Nossal, P. M. (1952). *Biochem. J.* **50**, 349.
Ochoa, S. (1952). *The Enzymes*, vol. 2, p. 929. Ed. by Sumner, J. B. & Myrbäck, K. New York: Academic Press.
Sacktor, B. (1953). *Arch. Biochem. Biophys.* **45**, 349.
Smolin, A. N. (1952). *Biochemistry, Leningr.*, **17**, 61.
Straub, F. B. (1942). *Hoppe-Seyl. Z.* **275**, 63.
Suzuki, H. (1920). *Sakura Kaishi*, **8**. [Quoted by Yamafuji (1937).]
Tsuji, Y. (1909). *Sanji Hokoku*, **35**, 1. [Quoted by Yamafuji (1937).]
Veiga Salles, J. B. & Ochoa, S. (1950). *J. biol. Chem.* **187**, 849.
Venkatachala Murthy, M. R. & Sreenivasaya, M. (1953). *J. sci. industr. Res.* B, **12**, 314.
Wyatt, G. R., Loughheed, T. C. & Wyatt, S. S. (1956). *J. gen. Physiol.* **39**, 853.
Yamafuji, K. (1937). *Tabul. biol., Hague*, **14**, part 1.

Enzymic Reduction of Sugar Phosphates in Insect Blood

By P. FAULKNER
Laboratory of Insect Pathology, Sault Ste. Marie, Ontario, Canada

(*Received* 6 *March* 1956)

The presence of carbohydrate and phosphorus compounds in insect blood is now well established and data on their distribution have been summarized by Buck (1953). Smolin (1952) obtained evidence for the presence of glucose 6-phosphate and smaller quantities of glucose 1-phosphate in acid extracts of silkworm (*Bombyx mori* L.) blood. Wyatt, Loughheed & Wyatt (1956), using more refined techniques, have confirmed the presence of glucose 6-phosphate in acid extracts of freeze-dried silkworm blood but were unable to find glucose 1-phosphate. Of the seven phosphorus-containing materials separated by Wyatt *et al.* (1956) from silkworm blood, only glucose 6-phosphate and inorganic phosphate have been identified.

The present report is concerned with the finding that certain sugar phosphates are reduced to polyhydric alcohol phosphates by an enzyme in silkworm blood.

EXPERIMENTAL

Preparation of silkworm blood. The dialysed silkworm blood used in this investigation was obtained as described previously (Faulkner, 1956).

Materials. Glucose, glucose 6-, fructose 6- and ribose 5-phosphates were purchased from Schwarz Inc., New York; galactose 6-phosphate and triphosphopyridine nucleotide (TPN) from Nutritional Biochemical Co., Cleveland, Ohio, and reduced TPN from the Sigma Chemical Co., St Louis, Missouri. The galactose 6-phosphate was found to be contaminated with material reacting as a ketose, and was purified by several reprecipitations of the barium salt from ethanol–water (5:1, v/v).

Sorbitol 6-phosphate was prepared by reduction of the sodium salt of glucose 6-phosphate with a sodium borohydride solution by the procedure of Abdel-Akher, Hamilton & Smith (1951). The anthrone reagent, which does not give a colour with sorbitol, was used to determine when the sugar phosphate was completely reduced. The product was converted into the barium salt and recrystallized three times from ethanol–water (3:1, v/v) at pH 8·5. Periodate oxidation of the product gave a formaldehyde: phosphorus ratio of 1·16:1. The theoretical value for sorbitol 6-phosphate is 1:1.

The concentration of sugar phosphate solutions was calculated from the organic phosphorus content by the method of Fiske & Subbarow (1925).

Methods. Sugar phosphates were determined colorimetrically by means of the reaction with anthrone, as follows. To the sample, made up to 0·5 ml., 2 ml. of the anthrone reagent (Fairbairn, 1953) was added. After thorough mixing the solution was heated for 12 min. in a boiling-water bath and cooled immediately, and the colour read at 625 mμ.

Pentose was determined by the method of Meijbaum (1939), with 0·033% in place of 0·1% $FeCl_3$.

Polyhydric alcohols were determined by the periodate-oxidation method of West & Rapoport (1949) with the following modifications. Half volumes of samples and reagents were used throughout, and the solutions were treated with periodate for 4 min. only. The samples were heated in a boiling-water bath for 5 min. after addition of the chromotropic acid reagent. Sorbitol or ribitol (adonitol) was used as a standard.

Pyruvate was determined by the method of Friedemann & Haugen (1943), ethyl acetate being used to extract the 2:4-dinitrophenylhydrazone.

Experiments in which a change of reduced TPN concentration was measured were carried out at room temperature in silica cells (1·0 cm. light path) in a Beckman DU spectrophotometer at 340 mμ.

RESULTS

Reduction of sugar phosphates in dialysed silkworm blood

Dialysed silkworm blood contains a TPN-linked dehydrogenase capable of reducing a number of sugar phosphates. The reaction, whose equilibrium favours the formation of reduced-sugar phosphate, may be followed spectrophotometrically by observing the decrease in optical density at 340 mμ. of

a mixture containing reduced TPN, sugar phosphate and enzyme. A typical experiment, in which glucose 6-phosphate was reduced, is illustrated in Fig. 1. During the first 2 min. of this experiment reduced TPN was incubated with dialysed blood, and it will be seen that there was little decrease in optical density, which indicates that no endogenous substrate was present. After 2 min., 5 μmoles of glucose 6-phosphate were added. At first reduced TPN was rapidly oxidized, but eventually an equilibrium was reached. The sugar phosphate and reduced TPN do not react in the absence of the enzyme preparation.

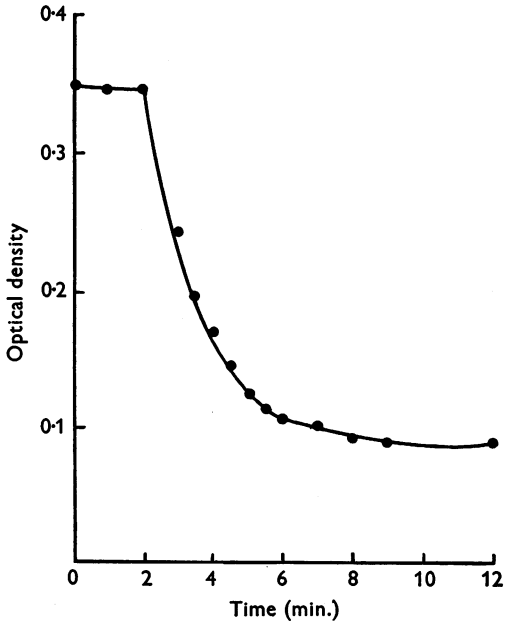

Fig. 1. Reduction of glucose 6-phosphate. Initial incubation mixture: tris buffer, pH 8·0, 20 mM; $MgSO_4$, 2·5 mM; reduced TPN, 0·12 mM; dialysed blood, 0·2 ml. After 2 min. 5 μmoles of glucose 6-phosphate was added. Final vol., 2·2 ml. Optical-density readings were corrected for dilution after addition of glucose 6-phosphate.

Table 1. *Rates of reduction of some sugars and sugar phosphates*

Incubation conditions as in Fig. 1, with sugar phosphate added to give final concn. 1·0 mM.

	Rate of reduction expressed in arbitrary units
Ribose 5-phosphate	1430
Galactose 6-phosphate	129
Glucose 6-phosphate	100
Fructose 6-phosphate	33
Glucose 1-phosphate	0
Glucose	0
Fructose	0

Several sugar phosphates are reduced in the presence of the enzyme, and a comparison of the rates at which the reaction proceeds with a number of substrates is given in Table 1. In these experiments enzymic activity was calculated for the period of 30 sec. following the addition of the sugar phosphate. The results show that, in order of decreasing rates, the following sugar phosphates are reduced: ribose 5-phosphate, galactose 6-phosphate, glucose 6-phosphate and fructose 6-phosphate. The rate of reduction of ribose 5-phosphate is more than ten times that of the next sugar in the series, galactose 6-phosphate. It is apparent that all the sugars that react possess a potential carbonyl group. Glucose 1-phosphate, which lacks this, is not reduced, but is hydrolysed to the free sugar by a specific enzyme in silkworm blood (Faulkner, 1955). Neither glucose nor fructose is reduced by the enzyme.

Coupling of the sugar phosphate reductase with the 'malic' enzyme

Dialysed silkworm blood contains a TPN-linked 'malic' enzyme whose equilibrium is in favour of pyruvate production (Faulkner, 1956). Experiments were therefore carried out to determine whether the oxidation of malic acid could be

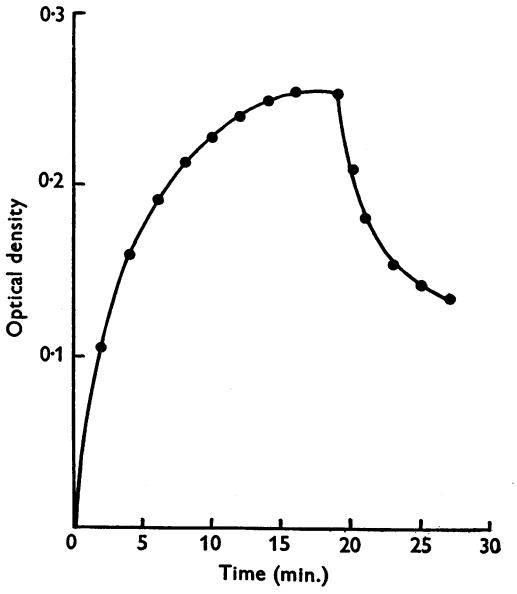

Fig. 2. Coupling of L-malate oxidation and glucose 6-phosphate reduction. Initial incubation mixture: tris buffer, pH 8·5, 10 mM; $MgSO_4$, 2·5 mM; L-malate, 2·5 mM; TPN, 0·09 mM; dialysed blood, 0·5 ml. At 19 min., 5 μmoles of glucose 6-phosphate was added. Final vol., 2·2 ml. Optical-density readings have been corrected for the dilution after addition of glucose 6-phosphate.

coupled to the reduction of sugar phosphates, dialysed silkworm blood being used as the enzyme source. The reactions visualized are:

$$\text{L-Malate} + \text{TPN}_{ox.} \rightarrow \text{pyruvate} + CO_2 + \text{TPN}_{red.},$$

$$\text{TPN}_{red.} + \text{sugar phosphate} \rightarrow \text{reduced-sugar phosphate} + \text{TPN}_{ox.}.$$

$$\text{Sum: L-malate} + \text{sugar phosphate} \xrightarrow{\text{TPN}} \text{pyruvate} + CO_2 + \text{reduced-sugar phosphate}. \quad (1)$$

Confirmation that these reactions take place was obtained in the following experiment (Fig. 2). TPN was completely reduced by incubation with dialysed blood containing L-malate; glucose 6-phosphate was then added and a new equilibrium for the concentration of reduced TPN was established. These results show that reduction of sugar phosphates occurs in a mixture in which reduced TPN is generated by the oxidation of malic acid.

By coupling the oxidation of malate with the reduction of sugar phosphate it was possible to obtain enough of the end products to investigate the chemical nature of the reduced-sugar phosphate and also to obtain data on the stoicheiometry of the reactions involved in its formation.

Pyruvate production

The conditions for maximum pyruvate production in the coupled reaction were investigated and the results are shown in Table 2. Maximum formation of pyruvate resulted when L-malate, TPN and glucose 6-phosphate were contained in the incubation mixture; in the absence of any of the components only small quantities of pyruvate were found. Results given in Table 2 also show that inorganic phosphate is not released during the reaction, and other experiments have demonstrated that the addition of inorganic phosphate to the reaction medium has no effect on the quantity of pyruvate synthesized; orthophosphate, therefore, does not appear to be essential for the reaction.

Most of the experiments on the reduction of sugar phosphates were carried out in open test tubes, i.e. aerobically. The results given in Table 3 show that approximately the same amounts of pyruvate are produced by the coupled system in air or under nitrogen.

An increase in pyruvate production is also observed when glucose 6-phosphate is replaced by fructose 6-phosphate in the coupled reaction, but not when replaced by glucose 1-phosphate (Table 4). These results confirm the observation that glucose 1-phosphate is not reduced by the silkworm-blood reductase.

Table 2. *Coupling of* L-*malate oxidation with glucose* 6-*phosphate* (*G* 6-*P*) *reduction*

Basic medium: tris buffer, pH 8·0, 20 mM; $MgSO_4$, 5 mM; dialysed blood, 0·5 ml. Additions: L-malate, 10 mM; TPN, 0·1 mM; glucose 6-phosphate, 2·5 mM. Total vol., 1·0 ml. Incubated for 2·5 hr. at 30°.

Additions	Pyruvate (μmoles)	Inorganic P (μmoles)
L-Malate + TPN + G 6-P	2·03	0·19
L-Malate + G 6-P	0·25	0·18
L-Malate + TPN	0·26	0·08
TPN + G 6-P	0·23	0·14

Table 3. *Pyruvate production under aerobic and anaerobic conditions*

Basic medium: tris buffer, pH 8·0, 20 mM; dialysed blood, 0·3 ml. Additions: L-malate, 10 mM from side arm at zero time; TPN, 0·1 mM; glucose 6-phosphate (G 6-P), 2·5 mM. Total vol., 1·0 ml. Incubated in Warburg flasks for 40 min. at 30°. Gas phase as indicated.

Additions	Pyruvate (μmoles)	
	N_2	Air
L-Malate + TPN	0·11	0·13
TPN + G 6-P	0·06	0·06
L-Malate + TPN + G 6-P	0·60	0·62

Table 4. *Pyruvate formation in presence of sugar phosphates*

Basic medium: tris buffer, pH 8·0, 20 mM; $MgSO_4$, 5 mM; L-malate, 10 mM; TPN, 0·1 mM; dialysed blood, 0·5 ml.; sugar phosphate additions, 5 mM. Total vol., 2·0 ml. Incubated for 60 min. at 30°.

Additions	Pyruvate (μmoles)	Net synthesis in presence of sugar phosphate (μmoles)
Nil	0·27	—
Glucose 1-phosphate	0·28	0·01
Glucose 6-phosphate	1·11	0·84
Fructose 6-phosphate	0·78	0·51

Production and identity of reduced-sugar phosphate

Two lines of evidence indicate that the reduced-sugar phosphate produced in the coupled reaction is a polyhydric alcohol phosphate.

(*a*) *Chromatography.* The phosphate ester generated when glucose 6-phosphate is reduced in the coupled reaction with malate oxidation has been separated from glucose 6- and fructose 6-phosphates by descending paper chromatography. Phosphates were detected on the paper by spraying with the molybdate reagent of Hanes & Isherwood (1949). The unknown ester separated from glucose 6-phosphate in solvent *B* containing *tert.*-butanol, picric and boric acids. However, the R_F of the unknown (0·50) was very close to that of fructose

6-phosphate (0·46) in this solvent (Table 5, solvent *B*). In order to distinguish the unknown phosphate ester from fructose 6-phosphate the incubation mixture was chromatographed with solvent *A* (De Ley, 1954) containing *tert.*-butanol and picric acid. In this solvent the newly formed phosphate ester had an R_F close to that of glucose 6-phosphate and quite distinct from fructose 6-phosphate (Table 5, solvent *A*). By the use of solvent *B*, which contained borate, the unknown phosphate ester was found to behave similarly to sorbitol 6-phosphate prepared chemically by reduction of glucose 6-phosphate.

(*b*) *Polyhydric alcohol phosphate analysis.* Polyhydric alcohols may be determined by controlled oxidation with periodic acid, followed by quantitative analysis of the liberated formaldehyde. The nature of the oxidation products when polyhydric alcohol solutions are treated with periodate is reviewed by Jackson (1944). One mole of an unsubstituted polyhydric alcohol yields 2 moles of formaldehyde by oxidation of the terminal $-CH_2.OH$ groups. Thus a polyhydric alcohol in which one of the terminal carbons has been substituted by phosphorylation would yield only 1 mol. prop. of formaldehyde. This reasoning has been followed in the calculation of the amounts of polyhydric alcohol phosphate produced by the enzymic reduction of sugar phosphates in silkworm blood.

The amounts of pyruvate and polyhydric alcohol phosphate formed when malate oxidation is coupled with the reduction of glucose 6-phosphate are compared in Table 6, where it will be seen that the molar ratio between the products, pyruvate and polyhydric alcohol phosphate, is almost 1:1 throughout the incubation period. This relationship is predicted by equation (1).

Two sets of data are given in Table 7, where the results refer to the reduction of ribose 5-phosphate. It will be seen that the maximum amount of ribitol phosphate is produced when the incubated mixture contains malate, TPN and ribose 5-phosphate. With heated enzyme, or with TPN or malate absent from the complete system, little ribitol phosphate is formed. The results in Table 7 also demonstrate that when 1 mole of ribose 5-phosphate is removed from the incubation mixture during the coupled reaction, approximately 1 mole of ribitol phosphate is produced.

Isomerase in silkworm blood

In order to determine the identity of the polyhydric alcohol phosphate produced by enzymic reduction of an added sugar phosphate, it was necessary to investigate whether the sugar phosphate underwent isomerization before reduction. Specifically, it was important to determine whether glucose 6- and fructose 6-phosphates were

Table 5. *Chromatography of sugar phosphates*

Solvent *A* contained *tert.*-butanol, 80 ml.; water, 20 ml.; picric acid, 4 g. Solvent *B* as for solvent *A* with 2 g. of boric acid added.

	R_F of phosphate esters	
	Solvent *A*	Solvent *B*
Glucose 6-phosphate (G 6-P)	0·28	0·31
Fructose 6-phosphate	0·39	0·46
Sorbitol 6-phosphate	—	0·50
L-Malate–G 6-P coupled reaction*	0·24, 0·28	0·30, 0·50
L-Malate–G 6-P coupled reaction, inactivated at zero time	0·25	0·31

* Incubation conditions for L-malate–G 6-P coupled reaction as in Table 2.

Table 6. *Stoicheiometry of pyruvate and polyhydric alcohol phosphate production*

Tubes contained: tris buffer, pH 7·5, 20 mM; $MgSO_4$, 5 mM; L-malate, 20 mM; TPN, 0·08 mM; G 6-P, 2 mM; dialysed blood, 0·5 ml. Total vol., 1·0 ml. Incubated at 30° for time indicated.

Time of incubation (min.)	Pyruvate found (μmoles)	Polyhydric alcohol phosphate found (μmoles)
30	0·23	0·22
60	0·42	0·42
90	0·60	0·58

Table 7. *Synthesis of ribitol phosphate*

Incubation conditions: tris buffer, pH 7·5, 20 mM; $MgSO_4$, 5 mM; L-malate, 20 mM; TPN, 0·08 mM; ribose 5-phosphate (R 5-P), 4 mM; dialysed blood, 0·5 ml. Total vol., 1·0 ml. Incubated at 30°, in Expt. 1 for 90 min.; in Expt. 2 for times indicated. In Expt. 1 tubes contained L-malate, TPN and R 5-P as indicated. All tubes in Expt. 2 contained complete incubation medium.

Expt. 1. Conditions for ribitol phosphate synthesis

Additions	Ribitol phosphate found (μmoles)
L-Malate + TPN + R 5-P	3·36
L-Malate + TPN + R 5-P*	0·07
L-Malate + R 5-P	0·36
TPN + R 5-P	0·30
R 5-P	0·10

* Heated enzyme present in mixture.

Expt. 2. Relationship between R 5-P uptake and ribitol phosphate formation

Incubation time (min.)	R 5-P found (μmoles)	R 5-P removed during enzymic reaction (μmoles)	Ribitol phosphate found (μmoles)
0	3·97	0·00	0·00
30	2·61	1·36	1·24
60	1·42	2·55	2·34
90	0·41	3·56	3·51
120	0·06	3·91	4·04

interconvertible and whether ribose 5-phosphate was converted into ribulose 5-phosphate by an enzyme in dialysed silkworm blood.

Isomerase activity, with glucose 6-phosphate, galactose 6-phosphate and ribose 5-phosphate as test substrates, was determined under the experimental conditions of Axelrod & Jang (1954). Controls, in which the reaction mixture was inactivated at zero time, were run. The qualitative results indicated that glucose 6-phosphate was converted into a keto sugar. However, ribose 5- and galactose 6-phosphates did not form keto sugars. Phosphohexoisomerase activity was investigated further by the method of Roe (1934) for keto sugar analysis. The results, shown in Fig. 3, indicate that glucose 6-phosphate is rapidly converted into fructose 6-phosphate initially, but that the rate of ketose formation decreases as the equilibrium position is approached.

It is not possible to predict whether sorbitol 6- or mannitol 6-phosphate is formed when glucose 6- or fructose 6-phosphate is reduced by the enzyme. If there were no conversion into fructose 6-phosphate, glucose 6-phosphate would be reduced to sorbitol 6-phosphate, and fructose 6-phosphate would form either mannitol 6- or sorbitol 6-phosphate, or a mixture of both. However, since galactose 6- and ribose 5-phosphates are not converted into keto sugars, only galactitol 6- and ribitol 5-phosphates can be formed when they are reduced, provided that no Walden inversion occurs in the configuration of the remaining hydroxyl groups.

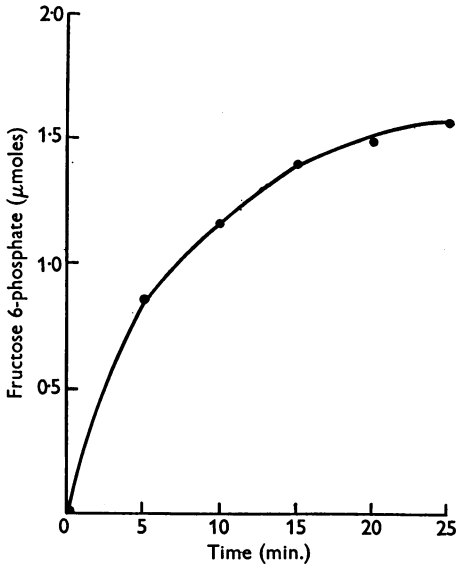

Fig. 3. Isomerase of silkworm blood. All tubes contained: tris buffer, pH 7·5, 40 mM; glucose 6-phosphate, 10 mM; dialysed blood, 0·2 ml. Total vol., 1·0 ml. Incubated at 30° for times indicated.

DISCUSSION

The polyhydric alcohol phosphate dehydrogenase of silkworm blood is somewhat similar in action to mannitol 1-phosphate dehydrogenase of *Escherichia coli* recently described by Wolff & Kaplan (1954, 1955, 1956). The bacterial enzyme, which is a DPN-linked dehydrogenase, appears to have a high substrate specificity and reduces fructose 6- to mannitol 1-phosphate. The TPN-linked enzyme of silkworm blood reduces both aldose and ketose phosphates possessing potential carbonyl groups. To date, it has not been possible to separate the dehydrogenase from the phosphohexoisomerase in silkworm blood, although this is desirable since only then will it be possible to determine with certainty whether the enzyme is specific for aldose phosphates.

Silkworm blood contains a 'malic' enzyme (Faulkner, 1956) and analyses have shown that malic acid (Tsuji, 1909) and glucose 6-phosphate (Wyatt *et al.* 1956) are also present; thus synthesis of polyhydric alcohol phosphates probably takes place in silkworm blood. It is possible that some of the unidentified phosphorus-containing compounds separated by Wyatt *et al.* (1956) from silkworm blood belong to the same class of compounds.

In the presence of reduced TPN, the equilibrium of the polyhydric alcohol phosphate dehydrogenase is in favour of synthesis of polyhydric alcohol phosphate. These substances, which are more highly reduced than is the parent sugar phosphate, may be regarded as possessing an energy-storage function and may assist in the carrying over of metabolic energy at certain stages of the insect's development.

SUMMARY

1. Dialysed silkworm blood contains a TPN-linked dehydrogenase that, in order of decreasing rates, reduces the following sugar phosphates to polyhydric-alcohol phosphates: ribose 5-, galactose 6-, glucose 6- and fructose 6-phosphates.

2. Synthesis of polyhydric alcohol phosphate can be accomplished by coupling the oxidation of malic acid with the reduction of the sugar phosphate in the presence of TPN and dialysed silkworm blood.

3. Phosphohexoisomerase is present in silkworm blood; no ribose phosphate-isomerase activity has been observed.

4. The possibility is considered that polyhydric alcohol phosphate formation in the insect assists in the storage of metabolic energy.

The technical assistance of Miss Lorna Callahan is gratefully acknowledged. This paper is Contribution no. 289, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

REFERENCES

Abdel-Akher, M., Hamilton, J. K. & Smith, F. (1951). *J. Amer. chem. Soc.* **73**, 4691.
Axelrod, B. & Jang, R. (1954). *J. biol. Chem.* **209**, 847.
Buck, J. B. (1953). *Insect Physiology*, p. 147. Ed. by Roeder, K. D. New York: John Wiley and Sons, Inc.
De Ley, J. (1954). *Enzymologia*, **17**, 55.
Fairbairn, N. J. (1953). *Chem. & Ind.* p. 86.
Faulkner, P. (1955). *Biochem. J.* **60**, 590.
Faulkner, P. (1956). *Biochem. J.* **64**, 430.
Fiske, C. M. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
Jackson, E. L. (1944). *Organic Reactions*, vol. 2, p. 341. New York: John Wiley and Sons.
Meijbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
Roe, J. M. (1934). *J. biol. Chem.* **107**, 15.
Smolin, A. N. (1952). *Biochemistry, Leningr.*, **17**, 61.
Tsuji, Y. (1909). *Sanji Hokoku*, **35**, 1. [Quoted by Yamafuji, K. (1937). *Tabul. biol., Hague*, **14**, part 1.]
West, C. D. & Rapoport, S. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 142.
Wolff, J. B. & Kaplan, N. O. (1954). *Bact. Proc.* p. 110.
Wolff, J. B. & Kaplan, N. O. (1955). *Methods in Enzymology*, vol. 1, p. 346. New York: Academic Press.
Wolff, J. B. & Kaplan, N. O. (1956). *J. biol. Chem.* **218**, 849.
Wyatt, G. R., Loughheed, T. C. & Wyatt, S. S. (1956). *J. gen. Physiol.* **39**, 853.

Vitamins in Germination

DETERMINATION OF FREE AND COMBINED INOSITOL IN GERMINATING OATS

By A. DARBRE and F. W. NORRIS
Department of Applied Biochemistry, University of Birmingham, and Department of Physiology, Biochemistry and Pharmacology, King's College, University of London

(*Received* 26 *March* 1956)

The earliest record of the occurrence of inositol in plant materials appears to be that of Vohl (1856), who isolated inositol from haricot beans. It has subsequently been found in all plants examined, and its combined form phytin, a calcium, magnesium and potassium complex of inositol phosphoric acids, also appears to be ubiquitous in the plant world. Inositol has been found in phospholipids of soya bean (Klenk & Sakai, 1939; Woolley, 1943); of ground nut (Hutt, Malkin, Poole & Watt, 1950); and maize (Scholfield, McGuire & Dutton, 1950). Hawthorne & Chargaff (1954) isolated, from soya bean phosphatides, sugars in combination with inositol monophosphate.

Quantitative studies of inositol have been limited hitherto by the lack of satisfactory analytical techniques and by the difficulty of distinguishing it from its isomers and congeners. The microbiological method is the most sensitive and accurate method at present available for the assay of *meso*-inositol, and in the following studies we have used *Schizosaccharomyces pombe* as assay organism on the lines developed by Northam & Norris (1952), with modifications introduced by Norris & Darbre (1956).

The study of vitamins of the B group in the germination of seeds necessarily waited on the growth of knowledge of the group and the development of satisfactory methods of assay. In the last 10–15 years the changes in content of a number of B vitamins in seeds during germination have been investigated by several workers. The literature has been reviewed by Norris (1947, 1950). In general, the reported changes, whether of increase or decrease of a particular vitamin after germination, have tended to become smaller as time went on. This is due largely to improvements in assay techniques, but also to greater care in planning experiments and assessing results. Burkholder (1943) reported an increase of inositol from 630 μg./g. dry wt. to 1290 μg./g. after 5–6 days' germination of oats. Taking into account the loss in dry weight of the seed on germination, from data given by Burkholder, we have calculated a real increase from 12·2 to 19·1 μg. of inositol/seed. This increase is in sharp contrast with the decrease reported in the present paper. Differences may be due in varying degrees to much higher temperatures and humidities in America than are normal in this country. The analytical procedure is open to question as it involved digestion with a papain–Taka diastase mixture, a method found to result in incomplete liberation of inositol (Jones, 1951).

The literature offers a wide choice of conditions for the liberation of inositol from phytin, whether by chemical (Bartow & Walker, 1938) or enzymic means (Peers, 1953). We believe that the sealed-tube method with N-HCl described below gives a