photographic emulsion. After exposure, the developed emulsion is protected with a coat of vinylite and mounted under coverslip.

Note added in proof: a dipping method of radioautography was also found in the German literature: Kolousek *et al., Acta Histochemica,* 1956, v3, 328.

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Utilization of Methylglyoxal in Animal Tissues. (23381)

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In spite of the difficulty of detecting methylglyoxal (MG) in the presence of the highly active enzyme glyoxalase, Neuberg and Kobel(1) isolated the compound from products of fermentation of hexose diphosphate (HDP) by dried yeast. MG is also formed from HDP by mixed preparations of muscle and pancreas (Tonniessen and Fisher(2), Ariyama(3)), and by acetone powders of rat liver (Artom(4)). Geiger and Rosenberg(5) detected MG in urine of polyneuritic dogs and in urine and cerebrospinal fluid of infants suffering from acute toxic dyspepsia; and Salem(6) could demonstrate MG in urine of thiamine-deficient rats. It appears therefore that MG is produced from HDP and can be detected under conditions, in which activity of the glyoxalase system is reduced or suppressed.

The aim of the present paper is to examine the possibility that MG could be converted enzymatically to HDP, *i.e.* to investigate whether the reaction: HDP→MG + Inorganic phosphate (Pi) can be considered as a reversible reaction.

Methods. MG was prepared from acetone by slight modification of the method of Riley, Morley and Friend(7). HDP was supplied by L. Light, Ltd., as the Ca salt. Fresh rat liver was weighed, ground with sand in a mortar, then extracted with one part of water and with occasional stirring for 30 minutes in the refrigerator. The mixture was centrifuged, and the supernatant used as a source of the enzyme. Pi was determined according to Fiske and Subbarow(8) and MG according to Salem(6). Two methods (a) and (b) were used to identify the compound resulting from incubation of liver preparation with MG: (a) Ba soluble and Ba insoluble phosphorylated compounds were fractionated, as described by Umbreit *et al.*(9), and hydrolysis curves were determined according to Lohmann(10); (b) descending one-dimensional paper chromatograms were made with trichloroacetic acid:acetone (35/65 v/v) as solvent. Molybdic acid method for phosphate esters (Hanes and Isherwood(11), Axelrod (12)) was used to detect HDP. Apparatus and working details were as described by Dent(13).

Results. Changes of Pi and MG during incubation: 1 ml of liver extract was incubated with 0.5 ml of 1:5 kidney extract for 2 hours at room temperature, to inactivate the glyoxalase system. Then 2 ml of 1% neutralized MG solution, 2 ml of borate-boric acid buffer, pH 7.0, and 0.2 ml toluene, as an antiseptic, were added. A similar sample was prepared, except that MG was omitted. Both samples were incubated at 37°C for 24 hours. The protein was precipitated with 10% trichloroacetic acid, and the amounts of Pi were determined on supernatants before and after incubation (with or without MG). From Table I, it is apparent that when MG was incubated

TABLE I. Inorganic P before and after Incubation, in 4 Experiments.

Before incubation	After incubation with methylglyoxal	After incubation without methylglyoxal
	mg	
.23	.12	.23
.22	.11	.22
.20	.11	.21
.16	.11	.18

with a mixture of liver and kidney extracts, there was a decrease of about 50% in the Pi initially present. In some experiments, in which the MG remaining after incubation was also measured, it was found that nearly one-fourth of the original amounts had disappeared. It should be noted that glyoxalase was completely inactive, as shown by determining enzyme activity in mixed liver and kidney extracts. The amounts of MG disappearing were about 25 times greater than the equivalent amounts of Pi disappearing during incubation. This lack of equivalence is possibly due to interference of other reactions, such as liberation of Pi from other phosphorylated compounds, or destruction of MG through other pathways. At any rate, the simultaneous disappearance of Pi and MG is in line with the hypothesis of a reaction in which both compounds are used.

Fractionation and hydrolysis of incubation products. By the method we have used(9), phosphorylated intermediates are separated into a Ba insoluble fraction, which contains ATP, ADP, HDP and phosphoglyceric acid, and a Ba soluble fraction, containing glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, phosphopyruvate and triose phosphates. Hydrolysis curves of the 2 fractions allow the probable demonstration of the compound(s) present. In our experiments, 5 ml of the mixture of liver and kidney extracts were incubated at 37° for 24 hr with 10 ml of borate-boric acid buffer, pH 7.0, 10 ml of 1% MG, and 1 ml toluene. The controls "before" and "after incubation" were prepared identically, except that 10 ml of distilled water were substituted for the solution of MG. In the experimental and control samples, the Ba soluble and Ba insoluble fractions were separated and hydrolyzed, and Pi was determined on aliquots taken at various time intervals. Fig. 1 records the hy-

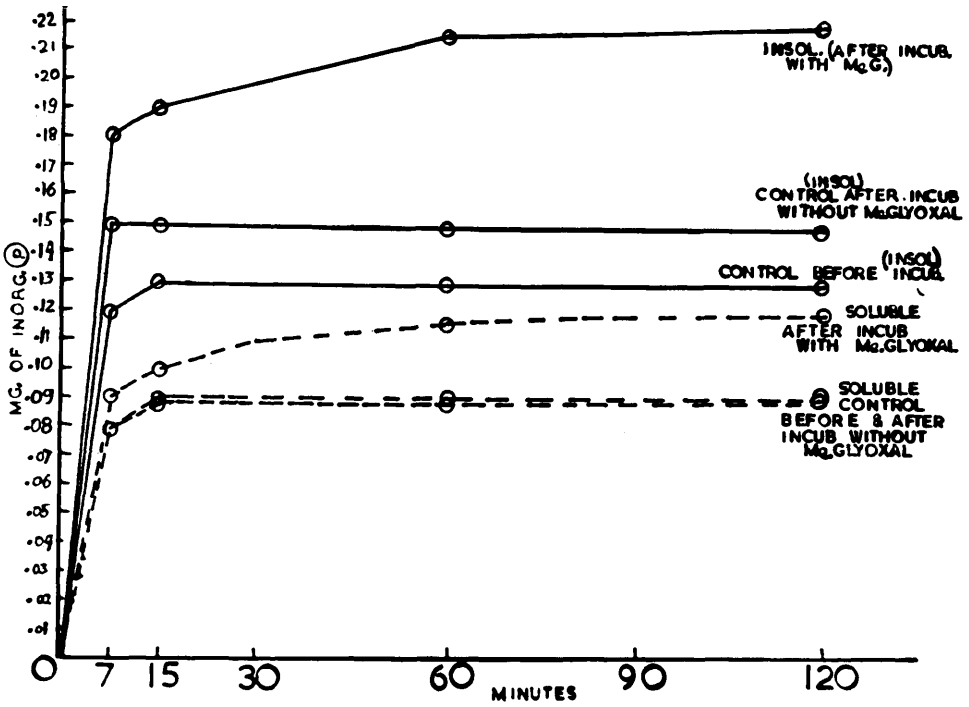

FIG. 1. Hydrolysis curves of barium soluble and insoluble fractions, before and after incubation with and without methylglyoxal.

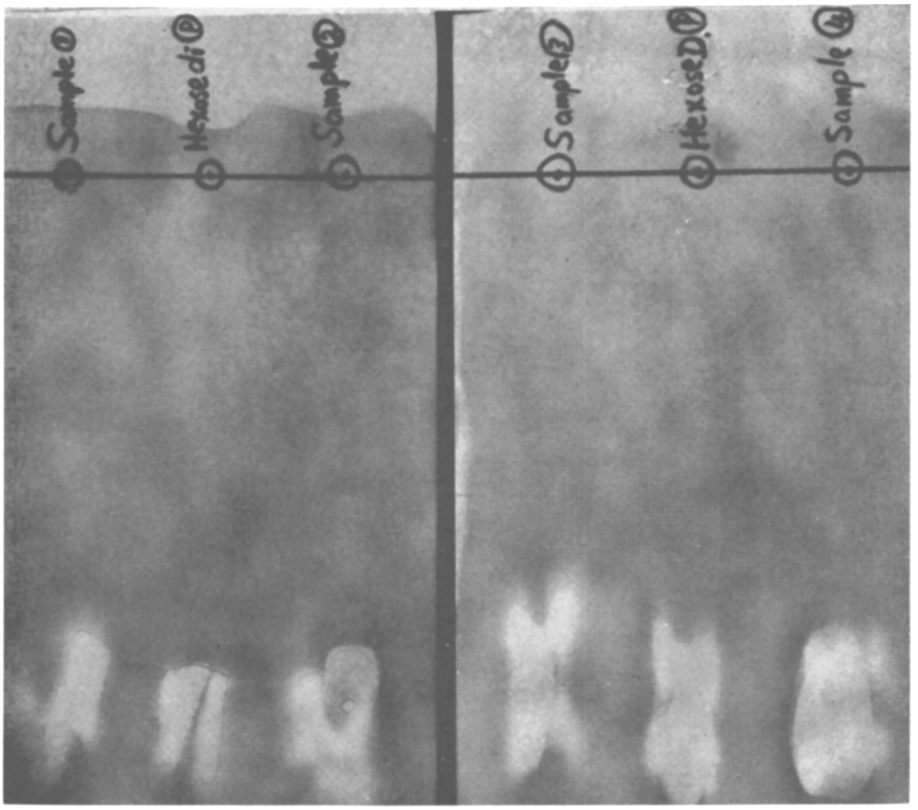

FIG. 2. Paper chromatograms of products of incubation of methylglyoxal with liver extracts (samples 1, 2, 3 and 4) and of solutions of pure hexosediphosphate (Hexosedi P). (No spots were detectable in the "controls" not incubated, or incubated without methylglyoxal.)

drolysis curves of the 2 fractions, as determined in one of our experiments. It is apparent that after incubation with MG, the increase in total hydrolyzable P of the Ba insoluble fraction was greater than that of the same fraction, incubated without MG. There was also an increase in total hydrolyzable phosphate of the soluble fraction after incubation with methylglyoxal, whereas there was no appreciable increase in the P of this fraction after incubation without MG. According to Umbreit *et al.*(9), 26.5% of HDP is hydrolyzed in the first 7 minutes, and then hydrolysis continues at a slower rate, whereas 46% of phosphopyruvic acid and triosephosphates is hydrolyzed in 7 minutes. The hydrolysis curve of the Ba insoluble fraction incubated with MG suggests therefore that HDP, or a P-containing compound with similar properties, increases during incubation with MG. The increase in the Ba soluble fraction curve might be due to formation of phosphopyruvic acid, or of triosephosphates, or both. It is possible that MG is converted to HDP through some steps including formation of triosephosphates. The slight increase in total hydrolyzable P of the Ba insoluble fraction after incubation without MG was unexpected. It may be noted that such increase occurred only during the first 7 minutes of hydrolysis.

Paper chromatography of incubation products. "Experimental" samples containing MG, liver and kidney extracts, borate buffer, and toluene were incubated at 37° for 24 hours. In addition, 2 "control" samples containing the extracts, the buffer and the antiseptic, but no MG were prepared. To one of these samples, trichloroacetic acid was added immediately, whereas the other control was incubated at 37° for 24 hours. After deproteinization and neutralization, small aliquots of the su-

pernatants from the experimental and the control samples were placed on paper. At the same time, aliquots of the commercial solution of HDP were also chromatographed. With the molybdic acid reagent, one blue spot on bleached background was detected in the chromatograms of each of the experimental samples, the position of the spot being identical to that observed in the chromatograms of the HDP solution. No spots were detectable in the chromatograms of the control samples, nonincubated, or incubated with MG. The chromatograms of 4 experimental samples (numbered 1 to 4) and of 2 samples of the HDP solution (indicated as hexosedi-P) are shown in Fig. 2.

Discussion. The present results give at least presumptive evidence for a conversion of MG into HDP. This conversion would be in line with the observation of Stoehr(14) that liver glycogen is formed from MG, fed at a carefully controlled level. Moreover, if D-lactate is formed from MG by glyoxalase and if the action of this enzyme is also reversible, as claimed by Dakin and Dudley(15), the following scheme may be tentatively suggested:

$$\text{D-lactate} \rightleftharpoons \text{MG} \rightleftharpoons \text{HDP} \rightleftharpoons \text{L-lactate}$$

This scheme would explain the finding of Dakin and Dudley(15) that both D- and L-lactic acid are formed when MG is incubated with crude preparations of the liver, and may perhaps account also for the partial utilization of D-lactate in the animal body (Cori and Cori(16)).

Summary. Methylglyoxal has been incubated with rat liver extracts in the presence of kidney antiglyoxalase. During incubation, methylglyoxal and inorganic P decrease, whereas total hydrolyzable P increases. The hydrolysis curve of the Ba insoluble fraction of liver incubated with methylglyoxal resembles that obtained from hexose phosphate. Paper chromatography also gave evidence for the formation of hexose phosphate from methylglyoxal.

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