

# INFLUENCE OF THE CHLORIDE ION ON THE CONTENT OF CARBOHYDRATES IN POTATO LEAVES<sup>1</sup>

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(WITH THREE FIGURES)

From the literature and from numerous experiments made at the Scientific Institute of Fertilizers, it is well known that high amounts of chlorides lower the percentage of starch in potato tubers. While the depressing influence of fertilizers containing chlorine on the total yield of potatoes is not always manifested, the decrease of the starch content in the tubers may be considered a constant attendant of the introduction of high amounts of chlorides.

There are some indications that the use of sulphate of potassium is preferable when fertilizing plants which are cultivated for carbohydrates (2). The depressing action of the chloride ion on the accumulation of starch in potato tubers may be called forth by various causes. It is known from literature (5) and from our experiments (1), that large doses of chlorides lower the content of chlorophyll in plants. MONTFORT (4), who observed a sharply decreased synthesis in leaves when immersed into chloride solutions, explains this phenomenon by the destruction and therefore by the decrease of the total amount of chlorophyll in those leaves. On the other hand the chlorides may also exert an influence on the activity of the amylase and therefore on the conversion of starch secondarily formed in the tubers. The increased conversion of starch under the influence of chlorides was observed by SCHMETZ (6), MONTFORT (4) and others. However, these authors confined themselves mostly to qualitative indices; they estimated the synthesis and the conversion of starch by the intensity of the iodine color test. Much attention has been given in this laboratory during recent years to the study of the physiological action of chlorine. It is the purpose of our work to show the influence of chlorine on the photosynthetic activity and on the accumulation of carbohydrates in the potato leaves. In this paper are presented the results of experiments concerning the influence of chlorides only on the content of carbohydrates. We considered two groups of carbohydrates, starch, and the total soluble sugars.

The experiments were conducted with an early variety of potato, "Epicure," during the years 1933 and 1934.

## Experiments of 1933

In 1933 field and pot experiments were carried out. The field experiments on the Dolgoprudni Experiment Station were started according to the

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following plan: Plot 1, control; fertilizers were given in the form of urea,  $\text{KNO}_3$ , and of double superphosphate; that is, without chlorine and without  $\text{SO}_4$ . Plot 2, nitrogen and potassium were given in the form of chlorides, the amount of chlorine per hectare being 195 kg.; the fertilizers were introduced the first year. Plot 3, analogous to plot 2, but the fertilizers were introduced during two consecutive years. Plot 4, nitrogen was given in the form of  $\text{NH}_4\text{Cl}$ , and potassium in the form of  $\text{K}_2\text{SO}_4$ , and the amount of chlorine was 150 kg. per hectare.

The nutritive substances were given in proportion of 60 kg.  $\text{K}_2\text{O}$ ,  $\text{P}_2\text{O}_5$ , and N per hectare.

The pot experiment was started in sand cultures according to the following scheme:

FERTILIZERS USED	PER KG.		PER POT	
	Cl-	$\text{SO}_4^-$	Cl-	$\text{SO}_4^-$
NPK .....	0.016	0.048	0.22	0.67
“ + Cl- .....	0.226	.....	3.16	.....
“ + Cl- .....	0.436	.....	6.10	.....
“ + $\text{SO}_4^-$ .....	.....	0.336	.....	4.70

The nutritive mixture of HELLRIEGEL was taken as a basis. The amounts of N, P, and K per pot were kept unchanged.

Planting of the field plants was carried out on May 29th; the planting in pots, on June 9th, one plant per pot.

The addition of chlorides caused a considerable decrease of the yield of tubers in the field experiment and a less abrupt decrease in tuber yield in the pot experiments.

At the time of the last harvesting on the 21st of August we had the following yields:

TABLE I  
YIELD DATA OF PLANTS OF THE POT EXPERIMENT

FERTILIZER USED	DRY WEIGHT OF LEAVES AND STEMS	FRESH WEIGHT OF TUBERS
NPK .....	<i>gm.</i> 19.33	<i>gm.</i> 365
“ + 3.16 gm. Cl- per pot .....	18.81	382
“ + 6.10 gm. Cl- “ “ .....	19.06	342
“ + 4.70 gm. $\text{SO}_4^-$ “ “ .....	18.22	365

TABLE II

FRESH WEIGHT OF THE TUBERS OF THE FIELD EXPERIMENT PER ONE PLANT

	<i>gm.</i>
Plot 1. Control .....	666
“ 2. 195 kg. Cl <sup>-</sup> one year .....	536
“ 3. 195 kg. Cl <sup>-</sup> two years .....	541
“ 4. 150 kg. Cl <sup>-</sup> + SO <sub>4</sub> <sup>=</sup> .....	616

No special symptoms of depression in the above-ground parts were observed in those experiments, with the exception of lighter coloring of the leaves in the plants of the series containing chlorides.

The percentage of chlorine in the plants varied according to the dose of chlorides introduced. Thus in the field experiment toward the end of vegetation, the percentage of chloride ion in the above-ground tissues of the plants of plot 1 was 0.04; of plot 2, 2.22; of plot 4, 1.94 per cent.

For analyses of carbohydrates the pot and field plants were harvested at four dates.

The plants were usually harvested at 8–9 A.M., were divided into two separate fractions (leaves, and stems) which were then carefully cut up into small pieces. An average sample was taken from the finely cut-up material and fixed in boiling alcohol. The separation of the carbohydrates into total sugars and starch was performed essentially according to the method suggested by Prof. A. KIZEL (3).

The principles of the method may be stated as follows: the soluble carbohydrates (sugars) are extracted with 82 per cent. alcohol. After evaporating the alcohol with partial vacuum at 40° C., the solution is cleared with lead acetate, and the excess lead removed with Na<sub>2</sub>SO<sub>4</sub>. After this the solution is hydrolyzed for 3 hours on a boiling water bath with 2 per cent. HCl.

Starch is determined in the residue from the alcohol extraction. Hydrolysis is performed by diastase. After complete saccharification (disappearance of reaction to I) the material is further filtered, and the filtrate is subjected to a 3-hr. hydrolysis with 2 per cent. HCl. In both cases the carbohydrates were determined as glucose by the method of BERTRAND.

In the field experiment triplicate and in the pot experiment duplicate samples were made.

At the last date of harvesting the field experiment, the starch was determined by the specific weight of the tubers. A marked decrease was obtained, as compared with the control plants, on the chlorine series. On the control, 14 per cent.; on the plot receiving Cl one year, 10.9 per cent.; on plot receiving Cl + SO<sub>4</sub>, 11.25 per cent.

In the following tables we present only the results of the determination

of carbohydrates for only one date, as at all the other dates the results were essentially the same.

TABLE III

CONTENT OF CARBOHYDRATES IN THE LEAVES AND STEMS OF POTATOES OF THE FIELD EXPERIMENT IN PERCENTAGE OF FRESH SUBSTANCE

FERTILIZERS USED	SOLUBLE CARBOHYDRATES (SUGARS)	STARCH	TOTAL CARBOHYDRATES	RELATIVE CONTENT OF STARCH
(a) leaves				
	%	%	%	%
NPK .....	0.48	0.08	0.56	14
“ + Cl- one year .....	0.16	0.17	0.33	51
“ + Cl- + SO <sub>4</sub> <sup>-</sup> .....	0.32	0.13	0.45	29
(b) stems				
NPK .....	1.21	0.45	1.66	26
“ + Cl- one year .....	0.52	0.22	0.74	29
“ + Cl- + SO <sub>4</sub> <sup>-</sup> .....	0.81	0.51	1.32	38

The total of carbohydrates in the leaves, as well as in the stems of the plants of the chlorine series is considerably reduced. If we assume the total of carbohydrates of each separate series as being 100, and calculate the content of starch in relation to the latter, then there is apparent a relatively increased content of starch in the leaves and stems of the chlorine variants, which is especially sharply expressed in the leaves.

TABLE IV

CONTENT OF CARBOHYDRATES IN THE LEAVES AND STEMS OF POTATOES OF THE POT EXPERIMENT IN PERCENTAGE OF FRESH SUBSTANCE

FERTILIZERS USED	SOLUBLE CARBOHYDRATES (SUGARS)	STARCH	TOTAL CARBOHYDRATES	RELATIVE CONTENT OF STARCH
(a) leaves				
	%	%	%	%
NPK .....	0.82	0.25	1.07	23
“ + 0.226 gm. Cl- .....	0.54	0.26	0.80	32
“ + 0.436 “ “ .....	0.50	0.31	0.81	38
“ + 0.336 “ SO <sub>4</sub> <sup>-</sup> .....	0.76	0.25	1.01	24
(b) stems				
NPK .....	0.82	0.22	1.04	17
“ + 0.226 gm. Cl- .....	0.93	0.19	1.12	17
“ + 0.436 “ “ .....	0.80	0.21	1.01	20
“ + 0.336 “ SO <sub>4</sub> <sup>-</sup> .....	0.93	0.22	1.15	19

In the leaves of plants from these pot experiments one observes the same response as in the case of leaves of plants in the field experiment; that is,

with a generally smaller total of carbohydrates in the plants rich in chlorine, the relative content of starch is found to be increased. On the other hand, more or less equal figures were obtained in the case of the stems for all variants.

These data are of interest, because they offer some possibility of separating the action of  $K^+$  from that of  $Cl^-$ . The plants of the field experiment on chlorine variants which showed an increase in chlorine content as compared with the control plants, showed also a parallel increase in content of potassium: the control, 1.7 per cent.; with a large amount of chlorine, 2.6 per cent.

On the other hand, in the pot experiments there was hardly any difference in the content of potassium between the plants of the variants NPK + 0.226 gm.  $Cl^-$ , and NPK + 0.336 gm.  $SO_4^-$  per pot. In the plants of this last variant the amount of potassium was even somewhat higher. This allows us to refer the differences in the content of carbohydrates observed in the above mentioned experiments, largely to the differences in the content of chlorine.

#### Experiments of 1934

Only pot experiments on sand cultures according to a plan analogous to that of 1933 were carried out.

The carbohydrates were determined only in the leaves. In these experiments of 1934 it seems more interesting to represent the content of carbohydrates by curves of the daily course of their accumulation.

For analyses the leaves were cut off at definite hours: at 5 A.M., at noon, and at 6 P.M.; they were then weighed and fixed in boiling alcohol. On July 27, the material of all four variants was fixed simultaneously. On August 17, and August 21, samples from plants of two variants were taken. The method of determining the carbohydrates was the same as in the experiments of 1933.

The results are presented in table V.

Most interesting are the data of August 27 when all eight samples were taken in one day, so that all four series might be comparable with one another.

First of all, stands out the decrease of the sum of carbohydrates in the leaves of plants of the chlorine variants at noon, and in the evening. Early in the morning, however, their content of soluble carbohydrates is even somewhat greater than in the control plants. Furthermore, if the total of carbohydrates is assumed to be 100, then the relative content of soluble carbohydrates (sugar) and starch may be expressed by the following values.

The relative content of starch in the leaves of plants in the chlorine variants is higher than in plants receiving no chlorine. The excess of starch content over the soluble carbohydrate content in the chlorine variants begins

TABLE V  
 CONTENT OF CARBOHYDRATES IN THE LEAVES OF POTATOES IN PERCENTAGE OF FRESH SUBSTANCE

DATE	FERTILIZER USED	5:00 A.M.			NOON			6:00 P.M.		
		SUGAR	STARCH	TOTAL	SUGAR	STARCH	TOTAL	SUGAR	STARCH	TOTAL
August 17	NPK	%	%	%	%	%	%	%	%	%
" "	" + 6.10 gm. Cl <sup>-</sup>	0.36	0.05	0.41	0.62	0.41	1.03	0.65	0.42	1.07
August 25	NPK + 3.16 gm. Cl <sup>-</sup>	0.64	0.01	0.65	0.07	0.20	0.27	0.14	0.33	0.47
" "	" + 4.70 gm. SO <sub>4</sub> <sup>-2</sup>	0.08	0.01	0.09	0.28	traces	0.28	0.22	0.30	0.52
August 27	NPK	0.49	0.01	0.50	0.42	"	0.42	0.47	0.30	0.77
" "	" + 3.16 gm. Cl <sup>-</sup>	0.24	0.02	0.26	0.77	0.71	1.48	0.56	1.44	2.00
" "	" + 6.10 gm. Cl <sup>-</sup>	0.26	0.07	0.33	0.65	0.74	1.39	0.41	1.37	1.78
" "	" + 4.70 gm. SO <sub>4</sub> <sup>-2</sup>	0.32	0.19	0.51	0.31	0.87	1.18	0.25	1.18	1.43
" "	"	0.41	none	0.41	0.74	0.56	1.30	0.61	1.31	1.92

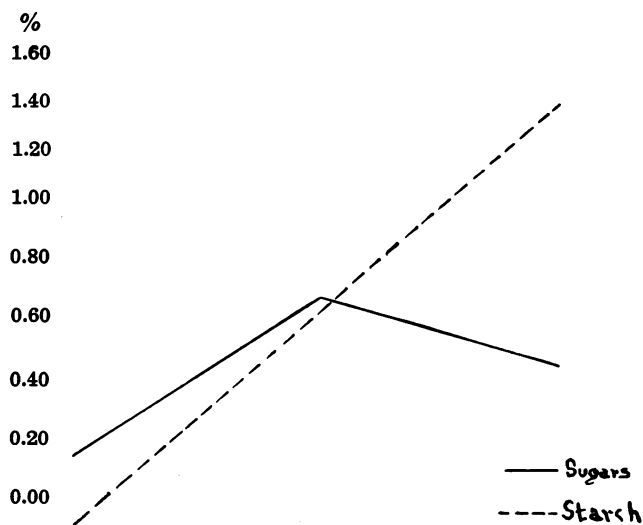


FIG. 1. Content of starch and sugars in per cent., with NPK fertilizers.

TABLE VI

RELATIVE CONTENT OF SUGAR AND STARCH IN THE LEAVES OF POTATOES ON AUGUST 27, 1934

FERTILIZERS USED	5:00 A.M.		NOON		6:00 P.M.	
	SUGAR	STARCH	SUGAR	STARCH	SUGAR	STARCH
NPK .....	%	%	%	%	%	%
NPK .....	90	10	52	48	27	73
“ + 3.16 gm. Cl <sup>-</sup> .....	78	22	47	53	23	77
“ + 6.10 gm. Cl <sup>-</sup> .....	63	37	28	72	17	83
“ + 4.70 gm. SO <sub>4</sub> <sup>=</sup> .....	100	00	56	44	31	69

at earlier hours than in the control plants. This is seen especially clearly from figures 1, 2, and 3. The decrease of the total content of carbohydrates in the leaves, the relatively greater accumulation of starch in them, and the greater content of soluble carbohydrates in the morning hours in the leaves of plants of chlorine variants, all allow us to assume a slackening of the photosynthesis in those leaves, with a consequent decrease in the formation of carbohydrates, and in addition, a weaker movement of the sugars from the leaves to the roots.

Direct measurements of the energy of photosynthesis in the leaves of the potato, conducted during these experiments, confirm this assumption concerning the decreased energy of photosynthesis in the plants receiving chlorine. But these results will be reported in another communication.

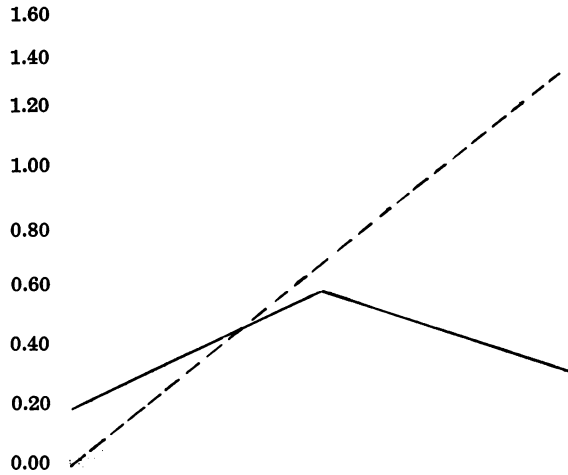


FIG. 2. Content of starch and sugars in per cent., with NPK + 3.16 gm. Cl.

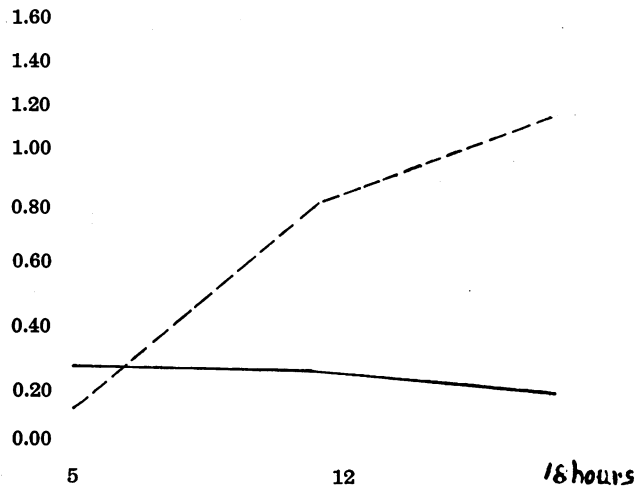


FIG. 3. Content of starch and sugars in per cent., with NPK + 6.10 gm. Cl.

The decrease of the amount of carbohydrates in the leaves of potatoes is obviously connected with the weakening of the photosynthesis activity due to a decrease in the amount of chlorophyll per unit of fresh leaf surface.

As regards the relative enrichment of the leaves with starch, we have as yet no data explaining the cause of this phenomenon. Either the movement of the starch to the tubers was retarded, or else the soluble carbohydrates were utilized more energetically in the respiratory process.

Studies of the influence of chlorine on the formation and hydrolysis of



starch, and on the activity of the enzymes concerned in carbohydrate metabolism are being carried out in our laboratory.

The general conclusions from the work presented in this paper are as follows:

1. Large doses of chlorides decrease the total sum of the carbohydrates in the leaves. This is obviously connected with a lowered content of chlorophyll per unit of leaf surface and a weakened photosynthetic activity.

2. The leaves of plants of the chlorine series are relatively richer in starch than the leaves of the control plants. Further investigations are necessary to explain this phenomenon.

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