

power of this instrument is remarkable, the central illumination being, it is claimed, fully 20 per cent and the illumination at margins of field 137 per cent greater than that obtained with other binoculars of the same power and aperture. Messrs. Barr and Stroud, Ltd., have introduced a novel element in the design of their binoculars by the use of bakelite for the usual enamelled metal parts, which has enabled the weight to be reduced. We have not space to mention more of the many other excellent and novel exhibits of this section, but one general observation should be emphasised.

We have already said that the scientific and optical instrument exhibits gained greatly this year by being, in great part, organised on a fairly representative single stand. But while this method of exhibition has its great and obvious advantages,

it can yield its greatest benefits only if it receives the whole-hearted co-operation of all, or nearly all, the firms in these important industries. We talk of mass action in chemical reactions; there is such a thing as mass mental effect, both as to quantity and quality, in exhibitions; and it is of great importance to realise that a visitor to the British Industries Fair, and more particularly, perhaps, a foreign visitor, cannot help getting from the exhibits of scientific and optical instruments a general net impression of what these British industries as a whole are like. For this reason it is important that, at least, all the leading firms in the industry should realise the opportunities the Fair offers for creating an impression of the scope and quality of British scientific and optical instruments, quite apart from any direct benefits that may accrue to individual firms.

The Function of Phosphate in Alcoholic Fermentation.¹

By Prof. ARTHUR HARDEN, F.R.S.

NATURE AND FUNCTION OF THE PHOSPHORIC ESTERS PRODUCED.

IF we next consider the exact nature of these phosphoric esters and the relation of their formation and hydrolysis to the decomposition of the sugar molecule, we are met with a singularly complex condition of affairs, which cannot yet be interpreted satisfactorily. The main facts seem to be as follows.

When fermentation of sugar by yeast preparations is carried out under suitable conditions in the presence of added inorganic phosphate, a rapid production of carbon dioxide and alcohol occurs and a phosphoric ester of a sugar accumulates, the amount of phosphate found in this form being approximately proportional in the ratio (CO_2/PO_4) to the increased production of carbon dioxide and alcohol caused by the addition of the phosphate (Kluyver and Struyk, it is true, have found lower ratios than this, but there is no doubt that high ratios, 0.8-1, are often observed).

The phosphoric ester produced, however, may consist mainly of the hexosediphosphate originally described by Young and myself or of the hexosemonophosphate described by Robison and myself and afterwards studied by Robison, or it may be a mixture of these in any proportions. In the case of fermentation by dried yeast (and possibly of other preparations), a further complication is afforded by the fact that a disaccharide-phosphoric ester (trehalosemonophosphate) may also be present.

This conclusion is founded in the first place on a large amount of experience which has been gained at the Lister Institute in preparing hexose-mono- and di-phosphate. These preparations are as a rule carried out by making repeated additions of phosphate and sugar to a fermenting mixture of yeast juice or dried yeast and fructose (or glucose). With dried yeast a large proportion of the diphos-

phate is usually obtained, and the relatively small amount of monophosphate produced contains a considerable proportion of trehalosemonophosphate. With yeast juice the results are very variable and no trehalosemonophosphate has so far been detected among the products. More precise experiments have been made by Lord Henley and myself in which the gas evolved after a single addition of phosphate was carefully measured and the proportions in which mono- and di-phosphates were produced were determined as accurately as possible. Unfortunately, the available methods are not very good as they rest on the solubilities of the different compounds in 10 per cent alcohol, and these are to some extent mutually affected in the presence of both compounds. Further, yeast, like Africa, is always yielding something new, and the recently discovered fact that pyrophosphates exist in yeast and by their formation from, or hydrolysis to, orthophosphates may cause disappearance or appearance of 'inorganic phosphate', adds another source of inaccuracy to the many previously known.

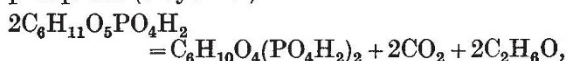
Allowance has, of course, to be made for the phosphorus compounds existing in the mixture at the moment of addition of inorganic phosphate and for the normal evolution of carbon dioxide which occurs throughout the experimental period in addition to the enhanced evolution due to the esterification.

In spite of these minor uncertainties, the somewhat surprising fact emerges, that whatever the nature of the phosphoric ester which accumulates, the carbon dioxide produced is approximately equivalent in the ratio $\text{CO}_2 : \text{PO}_4$ to the amount of phosphate which undergoes esterification. The full results are given in two papers published recently by Lord Henley and myself in the *Biochemical Journal*, and need not be quoted here in detail. These experiments indicate the wide variation which may occur in the nature of the hexosephosphate produced, whilst the ratio of $\text{CO}_2 : \text{PO}_4$ -esterified remains

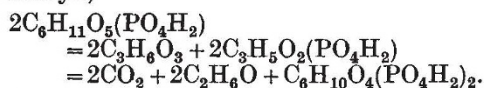
¹ Continued from p. 279.

constant and approximately equal to unity. Two extreme cases may be quoted, in one of which 13.5 per cent of the phosphate (PO_4) esterified was present as hexosediphosphate and 86.5 per cent as monophosphate and, in the other, 97 per cent as diphosphate and only 3 per cent as monophosphate; the CO_2/PO_4 -esterified ratios were 0.98 and 0.86 respectively.

I do not propose to discuss in any great detail the various theories which have been proposed to explain these complicated relationships. It would be natural to assume that the introduction of the phosphoric acid group into the sugar molecule forming a hexosemonophosphate might render this more accessible to decomposition into the compound (or compounds) containing three carbon atoms which are now accepted as an intermediate stage in the production of carbon dioxide and alcohol. The phosphate radical from one of these groups might then serve to convert another molecule of the monophosphate into the stable diphosphate (Meyerhof)



or two of the three-carbon groups containing each one phosphate group might unite with each other forming the stable diphosphate (Kluyver and Struyk)



Any monophosphate escaping these reactions would be found as a constituent of the mixed hexosephosphates resulting from the fermentation.

To add further to the difficulty of unravelling this complex tangle, it must be remembered that, whether glucose or fructose be fermented, the hexosediphosphate produced is probably a derivative of fructose, or at least yields fructose on hydrolysis, whilst the monophosphate is with equal probability a mixture of about 80 per cent of a glucosemonophosphate and 20 per cent of a fructosemonophosphate. It is obvious from this that whatever changes occur are not limited to the simple introduction or removal of a phosphoric acid group; fundamental changes occur in the constitution of the molecule of the sugar itself.

Attractive as is the theory of the intermediate character of any one of the hexosephosphates, it seems to me impossible at the moment to bring it into agreement with some of the facts which have just been related. The production of 70-80 per cent of the monophosphate, with an unaltered degree of formation of alcohol and carbon dioxide, renders it impossible that this ester should be "obviously nothing but a part of the intermediate product which has escaped the coupled decomposition—esterification reaction" (Meyerhof and Lohmann, *Biochem. Z.*, **185**, 155; 1927).

It appears to me that the fundamental idea expressed in the original equation of Harden and Young is nearer the truth than any alternative that has as yet been suggested. A coupled reaction of some kind occurs, as the result of which

the introduction of two phosphate groups into certain sugar molecules—either into the same molecule or one each into two different ones—induces the decomposition of another molecule. The introduction of these phosphate groups in the presence of muscle extract, and presumably in both muscle itself and yeast, is actually accompanied by a small evolution of heat (Meyerhof and Suranyi, *Biochem. Z.*, **191**, 106; 1927), and it is possible that this may have some significance for the occurrence of the coupled reaction. What are the conditions for the preferential formation of the mono- or di-ester we do not yet certainly know, although the work of Kluyver and Struyk suggests that dilution of the enzyme may be one factor in this.

The mechanism of the fermentation of the mono-ester has not yet been worked out in sufficient detail to afford valid evidence either for or against the theory, but Dr. Robison and I have made experiments (about to be published) which show that the monophosphate itself reacts with a further quantity of phosphate and that this reaction is accompanied by an enhanced production of carbon dioxide and alcohol.

The lack of exact chemical equivalence among the products (ester on one hand, carbon dioxide and alcohol on the other) is probably more easily explicable on this view than on any other.

SUGAR METABOLISM IN VEGETABLE AND ANIMAL ORGANISMS.

After the establishment of the important part played by phosphates and phosphoric esters in alcoholic fermentation, it was soon found by various workers that these compounds provided the clue to many other biological phenomena. The cozymase of alcoholic fermentation was found by Meyerhof to exert an equally important part in the respiration of yeast, and the important observation was made also by Meyerhof, that it occurred in muscle and was an essential factor in the carbohydrate metabolism of muscle, in which the intervention of a hexosephosphate had been proved by Embden. This phenomenon was shown to take place on lines quite similar to those of the respiration and fermentation of yeast, and in 1924, before the riddle of lactic acid formation had been completely solved, Meyerhof wrote ("Chemical Dynamics of Life Phenomena"): "It may indeed be considered a success of general physiology and its mode of experimenting, that the chemical dynamics of a highly-differentiated organ like the muscle could be partly revealed by the study of alcoholic fermentation of yeast".

A still greater success was to follow. An astonishing degree of similarity was shown to exist between almost every detail of the production of lactic acid by the muscle enzymes and of alcohol by the yeast enzymes, which extended to the identity of the phosphoric esters concerned, the accumulation of ester under similar conditions and even to the effect of arsenate on the process. After the publication of Meyerhof's preliminary papers in which these observations were recorded, I wrote

(Continued on p. 323.)

the following passage in concluding a short review of the work (NATURE, Dec. 18, 1926): "The striking similarity established by Meyerhof between the changes of carbohydrates in muscle and in the yeast cell is seen to be much closer than has been believed. The remarkable phenomena accompanying alcoholic fermentation are now duplicated in the case of lactic acid production, and it may reasonably be expected that most of the fermentative decompositions of the sugars will be found to be initiated in a similar manner."

Direct proof is still wanting in many cases, but some instances are known among bacteria (Virtanen), moulds (Euler and Kullberg), and higher plants (Ivanoff, Bodnar). It is not too much to say that the fundamental biological mode of attack on carbohydrates is that revealed by the study of alcoholic fermentation.

OSSIFICATION.

Another biochemical function of the hexose-phosphates which is shared by other hydrolysable phosphoric esters is that of a potential source of phosphate ions. I am happy to say that one of the most beautiful and important developments of this idea has been worked out quite independently at the Lister Institute by Dr. Robison as a direct

consequence of his work on the hexosemonophosphate of yeast juice. "During my investigation of the hexosemonophosphoric acid isolated from the products of fermentation", he said (*Biochem. J.*, 17, 286; 1923), "the hydrolysis of the ester by enzymes was studied. In some experiments in which the readily soluble calcium and barium salts were used as substrates, the progress of the hydrolysis was shown by the formation of a precipitate of sparingly soluble calcium or barium phosphate $C_6H_{11}O_5PO_4Ca + H_2O \rightarrow C_6H_{12}O_6 + CaHPO_4$."

The formation of this precipitate suggested to me the query whether some such reaction might conceivably be concerned in the deposition of calcium phosphate during the formation of bone in the animal body. In the first place I sought for an enzyme capable of effecting hydrolysis in the bones of growing animals."

The search was successful, a 'bone phosphatase' was found in the ossifying cartilage of young animals and a series of interesting and important investigations has followed; as a result of which I have little doubt that their author is on the highway to the biochemical explanation of the process of ossification—a good instance of the far-reaching and unexpected results flowing from observations made for quite a different purpose.

Obituary.

PROF. J. M. DUNCAN SCOTT.

DR. JAMES MATTHEWS DUNCAN SCOTT, professor of physiology in the University of Saskatchewan, died at Saskatoon on Jan. 28 last. Prof. Duncan Scott's career as a physiologist was a relatively short one. After taking an arts degree at St. Andrews, and qualifying in medicine with honours at Edinburgh, he joined the forces during the War and served in Egypt. At the conclusion of the War he suffered ill health for some years, owing to a troublesome frontal sinus infection which necessitated surgical intervention.

After practising for a time in South Africa, Duncan Scott felt called to undertake scientific work, which had always had a strong attraction for him, and in 1921 he proceeded to Cambridge, where he held a John Lucas Walker Studentship for research in pathology. His work at Cambridge was largely concerned with the regeneration of the red blood cells in anæmia. Becoming interested in physiology, and particularly in the teaching of it, he obtained in 1924 a teaching post at St. Bartholomew's Medical College as junior demonstrator, and afterwards became lecturer in physiology. There he continued some investigations which had been commenced at Cambridge in collaboration with Dr. Ffrangcon Roberts, on the situation and connexions of vagal and vasomotor centres in the medulla. This work he prosecuted with great assiduity and considerable skill, as a research scholar of the British Medical Association. As an outcome of his teaching work he also became interested in the physical chemistry of colloids, and held highly original, though not generally acceptable, views on that subject.

In 1926, Duncan Scott was invited to the University of Saskatchewan to occupy the newly created chair of physiology. This was an opportunity for the display of those fine qualities of orderliness and strict classification which had always characterised Duncan Scott. Before leaving England and proceeding to found and equip a physiological laboratory under conditions of relative isolation, he prepared a list, accurately classified down to the smallest detail, of every article which would be required in such a laboratory. He regarded his work there in a pioneering spirit, and as a field of high endeavour; he was, on the whole, well gratified with the results which came out of it so far as the organisation of the department was concerned. The duties of organisation and teaching naturally distracted him temporarily from his research work, though he never entirely lost touch with this, and when at the annual Physiological Congress at Boston in 1929, at which he gave two demonstrations, he expressed the opinion that the first labours of organisation had now been definitely completed, and would, he hoped, leave him free to continue his research work.

The passing of so courteous a colleague and of so keen a teacher and investigator will leave a gap among the physiologists of two continents. Dr. Duncan Scott leaves behind him a widow and three sons, to whom all his friends will extend their warmest sympathy.

MR. F. W. DOOTSON.

MR. FREDERICK WILLIAM DOOTSON, who died in Cambridge on Dec. 12, 1929, after a very short illness, was born in Manchester on Aug. 10, 1863.