense jungle of vegetation, but a few comparatively small areas of open water occur e.g. the cuttings formed by the Jordan etc; certain narrow waterways cut open by the Arabs for purposes of travel; and a few open pools or clearings of various extent which occur here and there. (Figure 28).

The temperatures vary according to the particular area under observation. In some of the large exposed areas of still water, the temperatures are similar to those of the lake, but in the channels or narrow/

narrow waterways and in the vicinity of these, the temperatures in the summer and autumn are comparatively low, indicating the presence of submerged springs. The following are some of the temperature records taken, showing a comparison between lake, swamp and channel water. The lake maximum temperatures are rather lower than normal, these being taken in a cool part of the lake for the sake of convenience and for concealment from marauders, as thermometers placed in a prominent position were unfortunately subject to surreptitious removal. The occasional temperature given for "Barid Pool", (a pool at the source of the Wadi el Barid), are probably more indicative of the temperatures prevailing in the lake at that time.

TABLE 4.

Temperatures in various habitats

Temperatures in habitats in °F.								
Date.	Lake		Barid Pool		Swamp		Channel	
	Max.	min.	max.	min.	max.	min.	max.	min.
31/10/35	67°F	62.5	71.6		65	63	64	63
2/11/35	69	62	th.stolen		65	61	64	61
3/11/35	69	61	-	-	64	62	64	61
7/11/35	71	63	-	-	65	60	63	61
11/11/35	-	-	-	-	60	56	63	59

It will be noted that the greatest constancy in temperature is to be found in the channel water, as might be expected. There must be a large volume of water discharged from the submerged springs as there is practically always a flow of water, cold to the touch, passing along the channels.

Mention has previously been made to the almost normal oxygen content of the water of the lake. On the other hand, however, the water of the swamp is distinctly subnormal in its oxygen content. This might be correlated with the source of the water and with the amount of decaying debris and of living respiring roots in contact with it.

Various estimations of the oxygen content were made and the method employed was that of Alsterberg (2), as this modification of the Winkler method is suitable where the water is rich in organic matter. Some of the results obtained are to be found in the following tables:-

TABLE 5.

3/11/35. Oxygen content of some habitats.

Habitat	Ml. O2 at N.T.P.
Swamp: (a) in channel	1.086
(b) among vegetation	1.212
Barid Pool near spring	5.241
Lake at El Almaniya	6.368

TABLE 6.

7/11/35. Oxygen content of some habitats.

Habitat.	Ml. O_2 at N.T.P.
Channel water	0.814
do. (repeated)	0.839
Phragmites at El Almaniya	1.055
do. (repeated)	1.023

TABLE 7.

Comparison of habitats on same day.

Habitat	Ml. O_2 at N.T.P.
Papyrus swamp (a) in channel	1.065
(b) among vegetation	0.834
Phragmites at El Almaniya	
(a) inner	1.86
(b) outer (i.e. next to lake)	6.317
Lake	6.296

Further differences between the water of the swamp and of the lake can be found in the water analysis/

analysis:-

TABLE 8.

Analysis of Channel water taken on 31st October 1935.

Results expressed in parts per 100,000.

Suspended solids	minute trace
Dissolved solids	27.0
Chloride (as Cl_2)	2.0
Free Ammonia	0.045
Albuminoid Ammonia	0.03
Nitrate (as N_2)	nil
Nitrite	very faint trace
O_2 consumed by permanganate (4 hrs. at 80°F.)	0.488
Ph. value	7.3
Temporary hardness	16.13
Permanent hardness	2.47

This channel water is a little harder than the lake water and this may be due to the greater percentage of spring water present. It is also more acid than the lake and this fact may be associated with the close contact of the channel water with the organic debris/

debris of the swamp and the attendant acid decomposition products. It is important to note that it is always neutral-alkaline, as this probably has a particular effect on the peat formation of the swamp.

Vegetation of the Swamp.

The seral communities found are for the most part comprised of sedges and reeds and the more important consocies are those dominated by (1) Cyperus Papyrus L. (2) Phragmites communis Trin. (3) Typha angustata Bory and Chaub. (4) Cyperus serotinus Rottb. (5) Sparganium erectum L. (6) Cladium mariscus (L) R.Br. (7) Jussiaea repens L.

Cyperus Papyrus consocies.

Typically, the C. Papyrus is growing in 2-8 feet of water. In this habitat the thick rhizomes are so intermingled as to form a submerged "raft" which lies a half to one foot below the surface of the water and which is usually strong enough to bear a man's weight without breaking. It is, however, extremely difficult to walk over, and each step causes a sinking and movement of vegetation for several yards around.

Intermingled with the roots, which grow vertically/

vertically downwards to the floor of the swamp, is a mass of dead and decaying plant debris mainly composed of old rootstocks etc. of the Papyrus. This mass of debris is continually increasing but the neutral or alkaline nature of the water and the moderate to low temperatures, retard decomposition somewhat, resulting thus in the formation of a mild form of peat. Obviously, as this consolidation to peat continues, the floor of the swamp is gradually raised. Indeed, during the summer months, one can find the many stages between the condition of a loosely-bound, submerged debris and one where the peat has become exposed above the water level; in many such instances as the latter the water table may be two to three feet beneath the surface of the peat. The C. Papyrus is found to continue to grow on this exposed peat and is apparently as healthy as when growing in the more aquatic habitats (Figure 29). With the advent of the rainy season, when, as has been mentioned, the water level may be raised three feet or more, this exposed peat would become re-inundated. This periodic immersion of mild peat with alkaline water is reminiscent of the fen type/

type of habitat.

The soil profile of the peat is simple, being mainly peat and organic debris down to the water level. There seems to be very little decay due to absolute rotting in this, but carbonisation of the plant remains seems to take place. There is a change in the nature of the peat at the water level (3 - 4 ft below the surface level), as here it has the appearance of fibrous material. It is at this point also (i.e. at the water table), that there is evolved a very obnoxious sulphurous odour.

The roots from the surface Papyrus penetrate to varying distances through the peat, in which they have a black appearance, but at the water table they are white and more tender, like the young roots of Papyrus growing in water.

The more obvious remains in the peat are stems of Polygonum sp. and Papyrus. Soil tests carried out with a B.D.H. Comparator showed that the peat was of a very definitely alkaline nature, it being most alkaline on the surface.

In the "floating" *C. Papyrus consocies, the/

* Not strictly floating, as the roots reach the floor of the swamp.

the number of different species represented are very few, although those which are present occur very frequently. Most of the species found here form a lower vegetation stratum shaded by the tall Papyrus (usually 12-15 feet high), but one or two of the taller species are able to reach the more direct light. The plants all grow in little accumulations of organic material which have come to be held by the matted rhizomes of the Papyrus. The species found are:-

Dryopteris Thelypteris (L.) A. Gray.

Jussiaea repens L.

Lycopus europaeus L.

Lythrum salicaria var. tomentosum DC.

Mentha aquatica L.

Polygonum lapathifolium L.

Polygonum scabrum Poir.

In those areas where the water level is below the level, of the peat (Fig. 29) the species associated with the C. Papyrus are:-

Alternanthera sessilis (L.) R. Br.

Bidens tripartita L.

Chenopodium urbicum L.

Galium elongatum Presl.

Hydrocotyle/

Hydrocotyle ranunculoides L.f.

Hydrocotyle vulgaris L.

Inula viscosa Ait.

Lippia nodiflora (L.) Rich.

Lythrum Salicaria var tomentosum DC.

Polygonum lapathifolium L.

Polygonum scabrum Poir

Polygonum tomentosum Willd.

Salix Babylonica L. (?)

Solanum nigrum L.

Sparganium erectum L.

Urtica dioica L.

Veronica Beccabunga L.

The roots of the C. Papyrus often reach the water table which may be three feet below the level of the peat. Of the other species mentioned above L. salicaria var tomentosum has a root depth of about 15 inches; S. nigrum about 9 inches; C. urbicum about 6 inches; while D. Thelypteris and A. sessilis are superficial.

It is not suprising, however, in view of the fen nature of the habitat, that one should find that the Papyrus has occasionally yielded dominance to Phragmites/

Phragmites communis and Cladium mariscus; indeed quite considerable areas are occupied by these consocies.

The artificial waterways which traverse the swamp are invariably fringed with Ceratophyllum demersum with, occasionally, the occurrence of Utricularia vulgaris L. The open pools, however, harbour, in addition, such species as,

Lemna minor L.

Lemna trisulca L.

Nasturtium officinale R.Br.

Nuphar luteum Sibth and Sm.

Nymphaea alba L.

Polygonum scabrum Poir.

Polygonum senegalense Meisn.

Polygonum tomentosum Willd. etc.

Many additional species are also found on the shallow banks of the River Jordan (Figure 30) where C. Papyrus, P. communis and C. mariscus are the dominant species viz:-

Eupatorium cannabinum L.

Plantago major L.

Rumex sp.

Scrophularia alata Gilib

Tetragonolobus sp.

Verbena officinalis L.

and most of the species previously mentioned as growing in moist habitats. (Figures 31 and 32).

The C. Papyrus occupies, in the broad sense, the whole of the swamp area, except for the periphery, where the Papyrus is replaced by other reeds. The seral changes, as exemplified by the communites occurring along the periphery, are those common to other reed swamps (Figures 33). In the deeper waters of the edge the characteristic consocies is one dominated by Phragmites communis (Figure 34). This zone is succeeded by a Typha angustata (Bory and Chaub.) consocies growing in about a foot of water, while in the shallower waters, consocies dominated either by Cyperus serotinus Rottb. or Sparganium erectum L. are observed. These consocies are generally followed by a broad zone, several yards wide, and terminating in cultivated ground, of Cynodon dactylon (L.) Pers F. (Figure 35).

THE PHRAGMITES COMMUNIS CONSOCIES.

The floristic composition of this is the same as that of similar P. communis consocies on the lake except that the twiner, Cymanchum acutum L. is more in evidence.

THE/

THE TYPHA ANGUSTATA CONSOCIES.

The T. angustata occasionally becomes co-dominant with Scirpus lacustris L. Other species present are:

Alisma Plantago-aquatica L.

Cynanchum acutum L.

Hydrocharis Morsus-ranae L.

Juncus Fontanesii Gay ex Laharpe

Lythrum Salicaria var tomentosum DC.

Marsilea diffusa Lepr. ex A. Por. (Figures 36 & 37)

Sium erectum Huds.

Utricularia sp. etc.

CYPERUS SEROTINUS CONSOCIES.

The species associated with this are:-

Butomus umbellatus L.

Cyperus alopecuroides Rottb.

Cyperus dives Del.

Cyperus longus L.

Fimbristylis ferruginea (L.) Vahl.

Scirpus maritimus L.

Utricularia sp. etc.

SPARGANIUM ERECTUM CONSOCIES.

This generally occupies a position similar to/

to the last consocies. Other species found in this zone are:-

Mentha aquatica L.

Spirodela polyrhiza (L.) Schleid.

Utricularia vulgaris L.

Zannichellia palustris L.

At Jahula (the north-west corner of the swamp) cultivation takes place right down to the edge of the swamp. The adjacent fields are so low-lying that they are flooded by the first rains of approaching winter. Here again are some large mounds, large enough to avoid complete immersion, left by virtue of the Arab's disdainful avoidance of unnecessary labour. On these mounds one finds species more characteristic of drier habitats.

Althaea officinalis L.

Helminthia echioides (L.) Gaertn.

Lythrum Hyssopifolia L.

Rubus ulmifolius Schott. etc.

Succeeding the purely swamp communities is the C. dactylon consociation except where, in marshy places, a community of Jussiaea repens L. with Iris Pseudacorus L. and typically moisture-loving plants occur/

occur. (Figures 38, 39, 40 & 41). The grass community is similar to that around the lake except that around the swamp the slope of the ground surface is more gradual and the grass zone much broader. (Figure 42).

In this C. dactylon consociation, along the west side at least, there is an occasional Juncus acutus and also some communities of Inula viscosa Ait. co-dominant with Juncus acutus L. (Figure 43). This latter community begins and ends rather abruptly in the few areas where it occurs. Species associated with the I. viscosa - J. acutus consociation are:-

Oenanthe media Griseb.

Plumbago europaea L.

Spirodela polyrhiza (L.) Schleid.

Teucrium scordioides Schreb.

The seral succession indicated above is based mainly on observation on the west side of the swamp. In this area the different zones are very clearly marked indeed. On the eastern side of the swamp, however, there seems to be a certain amount of confusion and one can only conclude that this is the outcome of the cultivation which takes place right down to the water's edge and probably also to the fact that there seems to be a more widespread tearing up of the Papyrus rootstocks etc., and using/

using these as fuel (Figures 44 and 45). There must be other factors operating, as on this east side it is not uncommon to find that one of the zones is apparently missing. For instance, the P. communis and the T. angustata may not be present, both of which consocies are characteristic features of the western side. As the P. communis is not nearly so well-developed on the east side of the lake as it is on the west, due no doubt to some factor such as silting, wind currents, etc., it would appear that the differences to be observed in the zonation of the east and west sides of the swamp (i.e. presence or absence of any consocies), are probably related to the previous history of the swamp, at a time when this area was, in fact, part of the lake, as undoubtedly it must have been. With the present day observable differences in the distribution of the lake vegetation (e.g. the P. communis). due to various edaphic factors, it might well be supposed that this would be reflected in the "edge effect" when these parts of the lake become transformed into swamp.

On the north side there is also a confusion of the vegetation but this is as a result of the partial drainage carried out by the Arab fellahin with a consequent lowering of the water level to below the surface/

surface of the ground with a reclamation of the land for agricultural purposes. In the most recently reclaimed land, Maize and Millet are sown between the old rootstocks of Papyrus, without very much preliminary ploughing. In such places, various typically swamp plants persist here and there but no doubt these will in time disappear.

The southern extremity of the swamp, of course, abuts immediately on the lake. Generally there is a sharp line of demarkation between the lake and swamp, the tall mass of Papyrus etc. ending abruptly. Where, however, there has been a large deposition of organic material, silt, etc., such as is found near the Jordan mouth and that of the Wadi el Barid, there occur groups of "islands" of vegetation. Near the Jordan these islands are generally comprised of P. communis but at the Wadi el Barid exit, the islands are anchored 'buoys' of Papyrus. It would appear that these latter are formed by a fragment of Papyrus breaking off from the main body of the swamp, floating out and becoming caught in the mud bank (Figure 46). This fragment would gradually grow out in all directions, to form the island, treacherous to walk on, but held in place by the Papyrus roots. Other plants often find a resting place in these islands and one generally finds that the Papyrus has/

has become co-dominant with Typha angustata and the island becomes fringed with Jussiaea repens. (Figure 47).

Such an island in expanding, would gradually grow outwards into the lake and also meet in another direction the advancing main body of the swamp. Thus there would be a more rapid encroachment of the lake by the swamp, in these places. There is no doubt that the Swamp is encroaching on the lake from the north and it would thus only be a matter of time before the whole of the basin of the Huleh plain would become one huge "Papyrus Swamp".

THE ALGAE AND DIATOMS.

Collections of these have been made from various habitats, by plankton tow-net, or hand net, or by hand collection. Specimens have been taken from the lake, the swamp (both in the channels and among the vegetation), on marshy areas, at the springs, and in the wadis etc. The collections which to the time of writing have been analysed show that there is a very large variety of species of Diatoms present, in the region of 600-900 different types.

DISCUSSION.

From/

From the foregoing study of the Huleh basin it is apparent this possesses a flora which has affinities with the Temperate zones, Europe, Siberia, the Caucasus, Asia minor, Asia, The Mediterranean, The Orient, Africa (tropical and sub-tropical), India etc. With regard to the species which are not of Indian or African affinities it is understandable that a migration in historical time could have taken place across the water sheds which ultimately connect the Huleh valley with the regions outside. In addition, the geographical position of Palestine makes possible the formation of such a mixed flora, as this country is a junction or meeting place of the phyto - and zoo - geographical regions described by Eig (5) and Bodenheimer (3) viz: (1) Euro-Siberian, (2) Mediterranean, (3) Saharo-Sindian, (4) Irano-Turanian.

The flora of the Huleh is therefore of Palaeartic origin although there has been a penetration of species of the Sudano-Deccanian region of the Ethiopian sub-kingdom. The presence of these latter elements, so far away from their normal region of distribution, and in a region which is otherwise of a Palaearctic nature, cannot be understood without reference/

reference to the geological history of the country; for even in historical time a migration of any but desert types has been virtually impossible in view of the fact that great desert tracts intervene between the Sudano-Deccanian region and Palestine.

Unless the species showing this Ethiopian affinity have been brought by artificial means, they must have migrated at a time when conditions were very different from those of the present day.

Tristram, when discussing the question, comes to the conclusion that such a migration would have been possible during the Miocene and Early Pliocene eras, when Palestine was yet separated from Europe, etc., by the Syrio-Persian Sea and its only land connection was with Africa. During that time there was a continuous fresh water system from the Jordan basin in the north to the lakes and rivers of Africa in the regions of the Nile basin, the Nyanza, the Nyassa and the Tanganyika lakes (and possibly the Red Sea) in the South. The climate over all that area was, at that time, warm and suitable for a migration of species from Africa, northwards into Palestine.

We may assume therefore that the hydrophytic flora of the Huleh region was, at first, Palaeotropic in character/

character. Later, migration of a Palaearctic flora from the north occurred, and, with the final transition to a more or less Mediterranean climate, the flora of the Huleh basin came to assume a predominantly Palaearctic character. When we consider that the area must have been indirectly affected during the glacial period of the northern regions and also (if we are to believe the early historians of the place) that the Huleh basin has, at periods, been completely dry, any Ethiopian relicts must have shown extreme tenacity of life to have survived to the present day.

Lake Huleh must also have been an important factor in the preservation of these Palaeotropic hydrophytic elements in the Jordan Valley as this is the only one of the three Jordan lakes which has remained continuously fresh. A study of the literature shows that during the Middle Diluvial period, volcanic eruptions separated Lake Huleh from Lake Tiberias. Towards the end of this period, a dry period caused an excessive shrinking of the waters of Lake Tiberias which thus became distinctly saline in nature. During this time therefore, the aquatic flora of Lake Tiberias and of the Dead Sea, (which had already become saline) must have disappeared or very nearly so. It was not until the Upper/

Upper Diluvial, when the waters of Lake Huleh broke through the basaltic bar, that Lake Tiberias became fresh once more and a recolonisation by an aquatic flora from the northern lake took place.

Of the species mentioned, most of the tropical or sub-tropical types (Index Kewensis) are already known for Palestine (Post (7)), viz:-

Alternanthera sessilis (L.) R.Br.
Cyperus alopecuroides Rottb.
Cyperus Papyrus L.
Cyperus pygmaeus Rottb.
Fimbristylis bis-umbellata (Forsk) Bub.
Fimbristylis ferruginea (L.) Vahl.
Glinus lotoides L.
Hebiscus Trionum L.
Jussiaea repens L.
Polygonum scabrum Poir
Polygonum tomentosum Willd.
Ricinus communis L.

A most important contribution of the expedition is the finding of Marsilea diffusa Lepr. ex A.Br. This species is of African and Ethopian distribution. The present record is the first note of its occurrence in Asia/

Asia and is probably the most northern record of the species. It is noteworthy that the specimens were found in only one locality of the Huleh swamp viz:- on the west side at Beisamum and was in the vicinity of a spring where there was no fear of drying. This colony of the Marsilea is either a relict of the pre-glacial age when it no doubt had a distribution much wider than the present day, or it has been artificially introduced by birds. It is impossible to decide this important point but zoological records of Huleh and the Jordan Valley lend colour to the conclusion that this species is indeed a link with the pre-glacial flora of the Huleh and is one of the "tropical outliers" referred to by Tristram, a condition made possible by the unique climate of the Jordan Valley.

The following record of species new for Palestine is made on a basis of Post's Flora of Syria, Palestine and Sinai, rewritten by Dinsmore (1933). The species marked with an asterisk * are recorded in Post for Syria and the Lebanon etc. but not for Palestine.

* Chenopodium urbicum L.

Cuscuta pedicellata Ledeb.

Cyperus dives Del.

Cyperus serotinus Rottb.

Dianthus/

Dianthus strictus Banks and Soland.

Eupatorium cannabinum L.

* Euphorbia petiolata Banks and Soland

Galium elongatum Presl.

Hydrocharis Morsus-ranae L.

Marsilea diffusa Lepr. ex. A. Br.

Polygonum tomentosum Willd.

Prosopis farcata (Banks and Soland)Eig.

* Pulicaria vulgaris Gaertn.

* Stachys longespicata Boiss and Kotchy.

Vallisneria spiralis L.

* Veronica Beccabunga L.

From the ecological point of view it is found that, in the swamp, the distribution of the consocies is normal in character and shows a seral succession typical of other reed swamps. In the lake, the distribution of the plant communities seems to obey some governing factors inherent to the lake. Probably the rate of silt deposit and/or the nature of the silt to be found in the different areas of the lake, are in the main responsible. The excessive growth of the plants is no doubt due to the sufficiency of salts and organic material and to the moderate to high temperatures all of which would induce vegetative growth.

The/

The Expedition is most grateful to the many people who gave us valued help. In particular we owe a very great debt of gratitude to Dr. and Mrs. Mer of the Malaria Research Station, Rosh Pinna, for their hospitality and for the great trouble they took to help us; we are also indebted to Professor F.S. Bodenheimer and his staff at the Hebrew University for their hospitality and assistance. The present writer wishes to record his thanks to Dr. Eig of the Hebrew University for permission to use the Herbarium for identification of many of the species, although the responsibility for the final naming of the specimens is completely with the staff of the British Museum to whom appreciation is now given. Our thanks are also due to Professor W. Stiles, F.R.S. and Professor H. Munro Fox, F.R.S., for so kindly giving us leave of absence from Birmingham University, and for much help in other ways, and to Professor J. Stanley Gardiner, F.R.S. We are indebted to many people for advice; in particular to Dr. J. Ramsbottom, Dr. W. Leach, Dr. G.S. Carter, Professor P.A. Buxton and Dr. W.H. Pearsall, while we acknowledge with thanks the loan of apparatus from the Zoological Department, Cambridge University; the Departments of Botany and Zoology, the University of Birmingham; the John/

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* These references are taken from Barrois' paper and have not been verified.

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Figure 1 Map of Lake Huleh and the Swamp.

Figure 2 Showing the Huleh Valley as seen from the eastern range of Syrian hills. The hills to the north-west (background, left) are those of Upper Galilee. In the foreground are seen the dried remains of the last spring's vegetation.

(3)

(4)

(5)

Figure 3 Lake Huleh as seen from Rosh Pinna, facing north-east. Mount Hermon in the background is concealed by the early morning clouds (6 a.m.).

Figure 4 The River Jordan flowing through the Huleh Swamp. The lower ranges of Mount Hermon in the background. The banks of the river are supporting a C. Papyrus consociation and this is fringed with Polygonum spp.

Figure 5 River Jordan passing through the narrow gorge in the south of the Huleh plain. Middle foreground - Jissr Banat Yacoub Bridge which leads to Damascus. Background - Lake Huleh and Mount Hermon.

Figure 6 Arab fellahin standing on a reed raft in one of the waterways of the Swamp. The surrounding vegetation is mainly C. Papyrus.

Figure 7 Reed rafts being prepared for a journey into the Swamp. These are tied behind the pilot raft or rafts. Dr. Mer in the photograph is sitting on a double raft, but where the waterways are narrow single rafts are used.

(8)

(9)

(10)

Figure 8 Travelling up the east side of the lake; Mr. Washbourn in foreground. Arab guide and "protector" leading. Middle foreground shows some specimens of the shrub Z. Spina-Christi.

Figure 9 The lake from the north-east. A wild Fig tree is on the left and is growing near a concealed stream.

Figure 10 The River Jordan before it enters the Swamp. Note the shallow banks which afford little protection during the flood periods. The animals are water buffalo.

(11)

(12)

(13)

Figure 11 Lake Huleh from the south-east. In the foreground may be seen the new shoots of the next springtime vegetation. The photograph was taken in December, 1935.

Figure 12 The eastern shoreline of the lake. Note the basaltic boulders and the open vegetation of the littoral zone. Succeeding this is the C. dactylon consociation and behind this the herb-shrub vegetation in the vicinity of the wadis.

Figure 13 Wadi passing across the grass zone. In the middle foreground is seen the herb-shrub vegetation, while on the hills in the background are seen a few oak trees.

Figure 14 A wadi on the east side of the lake. The photograph is taken in the herb-shrub vegetation. On the right sky-line is seen Ricinus communis while on the left is Nerium Oleander. Other species are V. Agnus-Castus, Verbascum sinuatum, etc..

Figure 15 A sunken wadi where Arundo Donax is the dominant species. The photograph shows the height of the reed as compared with the Arab man.

Figure 16 The west shoreline near Yesud ham Ma'ala. The sparse grass zone of C. dactylon is seen growing on the limestone pebbles and this is followed at this point (near the Wadi el Hindej) by the V. Agnus-Castus consociation. The further succession is not clearly marked in the photograph.

Figure 17 Photograph taken on the delta of the Wadi el Hindej and looking towards the mouth of the wadi. The dense vegetation on the flat tops of the spit on either side of the wadi are clearly seen. In the foreground, on the delta, the erect plants are mainly young specimens of V. Agnus-Castus and the prostrate species H. villosum

Figure 18 The vegetation of the littoral zone on the east side of the lake. A specimen of E. crusgalli is seen in the centre left. In the foreground are Cyperus flavescens, etc., and Alternanthera sessilis, Portulaca oleracea, Jussiaea repens, etc.

Figure 19 Ain el Mallaha. The large pool formed by this spring before it runs into the Wadi el Barid. The sources of the spring are behind the boulders on the left side of the pool. The vegetation in the pool is mainly T. angustata and J. repens.

Figure 20 Spring water (foreground) gushing from underneath the boulders at the spring Ain el Mallaha. In the shaded damp environment of the overhanging boulders is seen A. Capillus--Veneris.

Figure 21 A. Capillus-Veneris growing in the shade of a large limestone boulder at the spring Ain el Mallaha. Running spring water in the foreground

Figure 22 The cave in which the spring at Jahula originates. In this cave mosses are plentiful as also are vipers. A Capillus-Veneris on the rocks in the centre right of the picture.

Figure 23 A "Sulphur" spring at El Almaniya. In the top right-hand corner of the pool a few bubbles of sulphurous gases are seen bursting on the surface of the water.

Figure 24 The Phragmites communis consociation at El Almaniya. In the lake is the S.W. extremity of the N. luteum consociation. The present writer is seen collecting some P. nodosus. The littoral vegetation is very sparse at this point.

Figure 25 The N. luteum consociation. The hills of Upper Galilee are seen in the background.

Figure 26 The N. luteum consociation, looking north to Syria and the Lebanon. The swamp is seen in the middle background.

Figure 27 Colonies of P. nodosus growing out in the lake opposite the mouth of the wadi.

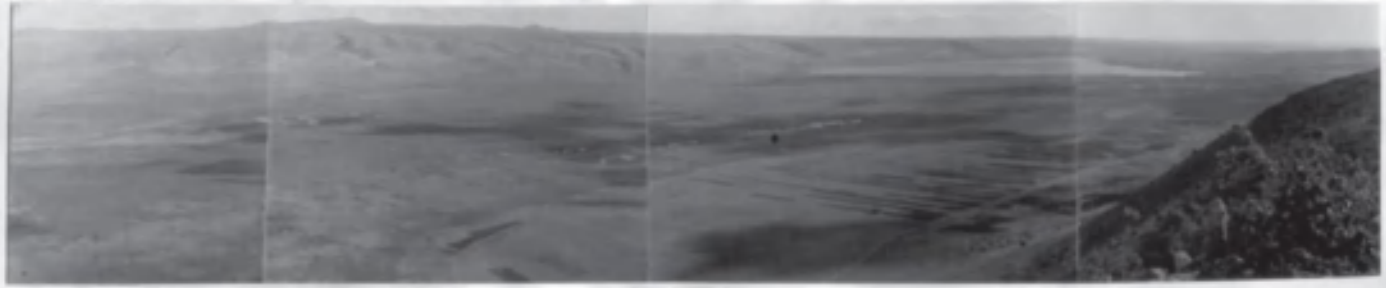


Figure 28 Panoramic view of the swamp and lake from the hills of Upper Galilee above Jahula. Note the open pools in the swamp. The middle foreground is cultivated ground, the newly-ploughed earth being evident in strips. The lighter vegetation in the left foreground, at the edge of the swamp proper, represents the Phragmites zone which extends up this western side of the swamp.

Figure 29 In the Papyrus swamp. The C. Papyrus is about 15 feet high. A few specimens of the fern D. Thelypteris and plants of Bidens tripartita are evident.

Figure 30 Polygonum spp. and J. repens fringing the Jordan as it passes through the Papyrus swamp. Looking north.

Figure 31 The River Jordan entering Lake Huleh. There is an extensive growth of *Polygonum* spp. and the lighter vegetation to the left of the river mouth (centre, right) is a colony of *Phragmites*. The darker vegetation in the photograph is *C. Papyrus*.

Figure 32 *J. repens* growing out from the banks of the Jordan as it flows through the swamp. Some of the yellow flowers are to be seen in the right foreground and centre.

Figure 33 The edge of the swamp near Mallaha. In the foreground is the lower-growing sedges such as C. serotinus etc. T. angustata is in the middle background, and Phragmites communis on the skyline. It is difficult to obtain a photograph of this type contrasting the different zones.

Figure 34 In the Phragmites zone of the swamp.

Figure 35 Looking northwards along the west side of the swamp. Left foreground shows some J. repens; right foreground and centre, C. serotinus etc. with T. angustata behind (centre, right). The water buffalo are grazing on the Cynodon dactylon consociation.

Figure 36 Marsilea diffusa in the T. angustata consociation, near Beisamun.

Figure 37 As in Figure 36.

Figure 38 A J. repens consociation in a marshy area near Mallaha. In the foreground is Iris pseudacorus and in the centre background, on the grass zone, is Juncus acutus. The Arab is standing next to a projecting wedge of Phragmites: the present writer (foreground, right) is negotiating the mud.

Figure 39 The area shown in Figure 38, after the first shower of rain. Note that the level of water now approaches the J. acutus. The J. repens is now under water except for the floating branches seen in the photograph.

Figure 40 Looking along the taller vegetation in Figure 38, centre, right. Note the J. repens in the left foreground is smaller-growing in the marshy ground as compared with that in the right foreground adjacent to free water. The tall vegetation in the centre right of the photograph is T. angustata. and J. acutus can be observed in the grass zone, centre and centre left.

Figure 41 A J. repens consociation on the east side of the swamp. The larger plants are specimens of Xanthium strumarium. This species is also frequent on drier ground. Note the open pool on the centre, left. Cyperus Papyrus (not shown) abuts directly on this pool, and the intermediate zones are absent.

Figure 42 The C. dactylon consociation. In the foreground is Centaurea calcitrapoides, succeeding the C. dactylon. In the background is a mass of dried Sparganium erectum which has withered because of the summer fall in the water level.

Figure 43 Looking north along the grass zone at the western side of the swamp near Beisamun. The swamp edge is on centre right of the photograph, and an I. viscosa - J. acutus consociation on the left. A few I. viscosa plants have penetrated into the grass zone.

Figure 44 An open pool on the east side of the swamp. In the foreground the vegetation is mainly J. repens with occasional plants of Nuphar luteum. Near the edge of the pool E. crusgalli becomes frequent. The pool leads direct to Papyrus swamp (middle).

Figure 45 A broad zone of C. dactylon on the eastern side of the swamp. The occasional larger plants in this grass zone are Xanthium strumarium. The grass leads back to an herb-shrub zone where Z. Spina-Christi etc. are the main species.

Figure 46 Where the mud bar in the north of the lake is near the surface. The aquatic vegetation is Ceratophyllum demersum with N. luteum. On the left foreground may be seen two young "islands" of C. Papyrus which are beginning to form. These will enlarge to the extent shown in the next figure.

Figure 47 Looking between two "islands" of vegetation in the lake near the swamp. The "islands" are seen on the right and left of the photograph and the main body of the swamp in the middle background. These "islands" are mainly composed of C. Papyrus and T. angustata, and are fringed with J. repens.

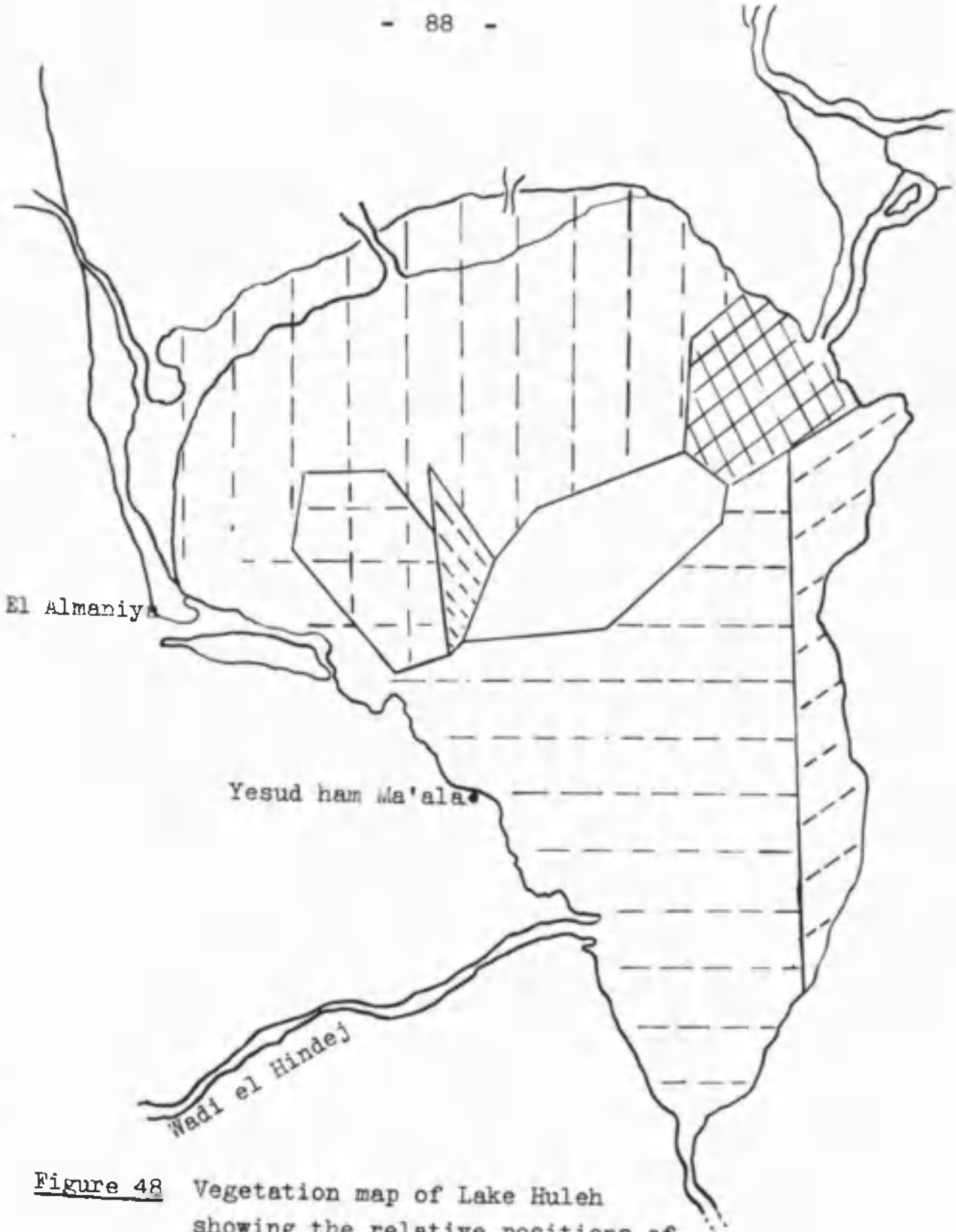


Figure 48 Vegetation map of Lake Huleh showing the relative positions of the main plant consociations. For description see overleaf.

Description of Figure 48

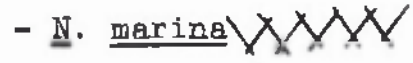
The plant consociations:

Nuphar luteum | | | |

M. spicatum — — — —

M. spicatum - *P. lucens* \ \ \ \

P. pectinatus / / / /

V. spiralis - *N. marina* 

The blank area in the centre of the lake is where no vegetation seems to grow. The water at this point is usually deeper than at other parts of the lake, and this may determine the absence of the plants in this area.