THE EFFECTS OF PHOSPHORUS SUPPLY ON THE RATES OF INTAKE OF PHOSPHORUS AND NITROGEN AND UPON CERTAIN ASPECTS OF PHOSPHORUS METABOLISM IN GRAMINEOUS PLANTS

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Summary

For experiments previously described, new data are presented relating to the intake of phosphorus by gramineous plants, its distribution within the plant, and its partition in the leaves between alcohol-soluble, nucleic-acid, and "residual" phosphorus.

Major determinants of the rate of intake of phosphorus by the plant are: (*a*) the demand set up by the growth and normal functioning of various plant parts, and (*b*) the concentration of the nutrient in the medium.

It is considered that the indirect effect of phosphorus treatment on growth, and hence on demand, is more important than the direct effects of external concentration of phosphorus on the rates of intake of that nutrient.

Even when growth is not limited by the supply of phosphorus, the rate of intake is limited by the maximal capacity of successive plant parts for accumulation.

For the oat experiment, rates of intake of phosphorus and nitrogen are expressed per unit weight of root system. With nitrogen, the supply of which was the same for all treatments, large initial effects of phosphorus treatment on the rates of intake are accounted for in terms of differences in the ratios of roots to shoots.

Phosphorus-deficient oat plants derived only 30 per cent. of their inflorescence phosphorus from other plant parts; those with an excessive supply derived no less than 93 per cent. of their inflorescence phosphorus from these sources.

The percentage phosphorus contents of the stems, leaves, and roots fell ultimately to lower values with a moderate supply of phosphorus than they did with the deficient supply. A more efficient re-utilization of phosphorus in the plants receiving the greater supply is favoured as an interpretation of this "dilution" effect.

From an examination of the data for protein-nitrogen and nucleic-acid phosphorus in the leaves of young plants, it is concluded that the effects of phosphorus treatment on protein-nitrogen content at this stage are due primarily to variation in nucleoprotein content.

With phosphorus deficiency, oat seedlings soon exhausted their seed reserves of phosphorus, and this was followed by a drastic change in the partitioning of leaf phosphorus, such that absolute nucleic-acid phosphorus was reduced to one-fifth of the value obtaining eleven days earlier, alcohol-soluble phosphorus decreased slightly, and the water-soluble fractions represented by "residual" phosphorus increased by 30 per cent. Total phosphorus was virtually unchanged in amount. During this same period of eleven days, the whole of the phosphorus intake from the medium was retained by the roots, and there was a stimulation of root growth relative to leaf growth. These facts suggest that the high root weight ratios found with phosphorus deficiency may be due to the fixation in organic forms of a greater proportion of the absorbed phosphorus, so that relatively little is available for shoot growth.

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Nucleo-cytoplasmic ratios of oat and Phalaris leaves are presented for a wide range of growth stage and nutrient treatment. No obvious correlations with growth data such as the relative leaf growth rates were revealed.

The literature relating to the partitioning of phosphorus within plant tissues is discussed and, where comparisons are relevant, this is in substantial agreement with the work reported here.

The theory of biological explanation is considered in relation to certain growth phenomena, and it is indicated that explanation should be sought at the biological as well as at the physico-chemical level of organization.

I. Introduction

The data presented in this paper all derive from two experiments in which phosphorus supply has been varied, and plants have been harvested and examined at a number of growth stages. The data for the main experiment – that with oats – have already been examined with respect to quantitative growth analysis (Williams 1936, 1939), the intake and distribution of nitrogen within the plant (Williams 1938), and the respiration of the leaves (Petrie and Williams 1938): the new data relate to the intake of phosphorus itself, its distribution within the plant, and its partition between inorganic and certain groups of organic compounds. As no extension of this work is contemplated, it is desirable to attempt some synthesis of the work, and to extract some general principles.

Additional data are also presented for an experiment with *Phalaris tuberosa* L., when two levels of phosphorus and nitrogen were supplied in all combinations. The growth analysis of this experiment appears elsewhere (Williams 1946).

In a general discussion of the upward movement and distribution of solutes, Hoagland (1944) stresses the need to envisage the plant as an integrated organism. Thus the growth and functioning of the roots are dependent upon metabolites normally deriving from the shoot. Likewise the distribution of solutes is not merely a necessary consequence of the movement of water containing these solutes. This latter point was illustrated by reference to work of Arnon, Stout, and Sipos (1940), where radioactive phosphate was used in a study of the movement of ions to developing fruit of the tomato plant. Treatment was applied when the plants already bore fruits of all sizes, and it was found that the radioactive phosphate accumulated to the greatest extent in the young growing fruit, and not in the regions of highest water loss. Here the control seemed to reside in the meristematic tissues having a high rate of metabolism.

Petrie (1934) also discusses the accumulation and redistribution of nutrients within the plant, and he contrasts the behaviour of potassium and calcium in these respects. The later-formed organs derive part, if not all, of their potassium from those formed earlier, but supplies of calcium are usually derived only from the external medium. The difference is attributed to the lesser mobility of the calcium ion, and to its almost irreversible chemical fixation in the tissues. Loss of potassium from a given organ and from the plant is thought to commence with the attainment of the maximum sap concentration attainable under the conditions, and this maximum is believed to decline with age. Petrie, however, is not concerned with the case where potassium is in short supply; under these

conditions he recognizes that the transfer of ions to other cells and organs might prevent the attainment of this maximum sap concentration.

The behaviour of nitrogen and phosphorus with respect to their accumulation and redistribution is undoubtedly more complex than that of potassium or calcium. The former are fixed organically in a variety of compounds, and this fixation is quite definitely reversible. An important point of difference between phosphorus and nitrogen is that, relatively speaking, the cell is able to accumulate much larger amounts of inorganic and soluble-organic compounds of phosphorus. To this extent phosphorus may have some features in common with potassium. Be that as it may, there would seem to be considerable scope for integrated control of the movement of nitrogen and phosphorus within the plant, and an attempt is made to develop this concept of integrated control in relation to the data presented here. The concept itself is consistent with Frank's (1935) definition of the problem of organic growth as "the measurement of the changing dimensions of structure-function activity of the organism, and the discovery of the sequence in which these changes occur."

The later sections of this paper are largely devoted to the discussion of alcohol-soluble, nucleic-acid, and residual phosphorus data for the leaf material of the two experiments mentioned above. This part of the paper could perhaps have been treated as an independent unit, though the data are relevant to an understanding of the redistribution of phosphorus within the plant. A review of the literature relating to the fractionation or "partition" of phosphorus within plant tissues is presented as part of the general discussion.

Details of the two experiments have been given before (Williams 1936, 1946); only the bare essentials are given here. The experimental plants were grown, five per pot, in water-washed sand (14 and 15 kg. per pot in the respective experiments) and with abundant water supply. In the oat experiment, phosphorus was applied as sodium acid phosphate. Nitrogen and phosphorus were applied to Phalaris as calcium nitrate and calcium acid phosphate respectively, calcium being then equalized for all treatments by the addition of appropriate amounts of calcium chloride.

The amounts per pot were:

A. Algerian Oats (*Avena sativa* L.)

Treatment	I (P_1)	0.008 g. P per pot.
„	II (P_2)	0.09 „ „ „ „
„	III (P_3)	0.6 „ „ „ „

B. *Phalaris tuberosa* L.

Treatment	P	N	Cl
P_1N_1	0.04 g.	0.4 g.	3.15 g. per pot.
P_1N_2	0.04 „	1.6 „	2.05 „ „ „
P_2N_1	0.24 „	0.4 „	3.06 „ „ „
P_2N_2	0.24 „	1.6 „	1.97 „ „ „

The treatment terrminology, P_1, P_2, etc., has been adopted as being more descriptive than that previously used.

Since the greater part of the discussion centres around the oat experiment, it will be helpful to give a statement of the stages of development reached by the plants at the successive harvests.

Harvest 1.—First leaf well through the coleoptile and developing rapidly.

Harvest 2.—First leaf mature (i.e. attained maximum area) and second leaf just showing.

Harvest 3.—First and second leaves mature and third leaves showing in many cases. Tillering commencing, especially in P_2 and P_3.

Harvest 4.—Fourth leaf mature and fifth leaf developing. Several tillers per plant, with more in P_2 and P_3 than in P_1.

Harvest 5.—Leaves at peak of their development and stems growing rapidly.

Harvest 6.—Flowering in P_1 but post-flowering in P_2 and P_3.

Harvest 7.—Full maturity, leaves dead.

II. Analytical Methods

Total phosphorus was estimated by the colorimetric method of Zinzadze (1931, 1935) after wet-ashing with nitric acid and perhydrol. In more recent work perchloric acid has been used in place of perhydrol. Appreciable blue colouration was found in blank estimations, and this was traced to siliceous impurities in the sodium bicarbonate (or anhydrous carbonate) used to neutralize the solutions prior to treatment with sodium metabisulphite. A constant excess of bicarbonate was therefore added, and the solutions were back titrated with normal sulphuric acid.

Methods for the estimation of the phosphorus-containing organic compounds of biological materials are far from satisfactory, and this in spite of their physiological importance. However, the method of Javillier and Allaire (1931) and Javillier and Colin (1933) for the estimation of nucleic-acid phosphorus (thought to be desoxyribose plus ribose nucleic acid) has been modified (Williams 1945) and was used in the present work.

Since this method requires the pre-extraction of the plant material with alcohol, the alcohol-soluble phosphorus was also determined. As a measure of phosphatide phosphorus, this latter is admittedly crude, for it has been shown by Jordan and Chibnall (1933) that considerable quantities of non-phosphatide phosphorus may be extracted by this solvent. More recent work by Rewald (1936, 1937*a*, 1937*b*) points to the inadequacy of any one solvent or solvent mixture for the extraction of phosphatides, and his work on "free" and "bound" phosphatides offers new opportunities in the study of phosphorus metabolism.

In the present investigation, the oven-dry leaf samples were extracted for 15 hours with absolute alcohol in Soxhlet extractors. The capacity of the upper vessels of the latter was 65 ml., and the siphoning rate approximately 9 times per hour. The selection of a 15-hour extraction period was arbitrary, for it was found that additional small increments of phosphorus were extractable up to, and probably beyond, a total period of 30 hours. In the samples tested, however, the amount of phosphorus extracted during the first 7.5-hour period was of the

order of ten times the amount extracted during the second period. The phosphorus contents of all extracts were determined by the method of Zinzadze (loc. cit.).

Jordan and Chibnall (1933) used ether alone for extracting phosphatides; thus a comparison of the alcohol- and ether-soluble phosphorus of 6 samples of leaf material from the present oat experiment is of interest (see Table 1). The extraction period was the same for each solvent. In rapidly-growing leaf material (harvest 4) the relative amount extractable with ether was negligible, whereas it increased to about 25 per cent. in senescent leaves (harvest 6). If these dis-

TABLE 1

ALCOHOL- AND ETHER-SOLUBLE PHOSPHORUS IN OAT LEAVES*

Solvent	Harvest 4		Harvest 5		Harvest 6	
	P_2	P_3	P_2	P_3	P_2	P_3
Anhydrous ether, E	4**	7**	8.2	10.7	8.1	25
Absolute alcohol, A	260	410	86	135	33	94
E/A x 100	<2	<2	9.5	7.9	24.5	26.6

* Parts per million. ** Very approximate values.

crepancies in time and between solvents are attributable mainly to "the extraction of water-soluble phosphorus by the boiling alcohol," as suggested by Jordan and Chibnall, then the data presented here are worthless as indices of phosphatide content. It is possible, however, that the bulk of the phosphatides present were insoluble in anhydrous ether, and this interpretation gains support from the finding of Halpern (1945) that phosphatides are so strongly held by proteins that certain solvents including ether do not extract any phosphatide at all from salmon roe. In either case the data must be interpreted with caution.

A third and very arbitrary phosphorus fraction, here styled "residual" phosphorus is obtained by the difference between total phosphorus and the sum of alcohol-soluble and nucleic-acid phosphorus. It is an index of those compounds which occur primarily in the aqueous phases of the cell, and would include inorganic and hexose-phosphates.

The method used for the estimation of protein nitrogen is described by Petrie (1937), the precipitant being tannic acid.

III. Presentation of Data and Discussion

The data relating to the intake and distribution of phosphorus within the plant as affected by phosphorus supply and stage of growth are summarized in the lower portion of Figure 1, where the absolute phosphorus contents of the roots, stems, leaves, and inflorescences of oats are plotted additively. The primary data are presented in Table 4, and parallel data relative to nitrogen for this same experiment are presented elsewhere (Williams 1938, Fig. 5 and Tables 3, 4, and 7).

(*a*) *The Rate of Intake of Phosphorus*

Watson and Petrie (1940, p. 331), when discussing the intake of nitrogen by the tobacco plant, visualized three major determinants of the rates: (*a*) the external concentration or supply of the nutrient; (*b*) the capacity of the roots

for intake; and (c) the capacity of the tissues as sinks for the nutrient. The third of these determinants may be restated as the demand for the nutrient set up by the growth and normal functioning of various plant parts, including the roots.

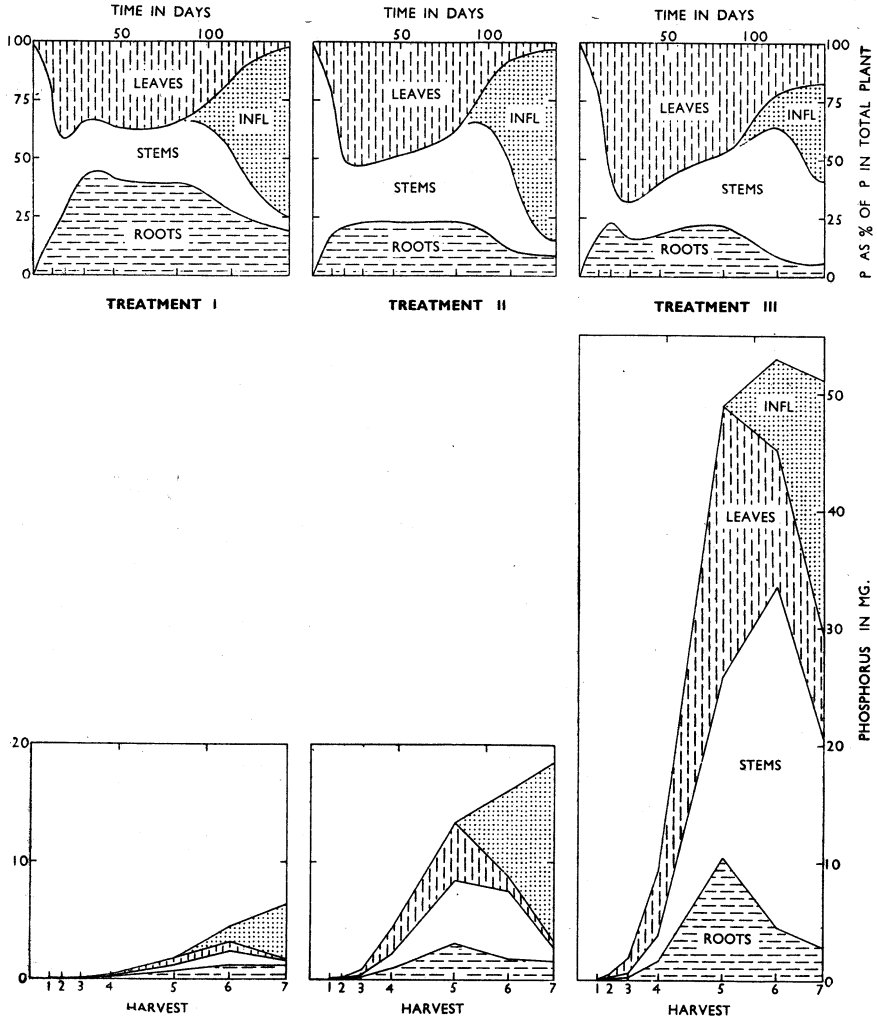

Fig. 1.—The intake of phosphorus by oats and its distribution within the plant. Above: The phosphorus present in different parts expressed as percentage of the total phosphorus in the plant. Below: The absolute phosphorus content of the whole plant and its parts. The treatments are low (P_1), medium (P_2), and high (P_3) supplies of phosphorus respectively.

In this form it also covers the second determinant, at least in the sense that this is developed by Watson and Petrie. It is considered that the influence of the external concentration of the nutrient on intake is more often subject to the controlling influence of the growth factors than otherwise; for this reason the internal or growth factor is considered first. The mean rates of intake of phosphorus and nitrogen per day are presented in Table 2.

(i) *The Internal or Growth Factor*

Each vegetative organ of a plant passes through phases of intake, relatively constant content, and of export of many of its mineral constituents.* Each organ thus has a certain capacity to accumulate nutrients such as phosphorus, and in its senescent phase it constitutes a potential source of these nutrients for younger plant parts. That the demand of younger organs for nitrogen must be regarded as the main factor causing loss of nitrogen from mature leaves has been suggested for barley by Walkley (1940), and for tobacco by Watson and Petrie (1940), and it is probable that export of phosphorus is initiated in the same way. At the same time, the demand of each organ for phosphorus is usually met in part, if

TABLE 2

MEAN RATES OF INTAKE* OF PHOSPHORUS AND NITROGEN BY OATS AS AFFECTED BY PHOSPHORUS SUPPLY AND AGE

Harvest Interval	Phosphorus			Nitrogen		
	P_1	P_2	P_3	P_1	P_2	P_3
1-2	−0.0004	0.0182	0.059	0.122	0.154	0.149
2-3	0.0023	0.058	0.131	0.170	0.416	0.325
3-4	0.019	0.208	0.441	0.474	2.80	2.65
4-5	0.039	0.250	1.098	1.58	2.08	2.21
5-6	0.087	0.087	0.129	0.93	−0.09	0.01
6-7	0.057	0.095	−0.067	−0.02	0.02	−0.20

* Mg. per day.

not entirely, by absorption from the external medium; this rate of intake by the roots, however, is restricted to the extent that phosphorus is more readily available within the plant itself.

Evidence for this general scheme may be adduced from the present experiment. Thus, during the early development of the seedling, the grain is the sole source of phosphorus, and for some time it remains an important alternative source, especially if the external medium is deficient in phosphorus. Between harvests 1 and 2 (days 11-18), the leaves and roots of P_1 derived all their phosphorus from the "stems" (which included the grain); whereas with P_2 and P_3, the "stem" fractions themselves showed net gains of phosphorus.

During the phase of rapid vegetative growth (between harvests 3 and 5), the roots, stems, and leaves all accumulated phosphorus rapidly, and the influence of external supply is very evident (see the absolute increments of Table 3). As between P_1 and P_2, however, much of the difference in intake is attributable to the very much greater rate of growth of the plant parts with P_2. This will be evident from an inspection of the increments of phosphorus per unit increase in dry weight of the parts (Table 3). Thus for the leaves of harvest-interval 3-4,

* In this discussion it is understood that we are concerned only with net change over periods of several days, though the same principles would doubtless hold for the interpretation of diurnal fluctuations such as those suggested by the work of Phillis and Mason (1942*a*) with leaves of the cotton plant. By contrast, studies with radioactive phosphorus (e.g. Biddulph 1941) are concerned with rates of translocation as such.

the absolute increase was more than fourteen times greater with P_2 than it was with P_1, but the increase per unit increase of dry matter was little more than twice as great with P_2 as it was with P_1. For interval 4-5 this treatment difference would seem to be due entirely to the growth factor, all three rates of increase per unit increase in dry weight being lower with P_2 than with P_1. With P_3, the rates of growth of the parts were little if at all greater than those with P_2 (Williams 1936), so that practically all of the difference in intake of phosphorus over this period could perhaps be attributed to differences of external supply. Even so, it is noteworthy that the absolute phosphorus increments for the three plant parts of P_3 are almost proportional to their respective weight increments, the increments per gram increase in dry weight being approximately equal within each harvest-interval, whereas they vary greatly in P_2. This suggests that a general state of

TABLE 3

INCREMENTS IN ABSOLUTE PHOSPHORUS CONTENT OF THE LEAVES, STEMS, AND ROOTS OF OATS FOR HARVEST-INTERVALS 3-4 AND 4-5

Increments (mg.)	Interval 3-4 Days 29-46			Interval 4-5 Days 46-82		
	P_1	P_2	P_3	P_1	P_2	P_3
Leaves	0.119	1.72	4.29	0.471	2.83	17.55
Stems	0.070	1.00	1.82	0.381	4.11	13.13
Roots	0.131	0.82	1.39	0.546	2.05	8.84
Increments (mg. per g. increment in dry weight)						
Leaves	1.37	2.99	7.58	0.61	0.50	2.74
Stems	2.45	4.38	7.26	1.00	0.88	2.79
Roots	1.31	3.56	7.10	0.93	0.69	2.31

saturation with phosphorus exists in the tissues, and that the sap concentration may be approaching the maximum attainable under the conditions (Petrie 1934, see above). In these circumstances phosphorus should be present in sap-soluble form to a relatively greater extent in P_3 than in P_2, and this is supported by the data for "residual" phosphorus in the leaves (see Table 6), "residual" phosphorus being an index of the phosphorus compounds of the aqueous phases of the cell. At all harvests, "residual" phosphorus as a percentage of total phosphorus is greater with P_3 than with P_2, and for harvests 3, 4, and 5 the mean values are 85 and 94 per cent. for P_2 and P_3 respectively. Under such conditions of saturation, the rate of intake of phosphorus with P_3 would be determined by the maximal capacities of the plant parts to accumulate this element, rather than by any direct effect of its concentration in the medium.

It will be noted that for P_1 and P_2 at both harvest-intervals the increments of phosphorus per unit increase in dry weight are greatest in the stems. This perhaps indicates a relatively greater meristematic activity in stem tissues over this period, when shoot elongation and inflorescence differentiation are proceeding. That a similar effect is not in evidence for P_3 is in accord with the suggestion of the last paragraph, that the rates of intake in this case are primarily determined by the maximal capacities of the several parts to accumulate phosphate in the cell sap.

The cessation of nitrogen intake (at harvest 5 with P_2 and P_3 and at harvest 6 with P_1) is not synonymous with the loss of capacity for intake in general, for phosphorus intake persisted to full maturity (harvest 7) with P_1 and P_2. Crowther (1934) found that nitrogen intake ceased at the time of rapid development of the inflorescence, and he suggested that competition for supplies of carbohydrate stopped the growth of the roots, and with it the intake of nitrogen.

In the present experiment, nitrogen intake did cease concurrently with the rapid development of the inflorescence and with the cessation of root growth as measured by dry weight change. However, the exhaustion of the supply of nitrogen was almost certainly the cause of the cessation of nitrogen intake in P_2 and P_3, and for P_1 an internal mechanism unrelated to carbohydrate supply has already been suggested (Williams 1938, pp. 78-9). This mechanism will be discussed, as it applies to phosphorus re-utilization, in a later section of this paper.

For phosphorus, and to a lesser extent for nitrogen, it would seem that the growth factor was the primary determinant of continued intake after the cessation of root growth.

(ii) *The External Factor of Supply*

The external concentration of phosphorus in sand cultures may not be related in any simple manner to the amounts of phosphorus added in solution, for it has been shown by Dunlap (1939) that phosphate retention is an important phenomenon in certain sands, and that the amount of this retention is determined by the kind of sand, the concentration of added phosphate, and the duration of treatment. Furthermore, it is now known that the total phosphorus content of the washed river-sand used for the present experiment with oats may have been of the same order as that applied as the highest treatment (600 mg. P per pot). Fortunately, little of this was available to the plants, since only 19.2 mg. of phosphorus per pot were absorbed by plants of P_1. It should be noted, however, that only 8 mg. were supplied in the culture solution and 0.5 mg. in the seeds, so that almost 11 mg. of phosphorus were derived from the sand. The ratios of maximum phosphorus absorbed to phosphorus supplied were 2.4, 0.61, and 0.27 for P_1, P_2, and P_3 respectively.

With P_1, the rate of phosphorus intake per day rose slowly to a maximum at harvest-interval 5-6, with the result that 71 per cent. of its total intake occurred after harvest 5, and 29 per cent. after harvest 6. With P_2 and P_3, only 27 and 8 per cent. respectively of the intake occurred after harvest 5, and rates of intake per day were at a maximum for harvest-interval 4-5. It is clear from the data that the presumed external concentrations of phosphorus were important, even if indirect, determinants of the rates of intake per day for the first half of the growing period.

(iii) *The Rate of Intake of Phosphorus per Unit Weight of Root*

Rates of nutrient intake per day are complicated by the size factor of the absorbing system; rates per unit weight of root are therefore presented as crude indices of the rates per unit area of absorbing surface. Kreyzi (1932) used the root weight basis in his study of the intake of phosphorus by plants in water cultures.

The instantaneous rate of intake of a mineral nutrient M is given by the equation,

$$I_M = \frac{1}{R}\frac{dM}{dt},$$

where R is the dry weight of the root system at that instant. The mean value of I_M for a finite time-interval may be calculated from the approximate formula

$$I_M = \frac{\log_e R_2 - \log_e R_1}{t_2 - t_1} \times \frac{M_2 - M_1}{R_2 - R_1}.$$

The errors involved in the use of this formula are discussed by Williams (1945) in connection with the parallel equation for net assimilation rate.

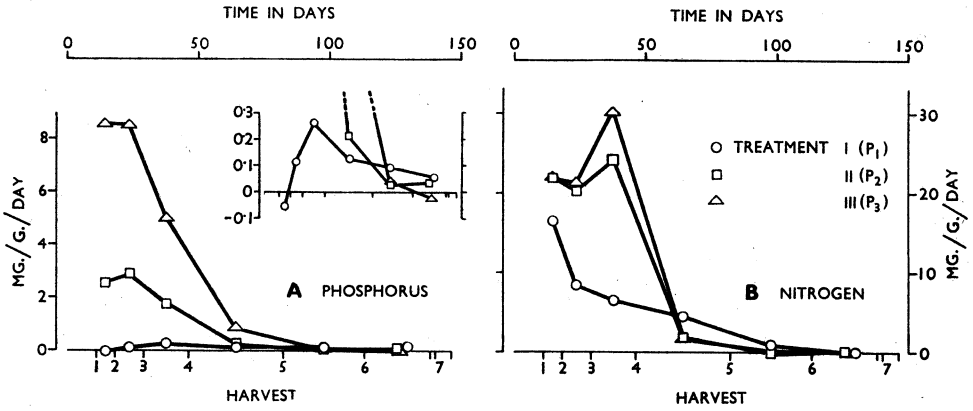

Fig. 2.—Rates of intake of phosphorus (A) and nitrogen (B) per unit weight of root in oats.

The values of I_M for phosphorus are presented in Figure 2A and the time trend for P_1 is shown more clearly in the inset of that figure. With this treatment, the rate rose from an initial negative value to a maximum of only 0.26 mg. per g. per day at harvest-interval 3-4. Thereafter the rate of intake fell steadily, and after harvest 5, became greater than the rates for P_2 and P_3. While the negative value requires experimental confirmation, it is conceivable that the internal concentration of phosphate in the roots (being derived from seed reserves) was so great that some was lost to the phosphorus-deficient medium. The rapid fall in the phosphorus content of all plant parts (see Table 4) and the presumed fall in root phosphate concentration would account for the observed upward trend in the rate of intake from the deficient medium. With P_2 there was a slight increase in the rate to a maximum of 2.82 mg. per g. per day at interval 2-3, but there was no increase in the rate with P_3. The importance of the concentration of phosphorus in the medium as a determinant of the rate of intake is again evident, even though the integrated control of the rate by the organism itself has been shown to be of primary significance.

The relatively high rates of phosphorus intake with P_1 for intervals 5-6 and 6-7 are an expression of the demand for inflorescence phosphorus (see later), though the high nitrogen status of plants of P_1 may also have played a part in maintaining these rates.

(*b*) *The Rate of Intake of Nitrogen per Unit Weight of Root*

This section properly belongs to an earlier paper (Williams 1938), but is included here because the basis of expression was not then considered. It is of special interest because it provides a definite example of control by the growth factor.

TABLE 4

ABSOLUTE AND RELATIVE AMOUNTS OF TOTAL PHOSPHORUS IN THE LEAVES, STEMS, ROOTS, AND INFLORESCENCES OF OATS

	Harvest	Phosphorus (% Dry Weight)			Absolute Phosphorus (mg.)		
		P_1	P_2	P_3	P_1	P_2	P_3
Leaves	1	0.596	0.596	0.596	0.0215	0.0215	0.0215
	2	0.296	0.784	1.87	0.0403	0.115	0.295
	3	0.140	0.743	2.27	0.0419	0.455	1.323
	4	0.138	0.342	0.899	0.161	2.17	5.61
	5	0.071	0.080	0.330	0.632	5.00	23.16
	6	0.051	0.029	0.240	0.765	1.24	11.69
	7	0.016	0.014	0.199	0.170	0.56	8.82
Stems	1	0.532	0.532	0.532	0.0606	0.0606	0.0606
	2	0.405	0.777	1.11	0.0304	0.653	0.097
	3	0.255	0.882	1.38	0.0288	0.212	0.313
	4	0.247	0.482	0.779	0.099	1.21	2.13
	5	0.114	0.108	0.307	0.480	5.32	15.26
	6	0.042	0.045	0.221	1.184	5.76	28.95
	7	0.014	0.012	0.166	0.407	1.29	17.70
Roots	1	0.365	0.365	0.365	0.0172	0.0172	0.0172
	2	0.232	0.459	1.23	0.0258	0.0459	0.117
	3	0.153	0.529	1.34	0.513	0.193	0.310
	4	0.137	0.380	0.776	0.182	1.01	1.70
	5	0.101	0.095	0.261	0.728	3.06	10.54
	6	0.103	0.061	0.158	1.246	1.78	4.55
	7	0.124	0.066	0.121	1.288	1.57	2.81
Inflorescences	6	0.152	0.178	0.233	1.338	7.28	7.76
	7	0.170	0.193	0.294	4.590	15.02	21.81
Whole Plant	1				0.0993	0.0993	0.0993
	2				0.0965	0.2264	0.509
	3				0.122	0.860	1.946
	4				0.442	4.39	9.44
	5				1.84	13.38	48.96
	6				4.533	16.06	52.95
	7				6.40	18.44	51.14

In spite of the fact that the supply of nitrogen was initially the same for each treatment, the data of Figure 2B show that phosphorus deficiency greatly depressed the rate of intake of nitrogen. The depression was most pronounced for harvest-interval 3-4. The subsequent reversal of this effect was due to the exhaustion of their nitrogen supply by the larger plants of P_2 and P_3. There was also an increase in the rate of intake with P_3 over that with P_2 for harvest-interval 3-4.

The early effects of treatment on nitrogen intake, however, were not primarily due to any direct effects of phosphorus on the mechanism of ion intake, but to its differential effect on the growth of roots and shoots. For the plants as a whole, the rates of increase of nitrogen per unit increase in dry matter were similar for all treatments (an average of 40.7, 47.1, and 45.1 mg. nitrogen per g. dry matter formed for P_1, P_2, and P_3 respectively over harvest-interval 1-4), but the implied uniform demands for nitrogen were met via the root system which varied greatly in size (dry weight) relative to the size of the whole plant. Thus, assuming a uniform requirement of 46.2 mg. (the value for P_2) per g. dry matter added over harvest-interval 3-4, the rates of intake per g. of root system would be 8.1, 24.3, and 31.6 mg. nitrogen per day for P_1, P_2, and P_3 respectively. The actual rates were 6.6, 24.3, and 30.4 mg. per g. per day. The further slight depression below theoretical with P_1 could be associated with depressed protein synthesis and the piling up of soluble nitrogen compounds; this occurred in the leaves (Williams 1938) and may be inferred for the roots also.

(*c*) *The Redistribution of Phosphorus within the Plant*

Reference to Figure 1 and Table 4 shows that, with all treatments, phosphorus accumulates rapidly in the roots and leaves, then in the stems, and finally in the inflorescences, and this is accomplished with varying degrees of redistribution of phosphorus. Reference has been made to the grain as a source of phosphorus for the seedling, and to the fact that each successive vegetative organ becomes a potential source of phosphorus for younger plant parts. In this way phosphorus is redistributed within the plant, and may be re-utilized many times during the life of an annual plant. Reid (1941) has shown that cells only 15 mm. from the root tips of cowpea seedlings may be losing phosphorus in this manner. Later the inflorescence, with its very great demand for phosphorus, becomes of increasing importance as a determinant of the redistribution of the element within the plant. The magnitude of this demand in oats may be gauged from the fact that the amounts of phosphorus in the inflorescence, expressed as percentages of the amounts in the whole plant at maturity, were 72, 82, and 43 for P_1, P_2, and P_3 respectively.

In Table 5, the net increments of phosphorus in leaves, stems, roots, and inflorescences are presented for harvest-intervals 5-6 and 6-7. For P_1, interval 5-6, all plant parts show net increases, and the inflorescences account for almost 50 per cent. of the total intake of phosphorus. In the sense that there was no net loss of phosphorus from the other plant parts, it may be postulated that the inflorescence phosphorus was derived entirely from the external medium. The picture is more complex with P_2 and P_3 over this interval; leaves and roots showed net losses of phosphorus, and stems and inflorescences net gains. In P_2 the stems received a total of 7.72 mg. of phosphorus: 5.04 mg. from the leaves and roots, and 2.68 mg. from the medium. Thus the proportion of stem and inflorescence phosphorus derived from the external medium may be regarded as 2.68/7.72 or 35 per cent. Similarly for P_3 the proportion becomes 3.99/21.45 or 19 per cent.

For harvest-interval 6-7, the leaves, stems, and roots of all treatments were losing phosphorus, hence there was no competition for such phosphorus as was

derived from the medium. For P_1 and P_2, the relative amounts of inflorescence phosphorus derived from the medium were 57 and 31 per cent. respectively. For P_3, however, all of the inflorescence phosphorus came from other plant parts, and the data indicate that some phosphorus may have been lost to the medium.

TABLE 5

INCREMENTS IN ABSOLUTE PHOSPHORUS CONTENT OF THE LEAVES, STEMS, ROOTS, AND INFLORESCENCES OF OATS FOR HARVEST-INTERVALS 5-6 AND 6-7

	Interval 5-6 (days 82-113)			Interval 6-7 (113-146)	(113-138)	(113-140)
	P_1 (mg.)	P_2 (mg.)	P_3 (mg.)	P_1 (mg.)	P_2 (mg.)	P_3 (mg.)
Leaves	0.13	−3.76	−11.47	−0.60	−0.68	−2.87
Stems	0.70	0.44	13.69	−0.77	−4.47	−11.25
Roots	0.52	−1.28	−5.99	−0.02	−0.21	−1.74
Inflorescences	1.34	7.28	7.76	3.25	7.74	14.05
	(*100*)	(*35*)	(*19*)	(*57*)	(*31*)	(*0*)
Whole plant	2.69	2.68	3.99	1.86	2.38	−1.81

The italic figures are estimates of the percentage amounts of inflorescence phosphorus derived from the medium (for explanation see text).

While the above analysis makes certain assumptions by virtue of the fact that only net increments are known, it nevertheless provides satisfactory comparative data upon which to build a tentative interpretation relative to the intake and distribution of phosphorus. Thus, in all, phosphorus-deficient plants derived only 30 per cent. of their inflorescence phosphorus from other plant parts, whereas those plants which had an excessive supply derived no less than 93 per cent. of their inflorescence phosphorus from these sources. In the latter case it is probable that the supply of phosphorus was still great, but that an abundant and more accessible supply was made available by the senescent breakdown of the protoplasm in the leaves and roots, and later in the stems of these plants. Evidence presented elsewhere (Williams 1938) suggests that this breakdown was caused by the demand of the inflorescence for nitrogen rather than phosphorus; this would contribute to the ready availability of the internal supply of phosphorus, and to conditions favouring a loss of phosphorus to the medium.

With phosphorous deficiency, on the other hand, the inflorescence phosphorus was more readily derived from the deficient medium than from the other plant parts. The nitrogen status was much higher in this case, and such senescent breakdown of the protoplasm as did occur in the leaves etc. may have been initiated by a demand for phosphorus rather than for nitrogen.

(*d*) *Relative Phosphorus Contents*

These contents (elementary phosphorus per cent. dry weight) are presented in Table 4 and in Figure 3 (leaves only). In leaves, stems, and roots of P_3 these relative contents attained very high maxima (2.27 per cent. for leaves) at harvest 3. For P_2 the maxima were much lower, and for P_1 the values fell rapidly from the

initial value. By harvest 5, however, the values for P_2 had fallen to the same level as those for P_1, and subsequently they fell still lower, especially in the roots. These phenomena are expressions of the "dilution" effect frequently encountered when a growth response is induced by an additional supply of the nutrient concerned. In these cases, the treatment appears so to stimulate the production of dry matter (primarily carbohydrates and proteins) that the increased quantity of the nutrient taken into the plant is thereby "diluted." Thus, for harvest-interval 4-5 (see Table 3) the intake of phosphorus by the leaves, stems, and roots was from 4 to 10 times greater with P_2 than with P_1, yet the rates of intake per gram increment of dry matter formed were consistently less with P_2 than with P_1.

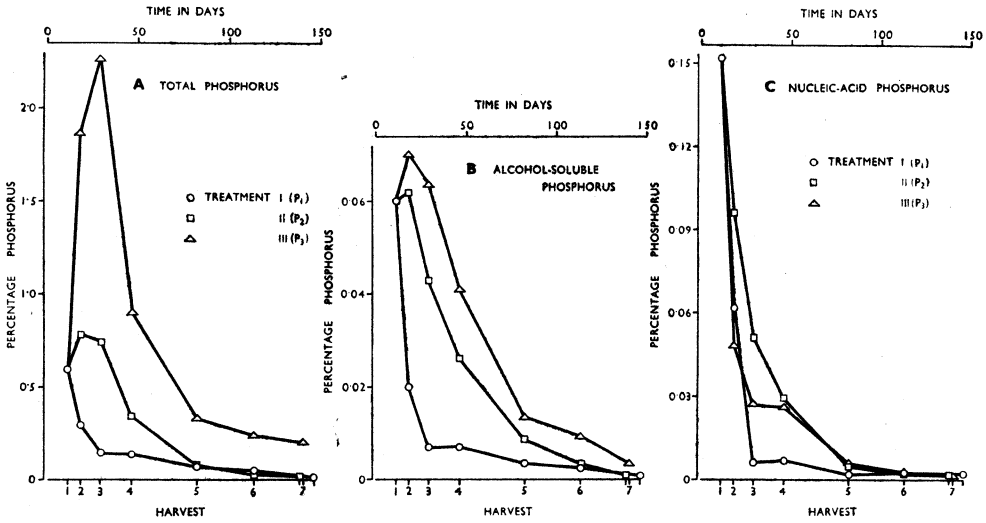

Fig. 3.—Total, alcohol-soluble, and nucleic-acid phosphorus as percentage of dry weight in leaves of oats.

The simplest interpretation of the "dilution" effect is the very literal one, stated above, that the production of carbohydrates etc. is so stimulated by treatment that the percentage content of the nutrient falls more rapidly than in the absence of treatment. Gregory and Richards (1929) found a slight though consistent increase in carbon assimilation with increased phosphorus supply at both low and high light intensities. The barley leaves studied, however, had only just attained maturity (full expansion) and it may be questioned whether they were very deficient in phosphorus. It is probable that carbon assimilation of individual leaves falls off rapidly with time in the case of phosphorus deficiency, as is indicated by measurements of the assimilation of the leaves as a whole. For the present experiment (Williams 1936) it was found that net assimilation rates (dry weight basis) were depressed by phosphorus deficiency during early stages of growth, and that a smaller proportion of the plant was concerned with the production of carbohydrates.

An alternative interpretation would be that the relative shortage of nitrogen with P_2 resulted in an earlier release of phosphorus from senescent plant parts,

and a more efficient re-utilization of this phosphorus. In this case, the demand per unit of new growth for phosphorus from the medium could have been less than with P_1 over the same period, and the phosphorus contents of the plant parts "diluted" by the phosphorus-poor tissues of the senescent individual parts. This second interpretation gains support from the fact that the inflorescences of P_2 were still able to obtain their phosphorus at a greater rate per gram increment of dry matter than was the case with P_1. These rates for harvest-interval 5-6 were 1.52, 1.78, and 2.33 mg. per g. for P_1, P_2, and P_3 respectively; for interval 6-7 the rates were 1.79, 2.10, and 3.43 mg. per g. of dry matter added to the inflorescences.

(e) Alcohol-Soluble, Nucleic-Acid, and Residual Phosphorus in the Leaves

The total, alcohol-soluble, and nucleic-acid phosphorus contents (relative) of the leaves are shown in Figure 3, and the absolute data (including residual phosphorus) are presented in Table 6. For reasons discussed (see Section II) a close examination of the time trends and treatment effects on percentage alcohol-soluble phosphorus – a crude index of phosphatide phosphorus – will not be attempted. It is sufficient to point to the general resemblances between these and the data for total phosphorus in the leaves.

TABLE 6

ABSOLUTE AMOUNTS OF NUCLEIC-ACID, ALCOHOL-SOLUBLE, AND RESIDUAL PHOSPHORUS IN THE LEAVES OF OATS (μG.)

		Treatment I P_1			Treatment II P_2			Treatment III P_3		
Harvest	Time in Days	Nucleic-Acid P	Alcohol-Soluble P	Residual P	Nucleic-Acid P	Alcohol-Soluble P	Residual P	Nucleic-Acid P	Alcohol-Soluble P	Residual P
1	11	5.5	2.2	13.8	5.5	2.2	13.8	5.5	2.2	13.8
2	18	8.5	2.7	29.1	14.2	9.2	91.8	7.6	11.1	276.0
3	29	1.7	2.1	38.1	31.0	26.6	397.0	15.9	37.1	1270.0
4	46	8.5	8.3	145.0	186.0	167.0	1820.0	165.0	255.0	5190.0
5	82	13.2	30.4	589.0	272.0	541.0	4190.0	382.0	948.0	21800.0
6	113	33.9	37.5	694.0	78.0	142.0	1020.0	120.0	458.0	11100.0
7	138	–	–	–	46.0	32.0	492.0	–	–	–
	140	–	–	–	–	–	–	59.0	155.0	8600.0
	146	21.4	5.3	146.0	–	–	–	–	–	–

The nucleic-acid phosphorus contents, on the other hand, present a strikingly different picture. These values fall rapidly with time with all treatments, and early values for P_3 are markedly depressed below those for P_2. Between harvests 1 and 3 the value for P_1 falls to a very low figure, but is maintained relatively constant thereafter.

For harvests 2, 3, and 4, striking similarities were noted in the effects of treatment, though not of time, on nucleic-acid phosphorus and protein-nitrogen contents (for protein-N see Williams 1938, Fig. 2, Exp. 2). This relation is shown in Figure 4, where protein-nitrogen and nucleic-acid phosphorus are plotted

respectively as the dependent and independent variables. Linear regressions are shown for each set of three values within harvest.

Two factors favour the suggestion that these approximately linear relations are due primarily to the biochemical association of the variables as nucleoproteins. Firstly, the treatments change position relative to each other from harvest to harvest (Fig. 4): if it were otherwise the relation could be merely an expression of an internal or time factor common to the variables. Secondly, the ratios of

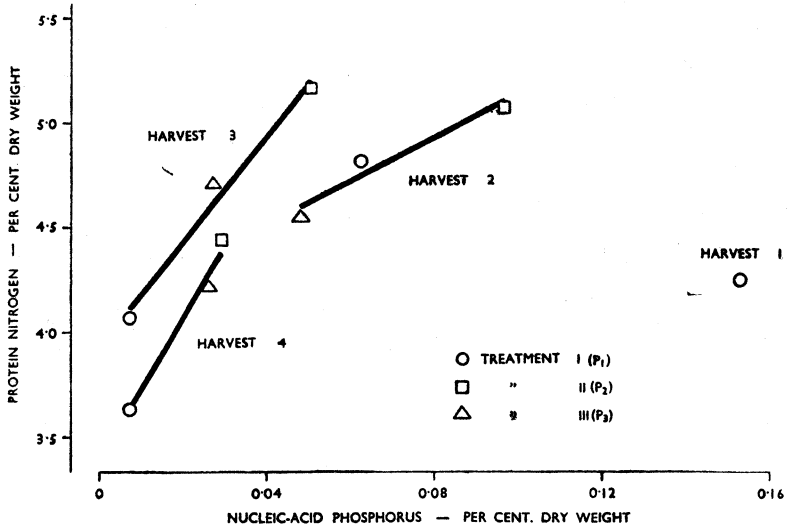

Fig. 4.—The relation of protein nitrogen to nucleic-acid phosphorus in the leaves of young oat plants.

nitrogen to phosphorus implied by the regressions (i.e. the b values) are 10.2, 25.1, and 34.5 for harvests 2, 3, and 4 respectively. These ratios are of the same order of magnitude as those for purified preparations of nucleoproteins. Greenstein (1944) gives analytical values for mammalian liver nucleoproteins, for which the nitrogen-phosphorus ratio ranges from 17 to 20. Bawden (1943) also gives data for plant viruses from which ratios ranging from 4 to 40 may be calculated; for the greater number, however, they approximate to 30. More recently Best (1948), working with tobacco mosaic virus, presented data for which the nitrogen-phosphorus ratios ranged from 30 to 33. The ratios derived from the regressions admittedly have a rather wide range, but it is very unlikely that the content of protein other than nucleoprotein would be entirely unaffected by treatment, as is assumed by this use of the regressions.

Because of the small amounts present in the tissues, the nucleic-acid phosphorus data for harvests 5, 6, and 7 are less reliable than those already discussed. The data for all three fractions may also be expressed as percentages of the total phosphorus present, and in Figure 5 this has been done for the alcohol-soluble and nucleic-acid phosphorus values for the oat experiment. In general, treatment effects are in the same direction for these two fractions, so that effects on residual phosphorus would be the reverse of these.

The values for alcohol-soluble phosphorus present a simple picture, with $P_2 > P_1 > P_3$ at all harvests. With nucleic-acid phosphorus the same order holds for harvest 3-6, but the percentages with P_1 are the greatest at harvests 2 and 7.

With P_1, a reduction in the concentration of residual phosphorus is associated with an accelerated hydroylsis of more complex forms (see below), and the maintenance of relatively low partition values for these forms. With P_3, on the other hand, the concentration of residual phosphorus rises to very high values at first and is associated with retardation of nucleic-acid synthesis; after this phase, the partition values for nucleic-acid and alcohol-soluble phosphorus increase somewhat, and then decline more or less continuously until the final harvest. In late senescence, after harvest 6, when phosphorus is being exported from the leaves, the partition values indicate an accelerated hydrolysis of phosphatides and a relative stability of nucleic acids (especially in P_1).

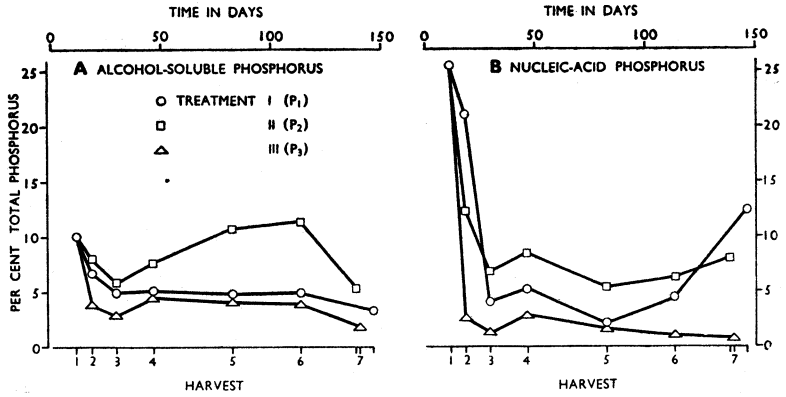

Fig. 5.—Alcohol-soluble and nucleic-acid phosphorus as percentage of total phosphorus in the leaves of oat plants.

The concept of a *partition index* for mineral elements in leaves was used by Phillis and Mason (1939, 1942*b*) and Mason and Phillis (1943), and was defined as the insoluble fraction of the element expressed as a percentage of the total amount in the leaf. For phosphorus, the insoluble fraction was that which was not pressed out with the sap, and was presumed to contain only "phospho-proteins and phospho-lipoids." If this assumption was sound, then the percentages of Figure 5 are components of the *partition indices* for oat leaves at seven stages of growth. Only at harvest 2, however, is there any suggestion of a linear correlation (negative) of the index with residual-phosphorus content; the *indices* are considerably lower with P_1 than with P_2 for harvests 3-6 inclusive, and, on the average, *indices* are much smaller for oats than for cotton. It is possible that compounds such as hexose phosphoric esters and adenyl pyrophosphate, which are less likely to be "sap-soluble" than is inorganic phosphorus, may have contributed materially to the "insoluble" fraction of Phillis and Mason. Furthermore, this contribution would increase with increasing phosphorus deficiency, so that a large proportion of the "residual" phosphorus in P_1 would be organically bound. This calls for experimental confirmation, and the whole question of the effects of age and of

phosphorus supply upon the partition of leaf phosphorus could well be elucidated using structurally uniform material and an experimental design similar to that of Walkley and Petrie (1941) for studying the relation between proteins and amino acids in a specified leaf.

(f) The Initial Growth Depression with High Phosphorus Supply

The absolute data for nucleic-acid phosphorus indicate that the synthesis of nucleic-acid was immediately retarded with P_3 and to a relatively greater extent than was leaf protein. These further facts necessitate a revision of statements previously made (Williams 1938, p. 77) with respect to the growth depression with high phosphorus supply. It was then established that treatment effects on protein nitrogen preceded those on water content, and, in the absence of any effect on soluble-nitrogen content, it was suggested that the detrimental effect of P_3 on protein synthesis and growth was through an effect on nitrogen intake rather than a direct effect on protein synthesis.

The rate of intake of nitrogen per day was certainly lower with P_3 than with P_2 (Table 2), but the rate per unit weight of root was at first unaffected by treatment and then became greater with P_3 for harvest-interval 3-4 (Fig. 2B). These effects have been examined in an earlier section, and they would seem primarily to be functions of the demand for nitrogen set up by the growth of the various plant parts and by differences in the growth rates of these parts relative to each other.

It now seems that synthesis of nucleoproteins is greatly retarded by high, perhaps excessive, phosphorus supply; this is at once in accord with a reduction in meristematic activity, a check in the rate of dry weight increase, and a reduction in intake of nutrients supplied in constant amount. Any attempt to seek cause-effect sequences between such attributes (structural as well as functional), however, is likely to lead to false emphasis, as in the writer's initial interpretation of the present case. Nevertheless, the retardation of nucleic-acid synthesis is more likely to precede the established effect on nitrogen intake than vice versa.

(g) Phosphorus Metabolism in Phosphorus-Deficient Leaves

With adequate (P_2) and excessive (P_3) supplies of phosphorus, the transition from the stage of dependence of the seedling upon seed reserves of phosphorus is rapid, but with deficient (P_1) supply it is slow, and the data warrant detailed examination (Fig. 6).

During harvest-interval 1-2, the dry weight of the leaves increased nearly fourfold, and that of the roots more than twofold. Much of the total increment may be attributed to carbon assimilation by the leaves, but the decrease in the weight of the "stem" fraction (which includes the residue of the grain) indicates continued translocation of materials from the endosperm. Over the same period, no phosphorus was absorbed from the medium, but 0.03 mg. per plant was distributed from the "stem" to the leaves and roots; the leaves obtaining twice as much as the roots.

During the next harvest-interval (2-3), the dry weight of the plant was more than doubled, that of the leaves was doubled, that of the roots was trebled, and that of the "stems" was increased by 50 per cent. At the same time, however, the

absolute-phosphorus contents of the leaves and "stems" remained stationary, and the whole of the phosphorus intake from the medium was retained by the roots. This fact has an important bearing on the effect of phosphorus treatment on root weight ratio, and will be discussed further.

Harvest-interval 3-4 was the period over which the rate of phosphorus intake per unit weight of root was at a maximum (Fig. 2A); the leaves, stems, and roots received phosphorus at rates of 1.37, 2.45, and 1.31 mg. per g. increase in dry weight respectively, and all parts grew rapidly.

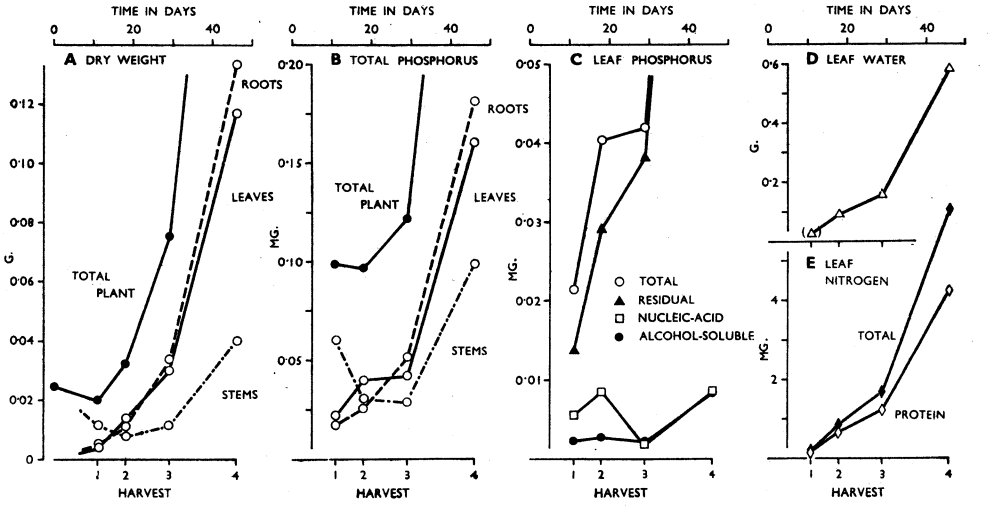

Fig. 6.—Absolute data relating to phosphorus-deficient oat seedlings (for explanation see text).

Thus with P_1, seed reserves of phosphorus were virtually exhausted 18 days after sowing, and absolute-leaf-phosphorus content was unchanged 11 days later. Leaf growth did not cease, however, as is shown by the increases in dry weight, protein, and water, and there were drastic changes in the partitioning of leaf phosphorus between the three fractions estimated. Thus, during this period (interval 2-3), nucleic-acid phosphorus was reduced to one-fifth of its initial amount; there was a slight decrease in the weight of alcohol-soluble phosphorus; and there was a 30 per cent. increase in residual phosphorus. When the fact of continued growth, with the production of new cells, tissues, and leaves, is taken into account, it will be seen that the hydrolyses here indicated may have been localized in the earlier-formed leaf-cells and tissues. On this basis, too, the resumption of net synthesis indicated by the data (interval 3-4) would primarily be a function of juvenile leaf growth at that time. There was, however, a retardation of total leaf growth between harvests 2 and 3, as is evident from the forms of the curves for dry weight, protein-nitrogen, and water (Figs. 6A, 6E, and 6D). It is tempting to speculate concerning the roles of ribonucleic and desoxyribonucleic acids in this sequence of net synthesis, hydrolysis, and synthesis of nucleic-acid phosphorus, but such speculation would scarcely be profitable since it is believed that the data refer only to total nucleic acid. Reference may

be made to Mirsky (1943) for a review of the literature concerning the localization of the two acids in the nucleus and the cytoplasm.

(*h*) *Root Weight Ratios*

This aspect of phosphorus nutrition was discussed by Williams (1936, p. 176) who found that the bulk of the experimental evidence indicated a depression in the root weight ratio with increasing phosphorus supply. Since the leaf and stem weight ratios were affected in a more variable manner, it was inferred that the depression could not be due to a positive stimulation of the growth of leaves or stems alone. This inference gains support from the data presented above in the section on phosphorus metabolism in phosphorus-deficient leaves. With P_1, the whole of the phosphorus intake for interval 2-3 was retained by the roots, and there was a concurrent stimulation of root growth relative to leaf growth (see Fig. 6): root weight became greater than leaf weight at harvest 3 and remained so for several weeks. The effect of treatment is well shown by a comparison of the relative growth rates of the leaves, R_L, with those of the roots, R_R, as these are affected by stage of growth and treatment (Table 7). For harvest-interval 2-3, R_R is greater than R_L for P_1, but the reverse is the case for P_2 and P_3: for interval 3-4, these rates are equal for P_1, but R_L remains the greater for P_2 and P_3.

TABLE 7

RELATIVE GROWTH RATE (G. PER G. PER DAY) OF THE LEAVES,* R_L, AND OF THE ROOTS, R_R, OF OATS

Harvest-Interval	P_1		P_2		P_3	
	R_L	R_R	R_L	R_R	R_L	R_R
1-2	0.190	0.123	0.201	0.108	0.211	0.101
2-3	0.072	0.100	0.130	0.118	0.119	0.081
3-4	0.080	0.081	0.138	0.117	0.139	0.132
4-5	0.057	0.047	0.064	0.069	0.067	0.081

* Leaf separations were made by cutting at the ligule, but with the youngest leaf of each axis only that portion was taken which had protruded from the sheath of its predecessor. This procedure may have introduced considerable error in the leaf and "stem" weights at harvest 1 (common to all treatments), but the error would be of rapidly diminishing significance for later harvests. Values of R_L for interval 1-2 were thus arbitrarily increased, though still reasonably comparable as between treatments. The relative shoot growth rates were 0.049, 0.062, and 0.070 g. per g. per day for P_1, P_2, and P_3 respectively.

These facts indicate that at least one factor contributing to an increased root weight ratio with phosphorus deficiency may be the fixation of a greater proportion of the absorbed phosphorus, so that relatively little is available for shoot growth: the roots, being nearer the supply, benefit at the expense of other parts of the plant. The stimulation of root growth relative to leaf growth, however, implies a diversion of a greater proportion of carbohydrate from the shoots, so that carbohydrate supply would set the limit to this tendency.

In his studies of root growth in Lemna, White (1937, 1938) interprets his results in terms of carbohydrate-nitrogen balance in the fronds, and he regards this interpretation as a particular case of a general phenomenon with respect

to the control of root development. This may be accepted for his own experiments, and for those experiments cited (White 1937, p. 652) in which carbohydrate level was obviously affected by treatment, but in those experiments in which the root weight ratio was increased by nitrogen deficiency, it seems probable that the roots, being nearer to the limited supply of nitrogen, may have benefited at the expense of other plant parts.

The difference between the two mechanisms lies in the fact that the roles of the roots and of the shoots respectively are emphasized – perhaps overemphasized. The relative importance of the mineral nutrient or the carbohydrate level tends also to be overstated in these attempts to seek cause-effect sequences. Once again, the remedy may lie in the restatement of the problem of organic growth, and a departure from the strictly analytic procedures which have been applied in the past.

(*i*) *Nucleo-Cytoplasmic Ratios*

Robertson and Dawbarn (1929) determined nucleic-acid nitrogen and coagulated nitrogen contents of various organs of the new-born lamb and the adult sheep, and they found that nucleo-cytoplasmic ratios based on these values fell with age in all organs examined. They also concluded that there must be an essential interdependence of nucleic-acid and protein synthesis in animal tissues. Huelin (1929) used the same methods (Robertson 1929) with wheat plants at three stages of growth, and found also that the ratio decreased rapidly with time.

The nucleo-cytoplasmic ratios of Table 8 were computed from the nucleic-acid phosphorus and protein nitrogen data of the oat experiment, using the factor 1.693 to convert nucleic-acid phosphorus to nucleic-acid nitrogen. The ratios cover a much wider range of growth stage and treatment than those of Huelin for wheat. During early growth, the ratios decrease rapidly from a high initial

TABLE 8

NUCLEO-CYTOPLASMIC RATIOS FOR OAT LEAVES (x 100)

Harvest	1	2	3	4	5	6	7		
Days from Sowing	11	18	29	46	82	113	138	140	146
P_1	6.44	2.25	0.24	0.34	0.12	0.25	–	–	0.61
P_2	6.44	3.32	1.69	1.13	0.91	0.76	0.66	–	–
P_3	6.44	1.83	0.99	1.08	1.39	0.88	–	0.72	–

value common to all treatments; only with P_2, however, does this trend continue on to full maturity. The initial decreases are greater with P_1 and P_3 than they are with P_2, but subsequent trends are more erratic. Treatment effects are largely a reflection of the effects on nucleic-acid content.

These nucleo-cytoplasmic ratios were examined in relation to the growth data of the experiment, and, in particular, to the relative leaf growth rates. No obvious correlations were revealed.

(*j*) *Total, Alcohol-Soluble, and Nucleic-Acid Phosphorus in the Leaves of* Phalaris tuberosa *L.*

The growth data for the experiment with Phalaris have been presented elsewhere (Williams 1946), and the phosphorus analyses of the leaf material were conducted in order to determine the effect of increased phosphorus supply at two levels of nitrogen. The experiment covered the period of rapid vegetative growth prior to stem elongation, and leaf dry weights were increasing throughout the period of investigation (day 69 to day 139). Relative and absolute values for total, alcohol-soluble, and nucleic-acid phosphorus, and for protein nitrogen are presented in Figure 7.

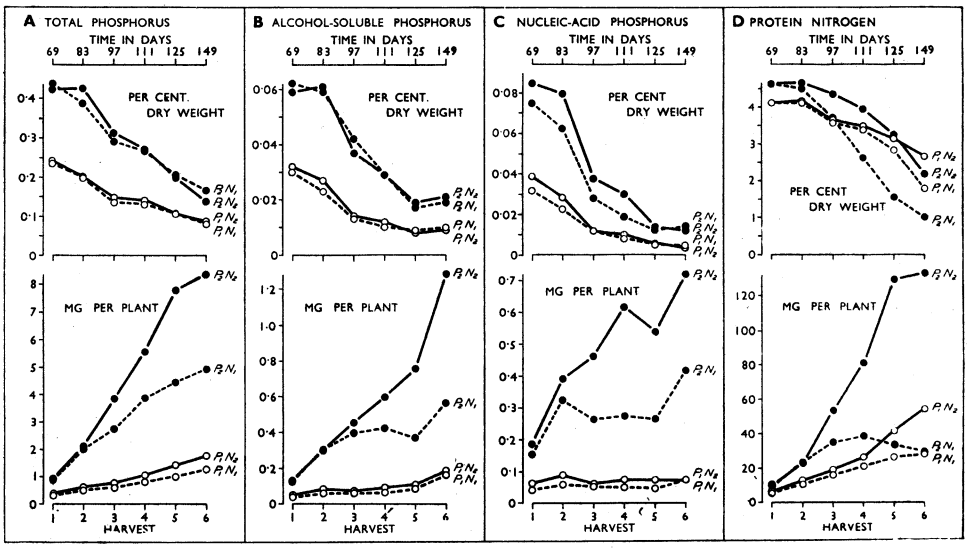

Fig. 7.—Relative and absolute amounts of total, alcohol-soluble, and nucleic-acid phosphorus, and of protein nitrogen in the leaves of Phalaris.

The sixfold phosphorus treatment, P_2, did little more than double the relative phosphorus content at any given harvest, and there was no clear-cut effect of the increased supply of nitrogen. The time trends in, and treatment effects on, the relative contents of alcohol-soluble phosphorus present a similar picture, except for increases with all treatments from harvest 5 to harvest 6. With nucleic-acid phosphorus, however, the time trends are more pronounced, and there are quite marked increases with N_2. Thus, P_1N_2 exceeds P_1N_1 at harvests 1 and 2, and P_2N_2 exceeds P_2N_1 for harvests 1 to 4 inclusive. As in the oat experiment, similarities of treatment effect on nucleic-acid phosphorus and protein-nitrogen contents are evident for early growth stages (harvests 1 and 2), and the ratio of nitrogen to phosphorus derived from a regression (*vide supra*) is approximately 11.5.

Although leaf weights were still increasing rapidly between harvests 5 and 6, there is a suggestion that the absolute phosphorus contents are approaching their maxima with P_2N_1 and P_2N_2. Protein nitrogen is at a maximum at harvest

4 with P_2N_1, and is probably so at harvest 6 with P_2N_2. Time trends in the absolute contents of alcohol-soluble and nucleic-acid phosphorus are somewhat erratic, though closer inspection, especially of the latter, reveals fairly consistent departures from the general trend. Thus, at harvests 2, 4, and 6 these departures tend to be positive, and at harvests 1, 3, and 5 they tend to be negative. Furthermore, these departures tend to be the reverse of those in protein nitrogen, in total phosphorus, and in relative leaf growth rate. When the growth rate is relatively high, the net synthesis of protein and the net intake of phosphorus are high, but the net increment in nucleic-acid phosphorus is small or even negative. Quite tentatively, it is suggested that rapid development of new leaf and stem tissues sets up a demand for phosphorus such that hydrolysis of nucleic acid, and to a lesser extent of phosphatides is hastened in the earlier-formed leaves, the whole being governed by the form of the steady-state relation between these constituents and their soluble phosphoric precursors.

The partition values for this experiment are given in Table 9. Those for alcohol-soluble phosphorus show no features of special significance, but those for

TABLE 9

ALCOHOL-SOLUBLE AND NUCLEIC-ACID PHOSPHORUS EXPRESSED AS PERCENTAGES OF TOTAL PHOSPHORUS IN THE LEAVES OF PHALARIS TUBEROSA L.

Harvest	Days from Sowing	Alcohol-Soluble P				Nucleic-Acid P			
		P_1N_1	P_1N_2	P_2N_1	P_2N_2	P_1N_1	P_1N_2	P_2N_1	P_2N_2
1	69	12.7	13.0	14.1	13.9	13.5	16.1	17.1	20.0
2	83	11.5	13.4	15.2	14.4	11.3	14.1	16.0	18.6
3	97	9.7	9.8	14.3	11.9	8.6	8.0	9.6	12.0
4	111	7.9	8.6	11.1	10.6	6.0	6.9	7.1	11.0
5	125	8.1	7.0	8.2	9.5	4.5	5.1	5.9	6.9
6	149	12.3	10.5	11.2	15.0	5.8	4.0	8.4	8.6

nucleic-acid exhibit the following characteristics: a general decline from a mean of 17 per cent. at harvest 1 to one of 6 per cent. at harvest 5; consistent increases with high phosphorus supply, these being more pronounced at N_2 than at N_1; and increases with high nitrogen supply, these being maintained until harvest 5 with P_2.

The nucleo-cytoplasmic ratios were also examined, but, as in the experiment with oats, these revealed no obvious correlation with relative leaf growth rate.

IV. GENERAL DISCUSSION

(*a*) *Phosphorus Fractionation in Plant Tissues*

A full review of the literature covering all aspects of phosphorus nutrition touched upon in this paper would be quite inappropriate, but a review of selected contributions to our knowledge of phosphorus fractionation within the plant may help to focus attention on a neglected field of plant-biochemical and physiological research. Available methods for the estimation of phosphatides, phosphoric esters, nucleic acids, and of nucleic-acid derivatives (such as adenyl pyrophosphate and certain enzymes) leave much to be desired, and lack of critical methods

greatly reduces the value of comparisons between existing sets of data. Procedures more recently elaborated by Arney (1939) and by Albaum and Umbreit (1943) would seem to be an improvement on those previously used, but they are still far too empirical.

Webster (1928) studied the phosphorus distribution in 11 types of grain. Phytin phosphorus tended to be the dominant form, and both inorganic and phosphatide phosphorus were present in very small proportions. The data give no satisfactory index of nucleic-acid content. Using more critical methods, Javillier and Colin (1933) give the following figures:

		Wheat Germ	Lentil Powder
As % of Total Phosphorus	Inorganic P	21	26
	Phytin P	42	51
	Phosphatide P	9	12
	Nucleic-acid P	28	11

Webster and Dalbom (1930) give data for field-grown mung beans at ten stages of growth. In all vegetative parts, organic-phosphorus content fell rapidly with time whereas inorganic-phosphorus content commenced at a low value and remained relatively constant. Expressed as a percentage of the total phosphorus, inorganic phosphorus increased from 10 to nearly 30 per cent. in the leaves; in the stems, it rose from 14 to 88 per cent., but fell again to 55 per cent. at maturity; whereas in the pods, inorganic phosphorus constituted only 14 per cent. of the total phosphorus present. Only traces of lipoid phosphorus (alcohol-ether extraction) were found in any plant parts. The high proportion of inorganic phosphorus in the stems at the time of rapid seed development suggests that phosphorus is translocated in this form. In the following year the same workers compared mung beans grown with and without superphosphate (600 lb. per acre), and at two stages of growth. Treatment had little effect upon the amount of growth, and the absence of any considerable effects upon the total or inorganic-phosphorus contents indicates that little extra phosphorus was taken into the plants.

Knowles and Watkin (1932), using field-grown wheat, determined phosphatide, phytin, and "inorganic" phosphorus, firstly, in the total shoots and later in ears and straw separately (9 growth stages in all). Their work is open to criticism in that they air-dried their material, thus giving greater opportunity for hydrolysis and other changes during drying. Furthermore, their "inorganic" phosphorus was obtained by difference and presumably includes the phosphorus of nucleic acid and of the simpler phosphoric esters. Absolute phosphorus per tiller increased with time in a manner similar to that of the shoots of oat plants receiving P_2 in the present experiments (see Fig. 1); almost 85 per cent. of this was located in the ear at final harvest. Alcohol-soluble and "inorganic" phosphorus were at their maxima soon after the appearance of the ears. Phytin phosphorus was also at a maximum in the straw at this time, but continued to form in the ear up to final harvest. The disappearance of "inorganic" phosphorus from the straw is difficult to reconcile with other data, and throws doubt upon the values given for phytin phosphorus in the straw (95 per cent. of the phosphorus present). The

phytin content of the ears (50 per cent. of the total) agrees substantially with other work. DeTurk, Holbert, and Howk (1933) found no phytin in the vegetative parts of maize, but it appeared in the seeds two weeks after pollination.

DeTurk, Holbert, and Howk (loc. cit.) and Burgevin and Guyon (1933) studied phosphorus fractionation in yellow dent corn and spring barley respectively. Their phosphorus fractions were obtained by successive extraction with absolute alcohol, acid alcohol, and dilute hydrochloric acid; these gave crude indices of phosphatide, inorganic, and esterified phosphorus (including phytin), and the residual phosphorus was an index of nucleic-acid phosphorus. DeTurk, Holbert, and Howk demonstrated the need for rapid drying of their material before extraction; that of Burgevin and Guyon was air-dried. The time trends in the data of DeTurk, Holbert, and Howk for the shoots (excluding ears when present) of two first-generation crosses of corn grown on untreated and phosphorus-treated soil may be summarized by the following mean partition values (per cent. total phosphorus) for the five sampling occasions:

	July 6	July 18	August 1	August 11	August 22
Inorganic P	27	34	35	39	33
Phosphoric-ester P	47	42	32	27	28
Phosphatide P	14	11	14	12	14
Nucleic-acid P	12	13	19	22	25

On the dry-weight basis, the phosphoric ester content fell rapidly with time, whereas the nucleic-acid content remained relatively constant. The two first-generation crosses differed markedly in their response to phosphorus: where the yield was increased, there were also increases in inorganic and nucleic-acid phosphorus, especially for the early sampling occasions; but in the case where there was a negligible increase in yield, the only consistent effect on the phosphorus fractions was a depression in the nucleic-acid content after the first harvest. Burgevin and Guyon found that, prior to earing, phosphorus treatment increased both the relative and absolute amounts of all four of their fractions in the shoots of barley. Inorganic phosphorus had the largest partition value and this value was slightly increased by treatment. Phosphorus intake by the shoots continued to the end of the experiment, and at maturity the dominant fraction was that soluble in dilute hydrochloric acid: at this stage this fraction would occur largely as phytin in the grain. Phosphatide phosphorus at no time exceeded 10 per cent. of the total phosphorus present.

While Strebeyko (1934) presents values for phosphorus fractions in the shoots of oats for only the first two of his five sampling occasions, his experiment has certain features in common with the present experiment with oats. There were five levels of supply of phosphorus* and his dry-weight, phosphorus, and nitrogen data include separate values for grain and straw for harvests 4 and 5. Some analyses for sulphur, potash, and protein nitrogen are given for early stages. Growth responses were considerable up to treatments 3, 4, and 5. The data confirm the following points: (i) phosphorus contents of the straw fall

* Treatments were 0, 0.02, 0.05, 0.10, and 0.20 g. P_2O_5 per pot for treatments 1-5 respectively; treatment 5 would approximate to P_2 of the present experiment.

ultimately to the same level with all but the highest supplies of phosphorus; (ii) with phosphorus deficiency, a greater percentage of the total intake is obtained during the second half of the growth period; (iii) for early growth stages, the nitrogen content of vegetative parts is at first increased, but then decreased by increasing phosphorus supply; (iv) for later growth stages, the nitrogen content of all parts is decreased by increasing phosphorus supply; and (v) the ratio of protein to total nitrogen tends to be decreased by phosphorus deficiency.

Strebeyko's phosphorus fractionation data may be restated in the form of partition values, thus:

	Day 35					Day 45				
Treatment	1	2	3	4	5	1	2	3	4	5
Mineral P	45	38	63	62	61	29	29	38	40	45
Phytin P	25	17	9	10	10	25	19	20	19	18
Insoluble Organic P	29	45	28	28	29	46	52	42	40	37

It is not clear where the phosphoric esters were included in these analyses, and the "insoluble organic" fraction would appear to include both phosphatides and nucleic acids. On a dry-weight basis phytin phosphorus is the only fraction which does not increase markedly with increasing phosphorus supply. In this, as in a later set of data (Strebeyko 1939), the partition values for insoluble organic-phosphorus tend to maxima with supplies in the range 0.0 to 0.04 g. P_2O_5 per pot. This is in accord with the pronounced maxima with P_2 in the partition values for alcohol-soluble and nucleic-acid phosphorus in the present experiment.

(*b*) *The Interpretation of Growth Data*

That growth in plants should be thought of as a complex and closely integrated process can scarcely be regarded as a novel idea, but few attempts have been made to develop its implications. Scientific method must, of necessity, abstract from this complexity, but biological interpretation should not end at the physico-chemical level of organization (Woodger 1929, Chapter VI). In this connection, research on plant hormones has made considerable contributions, for, as Went (1940) says, "the hormone concept transcends the differentation of the organism into cells; hormones integrate cells into an organism."

In the wider study of plant growth, however, where irreversible increase in volume is not the only attribute under consideration, the complexity is such that much might be gained by concentration on the higher rather than the lower relevant levels of organization. This has, in effect, been suggested by Bald (1946), who proposes "a principle of competition for the available metabolites between the various organs of the plant" as the basis for a general plan of growth for the potato plant. Similarly, when some specific function, such as the intake of a nutrient by the plant as a whole, is under discussion, the same principle of competition may be applied with advantage, without necessarily enquiring into the details of the mechanisms which set up the demand for available metabolites. Here again, the principle of competition between plant parts is no new idea (Went 1935), but relatively few sets of data in the literature are sufficiently comprehensive to make its application worth while.

The present paper is one of a series* which has been devoted to the study of certain of the physiological changes that occur in plants during ontogenesis. Stress is laid upon the interrelations of the attributes measured, and the ultimate task in the study of the growth is thought of as the elucidation of the integrated pattern which is characteristic of the living organism.

Experimental variation of this pattern has been achieved by varying the nutrient and water supplies, and by preventing the development of the inflorescence. The attributes measured include the dry-weights of the plant and its parts, the leaf area, and leaf respiration, the absolute and relative contents of water, nitrogen, protein-nitrogen, and phosphorus. To these are now added the phosphorus fractions considered above. Consideration has been given to the interpretation of the changes in dry-weight, leaf area and nutrient content of the whole plant and its composite parts (see especially Watson and Petrie 1940, pp. 323-35). This interpretation is tentatively made in terms of the processes of cell division, cell extension, accumulation of reserves, and differentiation, and of the known determinants of these processes. The interpretations thus tend to be rather too abstract, for little is yet known of the relations between these processes. However, in the case of discussions relating to the redistribution of nitrogen within the plant (Williams 1938, p. 78; Watson and Petrie 1940, p. 332), the idea of active competition between the plant parts is developed at some length. Use is made of units which are characteristic of the higher levels of organization in the living plant, and it is evident that the meristematic tissues are largely involved in the control of the movement of carbohydrate reserves and mineral nutrients within the plant. This is clearly in accord with the view of Hoagland, stated in the introduction to this paper, that the plant should be envisaged as an integrated organism; it implies that explanation should be sought at the biological as well as at the physico-chemical level of organization (Woodger loc. cit.).

An attempt has been made to apply these principles to the data under discussion, with the result that rates of intake of phosphorus were seen to be determined more by the internal factors of demand than by the external factor of supply. Then, too, specific effects of the variation of phosphorus supply on plant establishment, ratios of roots to shoots, and the redistribution of phosphorus within the plant are readily understood in terms of the competitive demand for nutrients by meristematic tissues.

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* *Gramineous plants*: Ballard and Petrie (1936); Petrie (1937); Petrie and Williams (1938): Williams (1936, 1938, 1939, 1946). *Tobacco*: Petrie and Arthur (1943); Petrie, Watson, and Ward (1939); Ward and Petrie (1940); Watson (1939); Watson and Petrie (1940). *Flax*: Tiver (1942); Tiver and Williams (1943).

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VI. References

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