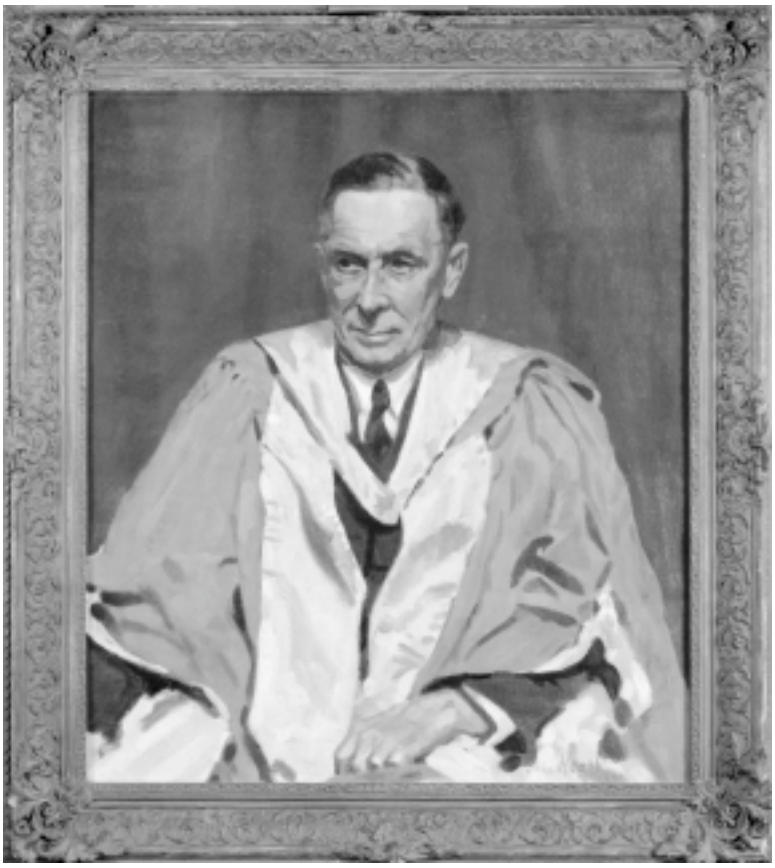
Liversidge Research Lecture

No. 2
1933

THE FUNCTION OF PHOSPHATES IN FERMENTATIONS OF SUGARS

W.J. YOUNG

The Royal Society of New South Wales



William John Young

This photograph of a portrait of W. J. Young is reproduced by permission of the University of Melbourne

Liversidge Research Lecturer No. 2, 1933

WILLIAM JOHN YOUNG 1878-1942

William John Young was born on 26 January 1878 at Withington, Manchester (U.K.). His early education was at Hulme Grammar School, Manchester, and then he studied at Owens College, Manchester, graduating B.Sc. in 1898. With the Levinstein (1899-1900) and the Dalton (1900-1901) Research Exhibitions he then did research in organic chemistry at Owens College and graduated M.Sc. in 1902. For the period 1900-1912 he was assistant biochemist at the Lister Institute of Preventive Medicine, London where he worked under (Sir) Arthur Harden (Nobel Laureate, 1929) on the chemistry of the yeast fermentation of sugars. The results of this very fruitful collaboration included the discovery of the coenzyme necessary for yeast fermentation, and the requirement of phosphates, particularly the involvement of hexose diphosphate in transformation of glucose into ethanol and carbon dioxide. In 1910 Young was awarded the degree of D.Sc. by the University of London. In 1913 he migrated to Australia to take up the position of biochemist at the Australian Institute of Tropical Medicine at Townsville. In 1920, Professor W.A. Osborne recruited Young as Lecturer in Physiology and Biochemistry in the Department of Physiology, University of Melbourne. He became director of the Department of Biochemistry, and was promoted to Associate Professor in 1924: he was appointed as the Foundation Professor of Biochemistry in 1938. His main interest became problems with the preservation and transport of food, and in 1926 A.C.D. (David) Rivett (later Sir David) obtained Young's leave of absence from the University to help the Council for Scientific and Industrial Research (CSIR) with studies of the biochemistry of cold storage of food. In 1928 he returned to his University duties, but remained a consultant to CSIR which set up a small Section of Food Preservation in 1931; this became the Division of Food Preservation and Transport in 1940, and is now the Division of Food Science Australia, Commonwealth Scientific and Industrial Research Organisation (CSIRO). He was chairman of the special committee concerned with citrus preservation from 1928-1934. In 1936 Young spent a year's study leave in Europe, visiting research institutes in Great Britain, France, Holland and Palestine. A considerable part of his time was spent at the Low Temperature Research Station of the Food Investigation Board at Cambridge, and at the branch laboratory in Covent Garden, London, investigating chemical changes in fruit during storage; the results of this were embodied in a report to CSIR. He also spent time at the Lister Institute, London, studying work going on there on the function of phosphates in carbohydrate metabolism. Also during that year, he attended the Seventh International Congress of Refrigeration at the Hague as Australia's representative; the Fifth International Congress of Microbiology, London, representing the University of Melbourne; the First Empire Dental Meeting of the British Dental Association, London, representing the University of Melbourne; the Empire Fruit Producers Conference, London by invitation; and the British Association for the Advancement of Science, Blackpool, by invitation.

William Young was Chairman of the Analytical group, Australian Chemical Institute during 1930-2, Chairman of the Australian College of Dentistry for the period 1931-8, and president of the Royal Society of Victoria during 1933-4; in 1937-8 he was the first President of the newly-formed Biochemical Group of the Australian Chemical Institute, and also the President of the Victorian Branch of the Institute. In 1939 he was President of the Physiology Division of the Australia New Zealand Association for the Advancement of Science (ANZAAS).

He died on 14 May 1942. A portrait of him painted by Charles Wheeler (see illustration), and paid for from the W.J. Young Memorial Fund, hangs in the Biochemistry Department, University of Melbourne. The residue of the Memorial Fund was used for the purchase of a W.J. Young book collection, for which a special bookplate was designed.

Honours and Awards

1933 Liversidge Research Lecture, Royal Society of New South Wales

1936 Member, Lister Institute of Preventive Medicine, London

1938 Fellow Australian Chemical Institute (FACI)

William Young was also elected to Fellowship of the Chemical, Physiological and Biochemical Societies of Great Britain, but the dates of admission were not given in the biographical sources quoted below.

Biographical Sources

(1) Marginson, M., 'Young, William John (1878-1942)', *Australian Dictionary of Biography,* 1990, **12**, 601.

(2) Maxwell, I., 'William John Young 1878-1942', *J. Chem. Soc.*, 1943, 44-45.

(3) Central Registry Files, CRF **59**, 1937; CRF **692,** 1942; and CRF **848,** 1943, University of Melbourne.

(4) Anon., obituary, 'Professor W.J. Young, D.Sc.', *Aust. J. Sci.*, 1942, **4**, 191.

(5) Anon., obituary, 'The late Professor W.J. Young (1878-1942)', *Journal and Proceedings of the Australian Chemical Institute,* 1942, **9**, 118-119.

(6) Vickery, J.R., The work of the Division of Food Preservation and Transport of the Council for Scientific and Industrial research', *Aust. J. Sci.*, 1940, **2**, 156-158.

(7) Vickery, J.R., 'William John Young: A Pioneer in Food Science in Australia', *Food Technology in Australia*, 1986, **38**, 158-159.

Scientific Publications of W.J. Young

A list of 40 publications up to 1937 was found in the Central files, University of Melbourne, but many important details were missing: these have been found, and, together with 8 additional references, are included here.

1. Thorpe, J.F. and Young, W.J., '*Cis*- and *trans*-αα,ββ-tetramethylglutaric acids', *J. Chem. Soc.*, 1900, 936-942.

2. Harden, A. and Young, W.J., 'Glycogen from yeast', *J. Chem. Soc*., 1902, **81**, 1224- 1233.

3. Thorpe, J.F. and Young, W.J., The αβ-dimethylglutaric acids, and the separation of *cis*- and *trans*- forms of substituted glutaric acids', *J. Chem. Soc*., 1903, **83**, 351-360.

4. Harden, A. and Young, W.J., 'Gahrversuche mit Pressaft aus obergahriger Hefe', *Chem. Ber*., 1904, (1), 1052-1070.

5. Harden, A. and Young, W.J., 'The influence of phosphates on the fermentation of glucose by yeast juice. Preliminary communication', *Proc. Chem. Soc*., 1905, **21**, 189-191.

6. Harden, A. and Young, W.J., 'Influence of sodium arsenate on the fermentation of glucose by yeast-juice'. (Preliminary notice), *Proc. Chem. Soc*., 1906, **22,** 283-284.

7. Harden, A. and Young, W.T., 'The alcoholic ferment of yeast-juice', *Proc. Roy. Soc.*, 1906, **77B**, 405-420.

8. Harden, A. and Young, W.J., 'The alcoholic ferment of yeast-juice. Part II. The coferment of yeast-juice', *Proc. Roy. Soc*., 1906, **78B**, 369-375.

9. Young, W.J., The organic phosphorus compound formed by yeast-juice from soluble phosphates. Preliminary notice', *Proc. Chem. Soc.,* 1907, **23**, 65-66.

10. Harden, A. and Young, W.J., 'The alcoholic ferment of yeast-juice. Part III. The function of phosphates in the fermentation of glucose by yeast-juice', *Proc. Roy. Soc*., 1908, **80B**, 299-311.

11. Harden, A. and Young, W.J., 'The fermentation of mannose and laevulose by yeast- juice' (Preliminary note)', *Proc. Chem. Soc*., 1908, **24**, 115-117.

12. Harden, A. and Young, W.J., 'The alcoholic ferment of yeast-juice. Part IV. The fermentation of glucose, mannose and fructose by yeast-juice', *Proc. Roy. Soc*., 1909, **81B**, 336-347.

13. Young, W.J., 'The hexosephosphate formed by yeast juice from hexose and phosphate', *Proc. Roy. Soc*., 1909, **81B**, 528-545.

14. Harden, A. and Young, W.J., 'The alcoholic ferment of yeast-juice. Part V. The function of phosphates in alcoholic fermentation', *Proc. Roy. Soc*., 1910, **82B**, 321- 330.

15. Harden, A. and Young, W.J., 'The function of phosphates in alcoholic fermentation', *Centralblat fur Bakteriologie, Parasitenkunde und Infektions-krankheiten*, 1910, **26**, 178-184.

16. Harden, A, Thompson, J. and Young, W.J., 'Apparatus for collecting and measuring the gases evolved during fermentation', *Biochem. J*., 1910, **5**, 230-235.

17. Harden, A. and Young, W.J., 'The alcoholic ferment of yeast juice. Part VI. The influence of arsenates and arsenites on the fermentation of the sugars by yeast juice', *Proc. Roy. Soc*., 1911, **83B**, 451-475.

18. Harden, A. and Young, W.J., 'Uber die zusammen setzung der durch Hefepressauft gebildeten hexosephosphorsaure. Part I.', *Biochemische Zeitschrift*, 1911, **32**, 173-176.

19. Young, W.J., 'Uber die zusammen setzung der durch Hefepressauft gebildeten hexosephosphorsaure. Part II.', *Biochemische Zeitschrift*, 1911, **32**, 177-188.

20. Harden, A. and Young, W.J., 'Der Mechanisms der alkoholischen Garung', *Biochemische Zeitschrift*, 1912, **40**, 458-478.

21. Harden, A. and Young, W.J.,'The preparation of glycogen and yeast-gum from yeast', *J. Chem. Soc*., 1912, **101**, 1928-1930; *Proc. Chem. Soc.*, 1912, **28**, 235.

22. Harden, A. and Young, W.J., 'The Enzymatic formation of polysaccharides by yeast preparations', *Biochem. J*., 1913, **7**, 630-636.

23. Young, W.J.,'A study of the nitrogenous metabolism in chyluria', *J. Trop. Med. Hyg.*, 1914, **17**, 241-244.

24. Taylor, F.H. and Young, W.J., 'The coastal climate of tropical Queensland: Meteorological observations taken at Townsville', *J. Trop. Med. and Hyg.,* 1914, **17**,. 225-227.

25. Young, W.J., 'A note on the black pigment in the skin of an Australian black', *Biochem. J*., 1914, **8**, 460-462.

26. Breinl, A. and Young, W.J., 'The occurrence of lead poisoning amongst north Queensland children', *Annals of Tropical Medicine and Parasitology*, 1914-1915, **8**, 575-590.

27. Young, W.J., 'Observations on body-temperature of Europeans in the tropics', *J. Physiol.*, 1914-1915, **49**, 222-232.

28. Young, W.J., 'The fixation of salvarsan and neosalvarsan by the blood after intravenous injection', *Biochem. J*., 1915, **9,** 479-484.

29. Young W.J., 'Studies in the antipyretic action of blood serum', *Biochem. J*., 1918, **12**, 499-515.

30. Young, W.J., 'The metabolism of white races living in the tropics. Part I. The protein metabolism ', *Annals of Tropical Medicine and Parasitology,* 1915, **9**, 91-108.

31. Breinl, A., and Young W.J., 'Tropical Australia and its settlement,' *Med. J. Australia,* 1919, (1), 353-361, 375-383, 395-404.

32. Young, W.J., 'The metabolism of white races living in the tropics. Part II. The composition of the urine', *Annals of Tropical Medicine and Parasitology,* 1919, **13**, 215-232.

33. Young, W.J., Breinl, A., Harris, J.J. and Osborne, W.A., 'Effect of exercise and humid heat upon pulse rate, blood pressure, body temperature and blood concentration', *Proc. Roy. Soc.*, 1919-1920, **91B**, 111-126.

34. Young, W.J., 'The metabolism of white races living in the tropics. Part III. The influence of external temperature and rate of cooling upon the respiratory metabolism', *Annals of Tropical Medicine and Parasitology,* 1919-1920, **13**, 313- 337.

35. Young, W.J., 'The extraction of melanin from the skin with dilute alkali', *Biochem. J.*, 1921, **15**, 118-122.

36. Osborne, W.A. and Young, W.J., 'The physiology and bio-chemistry of milk and milk products', *Proc. Soc. Chem. Ind. Victoria*, 1922, **22**, 850-860.

37. Cook, G.A., Love, E.F.J., Vickery,J.R. and Young, W.J., 'Studies on the refrigeration of meat. I. Investigations into the refrigeration of beef', *Aust. J. Exp. Biol. and Med. Sci*., 1926, **3**, 15-31.

38. Kerr,N.G. and Young, W.J., 'The action of certain fat-solvents on alcoholic fermentation', *Aust. J. Exp. Biol. and Med. Sci*., 1926, **3**, 177-185.

39. Kerr, N.G., and Young, W.J.,' The action of ether on the yeast cell. I - Carboxylase', *Aust. J. Exp. Biol. and Med. Sci*., 1926, **3**, 187-188.

40. Ranger, W. and Young, W.J.,' The export of oranges', C.S.I.R. Pamphlet, Melbourne,1928, **No.7**, 5-12.

41. Young, W.J. and Empey, W.A., 'The refrigeration of fish', *Journal of the Council for Scientific and Industrial Research,* 1929, **2**(2), 87-93; erratum, ibid., 150.

42. Young, W.J., 'The science of fruit preservation', *Proc. Soc. Chem. Ind. Victoria,* 1929, **29**, 148-153.

43. Young, W.J., 'A method for micro-Kjeldahl', *Aust. J. Exp. Biol. and Med. Sci.,* 1929, **6**, 315-316.

44. Osborne, W.A. and Young, W.J., ' Elementary Practical Biochemistry', third edition, 154 pp., W. Ramsay (Melbourne, 1930). (see also editions 1 & 2, and probably a fourth).

45. Young, W.J. , Bagster, L.S., Hicks, E.W., and Huelin, F.E., 'The ripening and transport of bananas in Australia', *C.S.I.R. Bulletin*, Melbourne,1932, **No. 64**, 52pp.

46. Young, W.J., 'The function of phosphates in the fermentation of sugars', *J. Proc. Roy. Soc. N.S.W.*, 1933, **67**, M1-M17. (The second Liversidge Research Lecture in the Royal Society of N.S.W. series).

47. Young, W.J., 'Some aspects of food preservation', *Proc. Soc. Chem. Ind. Victoria,* 1934, **34**, 924-929.

48. Young, W.J., 'Preparation and transport of Australian chilled meat', *Proc. Brit. Assoc. Refrig.*, 1936-7, **33**, 121-124.

THE FUNCTION OF PHOSPHATES IN FERMENTATIONS OF SUGARS*

W. J. YOUNG
Department of Biochemistry, The University of Melbourne, Victoria

I wish to express my appreciation of the honour which the Royal Society of New South Wales has done me in inviting me to give this the second Liversidge Research Lecture. We owe a debt of gratitude to Archibald Liversidge for the happy thought which inspired him to endow the lectures which bear his name. They help to keep green the memory of one who devoted thirty-five years of his life to furthering the study of chemistry at the University of Sydney, and to whom Science and the Scientific Organisations in Australia, and in New South Wales in particular, owe so much. In his will he directed that the purpose of the lectures should be to encourage research, so that after he himself had gone, he might still aid in inspiring others to investigate new territory in that science to which he acknowledged allegiance.

In my choice of subject I have endeavoured to keep in mind this desire, and propose to discuss a subject which has attracted a great deal of attention from biochemists, and of which our knowledge is still very far from being complete, in the hope that some of my audience may perhaps be able to rub clean the glass through which, at present, we see darkly.

During recent years, evidence has been gradually accumulating that when sugars undergo degradation by biological means, phosphoric acid is an essential agent in the reactions which take place.

The discovery of this role of phosphate was first observed in the fermentation of sugar to carbon dioxide and alcohol by yeast, and perhaps I may be permitted to give very briefly a few stages in the history of our knowledge of this fermentation.

Although the production of alcoholic liquors goes back beyond recorded history, it was not until 1837 that the vegetable origin of yeast was suspected. In this year three experimenters, quite independently and with different modes of attack, arrived at the conclusion that yeast was a living organism. These were Cagniard Latour, Theodore Schwann, and Kützing, and, as was to be expected, their publications were received with general incredulity.

Chief amongst the opponents of the view were the three great organic chemists of the time, Berzelius, Wohler, and Liebig, and in a number of the *Annalen* of that period is to be found an elaborate travesty by Wohler and Liebig, in which the idea is held up to ridicule in what would seem to us rather undignified language.

---

* Second Liversidge Research Lecture delivered to the Royal Society of New South Wales, November 8, 1933. Reproduced by permission of the Royal Society of New South Wales from *J. Proc. Roy. Soc. N.S.W.*, 1933, **67**, M1-M17.

For twenty years the theory made little headway, but in 1857 was commenced the long and rather bitter controversy between Liebig and Pasteur. The latter had accepted the theory and, in a series of researches extending until 1872, proved beyond doubt that fermentation was produced by the living cell in the course of its vital functions, and that there could be no fermentation without life.

As early as 1858 Moritz Traube had put forward the theory that all fermentations and putrefactions produced by living organisms were really brought about by enzymes, definite chemical substances elaborated by the cell. Traube was a genius whose ideas, in many ways, were greatly in advance of his times, and, in fact, his idea of fermentation as a series of oxidations and reductions is very much in accordance with our present view.

All attempts to obtain such an enzyme from yeast failed until 1897, when it was successfully accomplished by Edouard Buchner. By grinding the yeast with a mixture of sand and kieselguhr and thus rupturing the cells, he was able to press out their contents, the so-called "Hefepresssaft" or "Yeast Juice" a liquid which fermented sugars to CO_2, and alcohol in the entire absence of living cells. Since then, active preparations have been obtained from yeast in other ways, and the enzymic nature of fermentation has been definitely established.

The next advance was the discovery by Harden and Young that there are two other factors concerned in fermentation. One of these is the coenzyme (cozymase), a dialysable, heat-stable, substance necessary for fermentation, forming with the enzyme (apozymase) the fermenting complex. The other is the part played in fermentation by phosphates, and it is this discovery and the developments arising therefrom which I propose to discuss to-night.

When a soluble phosphate is added to a fermenting mixture of yeast juice and glucose, the rate of fermentation rises, sometimes as much as twenty times; this high rate continues for a time, depending on the quantity of phosphate added, and then falls to a rate almost equal to, but generally slightly higher than, that of the original mixture. During this period of increased activity the phosphate unites with the sugar to form esters, and when the rate has again fallen the phosphate is found almost entirely combined in these esters, and in a form not precipitable by magnesium solutions. During this period also the extra carbon dioxide evolved and the alcohol produced are equivalent to the phosphate esterified, in the ratio CO_2, or C_2H_6O to R_2HPO_4.

This equivalence is of special importance, as it shows that a definite action takes place between the phosphate and the sugar in which the esterification of the sugar with the phosphate is accompanied by the production of an equivalent amount of carbon dioxide and alcohol. If more phosphate be added the phenomenon re-occurs and may be repeated several times, whilst the same action was observed with the three fermentable hexoses, glucose, mannose and fructose.

When the action was discovered in 1909, it was thought that only one ester was formed, a hexose diphosphate, which was isolated by precipitation as the lead salt. Later on Harden and Robison obtained a hexose monophosphate, which Robison has since shown to be a mixture of three monophosphates, and in addition Robison and Morgan have isolated a phosphate derivative of the disaccharide trehalose. The diphosphate is separated from the others through the lower solubility of its barium salt, whilst the others have been separated through the different solubilities of their salts with brucine.

The following compounds have been definitely obtained.

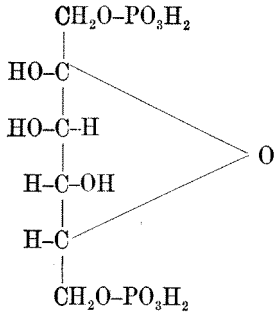
Hexose diphosphate or the Harden and Young ester.
$C_6H_{10}O_4(PO_4R_2)_2$

The free acid forms a strongly acid solution, which titrates as a tetrabasic acid with alkali to phenolphthalein, and reduces Fehling's solution. When heated with acid it readily hydrolyses with formation of phosphoric acid and the hexose fructose, so that it is a fructose diphosphate. The same compound is formed from the fermentation of glucose, mannose or fructose, so that in the cases of the aldoses some intramolecular rearrangement must have taken place. One phosphoric acid group is more easily split off than the other, yielding an intermediate monophosphoric acid ester usually known, after its discoverer, as the Neuberg ester.

When treated with phenylhydrazine in the cold it forms a hydrazone, and this on heating loses one phosphoric acid group yielding a compound which has been shown to be a derivative of the osazone of a monophosphate of fructose. The importance of this reaction is that it shows that the substance is a hexose diphosphate and not a triose monophosphate, and because it indicates that one of the phosphoric groups is attached to the carbon atom next to the carbonyl group of the fructose.

By conversion into the methyl hexosides and subsequent removal of the phosphoric acid groups by means of the enzyme phosphatase, Robison and Morgan obtained the methyl osides of fructo-furanose or γ-fructose. It is probably therefore a derivative of fructose with the phosphoric acid groups in the positions 1 and 6.

This diphosphate is almost invariably formed in the largest amount.

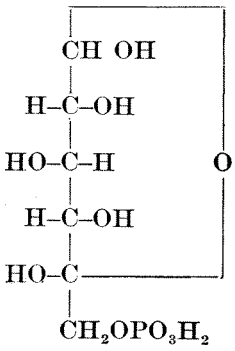


The Hexose monophosphates. $C_6H_{11}O_5PO_4H_2$

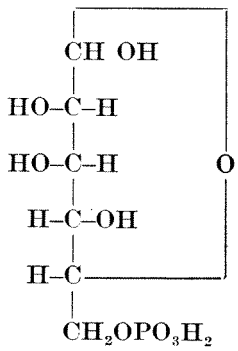
Fructose monophosphate, the Neuberg ester, was first obtained by partial hydrolysis of the diphosphate, but has since been obtained by fermentation. In addition, the aldose ester, generally known as the Robison ester, has now been shown by Robison to be a mixture of monophosphates of both glucose and mannose, and thus the three mono-esters are formed during the fermentation of any one of the three hexoses in the presence of phosphate.

They all yield the same osazone derivative as the diphosphate, the phosphoric group being therefore probably on the sixth carbon atom, whilst the fructose monophosphate is a derivative of the γ-sugar.

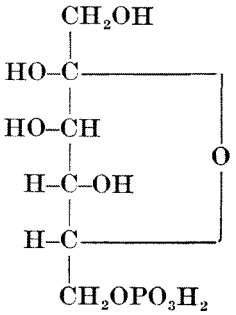
Glucose 6 monophosphate



Mannose 6 monophosphate



γ-Fructose 6 monophosphate



The trehalose compound is only found when the dried preparation "Zymin" is used, and its connection with fermentation is entirely obscure.

Turning now to the rôle of these compounds in alcoholic fermentation, there occur in yeast enzymes termed phosphatases, which hydrolyse the esters with formation of the sugars and free phosphate, so that when the zymase gradually loses its activity, free phosphate accumulates at the expense of the hexosephosphates.

Summarising the facts, we see that whilst there is still free phosphate present the high rate of fermentation continues, and when all the phosphate has been esterified it sinks to a constant value, which slowly falls off as the enzyme dies out. During this constant rate there are only traces of free phosphate present in the solution, and this only begins to accumulate when the zymase dies out, and then it does so at the expense of the esters.

The reaction between the phosphate and the sugars may be represented in the form of equations:

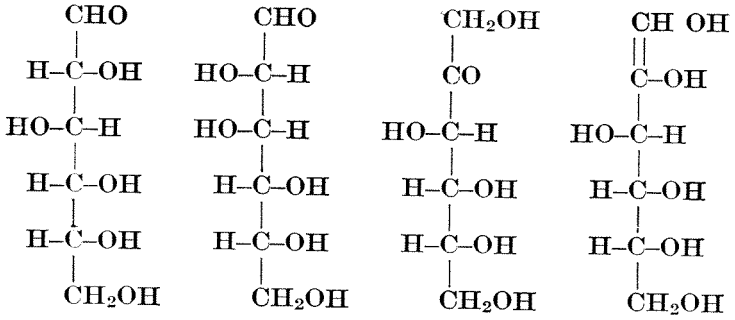
1. $2C_6H_{12}O_6 + 2PO_4HR_2 = C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O + 2\,CO_2 + 2C_2H_6O$

2. $3C_6H_{12}O_6 + 2PO_4HR_2 = C_6H_{11}O_5PO_4HR2 + 2H_2O + 2CO_2 + 2C_2H_6O$

Equation 1 was that originally suggested by Harden and Young when only the diphosphate was known. Equation 2 was added later to take in also the formation of the monophosphates. This assumes that these are formed independently of the diphosphate, and that their formation, like that of the diphosphate, is accompanied by the formation of an equivalent of carbon dioxide and alcohol, since these products are equivalent to the total phosphate esterified.

The theory that it is the enol form of the hexoses which is fermented was originally suggested by E. F. Armstrong, and again by Harden and Young, when the same diphosphate was found to be formed from all three sugars, and Robison's recent discovery that monophosphates of all three sugars are formed, lends further support to this view.

It will be remembered that glucose, mannose and fructose possess a common enolic form and that any one of the three is converted through this form into an equilibrium mixture of all three.



Glucose Mannose Fructose Enol

Robison has suggested that one phosphate group first attaches itself to number 1 carbon atom of the enol, and thence is transferred to number 6 carbon, in a similar manner to the wandering of the acetyl group in tetraacetyl glucose under the influence of alkali. The resulting 6-phosphated enol may then in part combine with a second phosphoric group to form fructose diphosphate, but some may pass into an equilibrium mixture of glucose, mannose and fructose monophosphates. In this way all four esters would be formed from the phosphated enol, CO_2 and alcohol being formed at the same time from more sugar.

On the other hand, it is known that phosphatase acting on the diphosphate removes the phosphoric groups in stages, producing the Neuberg ester as an intermediate, and Meyerhof has recently shown that an extract of animal muscle will convert either the aldose or ketose monophosphates into an equilibrium of both types. It is therefore possible that the diphosphate is first formed and the monophosphates arise by enzymic hydrolysis of this, the removal of the phosphoric group from carbon atom 1 being accompanied by intramolecular change through the enol. This also receives support from the fact that early this year, Robison and Macleod obtained a mixture of aldose and ketose monophosphates by the action of bone phosphatase on the diphosphate.

If this alternative be correct, equation 2 would not be required, since the formation of the monophosphates would not be accompanied by any fermentation products.

To complete the picture of fermentation we require two more equations showing the action of the enzyme phosphatase:

3. $C_6H_{10}O_4(PO_4HR_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2$

4. $C_6H_{11}O_5PO_4HR_2 + H_2O = C_6H_{12}O_6 + PO_4HR_2$

The phosphate set free in these reactions would then once more enter into equations 1 and 2.

Thus when all phosphate has been esterified, the constant rate of fermentation is determined by the rate at which it is again set free in the reactions 3 and 4.

If the fermentation of sugars by yeast can be represented by these equations, it should follow that no action should take place in the absence of phosphate. Hitherto it has not

been found possible completely to free any yeast preparations from phosphate, but in experiments in which it was greatly reduced, fermentation became correspondingly small, and was increased nearly a hundred times by adding phosphate.

There is thus very strong evidence that phosphate is indispensable for alcoholic fermentation.

In the equations which I have given, the hexosephosphates are in no sense intermediate bodies in fermentation, but a coupled reaction is suggested in which the phosphorylation of one molecule of sugar is accompanied by the decomposition of another.

There are other theories which assume these esters to be actual intermediate bodies. The one which has received much support is due to Meyerhof and his associates. According to this, during the period of rapid fermentation, an active form of monophosphate is first formed (equation I, *a*). Part of this then undergoes fermentation at a high rate, the sugar moiety of one molecule decomposing to CO_2 and alcohol, and the phosphoric acid uniting with another molecule to form the diphosphate (equation I, *b*). At the same time some of the active form passes into a stable form, which is the monophosphate of fermentation (equation I, *c*), whilst still another portion is esterifled to diphosphate by free phosphate still in the mixture (equation I, *d*).

The hexose diphosphate then undergoes decomposition in two ways. Part is directly fermented (equation II,*a*), giving CO_2 alcohol and free phosphate, which re-enters the cycle as in equation I,*a*. The rest is hydrolysed into hexose and free phosphate by the phosphatase (equation II,*b*), and these products also enter the cycle as in equation I,*a*.

It would take too long to give the evidence upon which this theory is based, but the main criticism brought against it by Harden is that the extra CO_2 and alcohol formed according to I, *b*, should be equivalent to the diphosphate formed in this action alone, and actually less than the total diphosphate formed, since some diphosphate is also formed in I, *d*, without any of these products, whereas, in actual fact, these products are equivalent to the total phosphate esterified, i.e., both mono and diphosphates.

I. *Phosphate Period.* Rapid Fermentation.

a. $C_6H_{12}O_6 + R_2HPO_4 = C_6H_{11}O_5(PO_4R_2)^* + H_2O$
b. $2C_6H_{11}O_5.PO_4R_2^* = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)$
c. $C_6H_{11}O_5.PO_4R_2^* = C_6H_{11}O_5.PO_4R_2$
d. $C_6H_{11}O_5.PO_4R_2^* + PO_4HR_2 = C_6H_{10}O_4(PO_4R_2)_2 + H_2O$

II. *Ester Period.* Slow Fermentation.

a. $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = 2CO_2 + 2C_2H_6O + 2R_2HPO_4$
b. $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4$

---

* = active form.

Other theories have propounded the preliminary formation of a triose monophosphate and a three carbon chain compound; two molecules of the former then condense to form a hexose diphosphate, whilst two of the latter break down to CO_2 and alcohol.

Let us now turn to another biological process in which carbohydrates are degraded anaerobically, and in which phosphates play a part similar to that in alcoholic fermentation, namely, the production of lactic acid during muscular activity in the animal.

The fuel from which animal muscle ultimately obtains its energy is largely carbohydrate, stored in the muscle cells as glycogen. During muscular work some of the glycogen is converted into lactic acid, a change which sets free energy but requires no oxygen, so that a muscle can do a limited amount of work without using oxygen. Subsequently some of the lactic acid or other carbohydrate intermediate is oxidised aerobically, and this action gives the necessary energy to rebuild up glycogen from the rest of the lactic acid and to restore the original condition of the system.

Embden was the first to obtain a hexosephosphate by the action of muscle-juice, and, considering it as a precursor of lactic acid, he called it "lactacidogen".

Since then it has been shown that minced muscle in the presence of sodium fluoride yields a hexose phosphate identical with the diphosphate of yeast fermentation, but in the absence of fluoride a monophosphate is formed, now known to be a mixture of aldose and ketose monophosphates similar to those obtained from yeast juice. Embden's lactacidogen is thus probably a mixture of the monophosphates of glucose, mannose, and fructose, and may arise as the result of partial hydrolysis of the diphosphate.

Muscle mince can readily convert glycogen into lactic acid, but it has no action on hexoses. The direct breakdown of a polysaccharide, built up of glucose units, to lactic acid without an intermediate stage of a hexose, is unthinkable, and it appears probable that the glycogen is first converted into a hexose in a more active form.

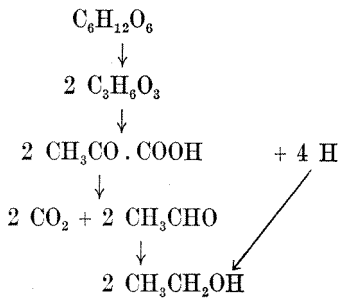
This idea is supported by the fact, discovered by Meyerhof, that a solution of the precipitate obtained by adding alcohol to autolysed yeast, enabled the muscle to produce lactic acid from hexoses. This activator or hexokinase, as it is termed, is thermolabile and is thought to convert hexoses into a more reactive form. In the presence of hexokinase and phosphate, minced muscle acts on sugars with a rapid production of lactic acid accompanied by the esterification of the phosphate, largely to hexose diphosphate. When all the phosphate has been esterified, the rate of acid production falls, but no free phosphate accumulates until all the sugar has been converted into lactic acid.

During the rapid initial high rate period, two molecules of phosphate are esterified for every molecule of sugar converted into lactic acid. The similarity of this reaction to that of alcoholic fermentation is at once apparent, and the equations previously given will fit the changes, the CO_2 and alcohol being replaced by lactic acid.

The changes which we have been considering so far are concerned with the first stage in fermentation, during which the hexose phosphate formation appears to be a necessary part, and we have said nothing about the intermediate steps in the change from the six carbon chain of the sugar to the final products, CO_2 and alcohol, or the three carbon compound, lactic acid.

Long before there was any experimental evidence it was believed that the first stage in the degradation of hexoses was the formation of a three-carbon compound, and various bodies were suggested as intermediate compounds. In later years, methyl glyoxal has found most favour as the immediate three carbon compound to be formed, but very recent work seems definitely to have ruled out this compound as an intermediate in the fermentation of sugars. It is almost certain that the next stage is pyruvic acid, and that this is acted upon by the enzyme carboxylase, yielding CO_2 and acetaldehyde, the latter then being reduced to alcohol.

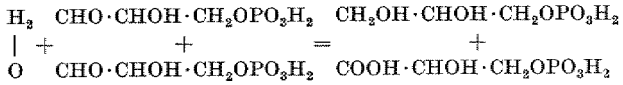
This can be represented in a crude way by the following scheme adapted from Neuberg, which shows the changes without introducing phosphate and as a series of oxidations and reductions:



The same scheme will fit the action in muscle, the pyruvic acid in the absence of the carboxylase being itself reduced to lactic acid.

Within the last few months a theory has been put forward by Embden and modified by Meyerhof, dealing with the mechanisms of these later stages in the anaerobic breakdown of carbohydrates, and which is really an extension of the scheme given above. The hexose diphosphate does not exist in quantity either in living yeast or in intact muscle, and Meyerhof considers that the accumulation of this compound in yeast-juice and in minced muscle results from the disorganisation of the cells.

The work of Lohmann had shown that muscle extract will act upon hexose diphosphate and convert some of it into a form more resistant to hydrolysis but with apparently the same composition. From this action Embden and Meyerhof have now obtained two compounds, one a monophosphate of glyceric acid termed phosphoglyceric acid, and the other α-glycero-phosphoric acid, two compounds which he considers as probable dismutation products from a triose monophosphate.



The phosphoglyceric acid is then broken down to pyruvic acid and free phosphoric acid, and the pyruvic acid reacts with the glycero-phosphoric acid to give lactic acid and possibly a triose monophosphate, again an oxidation-reduction action.

Embden's scheme may be shown thus:

1. Fructose diphosphate = 2 triose monophosphate = α-glycerophosphate +

phosphoglyceric.

2. Phosphoglyceric acid = pyruvic acid + phosphoric acid.

3. Pyruvic acid + glycerophosphoric acid = lactic acid + triose phosphate.

Meyerhof has applied the same ideas to the problem of alcoholic fermentation with some variation:

Initial Phase.

A. 1 hexose diphosphoric acid + 1 glucose + 2 phosphoric acid = 4 triose phosphoric acid = 2 α-glycerophosphoric acid + 2 phosphoglyceric acid

B. Phosphoglyceric acid = 2 pyruvic acid + 2 phosphoric acid = 2 acetaldehyde + 2 carbon dioxide + 2 phosphoric acid

Stationary Condition.

C. 2 acetaldehyde + 1 glucose + 2 phosphoric acid = 2 triose phosphoric acid = 2 alcohol + 2 phoshoglyceric acid + 2 acetaldehyde

As before, the fructose diphosphate is converted to phosphoglyceric and α-glycerophosphoric acids, and the phosphoglyceric acid to pyruvic acid. The enzyme carboxylase then comes into action and converts the pyruvic acid into CO_2 and aldehyde. The next stage is more difficult to explain since glycerophosphoric acid will not reduce aldehyde in the presence of yeast. He assumes, therefore, that this reduction is brought about by a triose phosphate which is itself at the same time re-oxidised to phosphoglyceric acid.

He divides the fermentation into two phases, the hexose diphosphate being only necessary to start the reaction, and produce the first quantity of triose ester. It is thus only required in traces.

The full account of these theories has not yet reached this country, and I have had to rely on the report in *Nature* of a lecture delivered by Meyerhof at Cambridge. It is, therefore, not safe to submit the theory to criticism, but it does not appear to offer any explanation of the esterification of the phosphate in yeast juice, nor of the equivalence observed in the fermentation products.

Other Actions in which Phosphates are Concerned

The fact that many bacteria acting on carbohydrates produce substances similar in type to the products of yeast, would suggest that phosphate might enter into these reactions also.

It is very difficult to obtain bacterial growths in sufficient quantities to filter off and be able to work with the separated organisms on the same scale as with yeast, but there is accumulating evidence that esterification does take place.

Thus Vertranen and his colleagues have shown that when the lactic acid producing organism, *S. lactis,* acts in the presence of phosphate on sugar, some of the phosphate becomes bound, and they obtained small quantities of a monophosphate from the mixture.

Barrenstein and Parry have also obtained a hexose monophosphate from assimilating *Elodea canadensis*.

Last year Jamieson, working in my own laboratory with *Bacillus acidophilus,* found that in the presence of toluene and phosphate, this organism converted glucose to lactic acid, and the free phosphate disappeared in considerable quantity. When the action ceased, the phosphate was gradually set free again, and could also be recovered at any time by hydrolysis with acids.

It thus seems very probable that the mechanisms of yeast fermentation and of muscle action on carbohydrates will throw light on the manner in which carbohydrates are decomposed in the living world.

Note by the compiler: there were no literature references cited.