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# OBSERVATIONS ON POTASSIUM DEFICIENCY IN *LEMNA MINOR* L.

by

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## 1. INTRODUCTION

Although extensive investigations dealing with the growth of *Lemna minor* under different conditions have been carried out, practically no attempt has been made to obtain quantitative data on photosynthesis and respiration of this species in connection with potassium deficiency. While there is general agreement in the effects of light on growth as reported by ASHBY (4), HICKS (10), ASHBY and OXLEY (5) and WHITE (14), the last investigator found notable differences mainly due to seasonal changes and differences in strains from different localities. This made it of interest to repeat these experiments for the strain used in this laboratory. The observations of WHITE (14) on the effects of potassium on growth and other metabolic activities have been extended by replacing K by Na, Rb, and Cs.

Photosynthesis and respiration were measured by means of a WARBURG apparatus. The measurements of photosynthesis were carried out at different levels of potassium deficiency in *Lemna* under different light intensities. PIRSON (12) made studies with *Chlorella*, by adding different kations to cells, which had been grown previously at different levels of potassium starvation, and measured the short term effect of addition of K on photosynthesis and respiration. A similar procedure was used with *Lemna* in the experiments described here.

## 2. MATERIAL AND METHODS

Stock cultures of *Lemna minor* were maintained in a light box under sterile conditions in 300 cc erlenmeyer flasks, containing 150 cc of a nutrient solution. This solution was based on that of GORHAM (7) containing $5.10^{-3}$ M $Ca(NO_3)_2$, 4 $H_2O$; $2.10^{-3}$ M $MgSO_4$, 7 $H_2O$; $5.10^{-3}$ M $KNO_3$; $1.10^{-3}$ M $KH_2PO_4$; 0.005 gr ferritartrate per litre, with the following addition of micro-elements: 2.86 mgr $H_3BO_3$; 1.81 mgr $MnCl_2$, 4 $H_2O$; 0.22 mgr $ZnSO_4$, 7 $H_2O$; 0.07 mgr $MoO_3$; 0.08 mgr $CuSO_4$, 5 $H_2O$.

For the purpose of growth experiments subcultures were made from the stock culture. A constant rate of growth under the new conditions was reached within 2 days. Dependent on the quantity of fronds needed in the growth experiments, the subcultures were used for inoculation at an age of 5–10 days. Growth experiments were made in 500 cc erlenmeyer flasks containing 250 cc of the nutrient solution mentioned above. Unless indicated otherwise, usually 25–50 fronds were inoculated per erlenmeyer under asceptic conditions, which then was stoppered by a cotton plug through which a glass tube was inserted for aerating the solution with air enriched with 5 % $CO_2$. Aeration for three 2-hour periods per day was sufficient to obtain exponential growth. Under these sterile conditions no bacterial or other pollutions

appeared before 25 days after inoculation. The average room temperature was 20–23 °C, the temperature of the solution 2–3 °C higher depending on duration of illumination and light intensity. The erlenmeyer flasks were illuminated on 2 sides by fluorescent daylight tubes giving a light intensity of 4–5000 lux. In the sodium, caesium and rubidium cultures, potassium was substituted by these elements respectively. In the caesium cultures only $CsNO_3$ was available, so in this case instead of $CsH_2PO_4$, $NaH_2PO_4$ was given. The same was done with the rubidium cultures, in which Rb was given as RbCl $5.10^{-3}$ M. Sodium nitrate and biphosphate were given in order to adjust the phosphate and nitrate to the same level as in the potassium series. Usually, 4–6 replicates were run in each experiment.

Growth was measured daily, by counting the number of fronds. In order to minimize the effect of different stages of development, a young frond was rated as $\frac{1}{4}$, a medium one as $\frac{1}{2}$, an older one as $\frac{3}{4}$, and a mature frond as 1. Thus, only the rate of reproduction was measured; changes in leaf area and thickness were not included in the measurements, unless indicated otherwise. Dry weight was measured by oven-drying 100–200 fronds in small weighing bottles. Some chlorophyll determinations were made by extracting the fronds in 90% ethanol. The extraction was repeated three times in a waterbath (between 50–60 °C) which was sufficient for total extraction. It may be mentioned that extraction of fronds from the sodium cultures was particularly difficult, especially in the later stages.

The chlorophyll concentration was measured in a BLEEKER colorimeter at wavelength 6650 Å.

Photosynthesis and respiration measurements of the fronds were made in a WARBURG apparatus. The apparatus was of the usual type, provided with a thermostate of 100 × 30 cm, which could be kept constant with an accuracy of 0.05 °C between 15 and 35 °C by means of an electric heating unit controlled by a thermo-relay and a constant water cooling system through a copper spiral. Below the glass bottom of the thermostat, two 140 W sodium lamps with a white reflector were mounted; the lamps were cooled by two small fans. In front of the thermostate a shaking frame was mounted on which 12 manometers could be placed. This frame is moved excentrically at a rate of 150 revolutions per min. with an amplitude of about 5 mm. Stirring of the waterbath was achieved by a horizontal stirrer with 6 blades. The conical vessels with flat bottoms had a volume of about 20 cc, and were provided with two sidearms of about 3–4 cc. The manometers carried BRODIE solution. Different light intensities were obtained by using filters, which were placed in metal holders fixed under the vessels. The light intensity was measured by a photocell, calibrated against a large surface thermopile. The position of the photocell was chosen in such a way as to put the sensitive surface at the same level as the *Lemna* fronds. The highest light intensity obtainable in this arrangement was about 60.000 ergs/cm²/sec.

Photosynthesis was usually measured in air containing 5% $CO_2$, at 25 °C. Dependent on the rate of photosynthesis, 10 to 25 fronds in 5 cc tapwater were used per flask (pH 5,5). In the experiments with K or Na additions, 0.2 cc KCl or NaCl were pipetted into the sidearms which addition, after tipping into the main part of the flask, resulted in a concentration of M/100. The gas exchange was computed from the readings observed in a single WARBURG vessel. The different solubilities of the gases cause a pressure change, from which the gas exchange can be deduced in accordance with the formula $x_{CO_2} = \frac{h\, k_{O_2}\, k_{CO_2}}{p\, k_{CO_2} - k_{O_2}}$

The photosynthetic quotient $\frac{O_2}{CO_2} = p$ was determed in some experiments with 2 vessels, each containing the same number of fronds, and containing 2 cc and 5 cc tapwater respectively. A rather constant value of 1.06 was obtained for $p$, which was considered representative and was used in the above formula.

Respiration was measured in the dark with 0.2 cc 15% KOH in the side arms, gas exchange representing $O_2$ uptake. Because of the relatively low gas exchange under these conditions, 30 fronds in 5 cc tapwater were used per flask.

### 3. GROWTH EXPERIMENTS

The different behaviour in growth of *Lemna minor* from different localities observed by WHITE (14) emphasizes the necessity of repeating his growth experiments for the strain used in this laboratory. An experiment with three replications was made for three light intensities. Cultures were grown in small glass tubes aerated with air plus 5 % $CO_2$. Different light intensities were obtained by

varying the distance from the fluorescent tubes. Relative rates of increase are given by the formula $\frac{\ln n_1 - \ln n_0}{t_1 - t_0}$ in which $n_1$, and $n_0$ represent the frond numbers at times $t_1$ and $t_0$ respectively. The relative rate of increase in frond number was constant throughout the experimental period of 10 days for each light intensity, indicating that no limitation of $CO_2$ occurred.

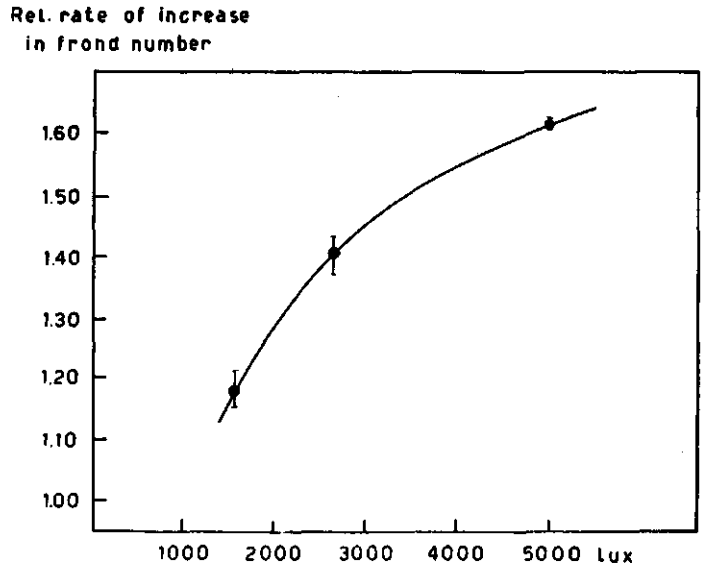


Figure 1. Effect of light intensity on the relative rate of increase in frondnumber of *Lemna minor* L.

In figure 1 the relative rate of increase in frond number is plotted against light intensity. The curve indicates a direct relationship between rate of frond multiplication and light intensity. Though too few points are recorded, it may be seen that the slope decreases progressively, in about the same way as was reported by WHITE (14). At present, however, no connection can be made with our measurements of photosynthesis at different light intensities on account of the few points measured in growth. It may be seen that our multiplication rates in general are much higher than those observed by WHITE (14). This is possibly due to differences in physiological strain, or in environmental conditions. In the range of light intensities used in our experiments, no differences in percentage dry weight were observed.

After these investigations on the relation between growth and light intensity, experiments were made with variations in nutrient solution. In order to obtain some more information on potassium metabolism in *Lemna*, potassium was substituted by sodium, rubidium and caesium. The composition of the nutrient solution of these series has already been given in Section 2.

Figure 2 represents growth of the K, Na, Rb and Cs cultures. The number of fronds (ordinate) is plotted on a logarithmic scale. It may be seen that K, according to the formula $N = N_o e^{rt}$ gives a straight line. Rb and Na show about the same multiplication rate during the first 5–8 days. Thereafter, the slope of the Na curve shows a logarithmic decrement; Rb, however, shows a lower

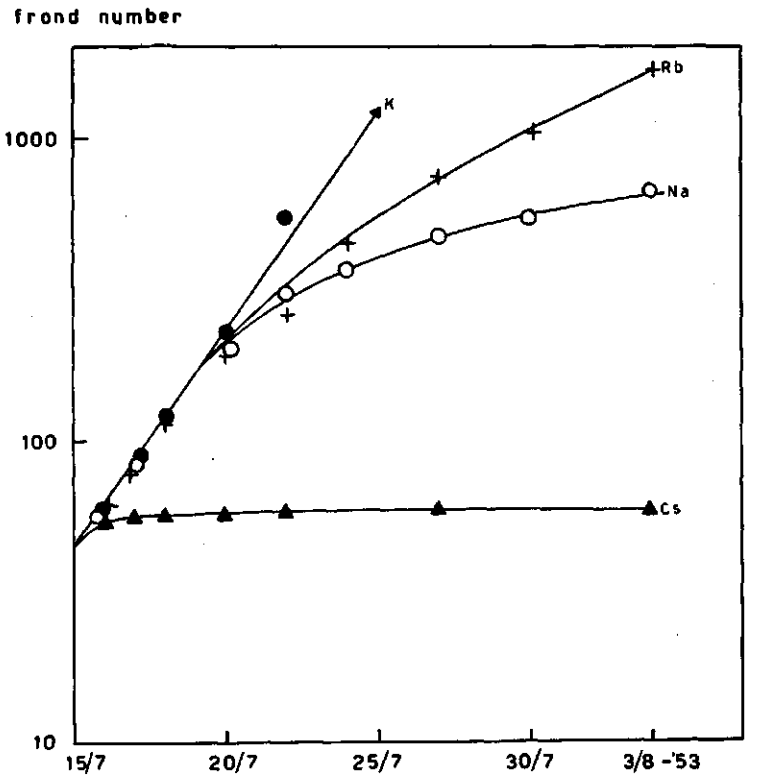


Figure 2. Growth of *Lemna minor* L. in potassium, sodium, rubidium and caesium cultures

exponential rate of growth than the K series. The identical rate of increase during the first 5–8 days in Rb and Na may be ascribed to a high potassium level originally present in the *Lemna* fronds, which level decreases with an increase in fronds until a potassium deficiency becomes manifest. Observations of WHITE (13) emphasize this idea, as in his experiments the decline of the exponential growth was dependent on light intensity. Lower light intensities result in a lower rate of increase, which in turn results in a longer period of exponential growth. It may be mentioned here that the fronds of the Rb series deviated strongly from the normal frond habit and were curled and compact. The Cs cultures show an inhibition even at the start of the growth experiments and after one day no growth at all was observed for a period of at least 18 days. PIRSON (12) observed an inhibition of growth in *Chlorella* by Cs concentrations of M/100.

In connection with these growth experiments, chlorophyll content, dry weight, starch content, frond area and rootlength were estimated at different ages of the series. Some results are summarised in Table I.

It is clear that a marked reduction in chlorophyll content with time occurs in the Cs and Na series. The lowest values for Cs are about 25% and for Na 50% of that in K. Per unit area, the chlorophyll content is still lower; for Cs and Na, the reductions are 33 and 75% respectively. The chlorophyll content in the Rb series is higher than that in the K series in some cases and lower in other ones. These differences must be ascribed to difficulties in separating the

TABLE I. The effect of K, Na, Rb and Cs nutrition on starch content, frond area, chlorophyll content and root length in growing cultures of *Lemna minor* L.

| Days after inoculation | Starch content | | | | Frond area as % of that in K | | | Chlorophyll content as % of that in K | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | K | Na | Rb | Cs | Na | Rb | Cs | Na | Rb | Cs |
| 3 | 1 | 1 | 1 | 3 | 112 | 105 | 90 | 106 | 126 | 65 |
| 5 | 2 | 3 | 2 | 4 | 98 | 95 | 70 | 76 | 122 | 41 |
| 7 | 2 | 4 | 3 | 4 | 100 | 105 | 81 | 75 | 76 | 22 |
| 9 | 1 | 3 | 3 | — | 87 | 73 | — | 63 | 115 | 27 |
| 12 | 3 | 4 | 2 | — | 64 | 80 | — | 50 | 82 | 27 |
| 15 | 3 | 4 | 2 | — | 60 | 76 | — | 47 | 91 | 27 |
| | | | | | Frond area K = 6.8 ± 0.6 mm²/frond | | | Chlorophyll content K = 0.185 ± 0.010/15 fronds | | |

| Days after inoculation | Dry weight/100 fronds as % of that in K | | Dry weight/ unit area as % of that in K | | Chlorophyll content unit area | | | Rooth length in cm/root | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Na | Rb | Na | Rb | Na | Rb | Cs | K | Na | Rb | Cs |
| 3 | — | — | — | — | 94 | 120 | 72 | 2,5 | 1.5 | 1.2 | 0.5 |
| 5 | 95 | 131 | 97 | 138 | 77 | 128 | 59 | 2.5 | 2.5 | 1.5 | 0.5 |
| 7 | 112 | 137 | 112 | 131 | 75 | 73 | 27 | 2.5 | 2.5 | 1.2 | 0.5 |
| 9 | 115 | 87 | 132 | 119 | 72 | 157 | 33 | 2.5 | 2.5 | 1.0 | |
| 12 | 138 | 96 | 215 | 120 | 78 | 103 | 33 | 2.5 | 1.5 | 1.0 | |
| 15 | 135 | 110 | 225 | 145 | 78 | 120 | 33 | 2.5 | 0,5 | 0.5 | |
| | Dry weight K = 13.2 ± 0.9 mg/100 fronds | | | | | | | | | | |

fronds. The content per unit area is, except on the 7th day, higher throughout the whole treatment, indicating, probably, that the fronds of the rubidium series were thicker.

Frond dry weight increases up to 30–40 % more for the Na series than for K. The dry weight per unit area in the Na series is higher and may amount to more than twice that observed in the potassium series. The interpretation of the results with Rb is somewhat difficult. No results could be obtained for Cs, since the number of fronds was too small to make accurate dry weight determinations.

Though no quantitative measurements of starch content have as yet been made, qualitative estimates were possible by staining the fronds in a solution of iodine in potassium iodide. The number 1 indicates no colour; 2: colour at the meristems; 3: pale blue over the whole area; 4: dark blue. From the table it can be seen that, in contrast to the K and Rb series a high starch content occurs in the Cs and Na series. There is a tendency in K and Rb cultures towards an increase in starch with an increase of age.

Frond area was measured from photographic contact prints of the fronds by means of a planimeter. A decrease in frond area (relative to the one obtained in K solutions) occurs in Na and Rb already after 7 days, so that relative areas of 60 % and 76 % result after 15 days. In the caesium series a similar decrease occurs already after 3 days. The lower values of the Na and Rb series may account for the higher dry weight and chlorophyll content values per unit area, compared with those per number of fronds.

It may be seen from the rough estimations of root length, that a reduction in root length occurs in the Na series after 9 days. Root length in the Rb series is smaller throughout the treatment, whereas Cs has only a few roots, none longer than 0.5 cm, thus indicating an inhibiting effect of this element on root growth.

## 4. RESPIRATION AND PHOTOSYNTHESIS STUDIES ON IN *LEMNA* RELATION TO POTASSIUM DEFICIENCY

Cultures grown in a sodium nutrient solution have a higher respiration than those in a normal potassium solution. The increase may amount to 200 %. In one case an increase was observed after three days. After seven days the increase is about twice that measured in the potassium series. No further decrease or increase in respiration is observed for periods up to 25 days.

The question arises as to whether this stimulated respiration could be reversed by addition of Na, K, Rb or Cs in short term experiments. In Table II the effects of the addition of different kations are given (final concentrations M/100).

TABLE II. The effect of Na, K, Rb and Cs addition on respiration of *Lemna minor* L., grown in a solution with sodium replacing potassium

| Hours after addition | Respiration (in % of sodium culture without addition) upon addition of | | | |
|---|---|---|---|---|
| | Na | K | Rb | Cs |
| 1 | 103 | 93 | 63 | 84 |
| 2 | 103 | 84 | 68 | 63 |
| 3 | 119 | 77 | 66 | 54 |
| 5 | 112 | 60 | 61 | 53 |

It can be seen that Na addition has a slightly stimulating effect. On the other hand, K, Rb, and Cs decrease the stimulated respiration of the sodium cultures within a few hours. It seems that Cs is the most effective of the three. In several cases the decrease results in the same level of respiration as obtained in *Lemna* grown in a nutrient solution prepared with K. As Na, K and Rb were given as chlorides and Cs as a nitrate, the effects of anions were investigated in order to see wether the effect of Cs could be ascribed to the nitrate additions. No differences were observed if Na was given as chloride, nitrate or sulphate.

From these experiments, it seems that the stimulation of respiration in Na cultures is, at least partly, reversed by K, Rb, or Cs addition. It is clear that Rb and Cs cultures probably have low respiration values also. In 7, 9, and 12 day cultures it was found that respiration was about the same as that of the potassium series. Usually, Rb cultures showed a slightly higher respiration and those with Cs a slightly lower one. In later stages the Cs cultures showed a further decrease in respiration, probably due to a shortage of assimilates or sugars since it was noticed that no growth took place. The effect of K addition was measured in Rb and Cs cultures of 7, 9 and 12 days. No increase or decrease in respiration within the limits of error was observed on potassium addition.

Besides this, also the influence of potassium deficiency on photosynthesis was studied. A survey of some results has been presented in Table III. Half-hourly measurements were made between 2–4 hours after addition, and averaged.

It follows from columns 3 to 5 that addition of K to a Na culture causes a marked increase in the rate of photosynthesis especially in older cultures. Such an increase is neither found upon addition of Na to Na cultures nor upon addition of K to K cultures.

The decrease in photosynthesis (colomn 2) is evident at 9 days, which decrease becomes progressively greater down to the low level of 11 % at 16 days. It may be repeated here, that after 5–8 days a decline in growth is observed in the so-

dium cultures. There seems to be a correlation in these experiments between the decrease in growth, the decrease in photosynthesis, and the evident increase of photosynthesis after potassium addition.

The photosynthesis data reported were all obtained under conditions of light saturation.

TABLE III. Rate of photosynthesis in sodium cultures of *Lemna minor* L., and the effect of K and Na addition in short term experiments

| Age of culture in days | Rate of photosynthesis of Na cultures in % of K cultures | Procentual change of actual rate upon addition of | | |
|---|---|---|---|---|
| | | Na to Na culture | K to Na culture | K to K culture |
| 3 | 113 | – | 6 | 0 |
| 7 | 95 | – | 7 | 7 |
| 9 | 50 | 0 | 31 | –3 |
| 12 | 45 | – | 32 | 1 |
| 16 | 11 | 5 | 150 | 20 |

Some experiments were made at different light intensities with 15 days old Rb, K, and Na cultures, with sodium or potassium chloride addition. It can be seen from figure 3 that no differences in rate of photosynthesis are observed

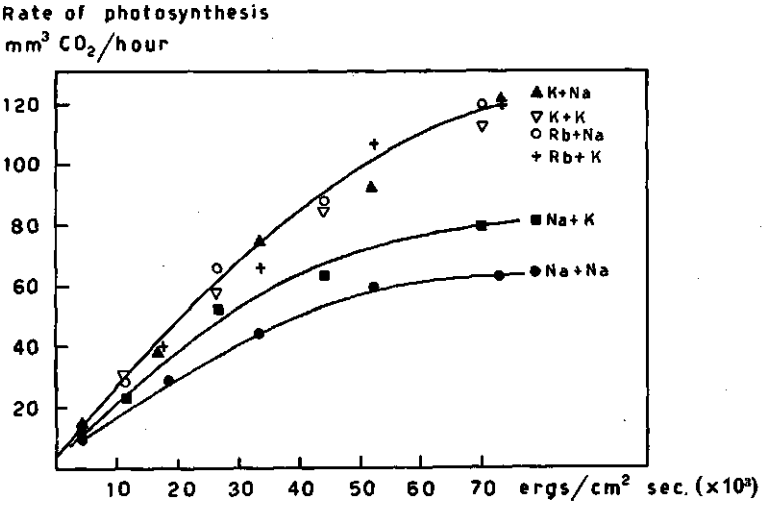


Figure 3. Rate of photosynthesis in sodium, rubidium and potassium cultures at different light intensities, and the influence of potassium addition on the rate of photosynthesis

between the Rb and K cultures, and even a potassium addition has no increasing effect on the rate of photosynthesis as compared with a sodium addition. Addition of potassium to the Na cultures, however, effects an increase in rate, as was already reported above. The increase is not restricted to light saturation, but is also observed under light limitation. The set of curves shown is of the same type as generally obtained in cases of urethane inhibition.

## 5. DISCUSSION

The various effects of potassium starvation on the metabolism of *Lemna* recorded in the present investigation are in general agreement with those reported by other authors. ALTEN and GOEZE (2) showed that a low potassium supply reduces the rate of photosynthesis and the chlorophyll content in wheat leaves and confirmed a similar finding for barley by GREGORY and RICHARDS (8). PIRSON (12) observed the same effects on *Chlorella* and *Ankistrodesmus*. Higher respiration rates were observed by PIRSON, and by ALTEN and GOEZE as a result of potassium deficiency. Some contradictory data, however, were observed in dry weight and starch content. JANSSEN and BARTHOLOMEW (11) report a decrease in starch in soybeans, cowpeas, sudangrass, clover and corn at low potassium levels. They note, however, that this relationship may be reversed in young plants. HARTT (9) and other investigators, on the other hand, have found accumulation of starch in certain species. These different findings show that the question of potassium deficiency is probably complicated by the effect of other ions present. The results reported here confirm those of WHITE with *Lemna minor*.

Because of its different behaviour towards various metabolic activities of the cells, the action of potassium is difficult to explain. We may assume that the role of potassium in photosynthesis is not that of a regulator of $CO_2$ uptake only (BUKATSCH), nor that it is operating in a $CO_2$ transfer system (ARENS). Although a decrease in photosynthesis due to K deficiency is observed both under light saturation and light limitation, it is at least partly reversible when potassium is added. Furthermore, the higher respiration rates at low potassium levels, which are readily reversed in short term experiments by K, Rb, or Cs addition, do not support the view of a regulation of $CO_2$ permeability only. The influence of potassium deficiency on photosynthesis and respiration may be compared with inhibition phenomena as observed, *e.g.*, with the urethanes. Inhibition of photosynthesis by these inhibitors occurs both in light saturation and in light limitation. Along with moderate inhibitions of photosynthesis, an increase up to 200 % in the rate of respiration is often observed, which effects may be counteracted by washing. The close resemblance between the effect of K deficiency and inhibition by the urethanes strongly suggests that the action may be similar in both cases.

Substitution of K by Cs inhibits growth of *Lemna* in the concentrations used in these experiments. Similar findings with Cs were obtained by PIRSON with *Chlorella* and by ALTEN and GOTTWICK with oats and maize. Rb, however, can replace K, though a lower rate results. The abnormal appearance of the fronds of the rubidium cultures of *Lemna* described in this paper is interesting. Root length also is smaller than in the potassium series. In higher plants no growth is obtained by substituting K by Rb (ALTEN and GOTTWICK [1]). On the other hand, in lower organisms, substitution can be fully realised, as has been observed in *Chlorella* by PIRSON. In *Lemna*, according to the above observations, the behaviour seems to be somewhat intermediate.

Some remarks may be made on the relation between potassium and carbohydrate metabolism. Photosynthesis and growth are reduced in the Na cultures after about 7 days. At the same time an increase in respiration and frond dry weight occurs; especially the latter fact indicates that the reduction in growth is not paralleled by an equal reduction in photosynthesis. This may lead to starch

accumulation. It is not clear why starch accumulation is observed already after 5 days. However, it is known that potassium plays an important role in starch hydrolysis. So it may be that hydrolysis of starch is affected more than starch accumulation. Before any more comments can be made, however, exact measurements of starch content are required. It should be remarked that these findings do not at all prove that the effect of K on starch hydrolysis is a direct one. It simply means that somewhere the path of starch utilisation is blocked. This might, *e.g.*, as well find its cause in some process belonging to protein synthesis.

SUMMARY

1. A study has been made of the growth and reproduction, respiration and photosynthesis of *Lemna minor* grown in a complete nutrient solution or in solutions in which potassium was substituted by sodium, rubidium or caesium.
2. Exponential growth has been obtained in the potassium and rubidium cultures, though in rubidium at a lower rate of increase. Sodium cultures show a progressive decline from exponential growth after 5–8 days. Complete inhibition is observed in the caesium cultures.
3. In the sodium cultures potassium deficiency causes a decrease in the rate of photosynthesis, in the chlorophyll content, the frond area and the rootlength, and an increase of respiration, frond dry weight and starch content. In the rubidium cultures, only a decrease in frond area and rootlength, and an increase of dry weight per unit area were observed as compared with potassium cultures.
4. The decrease in the rate of photosynthesis occurring upon potassium deficiency both under conditions of light saturation and light limitation can, at least partly, be reversed upon potassium addition in short time experiments.
5. The increase of respiration on potassium deficiency can be reversed in short time experiments on potassium, rubidium or caesium addition.

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