involved procedure for preparing the suitable starting material.

We have now observed[5] that chromic acetylacetonate, chromic and chromous salts of organic acids such as acetic and 2-ethylhexanoic can be reduced easily and converted into the hexacarbonyl under high pressure of carbon monoxide if pyridine or related bases are employed as a reaction medium.

Our method consists in dissolving (or suspending) any one of the above compounds in pyridine, containing catalytic amounts of halogens or halogenated substances. The mixture is then treated at 80–170° with an excess of powdered magnesium or zinc and 100–300 atm. of carbon monoxide. When soluble chromium compounds are employed the yields are as high as 80–90%.

We believe the base to play an essential role in the synthesis because intermediate pyridine-containing complexes are formed in the course of the reaction.

It is also noteworthy that the hexacarbonyl as such is still present in substantial quantities in the end-products of the reaction. This seems not to happen in the synthesis by the Grignard method, the hexacarbonyl, in this case, being produced uniquely after the hydrolysis of the reaction mixtures.[4]

Experimental.—Chromium acetylacetonate (17.5 g.), magnesium (4.5 g.) and 80 g. of a 2% solution of iodine in dry pyridine are charged in a stainless steel oscillating autoclave of 500-ml. capacity. After removal of air, oscillation is started and pure oxygen-free carbon monoxide is compressed into the vessel up to a pressure of 190 atm. The autoclave is then warmed to 160° within one hour and maintained at this temperature (± 2°) for six hours, while pressure drops from 325 to 285 atm. After cooling and venting, the hexacarbonyl and the other reaction products are quantitatively transferred into a 3-liter two-necked distillation flask with the aid of a large amount of water. On distillation of the mixture, the white hexacarbonyl is drained off. The distillate is filtered, washed with chilled methanol and paper dried. Sublimation of the powder at 70–75° at 15 mm. affords 9.0 g. of pure chromium hexacarbonyl in coarse crystals (yield 82%).

(5) Italian Patent Application No. 671, February 4, 1957 (to Montecatini S.p.A.).

DEPARTMENT OF INDUSTRIAL CHEMISTRY
POLYTECHNIC INSTITUTE OF MILAN
MILANO, ITALY

G. NATTA
R. ERCOLI
F. CALDERAZZO
A. RABIZZONI

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PHOSPHATE TRANSFER CATALYZED BY PHOSPHOGLYCERIC ACID MUTASE

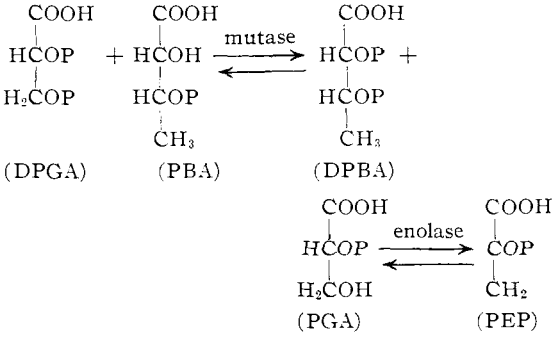
Sir:

The activation of partially purified muscle phosphoglyceric acid mutase by diphosphoglyceric acid (DPGA) was reported by Sutherland, *et al.*,[1] and we have undertaken the further study of this process with the crystalline enzyme, which was obtained from purified muscle enzyme[2] by employing the conditions found by Rodwell, *et al.*,[3] for the crystallization of the yeast mutase. In order to eliminate the possibility of DPGA contamination of the monophosphate substrate, synthetic phosphoenolpyruvate (PEP)[4] recrystallized as the cyclohexylamine salt[5] was used, and 2-phosphoglyceric acid (2PGA) was generated from it with crystalline enolase. Mutase activity was detected by its effect on the absorbance at 240 mμ of the equilibrated mixture, (PEP $\overset{\text{enolase}}{\rightleftharpoons}$ 2PGA $\overset{\text{mutase}}{\rightleftharpoons}$ 3PGA).

Synthetic DPGA[6] activated the mutase over the range 10^{-4} to 10^{-7} M, and the K_m for the conditions used was 2×10^{-6} M. The enzyme also exhibited activity under the same conditions when no DPGA was added. This activity was proportional to the amount of enzyme added and corresponded to 5% of the activity displayed when the enzyme was saturated with DPGA. The same order of activity was observed when the substrate was synthetic 3PGA,[7] although the presence of DPGA in trace amounts is here possible. In the latter case the activity was followed by the formation of PEP.

These results are indicative of two forms of the mutase, one which requires DPGA activation and one which does not. The enzyme, like phosphoglucomutase,[8] probably exists in a nonphosphorylated and a phosphorylated form. The latter species could exhibit activity in the absence of DPGA, and DPGA would activate by donating one of its phosphate groups to the nonphosphorylated form.

In experiments testing the specificity of DPGA as a donor of phosphate groups, we made use of the *erythro*-2,3-dihydroxybutyric acid monophosphates (DPBA)[9]; which have been shown to be active substrates for the mutase[2] but not for enolase.[5] The acceptance of phosphate from DPGA by PBA results in the formation of a mixture of the phosphoglyceric acid isomers (2PGA and 3PGA) which



are in part converted to PEP if enolase is present.

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In this experiment the formation of PGA from DPGA occurs only in the presence of PBA (Fig. 1, lines Ia, b) to an extent dependent on DPGA concentration if PBA is in excess (Fig. 1, lines IIa, b),

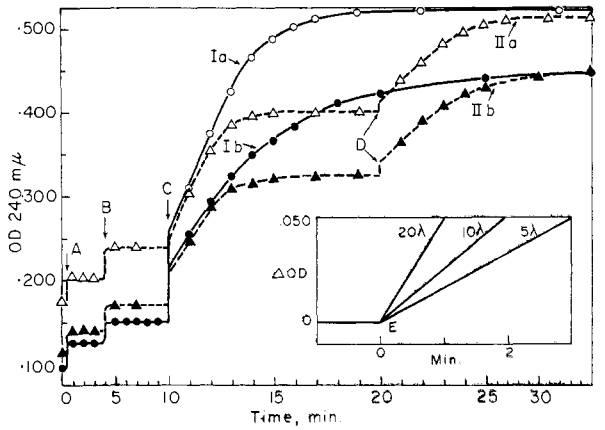

Fig. 1.—Spectrophotometric demonstration of the transfer of phosphate from DPGA to 2PBA and 3PBA: each 1 cm. cuvette contained 1 ml. of 0.1 *M* imidazole-HCl buffer, *p*H 7.0, 0.015 *M* in $MgCl_2$. To Ia and Ib was added 0.2 ml. of 0.015 *M* DPGA; to IIa was added 0.1 ml. of 0.15 *M* 2PBA; and to IIb, 0.1 ml. of 0.15 *M* 3PBA. At time A, enolase was added to each cuvette; then mutase was added at time B. At time C, 0.1 ml. of 0.15 *M* 2PBA was added to Ia, and 0.1 ml. of 0.15 *M* 3PBA was added to Ib. At the same time, 0.1 ml. of 0.015 *M* DPGA was added to both IIa and IIb. A further 0.1 ml. of DPGA was added to IIa and IIb at time D. Insert: Demonstration of the dependence of rate on mutase concentration. Cuvettes contain: 1 ml. of buffer, 0.1 ml. of 0.015 *M* DPGA, 0.1 ml. of 0.15 *M* 2PBA, 0.02 ml. of enolase and 1.76 ml. of H_2O. At E the quantities of mutase indicated on the curves were added and the 240 mμ adsorbance followed with the energy recording adaptor to the Beckman DU spectrophotometer.

and at a rate proportional to the mutase concentration, (Fig. 1, insert). The formation of PEP has been confirmed by paper chromatography of the reaction mixture in 2-propanol, ammonia, water (70:10:20). With the acid molybdate spray, spots corresponding to PEP, PGA-PBA (no separation) and a diphosphate corresponding to synthetic DPBA[10] are found. Synthetic DPBA can replace DPGA as a mutase activator, although ten times the concentration is required for comparable activity.

This series of reactions independently supports the conclusion[1] that DPGA donates one of its phosphate groups during its role in the enzymatic interconversion of the two monophosphoglyceric acid isomers. To show that DPGA donates directly to the enzyme would require the demonstration of phosphorus on the enzyme in equilibrium with the phosphorus on the substrate, but as the formation of a phosphate diester (between DPGA and PGA) as an intermediate in the phosphate transfer appears unlikely,[11] the series of reaction (a) and (b) satisfactorily explains the results.

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$$\text{(a)}\quad \text{DPGA} + \text{E} \rightleftharpoons \text{PE} + \text{PGA} \xrightarrow[\text{enolase}]{} \text{PEP}$$

$$\text{(b)}\quad \text{PE} + \text{PBA} \rightleftharpoons \text{E} + \text{DPBA}$$

$$\text{(a)} + \text{(b)}\quad \text{DPGA} + \text{PBA} \rightleftharpoons \text{DPBA} + \text{PGA} + \text{PEP}$$

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

LEWIS I. PIZER
CLINTON E. BALLOU

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THE MOLECULAR WEIGHT OF β-LACTOGLOBULIN

Sir:

The generally accepted molecular weight of β-lactoglobulin is in the vicinity of 35,000.[1,2,3,4] In the course of a detailed study of the association properties of this protein as a function of *p*H, it has been found that the weight of its disperse units is strongly dependent on *p*H.[5,6,7] In the *p*H regions below 3.5 and above 7.5, its sedimentation constant, measured at a protein concentration of 10 g./l., decreases from a value of $S_{20,w} = 2.85$ to $S_{20,w} = 2.25$, indicating that there occurs either a decrease in molecular weight or an expansion of the molecule, as in the case of serum albumin.[8,9,10,11,12]

In order to clarify this question, light scattering measurements were carried out on β-lactoglobulin as a function of *p*H. These show[7] that below *p*H 3.5 the molecule of β-lactoglobulin dissociates into units smaller than 35,000, probably into two portions of equal weight. These data are in qualitative agreement with the results of Rands and Tanford,[13] who also observed a similar decrease in the molecular weight of this protein at low *p*H. Because of the rapid re-equilibration of the dissociation with dilution, however, the light scattering data curve strongly upward in the low concentration range, and it is not possible to extrapolate them to a reliable value of the weight average molecular weight. As a result, ultracentrifugal determinations of the molecular weight were carried out by the Archibald technique,[14,15,16] under conditions of strong dissociation.

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