Thus at the shorter time-interval the main change is a general dephosphorylation resulting in an increase of the monophosphates at the expense of the triphosphates, whereas at the longer time-interval there appears to be a catabolic breakdown which results in the depletion in the triphosphates alone, an effect which is particularly marked with the 'ATP' peak.

Whether the dephosphorylation of UTP and GTP is secondary to a lowering of the ATP concentration or whether it is due to an increased enzymic dephosphorylation cannot be determined from the present results; nor is it possible to determine whether the dephosphorylation is a result or a cause of the mitotic inhibition. Nevertheless, the finding that treatment with colchicine leads to a very significant reduction in the concentration of ATP in the tissue is consistent with those schemes which envisage a role for ATP in mitosis and for the idea that the inhibitory action of colchicine on mitosis is effected by its action on the concentration of tissue ATP. In this connexion it is interesting to contrast the effect of Myleran on the liver acid-soluble nucleotides with that of colchicine. Although Myleran is a powerful inhibitor of mitotic activity it has almost no effect on the concentration of any of the individual acid-soluble ribonucleotides, and it would appear that this substance may well produce its inhibitory effect by a mechanism different from that of colchicine.

SUMMARY

1. The concentrations of the acid-soluble ribonucleotides in the livers of normal and partially hepatectomized rats and of normal and partially hepatectomized rats treated with either colchicine or Myleran have been measured.

2. Although the treatment with drugs had little effect on the majority of the nucleotides, colchicine caused the concentrations of NAD and AMP to increase and those of ATP, GTP and UTP to decrease.

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Vitamins in Germination

DISTRIBUTION OF INOSITOL DURING THE GERMINATION OF THE DWARF BEAN, *PHASEOLUS VULGARIS*

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Previous papers from these Laboratories discussed the determination of inositol in germinating oats (Darbre & Norris, 1956) and in the seed and young plants of the French bean (Darbre & Norris, 1957). Studies on inositol in germinating seeds have been described by Burkholder & McVeigh (1945*a, b*), Cheldelin & Lane (1943), Hinton (1942, 1947), Hinton, Peers & Shaw (1953) and Richardson & Axelrod (1957); but to date there is little information on the distribution of inositol in the dissected anatomical parts of the seed and young plant.

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The present work is an extension of that described by Darbre & Norris (1957) and is a comprehensive survey of the distribution of total and free inositol in six anatomical parts of the germinating seed and seedlings of *Phaseolus vulgaris* at six morphologically distinct stages of growth taking place in total darkness.

EXPERIMENTAL

Materials

The seeds used were of the dwarf French bean, *Phaseolus vulgaris* var. Improved Canadian Wonder.

Germination. The germination and early development of the bean seedling was divided into six stages: (1) Dormant ungerminated seed. (2) Steeped turgid seed (1–2 days). (3) Elongated radicle, 1.5 cm. (3 days). (4) Appearance of lateral roots; radicle 7.5 cm., plumule 0.8 cm. (5 days). (5) Extension of hypocotyl; plumule 1.2 cm., hypocotyl 6 cm. (7–8 days). (6) Exhaustion of cotyledons; hypocotyl 12 cm., epicotyl 5 cm. (12–14 days).

The seeds were rendered turgid by steeping in running water at 12–15° for 24–48 hr. They were then laid out on moist filter paper supported in vacuum desiccators and aerated by using a small compressor. For plants required at stage 4 and later, the seedlings were transferred to layers of gauze pegged out on large photographic trays containing water about 1 in. deep. Complete darkness was maintained throughout germination.

Sampling. The seedlings were removed from the germinating trays at the required stage of germination and dissected into their various anatomical parts. The number of each part taken, usually in excess of 100, provided a representative sample from which the mean weight per part could be determined. The samples were weighed, quickly freeze-dried, ground in a mortar and pestle and reweighed. The dry powders were stored in sealed bottles until required for moisture and inositol assay.

Methods

Moisture. After many trials with different weights, temperatures and times, the following procedure was found to give satisfactory and reproducible results: 0.25 g. of sample, in a small glass dish, was heated in an electric oven at 100° for 6 hr.

Inositol. Total and free inositol were determined by microbiological assay, *Schizosaccharomyces pombe* being used as test organism (Norris & Darbre, 1956; Darbre & Norris, 1957). Total inositol was measured after hydrolysis of the sample with N-HCl at 123° for 48 hr.

All contents are reported as $\mu\text{g.}$ of inositol/g. dry wt. or as $\mu\text{g.}$ of inositol/mean wt. of dissected part.

RESULTS

The dry weights and inositol contents of the bean plants throughout the six stages of germination are shown in Table 1.

Whole plant. The total inositol content of the plant falls after stage 3 until, at stage 6, 40 % has disappeared. Simultaneously there is a 'respiration loss' of 30 % in the dry weight of the plant. The free inositol content rises quite sharply in the early stages, and, at stage 6, over 50 % of the total inositol content is in the free state. There is a concomitant loss of bound inositol.

Cotyledons. The dry weight of the cotyledons remains fairly constant until stage 3, after which loss commences until only about 25 % of the original weight remains at stage 6. The total inositol content ($\mu\text{g.}$ /part) also decreases from stage 3, and during the later stages of development free inositol increases greatly at the expense of bound forms.

Plumules. No change in dry weight of the plumules occurs until stage 3, but thereafter it increases quite rapidly, to 23 times the original, at stage 6. The total inositol content also increases from stage 3, owing largely to a rise in free inositol content, the bound forms making a relatively small contribution.

Roots. Dry weight of the roots increases after stage 3 with a concomitant rise in total inositol content. The free and bound forms both show an increase, but, contrary to the observation made with the plumules, by far the greater part of the increase in total inositol is due to bound forms.

Testas. The leaching action of the water in the steeping process accounts for a 54 % loss of total inositol in the testas. High colour and turbidity of extracts prohibited assay of free inositol in the testas of ungerminated beans, and in the later stages of germination the amounts of free inositol were beyond the lower limits of assay. After stage 2 the total inositol content and dry weight remain virtually unchanged.

Epicotyls and hypocotyls. These structures could be dissected only at the later stages of germination. The rapid elongation of the plant which takes place at stages 5 and 6 is amply reflected in the increase in dry weight of the hypocotyl between these stages. There is a parallel rise in total inositol content, and, as in the plumules, the increase is largely due to free inositol. The epicotyls, at stage 6, also contain a high proportion of free inositol.

DISCUSSION

The total inositol content of the whole bean shows a marked fall during the first 2 weeks of growth. This phenomenon has been observed during the germination of cabbage, maize, mung bean, pea, soya bean and wheat when grown in the light, and in etiolated pea and mung-bean seedlings (Richardson & Axelrod, 1957) and in oats (Darbre & Norris, 1956). Several authors have described changes in inositol content during germination and early development of seedlings (Burkholder, 1943; Cheldelin & Lane, 1943); but valid and convincing results had to await the development of satisfactory methods for the extraction and assay of inositol.

Recently, pathways whereby inositol is incorporated into the cycles of carbohydrate metabolism have been described for the rat kidney (Charalampous, Bumille & Graham, 1958), and for *Schwanniomyces occidentalis* (Sivan & Hoffmann-Ostenhof, 1961), but little is known of its fate within the plant. Although Fernandez, Izquierdo & Martinez (1944) reported the presence in *Lactuca virosa* of an enzyme, cyclase, which would effect the conversion of glucose into inositol, this

Table 1. *Dry weight and inositol content of anatomical parts of the bean at six stages of growth*

Stage	Part	Dry wt./part (g.)	Inositol					
			Total		Free		Bound	
			($\mu\text{g./g.}$)	($\mu\text{g./part}$)	($\mu\text{g./g.}$)	($\mu\text{g./part}$)	($\mu\text{g./g.}$)	($\mu\text{g./part}$)
1	Plumules	0.0007	10 980	8	1 510	1	9 500	7
	Cotyledons	0.4852	3 960	1 921	260	126	3 700	1 795
	Radicles	0.0035	7 750	27	2 240	8	5 510	19
	Testas	0.0403	1 030	42	—	—	—	—
	Total	0.5297	—	1 998	—	135	—	1 821
2	Plumules	0.0006	10 940	7	1 460	1	9 480	6
	Cotyledons	0.4657	4 180	1 947	190	88	3 990	1 858
	Radicles	0.0031	7 690	24	1 270	4	6 420	20
	Testas	0.0446	380	17	0	0	380	17
	Total	0.5140	—	1 995	—	93	—	1 901
3	Plumules	0.0006	10 060	6	3 000	2	7 060	4
	Cotyledons	0.4829	4 340	2 096	550	265	3 790	1 830
	Radicles	0.0036	6 320	23	2 080	7	4 240	15
	Testas	0.0372	310	12	0	0	310	12
	Total	0.5243	—	2 137	—	274	—	1 861
4	Plumules	0.0030	7 370	22	4 610	14	2 760	8
	Cotyledons	0.4301	4 050	1 742	930	400	3 120	1 342
	Roots	0.0177	3 760	67	1 410	25	2 350	42
	Testas	0.0351	320	11	0	0	320	11
	Total	0.4859	—	1 842	—	439	—	1 403
5	Plumules	0.0064	6 150	39	3 930	25	2 220	14
	Cotyledons	0.3739	4 050	1 514	1 420	531	2 630	983
	Hypocotyls	0.0256	4 060	104	1 899	48	2 170	56
	Roots	0.0205	3 820	78	1 560	32	2 250	46
	Testas	0.0371	310	12	0	0	310	12
	Total	0.4635	—	1 747	—	636	—	1 111
6	Plumules	0.0185	8 370	161	6 600	122	2 130	39
	Epicotyls	0.0303	4 180	127	2 530	77	1 650	50
	Cotyledons	0.1329	3 190	424	1 310	174	1 880	250
	Hypocotyls	0.0854	3 330	284	2 180	186	1 150	98
	Roots	0.0682	2 970	203	820	56	2 150	147
	Testas	0.0364	280	10	0	0	280	10
	Total	0.3717	—	1 209	—	615	—	594

claim has not been substantiated. Fischer (1945) suggested that phytin is a storage form for carbohydrate as well as phosphate and intimated that inositol could be easily converted into glucose. A metabolic link between inositol and the sugars and sugar acids associated with pectin and hemicelluloses is indicated by the production from labelled inositol in strawberry fruit and parsley leaves of D-galacturonosyl units of pectin, and other products such as D-xylose and the D-arabinosyl and D-xylosyl residues of hemicelluloses. It is suggested that inositol is converted into D-glucuronic acid in plant tissues (Loewus, Kelly & Neufeld, 1962).

The relatively small amount of phytin inositol in the bean compared with total reserve carbohydrates (Greenshields, 1961) suggests that the metabolic removal of inositol during the germination and early development of the plant under etiolated

conditions is a function of cellular economy rather than cellular necessity.

The leaves and roots show an increase in their respective total inositol contents, but whereas in the leaves this rise is due to an influx of free inositol, that in the roots is due to bound forms. This may reflect the functional differences, in the physiological sense, of the two structures. It is well known that inositol hexaphosphate appears to have a particular affinity for Ca^{2+} , Mg^{2+} and K^{+} ions in the phytin complex. The root system is involved in the ion uptake of the plant, and the presence of relatively large amounts of inositol phosphates in the roots may be exerting an influence on the ionic balance.

The overall picture obtained in this study is of the release, within the cotyledons, of inositol from phytin by the enzyme phytase, some properties of which will be reported later. Subsequently, ino-

sitol is drained away from the cotyledons and translocated to the growing parts of the plant. Whether inositol is translocated in the free state, or in some combined form, is not known at present, but the metabolic fate of inositol during the early development of the bean seedling seems to depend on the site to which translocation takes place.

SUMMARY

1. The inositol content of each anatomical part of the bean seedling has been determined, by microbiological assay, at each of six stages during the early development of the plant.

2. The total inositol content of the bean seedling decreases by 40 % during the first 2 weeks of growth in the dark.

3. Inositol is liberated from its bound forms (phytin) in the cotyledons and translocated to the growing parts of the plant.

4. The inositol content of the plumules increases 20-fold during early development of the plant, the greater part of the increase being due to free inositol.

5. The increase in content of the roots (about eightfold) is due mainly to bound forms of inositol.

6. The testas do not appear to play any part in the metabolism of inositol during germination.

7. The hypocotyls show an increase in total inositol content, mainly due to an influx of free inositol. The epicotyls behave similarly.

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Phytase and Acid Phosphatase in the Dwarf Bean, *Phaseolus vulgaris*

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A comprehensive review of phytase has appeared (Sloane-Stanley, 1961) and the acid phosphatases have also been discussed (Morton, 1961). In the present paper some properties of phytase and a purified acid phosphatase in the dwarf French bean are presented, together with some observations on these activities in the early stages of metabolism of the developing plant.

EXPERIMENTAL

Materials

The plant used was the dwarf French bean, *Phaseolus vulgaris* var. Improved Canadian Wonder.

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Enzyme substrates. A commercial preparation of sodium phytate was recrystallized from water. The crystalline product was dried *in vacuo* for several days and ground to a white powder, which was moisture-free and contained no free phosphate. Chromatography revealed the presence of only one P-containing compound. This preparation was used as a substrate (1 mM) for phytase assays.

Sodium phenyl phosphate was used as substrate for acid-phosphatase assays.

Diethylaminoethyl(DEAE)-cellulose was prepared according to the method of Peterson & Sober (1956) and stored as a dry powder until required.

Calcium phosphate gel was prepared according to Dixon & Webb (1958) and stored for at least a month before use.

Buffer systems. Stock buffer solutions were prepared according to the data of Gomori (1955) except that concentrations were increased to 1.0 M. The following systems were used, over the pH ranges indicated: pH 2.0-3.5, glycine-