it seems reasonable to establish this stimulation by $PO_4^{=}$ and $SO_4^{=}$ as occurring on either the *I* or the *D* form of transglucosylase. On the other hand, Mg^{++} and Ca^{++} appear to stimulate only the *I* form. In paper V it will be demonstrated that phosphate and sulfate ions competitively inhibit the activation by glucose-6-P of the particulate *D* form of transglucosylase from dog muscle (Rosell-Perez and Larner, paper in preparation).

This study on the preparation of two forms of transglucosylase from rabbit muscle, together with the previous one on rat-skeletal muscle (Rosell-Perez *et al.*, 1962), support the hypothesis of the existence of both forms in these mammalian species. This hypothesis was proposed to explain the action of insulin on this system (Villar-Palasi and Larner, 1960).

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REFERENCES

Brown, D. H., and Kornfeld, R. (1960), Abstracts of Papers, 135th Meeting of the American Chemical Society, Sept., 1960, p. 78 c.
Hauk, R., and Brown, D. H. (1959), *Biochim. Biophys. Acta 33*, 556.
Kornfeld, R., and Brown, D. H. (1962), *J. Biol. Chem. 237*, 1772.
Larner, J., Villar-Palasi, C., and Richman, D. J. (1959), *Ann. New York Acad. Sci. 82*, 345.
Peterson, E. A., and Sober, H. A. (1962), *Methods Enzymol. 5*, 3.
Rosell-Perez, M. (1962), *Fed. Proc. 21*, 206.
Rosell-Perez, M., and Larner, J. (1961), *Fed. Proc. 20*, 193.
Rosell-Perez, M., and Larner, J. (1962), *Biochemistry 1*, 769.
Rosell-Perez, M., and Larner, J. (1963), *Fed. Proc. 22*, 463.
Rosell-Perez, M., and Larner, J. (1964), *Biochemistry 3*, 81 (this issue).
Rosell-Perez, M., Villar-Palasi, C., and Larner, J. (1962), *Biochemistry 1*, 763.
Villar-Palasi, C., and Larner, J. (1960), *Biochim. Biophys. Acta 39*, 171.
Villar-Palasi, C., and Larner, J. (1961), *Arch. Biochem. Biophys. 94*, 436.

Studies on UDPG-α-glucan Transglucosylase. V. Two Forms of the Enzyme in Dog Skeletal Muscle and Their Interconversion*

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UDPG-α-glucan transglucosylase (transglucosylase) extracted from fresh skeletal muscle of dog is highly dependent on glucose-6-phosphate for activity. High-speed centrifugation yielded a particulate fraction that had an absolute requirement for the sugar phosphate. It behaved kinetically like the dependent or *D* forms that had been prepared previously from stored frozen rat- and rabbit-skeletal muscle. The *D* form was converted totally to an independent or *I* form by incubating crude extracts for 40–60 minutes at 30° in the presence of mercaptoethanol. This sulfhydryl reagent increased the rate and the yield of the *I* form in the *D*-to-*I* conversion. The kinetic behavior of the *I* form obtained after transformation was similar to the *I* forms of other species studied. The reverse conversion of the *I* to the *D* form was obtained after adding ATP-Mg^{++}. This conversion was enhanced markedly by the addition of adenosine3′, 5′-cyclophosphate to the ATP-Mg^{++} system. UTP-Mg^{++}, in similar concentrations, produced an effect that was three times greater than ATP-Mg^{++}. However, the effect of UTP-Mg^{++} was accompanied by a parallel decrease in the activity measured with glucose-6-phosphate. Because of these and other facts, a scheme of interconversion through an inactive intermediate is postulated. Studies of the inhibitory action of *p*-mercuribenzoate showed that, as in rat, the *I* form was more sensitive than the *D* form to this inhibitor.

UDPG-α-glucan transglucosylase (transglucosylase)[1] has been prepared from the skeletal muscle of a number of different species (e.g., Villar-Palasi and Larner, 1958; Hauk and Brown, 1959; Robbins *et al.*, 1959; Trivelloni, 1960; Takeuchi and Glener, 1960; Hess and Pearse, 1961a,b; Traut, 1962; Kornfeld and Brown, 1962; Rosell-Perez and Larner, 1962, 1963, 1964).

Rosell-Perez and Larner (1961a) and Rosell-Perez *et al.* (1962) demonstrated that two forms of this enzyme could be prepared from rat muscle and differentiated kinetically. Rosell-Perez and Larner (1962) also showed that the form of the enzyme that was prepared from the skeletal muscle of frog and toadfish exhibited differences from that of rat with respect to kinetic behavior toward UDPG with and without glucose-6-P and Mg^{++}. The form of the enzyme from toadfish was highly dependent on glucose-6-P for activity, whereas the enzyme from frog was absolutely dependent. Recently, these authors (Rosell-Perez and Larner, 1963, 1964) isolated, purified, and characterized kinetically both forms, the independent or *I* form and

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[1] Abbreviations used in this work: UDPG, uridine diphosphoglucose; Tris, tris(hydroxymethyl)aminomethane; glucose-6-P, glucose 6-phosphate; transglucosylase, UDPG-α-glucan transglucosylase; TPN, nicotinamide adenine dinucleotide phosphate; DEAE-cellulose, diethylami-_oethyl-cellulose.

TABLE I
PURIFICATION OF DOG-MUSCLE TRANSGLUCOSYLASE

Steps	Volume (ml)	Total Activity (units/ml)[c]		Specific Activity (units/mg protein		Recovery (%)	
		−Glucose-6-P[a]	+Glucose-6-P[b]	−Glucose-6-P[a]	+Glucose-6-P[b]	−Glucose-6-P	+Glucose-6-P
1. Homogenate (1:10, w/v)	30	0.0102	0.112	0.00057	0.00625	100	100
2. Supernatant fluid (International)	28	0.0086	0.125	0.00146	0.021	79	100
3. 100,000 × *g* particulate fraction resuspended	23.3	0.0055	0.139	0.0061	0.154	42.1	96.5

[a] Activity measured without glucose-6-P. [b] Activity measured with added glucose-6-P. [c] Unit and specific activity used as defined by the recommendations of the Commission on Enzymes of the International Union of Biochemistry.

the dependent or *D* form, from skeletal muscle of rabbit. Friedman and Larner (1963) showed that the two forms of transglucosylase from skeletal muscle of rat are interconvertible by a mechanism that involves a phosphorylation-dephosphorylation sequence. In the phosphorylation reaction the terminal phosphate group of ATP was incorporated into the enzyme in the presence of Mg^{++}, whereas in the dephosphorylation reaction inorganic phosphate was released from the enzyme.

In the present paper, the enzyme obtained from the skeletal muscle of another species, the dog, has been studied. The enzyme present in dog skeletal muscle is unusual in that it may be extracted chiefly as a *D* form, highly dependent on glucose-6-P for activity. The kinetics of the UDPG concentration dependence with and without glucose-6-P and Mg^{++} are similar to those of *D* forms studied in other species. The system of conversion of the *D* to the *I* form, stimulated by mercaptoethanol, is so active that a total conversion can be obtained in short incubation periods. The kinetics then show a total change and are those of the *I* form. Reconversion to the *D* form can be obtained by ATP-Mg^{++} addition and also by the addition of UTP-Mg^{++}. The inhibition by *p*-mercuribenzoate and its prevention by mercaptoethanol have also been studied.

MATERIALS AND METHODS

Analytical.—The methods for the determination of transglucosylase and for the measurement of glucose-6-P were described in paper IV (Rosell-Perez and Larner, 1964) of this series. In the kinetic studies, the UDPG concentrations that were used are indicated in the respective tables and figures.

Chemicals.—ATP, UTP, and UDPG were obtained from the Sigma Chemical Company. Adenosine 3′,5′-cyclophosphate was a generous gift from Dr. E. W. Sutherland, Jr. DEAE-cellulose was obtained from the Brown Company, glycogen and glucose-6-P from Nutritional Biochemicals Co., Versene from Fisher Scientific Co., mercaptoethanol from Eastman Kodak Co., Tris from J. Frederick Smith Chemical Co.

EXPERIMENTAL

Enzyme Preparations.—After intravenous injection of lethal doses of sodium seconal, the dogs were exsanguinated rapidly by section of the neck vessels. Samples of muscle (5–10 g) were taken quickly from the leg. These were kept on ice while the connective tissue was dissected as completely as possible. The muscle was minced with scissors and then homogenized (1:10, w/v) in cold 0.05 M Tris–0.005 M Versene buffer (*p*H 7.8) in a motor-driven homogenizer which had been prechilled in ice (step 1). The cellular debris was eliminated by centrifuging the homogenates in an International Refrigerated Centrifuge at 3,000 rpm for 20 minutes (step 2). An equal volume of 1.0 M NaCl–0.05 M Tris–0.005 M Versene (*p*H 7.8) was added to the supernatant, which was then centrifuged at 100,000 × *g* in a Spinco ultracentrifuge (Model L) for 2 hours (step 3). All the activity was found in the glycogen pellet. The pellet was resuspended in 0.05 M Tris–0.005 M Versene–0.05 M mercaptoethanol buffer (*p*H 7.8). The purification and recovery of the enzyme are shown in Table I.

The initial ratio of activities determined with and without glucose-6-P was 11 in the homogenate (step 1) and 14.5 in the supernatant fluid from the International Centrifuge. The ratio became 25.2 in the 100,000 × *g* particulate fraction (step 3). In this case the *D* activity was obtained by the direct centrifugation of fresh crude extracts without storing the frozen muscle as had been necessary previously with muscle from rat and rabbit. This marked dependence on added glucose-6-P was similar to the dependence reported in muscle from toadfish and frog (Rosell-Perez and Larner, 1962). Nevertheless, certain differences will be pointed out later.

Effect of Increasing Enzyme Concentration.—As shown in Figure 1, a linear relationship existed between the amount of enzyme and the amount of radioactivity incorporated into glycogen.

Effect of the Time of Incubation in the Assay.—Figure 2 shows that with increased time of incubation there was an increased amount of radioactivity incorporated into glycogen. The reaction was linear during the first 15 minutes of incubation. Incubation times of 5–10 minutes were used accordingly for all experiments.

Effect of Substrate Concentration in the Presence and Absence of Activators.—The 100,000 × *g* particulate fraction was used for kinetic studies of the UDPG concentration dependence. The high ratio of activities with and without glucose-6-P demonstrated that essentially only the *D* form was present. The enzyme was characterized kinetically in a manner similar to the enzyme from rat (Rosell-Perez *et al.*, 1962), rabbit (Rosell-Perez and Larner, 1964), frog, and toadfish (Rosell-Perez and Larner, 1962).

Figure 3 presents the reciprocal plots relating enzyme activity to increasing concentrations of UDPG with and without glucose-6-P, with and without Mg^{++}, and in the presence of both together. As was noted in all the *D* forms of transglucosylase studied up to the present, little activity was detected in the absence of glucose-6-P even in the presence of Mg^{++}. The

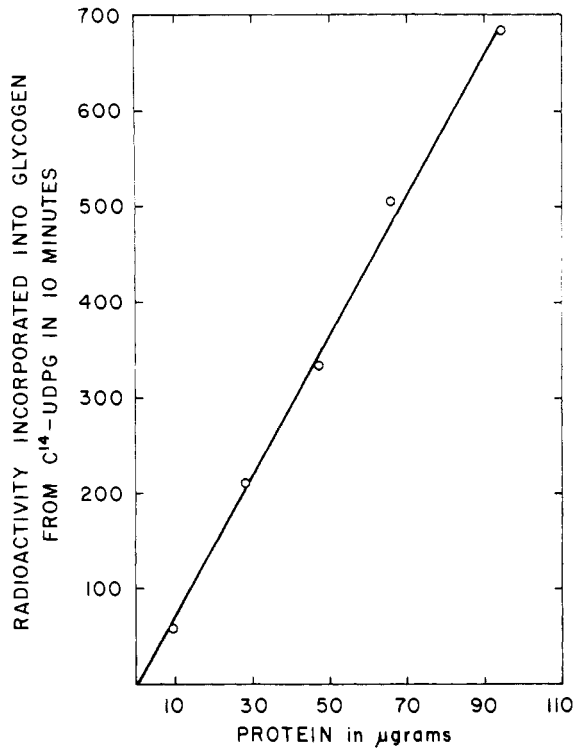

FIG. 1.—Relationship between enzyme concentration and activity. The reaction conditions were as stated under Materials and Methods. The enzyme source was a 100,000 × *g* particulate fraction (Step 3, Table I) resuspended in 0.05 M Tris–0.005 M Versene–0.05 M mercaptoethanol. Time of incubation was 10 minutes at 30°.

presence of glucose-6-P produced a 10- to 50-fold rise in activity in different preparations, and the addition of Mg^{++} (0.008 M) slightly enhanced this activity. The K_m values for UDPG were 3.5×10^{-4} M in the presence of glucose-6-P and 2.1×10^{-4} M with added Mg^{++}. The value estimated in the absence of glucose-6-P was 1.0×10^{-2} M. The 10-fold change in *V* at saturating concentrations of UDPG is shown in Figure 3.

Action of Mercaptoethanol on the Conversion of the D to the I Form.—In previous experiments it had been shown that when rat or rabbit muscle extracts were incubated with mercaptoethanol, a transformation of the *D* to the *I* form occurred. This method was used to prepare the *I* form in these species. On the other hand, when extracts of muscle from frog and toadfish were incubated under similar conditions, a rather different activation occurred in which the total activity (measured with glucose-6-P) increased with little or no conversion of the *D* to the *I* form. Since the enzyme from dog muscle was highly dependent on glucose-6-P for its action, especially after isolation in the 100,000 × *g* particulate fraction, it became of interest to examine the conversion of this transglucosylase in the presence of mercaptoethanol.

The enzyme (step 2) was incubated in the presence of 0.05 M mercaptoethanol at 30°. In Figure 4, the results of such an experiment are shown. During the 50 minutes of incubation, the *I* activity increased markedly. The total activity measured with glucose-6-P did not change significantly. Thus the ratio of activities with and without glucose-6-P decreased from 16.9 at 0 time to 1.1 at the end of the 50-minute incubation period. This indicates that a complete

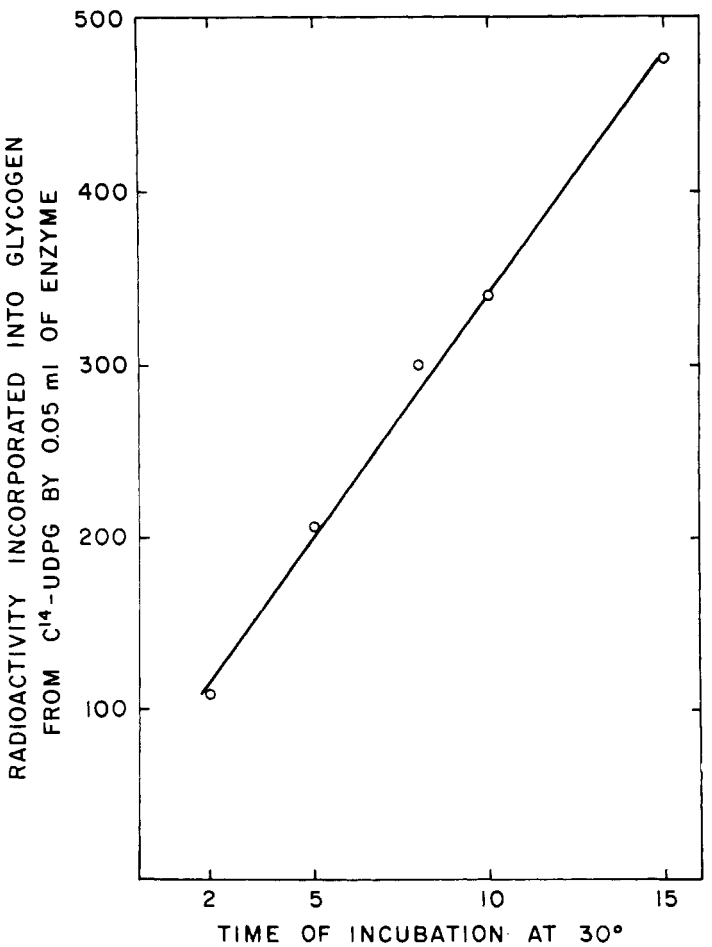

FIG. 2.—Activity of transglucosylase as a function of time. The enzyme source was the same as in Fig. 1; 0.05 ml of the suspension (48 mg of protein) was used.

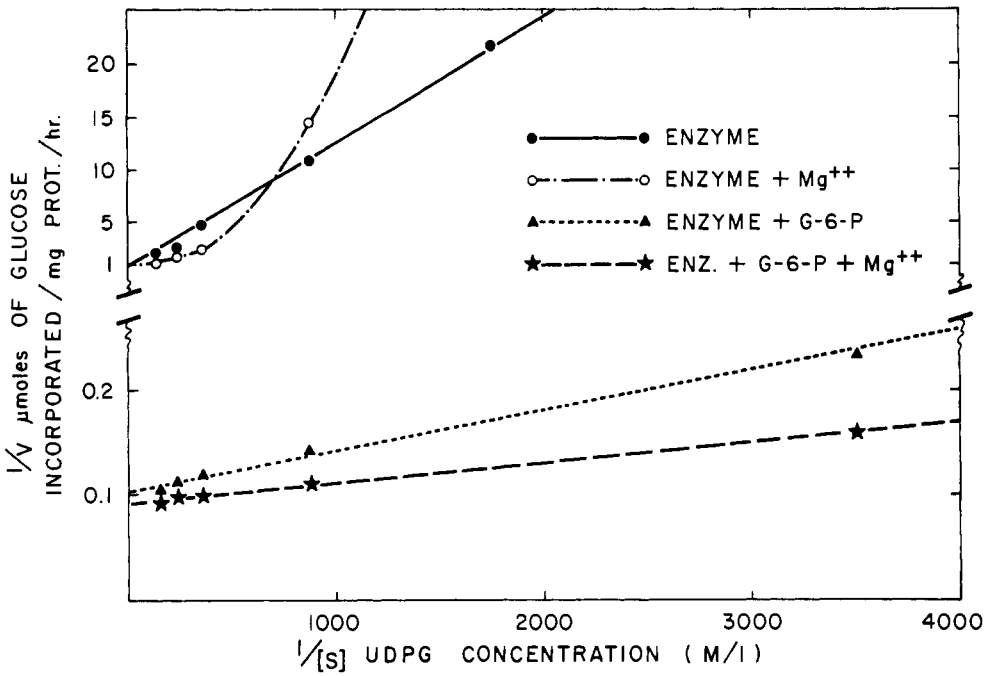

FIG. 3.—Reciprocal plot of the UDPG concentration dependence with transglucosylase from dog muscle (*D* form). The enzyme source was a 100,000 × *g* particulate fraction resuspended in buffer as cited in Fig. 1. The concentration of $MgCl_2$ was 0.008 M and of glucose 6-phosphate, 0.01 M.

transformation of the *D* form to the *I* form had occurred (Rosell-Perez *et al.*, 1962; Friedman and Larner, 1963). The comparison of the two curves of *I* activity, with and without mercaptoethanol, clearly demonstrates the increased rate and amount of transformation in the presence of this sulfhydryl compound.

The concentration dependence of mercaptoethanol for the *D*-to-*I* transformation is shown in Table II. It can be seen that with increasing concentrations of mercaptoethanol an increased rate, as well as an increased yield of transformed enzyme, was observed. A control experiment showed clearly that the transformation could not be accounted for by the formation of glucose-6-P during incubation. Table III shows that glucose-6-P did not increase during the incubation. The glucose-6-P concentrations observed are approximately one-tenth the K_m for glucose-6-P that we have determined (Rosell-Perez, M., and Larner, J., paper in preparation).

Enzyme Kinetics after the D-to-I Transformation.—The question arose as to whether the transformation observed with mercaptoethanol was producing an *I* form with kinetic characteristics similar to those obtained with enzyme preparations from rat (Rosell-Perez *et al.*, 1962) and rabbit (Rosell-Perez and Larner, 1964). A crude enzyme preparation was incubated for 60 minutes in the presence of 0.05 M mercaptoethanol. The initial ratio of activities in the presence and absence of glucose-6-P was 7.8. After incubation, the ratio was 1.0 or, in other words, no stimulation with glucose-6-P was detected.[2]

Studies of the UDPG concen ration dependence with and without glucose-6-P and Mg^{++} were done with the converted enzyme. In Figure 5 the results of these studies are shown. Both glucose-6-P and Mg^{++} produced a decrease in the K_m of the enzyme for UDPG with no change in the *V*. This is exactly the behavior of the *I* forms of transglucosylase prepared from rat and rabbit skeletal muscle (Rosell-Perez *et al.*, 1962; Rosell-Perez and Larner, 1964). In Table IV, the kinetic constants are presented.

Presence of the I-to-D Conversion Systems.—After the establishment of the *D*-to-*I* conversion it became of interest to study the *I*-to-*D* system, especially since the enzyme was extracted from muscle chiefly as the *D* form. An experiment in which this system was demonstrated is shown in Figure 6. When a crude enzyme preparation (step 2) was incubated for 30 minutes at 30° in the presence of mercaptoethanol, the *D*-to-*I*

TABLE II
THE ACTION OF MERCAPTOETHANOL IN THE DOG-MUSCLE TRANSGLUCOSYLASE CONVERSION REACTION[a]

Mercaptoethanol Concentrations (moles/liter)	C^{14}-Glucose (in cpm) Incorporated into Glycogen after Stated Preincubation Time (minutes)				
	0	10	20	30	40
0	67	247	340	356	378
5×10^{-3}	68	310	370	412	412
1×10^{-2}	69	330	392	447	447
5×10^{-2}	108	369	450	489	491

[a] Conditions: The mercaptoethanol was added to the enzyme preparations, and after the preincubation periods at 30° activity was tested as in the standard procedure.

TABLE III
GLUCOSE-6-P CONCENTRATION DURING THE DOG-MUSCLE TRANSGLUCOSYLASE CONVERSION REACTION

Time (minutes)	Glucose-6-P (mole/liter $\times 10^{-5}$)
0	5.2
10	6.5
20	4.8
30	4.0
60	4.0[a]

[a] During this time period, transglucosylase *I* activity increased 6.4-fold.

[2] On the basis of the kinetics of the *I* form, a small stimulation by glucose-6-P would have been expected. Traces of inorganic phosphate present could conceivably mask this stimulation.

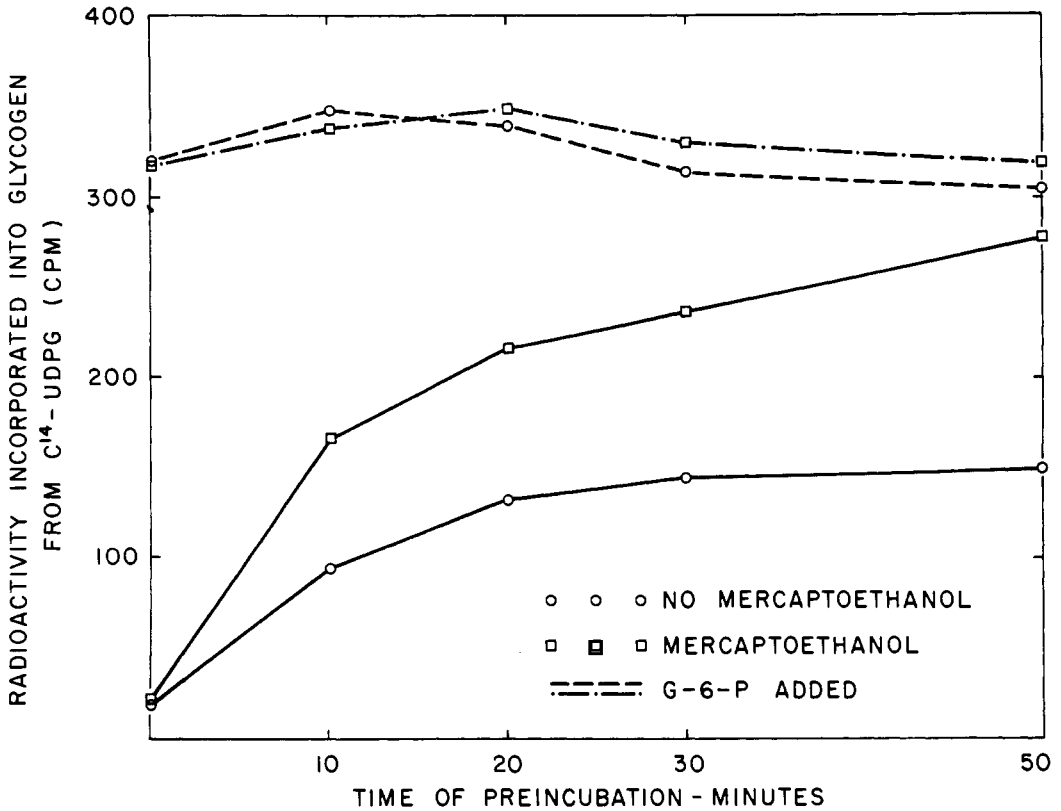

FIG. 4.—The effect of mercaptoethanol on the *D*-to-*I* transformation reaction. The enzyme preparation was a supernatant fluid (Step 2, Table I). The concentration of mercaptoethanol (when added) was 0.05 M.

TABLE IV
KINETIC CONSTANTS DETERMINED FOR THE TWO FORMS OF DOG-MUSCLE TRANSGLUCOSYLASE

Species	Form	Constants	Additions			
			None	Mg^{++}	Glucose-6-P	Glucose-6-P + Mg^{++}
Dog	*I*	K_m[a]	1.9×10^{-3}	2.4×10^{-4}	6.6×10^{-4}	2.3×10^{-4}
		V[b,c]	0.037	0.037	0.037	0.037
	D	K_m	1×10^{-2}	—	3.5×10^{-4}	2.1×10^{-4}
		V	0.016	0.016	0.16	0.19

[a] K_m is given as concentration of UDPG in moles/liter. [b] V is given in units as defined by the recommendation of the Commission on Enzymes of the International Union of Biochemistry. [c] Units/mg protein.

conversion was essentially complete. The addition of ATP-Mg^{++} (0.005 M–0.01 M) at that point brought about an *I*-to-*D* transformation. Five minutes after the addition, both enzyme activities measured with and without glucose-6-P showed a parallel decrease. Following the incubation the *I* activity continued to decrease whereas the total activity rose slowly and leveled off. The implications of this experiment will be discussed later.

Other Conditions Influencing the I-to-D Transformation.—Since it had been shown (Friedman and Larner, 1963) that the terminal phosphate group of ATP was introduced into the enzyme during the *I*-to-*D* conversion, presumably by a kinase reaction, it was of interest to study the effect of adenosine 3′,5′-cyclophosphate on this reaction. The influence of adenosine 3′,5′-cyclophosphate on the ATP-Mg^{++} reaction and the effect of UTP-Mg^{++} on the transformation are shown in Figure 7. It is clear that in the presence of the cyclic nucleotide the action of ATP-Mg^{++} was enhanced markedly. No action due to the adenosine 3′,5′-cyclophosphate alone was detected. The addition of UTP-Mg^{++} at a concentration equal to that of ATP-Mg^{++} produced an effect three times greater than with ATP-Mg^{++}. In contrast to ATP-Mg^{++}, which produced either no or only a small partially reversible decrease in the activity measured with glucose-6-P, the addition of UTP-Mg^{++} produced a marked decrease in the activity measured with added glucose-6-P.

The adenosine 3′,5′-cyclophosphate also enhanced the UTP-Mg^{++} reaction. In the presence of 6×10^{-4} M adenosine 3′,5′-cyclophosphate, a concentration of 1×10^{-3} M UTP produced the same effect as 5×10^{-3} M UTP in the absence of cyclic nucleotide. The concentration of Mg^{++} was always two times that of the triphosphate.

Inhibition by p-Mercuribenzoate.—The inhibitory action of *p*-mercuribenzoate was studied on the two forms by measuring its effect on two different enzyme preparations from dog muscle. In the first experiment, the inhibition by *p*-mercuribenzoate was tested on a crude enzyme preparation (step 2). In the second experiment the same preparation was preincubated without mercaptoethanol for 60 minutes in order to convert some of the *D* form to *I* form. It was shown

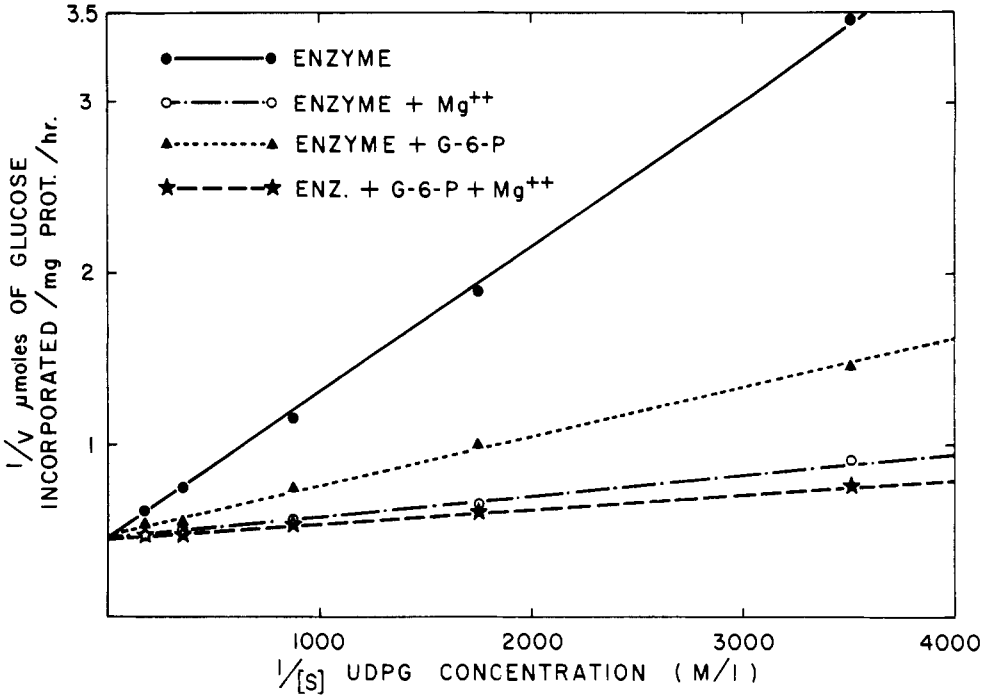

FIG. 5.—Reciprocal plot of UDPG concentration dependence with transglucosylase activity from dog muscle (*I* form). The enzyme source was the preparation incubated as shown in Fig. 4. Concentrations of glucose 6-phosphate and Mg^{++} were the same as in Fig. 3.

previously (Fig. 4) that in these crude preparations the conversion occurred without mercaptoethanol, but was not as rapid or complete.

It can be observed in Table V that the *I* activity in both types of experiments was more sensitive to the *p*-mercuribenzoate. A concentration of 4×10^{-5} M produced 71.4 and 82.2% inhibition of the *I* form and only 45 and 42% inhibition of the *D* form. A higher concentration of *p*-mercuribenzoate (1.2×10^{-4}) produced 100 and 96.2% inhibition of the *I* form as compared to 93.5 and 82% inhibition of the *D* form. The presence of 0.05 M mercaptoethanol counteracted the inhibition by *p*-mercuribenzoate.

TABLE V
INHIBITION OF DOG-MUSCLE TRANSGLUCOSYLASE ACTIVITIES BY *p*-MERCURIBENZOATE[a]

p-Mercuribenzoate Concentration (moles/liter)	Preparation A (Not Incubated) Inhibition (%)		Preparation B (Incubated) Inhibition (%)	
	I	*D*	*I*	*D*
0 (Control)	—	—	—	—
4×10^{-5}	71.4	45	82.2	42
1.2×10^{-4}	100	93.5	96.2	82
4×10^{-4}	100	98	100	94
4×10^{-4} + 0.05 M mercaptoethanol	—	—	—	—
0 + 0.05 M mercaptoethanol (control)	—	—	—	—

[a] As described in the text.

DISCUSSION

Since previous studies (Rosell-Perez *et al.*, 1962; Rosell-Perez and Larner, 1962, 1964) demonstrated a variation in transglucosylase activity present in several species, the study of the enzyme in a higher mammal was of interest. The high ratios of activity with and without glucose-6-P initially present in the homogenates of dog muscle suggested the possibility of obtaining particulate enzyme preparations relatively free of the *I* form, as had been done with the enzyme from toadfish and frog muscle (Rosell-Perez and Larner, 1962). Thus a three-step preparation procedure almost identical to that described for the enzyme from toadfish and frog was used. This procedure has the advantage of avoiding the introduction of ammonium sulfate, which itself weakly stimulates transglucosylase (Rosell-Perez, 1962) and thus complicates the determination of the sensitivity of the enzyme to glucose-6-P.

In kinetic studies on the UDPG concentration dependence, the lack of stimulation by Mg^{++} alone, the low activity in the absence of glucose-6-P, and the marked change in *V* when this sugar phosphate was added (Rosell-Perez *et al.*, 1962) clearly defined this form of transglucosylase as a *D* form. These characteristics have been demonstrated repeatedly in *D* forms prepared from rat (Rosell-Perez *et al.*, 1962), rabbit (Rosell-Perez and Larner, 1964), toadfish, and frog (Rosell-Perez and Larner, 1962).

When the *D* form was converted to an *I* form (Fig. 7), the kinetics were changed. At low concentrations of UDPG, Mg^{++} alone stimulated, as did glucose-6-P. No change in *V* was noted when either Mg^{++} or glucose-6-P was added, but the K_m for UDPG was decreased. Similar kinetics have been observed in *I* forms of all other species studied thus far.

The action of mercaptoethanol in the conversion of the *D* to the *I* form was first detected in a rat-muscle enzyme fraction which had been precipitated with ammonium sulfate and then lyophilized (Rosell-Perez *et al.*, 1962). A similar preparation was used to prepare the *I* form of the enzyme from rabbit muscle (paper IV). In these preparations no significant conversion was detected without added mercaptoethanol; this suggested the complete dependence of the conversion system on the sulfhydryl compound. In fresh rat muscle preparations, the conversion occurred without added mercaptoethanol (Friedman and Larner, 1963). This was true also with dog muscle (present work).

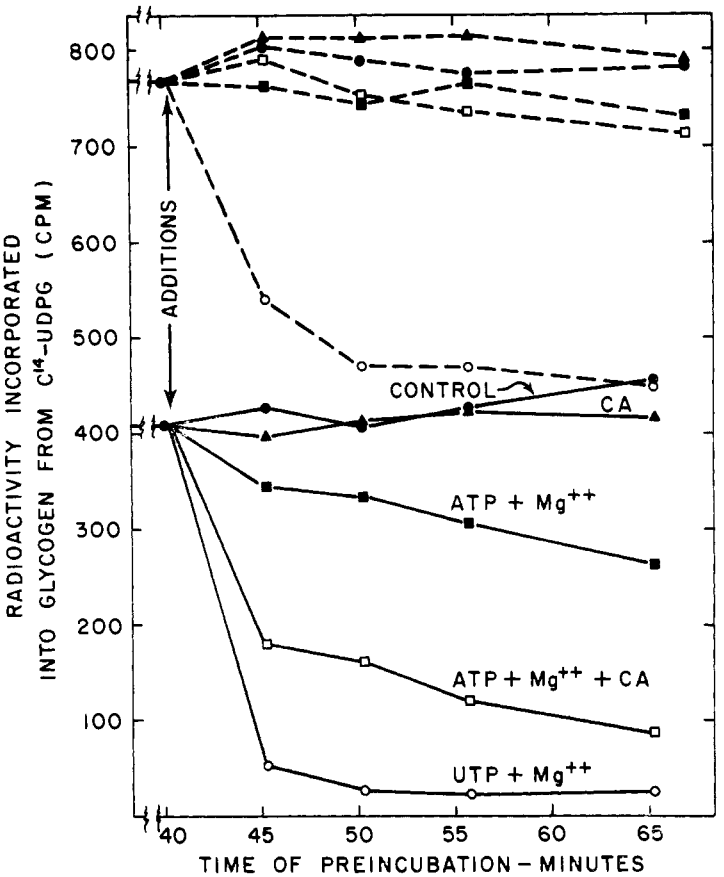

FIG. 6.—The conversion reactions of transglucosylase from dog muscle. The concentration of mercaptoethanol was 0.05 M. The concentration of ATP and Mg^{++} are given in the text.

However, as is shown here, the conversion reaction even in fresh muscle is greatly enhanced both in terms of rate and in final yield of *I* form in the presence of mercaptoethanol. In the conversion of the enzymes of fresh rat muscle, it was shown that the release of P^{32} as inorganic phosphate from the P^{32}-labeled enzyme correlated exactly with the conversion of the *D* to the *I* form (Friedman and Larner, 1963). Presumably the reaction proceeds in fresh muscle without added mercaptoethanol because of the presence of endogenous sulfhydryl compounds; these are largely removed when the ammonium sulfate–treated lyophilized preparations are prepared.

In the conversion of the *I* to the *D* form in rat muscle, the terminal phosphate group of ATP was incorporated into the enzyme. In these experiments when ATP-Mg^{++} was added no significant decrease in total enzyme activity was noted when measured with glucose-6-P. In the present work an early decrease in total activity was observed (Fig. 6) when ATP-Mg^{++} was added, and this trend was reversed partially with incubation time. This phenomenon has also been observed with other specific preparations from rat muscle.[3] What is implied here is a transformation of the *I* form or even possibly the *D* form to an intermediate form that is inactive even in the presence of glucose-6-P.

The experiments with UTP-Mg^{++} are consistent with this hypothesis. As was shown, UTP-Mg^{++} was more potent than ATP-Mg^{++} when compared at equal molar concentrations. A marked decrease in total activity was noted with UTP-Mg^{++}. Since the enzyme that remained at the end of the incubation with UTP-Mg^{++} was almost completely dependent on added glucose-6-P for activity, it would appear that all the *I* form had been transformed to an inactive species. Another possibility would be that the *I* form was converted to the *D* form along with a conversion to an inactive species.[4] This evidence for a third or inactive species is also in keeping with a previous observation. When toadfish and especially frog muscle preparations were incubated with mercaptoethanol, little or no conversion of *D* form to *I* form occurred. Instead, an increase in total activity was observed. The explanation suggested was that the increase in activity might be due to an activation of the enzyme from an inactive enzyme or an inactive precursor(s) (Rosell-Perez and Larner, 1962).

The effect of adenosine 3′,5′-cyclophosphate in this system is of interest. The effect noted, namely the enhanced reactivity of ATP and UTP, would appear superficially to resemble the known effects of this cyclic nucleotide in the muscle phosphorylase–activating system (Krebs and Fischer, 1962).

In Table IV the kinetic constants for the enzyme are presented. Two general features of the *D* form can be seen. One is the poor affinity for UDPG in the absence of glucose-6-P, and the other is the great increase in *V* that occurs in the presence of this cofactor. Mg^{++} does not stimulate in the absence of glucose-6-P. The *I* form is characterized by a decrease of the K_m for UDPG in the presence of Mg^{++} or glucose-6-P with no change in *V*. This is a common pattern which has been observed in rat, rabbit, and

[3] D. L. Friedman, unpublished observations.

[4] An experiment has been performed which suggests that the inactive species may be less phosphorylated than the *D* form. After adding UTP-Mg^{++} and incubating until the *I*-to-*D* conversion was complete with the accompanying decrease in *D*, ATP-Mg^{++} was then added. Under these conditions, a small but definite increase in *D* activity was observed.

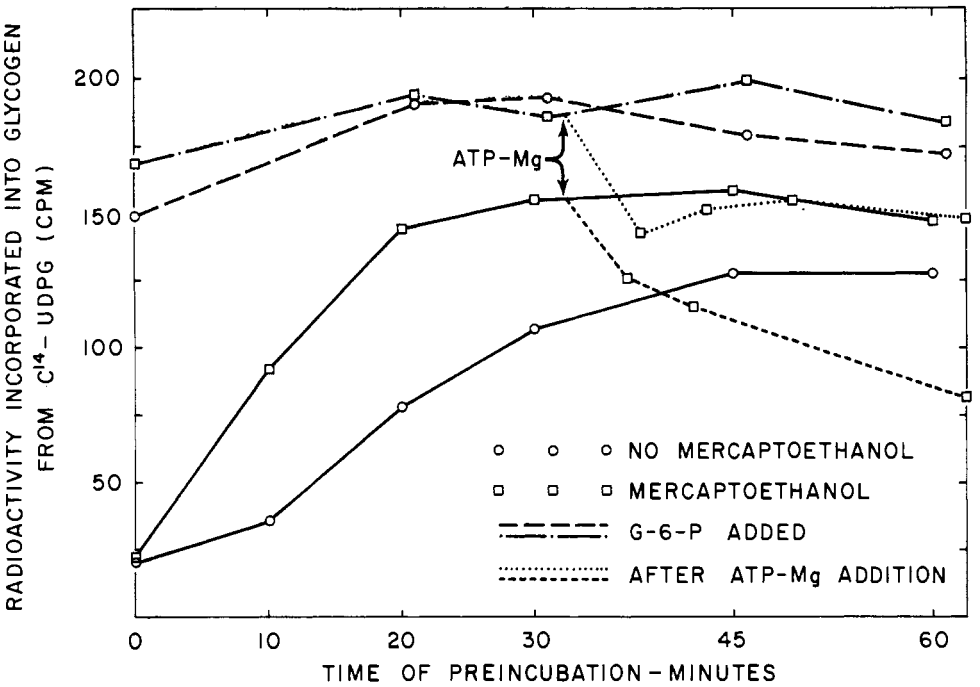

FIG. 7.—The effect of the addition of ATP-Mg^{++} and UTP-Mg^{++} on transglucosylase from dog muscle. The enzyme was preincubated for 40 minutes prior to the experiment. The effect produced by the addition of adenosine 3′,5′-cyclophosphate with and without ATP-Mg^{++} is shown. The concentrations of ATP and UTP were 5×10^{-3} M. The concentration of Mg^{++} was 1×10^{-2} M, and of the cyclic nucleotide 3.3×10^{-4} M. Full lines represent the activity without glucose 6-phosphate and the dotted lines represent activity with the compound added.

dog. The enzymes from toadfish and frog are apparently different from the other species studied with regard to the change observed in the V when Mg^{++} is added together with glucose-6-P. This may be explained as follows. We have observed in enzyme preparations from frog that the K_m for glucose-6-P was lowered 10-fold in the presence of Mg^{++}. The values were 2 to 3 $\times 10^{-3}$ M without Mg^{++}, so our test mixtures contained just enough glucose-6-P to give half maximum activity. Therefore, the addition of Mg^{++} made the concentrations of glucose-6-P fully effective. This fact can account for the doubling of the V observed.

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REFERENCES

Friedman, D. L., and Larner, J. (1963), *Biochemistry 2*, 669.
Grillo, T. A. I., and Ozone, K. (1962), *Nature* (London) *195*, 902.
Hauk, R., and Brown, D. H. (1959), *Biochim. Biophys. Acta 33*, 556.
Hess, R., and Pearse, A. G. E. (1961a), *Proc. Soc. Exptl. Biol. Med. 107*, 569.
Hess, R., and Pearse, A. G. E. (1961b), *Enzymol. Biol. Clin. 1*, 15.
Kornfeld, R., and Brown, D. H. (1962), *J. Biol. Chem. 237*, 1772.
Krebs, E. G., and Fischer, E. H. (1962), *Advan. Enzymol. 24*, 263.
Leloir, L. F., Olavarria, J. M., Goldemberg, S. H., and Carminatti, H. (1959), *Arch. Biochem. Biophys. 81*, 508.
Robbins, P. W., Traut, R. R., and Lipmann, F. (1959), *Proc. Nat. Acad. Sci. U. S. 65*, 6.
Rosell-Perez, M. (1962), *Fed. Proc. 21*, 206.
Rosell-Perez, M., and Larner, J. (1961a), *Fed. Proc. 20*, 198.
Rosell-Perez, M., and Larner, J. (1961b), Abstracts of Papers, 140th Meeting of the American Chemical Society, September, 1961, 12D.
Rosell-Perez, M., and Larner, J. (1962), *Biochemistry 1*, 769.
Rosell-Perez, M., and Larner, J. (1963), *Fed. Proc. 22*, 463.
Rosell-Perez, M., and Larner, J. (1964), *Biochemistry 3*, 75 (this issue).
Rosell-Perez, M., Villar-Palasi, C., and Larner, J. (1962), *Biochemistry 1*, 763.
Takeuchi, T., and Glener, G. G. (1960), *J. Histochem. Cytochem. 8*, 227.
Traut, R. R. (1962), Ph.D. dissertation (unpublished), The Rockefeller Institute.
Trivelloni, J. C. (1960), *Arch. Biochem. Biophys. 89*, 149.
Villar-Palasi, C., and Larner, J. (1958), *Biochim. Biophys. Acta 30*, 449.
Villar-Palasi, C., and Larner, J. (1960), *Biochim. Biophys. Acta 39*, 171.