force at 1.26°K . The steep fall of the curve in the beginning is due to the fact that in the vacuum of the small Dewar flask a trace of helium (10^{-7} mm.) has been left intentionally for thermal contact. This helium gas is condensed under development of the heat of condensation. When the pressure of the helium gas is still lower, the curve has a different form and heating up with the time goes extremely slowly. The cold has been caught in a trap. The vacuum becomes practically absolute and heat is no longer transmitted.

The forces are directly proportional to the moments and the only thing we have to find out is to which temperatures these moments correspond. The connexion between temperature and moment has been determined between 7.2°K . and 1.3°K . The curve representing this connexion was extrapolated linearly, though this gives a stronger increase of the moment than the curve itself does. That is why we can only give an upper limit of the temperature reached.

The first experiments were made in March and April of this year with cerium fluoride and gave as the upper temperature limit 0.27°K . More recent experiments with dysprosium ethyl sulphate gave the upper limit 0.17°K . Finally, the experiments of July last made with cerium ethyl sulphate gave as the upper temperature limit 0.085°K .

It is possible that with the same experimental arrangement much lower temperatures can be reached. The choice of the right substance will decide further results. I am convinced that with the above-described arrangement the theoretical limit can be reached.

Up to the present, the lowest temperatures had been reached with the aid of liquid helium, boiling under very low pressures. The results of this method evidently depend upon the capacity of the pumps used and upon the perfection of the thermal insulation.

With this method Kamerlingh Onnes reached

in October, 1921, a temperature of 0.82°K . Keesom worked with diffusion pumps of a capacity fifteen times higher than that of the pumps Kamerlingh Onnes had. In 1929 he reached a temperature of 0.71°K . It is, however, difficult to proceed much further in this way.

The conception of temperature is based upon the properties of the ideal gas. The temperature is determined with the aid of the helium thermometer (several corrections being applied). At the extremely low temperatures reached recently no gas thermometric measurement of the temperature is possible, so far as I can see.

A thermometric scale based upon another process must be fitted to the absolute temperature scale; only a magnetic scale can be used for this purpose.

Just as was done in gas thermometry, we shall have to find some substances, which within a considerable range of temperature show the same behaviour; a highly developed theory will then enable us to fix the temperature with the same accuracy by means of magnetic thermometry as by the use of gas thermometric methods.

A reservation must be made, however, for the case that at very low temperatures the substances used might become ferro-magnetic or might show a new kind of ferro-magnetism. In this case little might be said about the temperature.

Another great difficulty is this: if by means of the magnetic method we wish to cool down other substances, the question of the thermal contact becomes urgent. The radiation is negligible, and also the vapour pressure of the helium becomes so small that thermal contact by means of gaseous helium can scarcely be considered.

The experiments were made in collaboration with Dr. E. C. Wiersma, conservator of the Laboratory, to whom I express my warmest thanks for his help and for many suggestions. To Prof. H. A. Kramers of Utrecht I am indebted for his valuable theoretical assistance.

Intermediate Products and the Last Stages of Carbohydrate Breakdown in the Metabolism of Muscle and in Alcoholic Fermentation*

By DR. OTTO MEYERHOF

FROM these new results we see that the assumption of the rôle of methylglyoxal as an intermediate product in the splitting of carbohydrates becomes superfluous. Upon the discovery of methylglyoxalase by Dakin and Neuberg, the whole biochemical world was seized with the idea that lactic acid formation must take place with methylglyoxal as an intermediate. This was also applied to the formation of pyruvic acid during alcoholic fermentation.

In the meantime, in our own laboratory the 'stock' of methylglyoxal had sunk to a low level. Lohmann's discovery of the part played by glutathione as the co-ferment of methylglyoxalase sent them

down to rock bottom. In addition to the many important functions in the biochemistry of the cell which have been ascribed to Hopkins' glutathione, Lohmann has added a new one of great interest, namely, that glutathione acts as a true co-enzyme of methylglyoxalase. Now, after adding adenylypyrophosphoric acid and magnesium ion, glycogen was easily transformed into lactic acid in muscle extracts from which the glutathione had been dialysed away so that they could not convert methylglyoxal. It was also observed in new experiments that, with the same conditions, but in the presence of sulphite, pyruvic acid is formed, although, even in the presence of glutathione only traces of pyruvic acid are formed from

* Continued from p. 340.

methylglyoxal. We now recognise the formation of lactic acid as a true oxidation-reduction process in which the previous puzzling rôles of pyruvic acid as well as that of the esterification of the hexoses receive a new significance.

This observation is important also in deciding the question as to exactly where the two processes of alcoholic fermentation and lactic acid formation leave their common path. This question has occupied me for the past three months. Neuberg has assumed pyruvic acid as an intermediate in his well-known fermentation scheme, basing his hypothesis upon the discovery of carboxylase. Although this part of the scheme is now to be regarded as proven, one must also here remove the erroneous assumption that the pyruvic acid is derived from methylglyoxal. Neuberg himself had in the meantime shown for dry yeast, exactly as Embden had for muscle tissue, that phosphoglyceric acid can be transformed into pyruvic acid. It follows then, as can readily be shown, that in fresh yeast extract phosphoglyceric acid would be briskly decomposed into acetaldehyde and carbon dioxide. On the other hand, Nilsson had isolated, so early as 1930, phosphoglyceric acid from dry yeast in the presence of fluoride, hexose di-phosphate, and acetaldehyde.

In maceration juice containing fluoride, exactly in the same manner as in muscle extract, hexose di-phosphoric acid is actually converted into Lohmann's 'unhydrolysable ester', consisting of equimolecular portions of glycerophosphoric acid and phosphoglyceric acid. The phosphoglyceric acid, added to fresh yeast extract, decomposes, as shown, into carbon dioxide, phosphoric acid and acetaldehyde. However, α -glycerophosphoric acid does not react further and, since added sugar or hexose di-phosphoric acid is completely fermented by yeast, this stable compound cannot be formed during fermentation. The reduction of the aldehyde, therefore, is not accomplished by the glycerophosphoric acid, but in another manner. Nilsson in Euler's institute had already come across this reaction, but he failed to recognise its importance. Apart from the presence of carboxylase, this peculiar reaction is the principal difference between alcoholic fermentation and lactic acid production.

Nilsson established that when fluoride, glucose, hexose di-phosphate, and acetaldehyde are added simultaneously to dry yeast, with no fermentation taking place whatsoever, aldehyde is reduced to alcohol and sugar is simultaneously esterified. He identified the resulting ester as probably being phosphoglyceric acid. His observation was correct. I have followed the reaction further quantitatively and found two noteworthy fermentation equations.

When we add only hexose di-phosphoric acid to fluoride containing maceration juice, the ester is slowly converted into phosphoglyceric acid and glycerophosphoric acid, at the same rate at which it is fermented in the absence of fluoride. If

glucose alone, instead of hexose di-phosphoric acid, be added to maceration juice containing fluoride, no esterification takes place; for as I had observed earlier, glucose alone in the presence of fluoride is not esterified. It is different, however, when glucose and hexose di-phosphate are added simultaneously. In this case, glucose is esterified, but only to a certain extent. The reaction stops when a quantity equimolecular to the hexose di-phosphate has been esterified. The reaction is rapid, proceeding again with the same velocity as sugar fermentation in the absence of fluoride. Also this reaction produces a mixture of esters, consisting of almost equal quantities of glycerophosphoric acid and phosphoglyceric acid. This is shown in equation A in Table 2.

TABLE 2. New fermentation equations (with sodium fluoride).

A.	1 Hexose di-phosphoric acid	=	2 α -Glycerophosphoric acid
	+ 1 Glucose		+ 2 Phosphoglyceric acid
	+ 2 Phosphoric acid		
B.	2 Acetaldehyde	=	2 Alcohol
	+ 1 Glucose		+ 2 Phosphoglyceric acid
	+ 2 Phosphoric acid		
	(Hexose di-phosphoric acid)		

Addition of acetaldehyde changes this system altogether. The reaction proceeds further, aldehyde becomes alcohol and sugar is simultaneously esterified, but all the glucose that enters into the reaction is now converted into phosphoglyceric acid. Glycerophosphoric acid appears only to the extent that hexose di-phosphate is decomposed. The esterification and oxidation of the glucose proceeds until all the aldehyde has been converted into alcohol. As in this case the extent of the reaction depends not upon the quantity of hexose di-phosphate, but upon the amount of acetaldehyde added, the concentration of the hexose di-phosphate can be reduced to a large degree and yet accomplish the conversion of the sugar until the reaction is stopped by exhaustion of the aldehyde supply. In this manner an extremely small quantity of hexose di-phosphate, less than a tenth of a milligram per cubic centimetre of fermentation mixture, can lead to the esterification of fifty or a hundred times as much glucose. If one uses only a trace of hexose di-phosphate, then equation B in Table 2 is exactly satisfied. Here the hexose di-phosphate does not enter into the equation. It acts like a true catalyst. Actually, in the presence of acetaldehyde, hexose di-phosphate added in excess does not enter into the reaction, taking no part whatsoever in the course of reaction B. The velocity of reaction B is exactly as great as the maximum fermentation velocity of sugar. The necessary quantity of hexose di-phosphate is not larger than that which I found necessary fifteen years ago to initiate the fermentation, that is, to abolish the induction period, in maceration juice.

To explain further these peculiar intermediate reactions, I should like to add two more points.

(1) In place of hexose di-phosphoric acid, glyceric aldehyde phosphoric acid can be used. It behaves in both equation A and equation B practically the same as hexose di-phosphate. If it be assumed that

only *one* optical antipode enters into reaction, then the quantities of organic phosphorus necessary to accomplish the same amount of esterification are approximately the same. Thereby, the velocity of the rearrangement of the glyceric aldehyde phosphoric acid is greater than that of hexose di-phosphoric acid, while on the other hand the system glucose plus hexose di-phosphate reacts somewhat more rapidly than the system glucose plus glyceric aldehyde phosphoric acid. The glyceric aldehyde phosphoric acid can also be used to abolish the induction period. There is thus nothing to contradict the supposition that glyceric aldehyde phosphoric acid is formed as an intermediate product.

(2) The rôle of the acetaldehyde in the progress of the esterification consists in the oxidation of the momentary reaction products. One can therefore replace the acetaldehyde fairly well with other reducible systems, for example, methylene blue. Thus, the esterification can be conducted in accordance with equation B, using methylene blue instead of acetaldehyde. In this case, the oxidation product seems to be also phosphoglyceric acid. Therefore, methylene blue can also be used similarly to acetaldehyde as an activator of fermentation. The aldehyde reacts obviously with a nascent triose phosphoric acid produced from the reaction between glucose and hexose di-phosphate. At the same time the aldehyde prevents the rearrangement of the triose phosphoric acid into glyceric-phosphoric acid, so that the entire triose phosphoric acid is oxidised to phosphoglyceric acid. Everything gathered together results in the fermentation scheme shown in Table 3.

Here, in accordance with data obtained from kinetic studies it is necessary to separate the initial period of fermentation from the stationary state. In this case also, fluoride alone inhibits reaction B, while iodoacetic acid does not inhibit B, but does inhibit reactions A and C.

This new scheme will be seen to agree with all known facts. It explains the esterification of the phosphates, the characteristic catalytic rôle of the hexose di-phosphoric acid and the part played by acetaldehyde in determining the initial period. It shows fermentation further as an oxidation-reduction process in which most probably the dismutation of two aldehydes, acetaldehyde and glyceric aldehyde phosphoric acid, play the crucial parts. Finally, it brings the carboxylase into the process in an easily conceivable manner. The difference between alcoholic fermentation and lactic acid formation is to be found in the fate of the pyruvic acid. While pyruvic acid in muscle extract is reduced to lactic acid by the glyceric-phosphoric

TABLE 3. New fermentation scheme.

INITIAL PHASE		
A.	1 Hexose di-phosphoric acid = + 1 Glucose + 2 Phosphoric acid	4 Triose phosphoric acid = 2 α -Glycero-phosphoric acid + 2 Phosphoglyceric acid
B.	2 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 Acetaldehyde + 2 Phosphoglyceric acid

acid, in yeast, it is split up into carbon dioxide and acetaldehyde, which acetaldehyde is then reduced. This reduction, however, is not accomplished by the glyceric-phosphoric acid, but by some precursor, probably the glyceric aldehyde phosphoric acid. This circumstance determines the peculiar disproportionality of the reaction chain and compels us to set up one equation for the initial period and another for the stationary condition. The periods of induction and rising fermentation are, as well known, not present in lactic acid formation.

The recognition of these facts, which are all more or less connected with the Embden scheme of lactic acid formation, has brought us a good step forward and I think that now a large number of previously known facts may be fitted into a unified picture.

The British Association at Leicester

ON the eve of a meeting of the British Association, the members about to converge on the chosen site begin to take a lively interest in its merits and demerits.

A booklet of a hundred pages entitled "A Scientific Survey of Leicester and District", prepared for the Leicester meeting, partly answers this and shows very clearly the inescapable advantages and disadvantages of position revealed in the accounts of the geology and climate; the natural features that result in its flora and fauna; and the development by man of its economic possibilities described under farming and under industries of Leicester. This association of factors and interlocking of cause and effect is well brought out by

the editor of the booklet, Dr. P. W. Bryan, in his account of Leicester in its regional setting.

The corporate work of the citizen is described by the Town Clerk in a brief résumé of the rapid increase of activities since the establishment in 1835 of the new municipal body, particularly in relation to trading undertakings. Education, as a very special part of municipal responsibilities, is presented by the director of education, Mr. F. P. Armitage.

These accounts give prominence to the extreme administrative activity and forward thinking which resulted in the reorganising and in particular the re-grouping by 1929 of all the council schools in Leicester, in such a way as to make possible