covered by acid hydrolysis of the ester. This is likely, for Furth and Marian[3] have shown that only one-third of the theoretical glucose reducing value is obtained by hydrolysis of yeast hexose phosphate.

If the total carbohydrate loss (C) be assumed to be due to conversion to lactic acid (LA) and to hexose phosphate (HP), then C=LA+HP, and the equation $C=LA/2+x(H_3PO_4)$ (each quantity being expressed in mMols.) should indicate the molar ratio of phosphoric acid to hexose in the hexose phosphate. Column 6 gives the values for X in the experiments in which glycogen was added to the extract. It seems significant that these values lie between 1 and 2, and a logical explanation would be that a mixture of mono and diphosphate was formed.

The results support the hypothesis that glycogen passes through a carbohydrate-phosphate complex when it is transformed into lactic acid in muscle, and confirm the observations of Embden[1] and Meyerhof.[2]

This is a preliminary report.

[1] Embden, Gus., and Haymann, Clare, *Zt. f. Physiol. Chem.*, 1924, cxxxvii, 154.
[2] Meyerhof, Otto, *Biochem. Zt.*, 1926, clxxviii, 395, 462.
[3] Furth, O., and Marian, J., *Biochem. Zt.*, 1926, clxvii, 123.

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Observations on Glyoxals, Their Determination and Behavior.

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Shaffer[1] observed that the addition of alkali cyanide in small amounts to glyoxal produces in slightly alkaline solution a very highly reducing substance, which rapidly reduces many dyes and other reagents; and he suggested that this reaction be utilized for a quantitative method for the determination of glyoxals. Among the reagents which develop color on reduction are the complex phosphotungstic acids, and of these the arseno phospho-tungstic reagent used by Benedict[2] for uric acid was found to be well suited for our purpose. Without cyanide, glyoxal or methyl glyoxal scarcely reduces this reagent, but with cyanide reduction is rapid and proportional to

the glyoxal present. Six hundredths mg. or more glyoxal may thus be determined with an accuracy of ±5 per cent. To the glyoxal solution (preferably about 0.6 mg.) are added 2 cc. of Benedict's reagent, followed by 1 cc. of M NaCN and 5 cc. of M Na_2CO_3 in the order named. A similar mixture is made with 10 cc. 0.001 M glyoxal (0.58 mg.) as a standard. After 10 minutes at room temperature the mixtures are diluted to 100 cc. and read by colorimeter.

The notable effect of cyanide, in markedly increasing the reducing power, is not limited to glyoxals, having been observed also with uric acid and with cystin, but is exceptionally striking with the glyoxals.

With this method it is possible to follow the transformation of glyoxals by alkali, by tissue extracts (glyoxalase) and by cyanides. The following observations have been made. In pure buffer solutions at pH below 7 to 8 glyoxal and methyl glyoxal are quite stable; at higher pH the conversion to the corresponding hydroxy acid occurs, becoming rapid and complete at pH 12.5. At intermediate reactions at which the conversion is relatively slow, less lactic acid appears, then corresponds to the methyl glyoxal destroyed.

A comparison of the relative glyoxalase activity of filtered 20 per cent water extracts of various tissues on added methyl glyoxal shows that liver is most active, brain, skeletal muscle, heart muscle, lung, spleen, kidney and blood being about half as active. Simultaneous determinations of glyoxal and of lactic acid show that under the influence of tissue extracts methyl glyoxal is quantitatively converted to lactic acid. The optimum pH concentration for the glyoxalase activity is pH 7.0.

Meyerhof[3] has stated that dilute hydrocyanic acid converts methyl glyoxal to lactic acid. Our observations are in conflict with this statement. The action of cyanide is catalytic, a concentration of 10^{-5} M converting a much larger amount of methyl glyoxal rapidly in neutral buffer (where the glyoxal without cyanide is stable); but the product is not lactic acid. An acid is formed in amount about half the theoretical. The acid is volatile, is neither formic nor acetic nor pyruvic, but its identity is not established. The reactions by which methyl glyoxal is transformed under the catalytic influence of cyanide and by alkali or tissue extracts are therefore quite different. With cyanide a very much higher reducing intensity is attained than with either alkali or tissue extracts.

[1] Shaffer, P. A., unpublished.

[2] Benedict, S. R., *J. Biol. Chem.*, 1922, li, 187.

[3] Meyerhof, O., *Biochem. Z.*, 1925, clix, 432.