Preparation of a monoclonal antibody to rat liver glucose-6-phosphate dehydrogenase and the study of its immunoreactivity with native and inactivated enzyme

A monoclonal antibody of the IgG class was prepared against rat liver glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate:NADP+ 1-oxidoreductase, EC 1.1.1.49) by the hybridoma technique. This antibody does not affect the catalytic activity of the enzyme and shows crossreactivity with the palmitoyl CoA-inactivated G6PD. By solid phase radioimmunoassay, the presence of crossreacting materials in comparable amounts was determined in liver homogenate supernatants from rats that had been starved and refed a high-sucrose diet (containing a high level of G6PD activity) and from rats that had been starved and re-fed a high-fat diet (containing a low level of G6PD activity). These findings indicate that G6PD is present in an inactive form in rats fed a high-fat diet. The monoclonal antibody will facilitate isolation and characterization of the inactive variant G6PD.

The level of glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate:NADP+ 1-oxidoreductase, EC 1.1.1.49) activity decreases in the livers of starved rats and increases markedly when they are re-fed a high-carbohydrate diet (1-11). This increase in activity was reported to be 5-10 times the level found in untreated control animals and 10-15 times the starvation level (11-13). However, upon refeeding starved animals with a diet in which carbohydrate was replaced by fat at the same protein/calorie ratio, G6PD was severely repressed, resulting in a level 1/10th of that of the G6PD activity found in control animals re-fed a high-sucrose diet (12-16). One of the hypotheses concerning the nutritional regulation of liver G6PD was its direct inactivation by fat metabolites (16), and attempts have been made to determine whether or not an inactive form of the enzyme was present in the repressed animals. Goat and rabbit antiserum were prepared against rat or mouse liver G6PD, and immunochemical methods were applied to see whether or not the amount of crossreacting materials varied in parallel with the levels of enzyme activity found in animals maintained under various nutritional conditions. Unfortunately, conflicting results have been reported. Although some investigators have found an increase in the amount of crossreacting material that was proportional to the increase in the level of G6PD activity (17, 18), others observed that the same amount of protein was immunoprecipitated from liver preparations showing large variations in the level of G6PD activity (16, 19). This discrepancy in results may be due to the use of antibodies specific for different determinants on the enzyme.

Therefore, the existence of a catalytically inactive enzyme species cannot be either demonstrated or ruled out when the antibodies that were used crossreacted strictly with the active form of the enzyme, as shown by the loss of immunoreactivity of in vitro palmitoyl CoA-inactivated enzyme (20). The major problem in elucidating this conflict regarding the presence of an inactive enzyme species in the livers of animals fed a high-fat diet was the difficulty in obtaining a monospecific antibody that crossreacts equally well with the active and inactive forms of the enzyme. In an attempt to obtain such a reagent in large amounts and with reproducible characteristics, the hybridoma technique was applied to produce monoclonal antibody against rat liver G6PD.

EXPERIMENTAL PROCEDURES

Materials. Glucose 6-phosphate, 6-phosphogluconate, NADP+, bovine serum albumin, agarose, palmitoyl CoA, polyethylene glycol (PEG) 1,000, hypoxanthine, thymidine, amethopterine (methotrexate), and DEAE-cellulose were from Sigma. PEG 6,000 was from Baker. Dulbecco's modified Eagle's medium, sodium pyruvate, National Cancer Tissue Culture 109, and fetal calf serum were from Microbiological Associates (Bethesda, MD). Rabbit anti-mouse IgG and bovine sera were from Miles Laboratories, and Na25I was from Amersham. All other chemicals were reagent grade. Flexible polyvinyl chloride microtititer plates were from Dynatech, and tissue culture disposable labware was Costar or Falcon. Rat liver homogenate supernatants and goat and rabbit anti-G6PD-sera were prepared at The Oklahoma Medical Research Foundation (Oklahoma City, OK). SP2/0-Ag14 mouse myeloma cells (21) were obtained from the laboratory of John Cebra, Department of Biology, University of Pennsylvania. C3H/HeJ mice were from The Jackson Laboratories.

Purification of Rat Liver G6PD. G6PD was purified from rat liver homogenate supernatant by protamine sulfate precipitation, ammonium sulfate fractionation, ion-exchange chromatography with DEAE-cellulose and affinity chromatography with Cibacron blue-agarose and NADP+ -agarose as described (20).

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PCG, 6-phosphogluconate dehydrogenase; PEG, polyethylene glycol; RIA, radioimmunoassay; P/NaCl, 0.05 M phosphate buffer, pH 7.4/0.9% sodium chloride.

† High-carbohydrate diet: 70.5% sucrose/20.5% casein/5% corn oil/4% minerals/vitamins.

‡ High-fat diet: 92.2% lard/33.8% casein/8.3% corn oil/5.7% minerals/vitamins.

ABSTRACT A monoclonal antibody of the IgG class was prepared against rat liver glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate:NADP+ 1-oxidoreductase, EC 1.1.1.49) by the hybridoma technique. This antibody does not affect the catalytic activity of the enzyme and shows crossreactivity with the palmitoyl CoA-inactivated G6PD. By solid phase radioimmunoassay, the presence of crossreacting materials in comparable amounts was determined in liver homogenate supernatants from rats that had been starved and refed a high-sucrose diet (containing a high level of G6PD activity) and from rats that had been starved and re-fed a high-fat diet (containing a low level of G6PD activity). These findings indicate that G6PD is present in an inactive form in rats fed a high-fat diet. The monoclonal antibody will facilitate isolation and characterization of the inactive variant G6PD.

The level of glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate:NADP+ 1-oxidoreductase, EC 1.1.1.49) activity decreases in the livers of starved rats and increases markedly when they are re-fed a high-carbohydrate diet (1-11). This increase in activity was reported to be 5-10 times the level found in untreated control animals and 10-15 times the starvation level (11-13). However, upon refeeding starved animals with a diet in which carbohydrate was replaced by fat at the same protein/calorie ratio, G6PD was severely repressed, resulting in a level 1/10th of that of the G6PD activity found in control animals re-fed a high-sucrose diet (12-16). One of the hypotheses concerning the nutritional regulation of liver G6PD was its direct inactivation by fat metabolites (16), and attempts have been made to determine whether or not an inactive form of the enzyme was present in the repressed animals. Goat and rabbit antiserum were prepared against rat or mouse liver G6PD, and immunochemical methods were applied to see whether or not the amount of crossreacting materials varied in parallel with the levels of enzyme activity found in animals maintained under various nutritional conditions. Unfortunately, conflicting results have been reported. Although some investigators have found an increase in the amount of crossreacting material that was proportional to the increase in the level of G6PD activity (17, 18), others observed that the same amount of protein was immunoprecipitated from liver preparations showing large variations in the level of G6PD activity (16, 19). This discrepancy in results may be due to the use of antibodies specific for different determinants on the enzyme.

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Enzyme Assay. G6PD activity was assayed by measuring spectrophotometrically the change in light absorption at 340 nm at 30°C. A 10-μl aliquot of sample was added to 1 ml of reaction mixture A (4 μmol of glucose 6-phosphate and 1 μmol of NADP⁺ in 0.1 M Tris, pH 7.6/0.0335 M MgCl₂) as described (20). Whenever 6-phosphogluconate dehydrogenase (PGD; EC 1.1.1.44) was present, G6PD activity was calculated from the difference between the total activity of G6PD plus PGD and the activity of PGD alone. PGD was measured first by adding 10 μl of sample to 1 ml of reaction mixture (4 μmol of 6-phosphogluconate and 1 μmol of NADP⁺ in 0.1 M Tris, pH 7.6/0.0335 M MgCl₂). The total activity of G6PD and PGD was determined by adding 4 μmol of Glc-6-P to the above mixture.

Inactivation of G6PD by Palmitoyl CoA. A pure solution of G6PD was incubated with a solution of 0.5 mM palmitoyl CoA in 5 mM Tris, pH 8.0/2 mM 2-mercaptoethanol in a water bath at 37°C until the enzyme was completely inactivated. For the control, a solution of enzyme was incubated in parallel under the same conditions in an equivalent amount of Tris buffer (used to dissolve the palmitoyl CoA).

Immunization. A C57BL/6 mouse, age 8 wk, was injected subcutaneously at 1-wk intervals with pure rat liver G6PD (50 μg each time). The first injection was given in an equal volume of Freund's complete adjuvant, and the following injections were given in Freund's incomplete adjuvant. The production of immunoglobulins was tested by radioimmunoassay (RIA) of the mouse serum using 125I-labeled rabbit anti-mouse IgG as a second antibody. A booster was given to the mouse 3 days before the removal of its spleen for cell hybridization.

Preparation of Hybridomas. The mouse spleen cells were fused with an equal number of SP2/0-Ag14 myeloma cells in the presence of PEG 1,000 as described by Kennett et al. (22). After the fusions, the cells were resuspended in a selective growth medium, Dulbecco's modified Eagle's medium containing hypoxanthine, amethopterin, and thymidine prepared as described (23), and were cultured at 37°C in humidified 5% CO₂/95% air.

Screening of Hybridomas. Supernatants from growing cultures were tested for anti-G6PD activity by solid-phase RIA adapted from a described procedure (24). Flexible polyvinyl chloride microtiter plates were coated with G6PD by incubating each well with 100 μl of a solution of pure G6PD (5 μg per ml) in 0.05 M phosphate buffer, pH 7.4/0.9% sodium chloride (P/NaCl). The next day the plates were washed with P/NaCl containing 0.15% bovine serum and 0.02% sodium azide (washing solution), then incubated for 1 hr at room temperature with 100 μl of P/NaCl containing 1% bovine serum albumin and 0.02% sodium azide, and washed a second time as before. After incubation with various volumes of hybridoma culture supernatant (10, 20, 30, and 40 μl) overnight at room temperature, the plates were washed as described earlier, and each well was incubated with 50 μl of 125I-labeled rabbit anti-mouse IgG for 6 hr at room temperature. The plates were washed extensively with the washing solution, and the wells were cut out and assayed for radioactivity. Supernatants from SP2/0-Ag14 myeloma cell cultures were used as negative controls.

Radiiodination. 125I-Labeled IgG fraction from rabbit anti-mouse IgG was prepared by the chloramine-T procedure (25). The IgG fraction from rabbit anti-mouse IgG (100 μl; 1 mg/ml) was mixed with 1 mCi (1 Ci = 3.7 × 10¹⁰ becquerels) of Na¹²⁵I in the presence of 10 μl of chloramine T (1 mg/ml) for 2 min. The reaction was stopped by the addition of 10 μl of sodium metabisulfite (1 mg/ml). P/NaCl containing 10% bovine serum and 0.02% NaN₃ was used as a carrier, and the mixture was applied to a column of Dowex Ag 1 × 2 and DEAE-cellulose that had been equilibrated with the carrier. The column was washed with the equilibrating solution, and a total volume of 3 ml was collected. A 1.30 dilution of this solution in the carrier was used in radioimmunoassays.

Cloning of Hybridomas. Hybridomas producing anti-rat liver G6PD were cloned on 0.5% agarose in Dulbecco's modified Eagle's medium with hypoxanthine and thymidine. Growing clones were picked up and transferred with fine-point Pasteur pipettes into wells of a 96-well plate and grown in Dulbecco's modified Eagle's medium with 20% fetal calf serum. Supernatants from growing cultures were again tested for anti-rat liver G6PD.

Immunosassay of G6PD. The crossreactivity of the monoclonal antibody with pure active G6PD, palmitoyl CoA-inactivated G6PD, and rat liver homogenate supernatants was tested by RIA as described before but with plates coated with the corresponding antigens.

Effects of Monoclonal Antibody on G6PD Catalytic Activity. Pure solutions of G6PD (100 μl) were incubated overnight at 37°C with various amounts (10, 20, 30, 40, and 50 μl) of culture supernatant from hybridomas producing anti-rat liver G6PD antibodies. G6PD activities of the mixtures were measured as described above. SP2/0-Ag14 culture supernatant and goat and rabbit preimmune sera and anti-G6PD antisera were also incubated with the active enzyme as controls.

Competitive Binding Immunoassay. Hybridoma culture supernatant (2 vol) was preincubated with liver homogenate supernatant (1 vol) from rats that had been starved and then fed the high-sucrose diet or refed the high-fat diet. The rat liver homogenate supernatants were diluted to the same protein concentration before use. As the control, hybridoma culture supernatant was incubated with P/NaCl containing 10% bovine serum and 0.02% NaN₃. The resulting mixtures were used in RIAs as described earlier.

Partial Purification of Monoclonal Antibodies. An antibody preparation designated GDR151, as from culture medium of hybridoma clone GDR1, was precipitated by addition of ammonium sulfate to 50% saturation. The pellet was separated by centrifugation at 24,000 × g for 20 min and resuspended in a minimal amount of P/NaCl. The resulting solution was dialyzed overnight against two changes of 5 mM phosphate buffer (pH 6.5), and the IgG antibodies were separated by an adaptation of the Stanworth method (26, 27). The dialyzed solution containing the antibodies was passed slowly through a column of microgranular preswollen DEAE-cellulose that had been equilibrated with 5 mM phosphate buffer (pH 6.5). The void fraction containing the immunoglobulins was collected and concentrated by precipitation with ammonium sulfate to then saturation. The pellet was separated by centrifugation, resuspended in a minimal amount of P/NaCl and dialyzed against P/NaCl containing 0.02% NaN₃. Aliquots of the dialyzed solution were stored frozen until use (designation GDR152).

Immunoprecipitation of G6PD. Solutions containing G6PD were incubated with the partially purified monoclonal antibody (GDR152) for 1 hr at 37°C, followed by 1 hr at 4°C. The enzyme was precipitated with the equilibrating solution, and a total volume of 3 ml was collected. A 1.30 dilution of this solution in the carrier was used in radioimmunoassays.

1 The system used to designate these clones that produced specific antibody to a single enzyme on rat liver soluble G6PD is: GD = G6PD, R = rat, P = preclone. The clones were then numbered successively. Thus, a preclone (PGDR1) produced clones GDR1, GDR2, GDR3, GDR4, etc. A separate number was used for each clone because, even though they were produced from one preclone and assumed to be the same, they could be different. In order to designate the antibody preparations, supernatants from the first preclone used was designated PGDR1; then other antibody preparations became GDR151 (for the first supernatant preparation obtained from GDR1) and GDR152 (for GDR151 that was put through the fractionation steps of Stanworth (26) and ammonium sulfate fractionation.)
antibody complexes were precipitated by incubation with rabbit anti-mouse IgG or by a modification of the Moidment et al. method (28); PEG 6,000 was used for precipitation of soluble immune complexes. The immunoprecipitates were resuspended in 100 μl of P2/NaCl and assayed for G6PD activity.

**RESULTS**

**Immunization of the Mouse.** IgG immunoglobulins against rat liver G6PD were detected in mouse serum one week after the 3rd injection of the enzyme to the animal. At a dilution of 1:100, the mouse antiserum showed 8.5 times more cpm compared to the preimmune serum (Table 1).

**Preparation of Hybridomas Producing Anti-G6PD Antibodies.** Two weeks after the fusion, 14 of 384 wells showed growing cultures, one of which was positive for the production of anti-rat liver G6PD antibodies (Table 2). Cells from the positive well were cloned on soft agarose, and the supernatants from 54 of 55 cultured clones were positive for crossreactivity with G6PD. When tested by RIA, the supernatants of each of the positive cultures also showed crossreactivity with pooled liver homogenate supernatants from rats that had been starved and refed either a high-sucrose or a high-fat diet. The results obtained from the supernatants of four clones are reported as examples (Table 3). Clone GDR1 was selected for further study, and the other clones were frozen and stored in liquid nitrogen.

**Effect of GDR1 Antibody on G6PD Catalytic Activity.** The same number of enzyme units (0.3 units/ml) was observed when the enzyme was incubated with SP2/0-Ag14 cell supernatant, goat preimmune serum, or rabbit preimmune serum. There was also no decrease in catalytic activity when the enzyme was incubated with clone GDR1 supernatant preparation GDR1S1. In contrast, a 90% decrease in enzyme activity was observed with 10 μl of rabbit anti-G6PD immune serum.

**Competitive Binding Assays.** RIA were performed on plates coated with purified G6PD enzyme and clone GDR1 culture supernatant (GDR1S1) that had been preincubated with either 10% bovine serum in P2/NaCl or with rat liver homogenate supernatant. A significant decrease in binding of the antibody to the immobilized enzyme was observed after preincubation of the clone GDR1 culture supernatant with rat liver homogenate supernatants (Table 4). These results showed that the reacting material in rat liver homogenate supernatants shared the same antigenic determinant as pure G6PD and that the antigen was present in a relatively comparable amount in the fat-refed and the sucrose-refed animals. In contrast, there was 6 times more enzyme activity for the same protein concentration (10.25 mg/ml) in the liver homogenate supernatant from sucrose-refed rats than was detected in fat-refed animals (data not shown).

**Crossreactivity of Monoclonal Antibody with Palmitoyl CoA-Inactivated G6PD.** When the supernatant from the GDR1 clone was tested on plates coated with palmitoyl CoA-inactivated enzyme, crossreactivity was observed. The titration curve obtained with 10, 20, 30, and 40 μl of hybridoma culture supernatant (Table 5) showed 1.3, 3.1, 4.1, and 3.2 times the number of cpm displayed by the SP2 culture supernatant. Crossreactivity was also demonstrated with a sample of pure G6PD that had been inactivated by repeated freezing and thawing (Table 5).

**Immunoprecipitation of G6PD.** By second antibody. Liver homogenate supernatant (200 μl) derived from starved and then sucrose-refed rats was incubated in a microcentrifuge tube with 50 μl of the dialyzed 50% ammonium sulfate fraction of monoclonal antibody preparation GDR1S2 at 37°C for 1 hr. Rabbit anti-mouse IgG (100 μl; 1.6 mg/ml) was added, and the mixture was incubated 1 hr at 37°C, followed by 4 hr at 4°C. After centrifugation at 4,000 × g for 10 min, the supernatant was assayed for G6PD activity. A decrease in enzyme activity was observed as compared to a control sample that had been incubated under the same conditions but with P2/NaCl instead of monoclonal antibody. A further decrease in enzyme activity was obtained by addition of 20 μl of rabbit anti-mouse IgG incubation at 37°C for 1 hr and at 4°C overnight, and subsequent centrifugation (Fig. 1). G6PD activity could not be detected when the immunoprecipitate was resuspended.

**Table 1. RIA of mouse preimmune and immune sera**

<table>
<thead>
<tr>
<th>Mouse sera</th>
<th>1:10</th>
<th>1:100</th>
<th>1:500</th>
<th>1:1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmune</td>
<td>667</td>
<td>580</td>
<td>769</td>
<td>909</td>
</tr>
<tr>
<td>Immune</td>
<td>2997</td>
<td>4940</td>
<td>3409</td>
<td>2965</td>
</tr>
</tbody>
</table>

**Table 2. RIA of supernatant from preclone PGDR1**

<table>
<thead>
<tr>
<th>Culture</th>
<th>10 μl</th>
<th>20 μl</th>
<th>30 μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP2/0-Ag14</td>
<td>588</td>
<td>786</td>
<td>609</td>
</tr>
<tr>
<td>GDR1S1</td>
<td>4197</td>
<td>5681</td>
<td>5765</td>
</tr>
</tbody>
</table>

* cpm were obtained from the titration of unfractonated antibodies in the culture supernatant of hybridoma GDR1 (before cloning) as measured on a microtiter plate coated with pure G6PD.

**Table 3. Immunoreactivity of monoclonal antibodies with rat liver homogenate supernatants**

<table>
<thead>
<tr>
<th>Culture</th>
<th>10 μl</th>
<th>20 μl</th>
<th>30 μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>SP2/0-Ag14</td>
<td>366</td>
<td>289</td>
<td>312</td>
</tr>
<tr>
<td>GDR1</td>
<td>1247</td>
<td>1504</td>
<td>1447</td>
</tr>
<tr>
<td>GDR2</td>
<td>924</td>
<td>1247</td>
<td>1801</td>
</tr>
<tr>
<td>GDR3</td>
<td>1084</td>
<td>1365</td>
<td>1696</td>
</tr>
<tr>
<td>GDR4</td>
<td>1165</td>
<td>1602</td>
<td>1614</td>
</tr>
</tbody>
</table>

RIA of culture supernatant of four clones producing antibodies against rat liver G6PD (clones: GDR1, GDR2, GDR3, and GDR4); 10, 20, and 30 μl of unfractonated culture supernatant were incubated in wells of a microtiter plate that had been coated with liver homogenate supernatant from starved rats refed the sucrose diet (S) and from starved rats refed the fat diet (F).

**Table 4. Competitive binding assay of rat liver homogenate supernatants**

<table>
<thead>
<tr>
<th>Preincubation</th>
<th>RIA of supernatant, cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% bovine serum/0.02% NaN3 in P2/NaCl</td>
<td>2343</td>
</tr>
<tr>
<td>Liver homogenate supernatant (S)</td>
<td>1460</td>
</tr>
<tr>
<td>Liver homogenate supernatant (F)</td>
<td>1546</td>
</tr>
</tbody>
</table>

RIA of GDR1 clone unfractonated supernatant after preincubation with bovine serum or liver homogenate supernatants from starved and sucrose-refed rats (S) or starved and fat-refed rats (F). The plate was coated with pure G6PD, and competition was demonstrated by both liver homogenate supernatants at the same protein concentration (10.25 mg/ml), although the enzyme activity was 6 times higher in S than in F homogenates.
Table 5. Immunoreactivity of inactivated G6PD with monoclonal antibody

<table>
<thead>
<tr>
<th>Antigen</th>
<th>RIA of supernatant, cpm of $^{125}$I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µl</td>
</tr>
<tr>
<td>Palmitoyl CoA-inactivated G6PD</td>
<td>SP2/0-Ag14</td>
</tr>
<tr>
<td>Freezing/thawing-inactivated G6PD</td>
<td>SP2/0-Ag14</td>
</tr>
<tr>
<td>GDR1</td>
<td></td>
</tr>
</tbody>
</table>

Microtiter plate was coated with palmitoyl CoA-inactivated enzyme or G6PD inactivated by repeated freezing and thawing. Both antigens showed crossreactivity with GDR1 monoclonal antibody preparation GDR1S2.

By PEG. Samples of rat liver homogenate supernatant (200 µl each) were incubated with 20, 30, and 40 µl of partially purified monoclonal antibody (GDR1S2). After 1 hr of incubation at 37°C and 1 hr at 4°C, 10% of a PEG-6000 solution (25% wt/vol in 0.05 M Tris, pH 8/0.14 M NaCl) was added, and the mixture was incubated with continuous mixing at room temperature for 2 hr. The supernatant obtained by centrifugation at 4,000 × g for 10 min was collected and incubated at 4°C for 48 hr. The enzyme–antibody complex was precipitated by centrifugation (4,000 × g for 10 min), and the supernatant was assayed for G6PD activity. Increasing amounts of G6PD were precipitated by increasing amounts of monoclonal antibody (Fig. 2). The determination of G6PD in suspensions of the immunoprecipitates showed that G6PD activity was measurable after PEG precipitation of enzyme–antibody complexes. In contrast, measurement of PGD in the supernatant showed that PGD was present in equal amounts in the supernatants of both assay and control samples.

*Fig. 1. Immunoprecipitation of G6PD by monoclonal antibody and IgG fractions of rabbit anti-mouse IgG as a second antibody. Supernatant (100 µl) from the homogenate of livers from starved and then sucrose-refed rats was incubated with 50 µl of a 50% ammonium sulfate fraction of clone GDR1 culture supernatant (preparation GDR1S2) and IgG fractions of rabbit antiserum to IgG (100 µl and 120 µl). Control sample incubated with P/NaCl and IgG fractions of rabbit antiserum to mouse IgG. ○, assay sample incubated with monoclonal antibody and IgG fractions of rabbit antiserum to mouse IgG. •, assay sample incubated with monoclonal antibody and IgG fractions of rabbit antiserum to mouse IgG. G6PD was assayed in the supernatant after centrifugation. The amount of the second antibody was critical for precipitation of the enzyme–antibody complex, which was otherwise soluble.*

**DISCUSSION**

Considerable confusion regarding mechanisms underlying modulations in liver G6PD activity by carbohydrates and fats in animal diets has arisen. Conflicting data obtained with hyperimmune sera having different immunospecificities may be responsible for these ambiguities. The hypothesis of G6PD inactivation by direct interaction of G6PD with fat metabolites, as postulated by Kelley et al. (16), has required procurement of a reagent capable of detecting inactive forms of the enzyme.

The present study shows that it is possible to prepare a monoclonal antibody directed against an antigenic determinant not related to the G6PD active site, in that antigen–antibody complexes retain G6PD activity. Additionally, immunoactivity is retained after inactivation of G6PD activity by treatment with palmitoyl CoA or by freezing and thawing. Because the hybridoma technique allows the production of this antibody in large amounts, it is now possible to isolate and characterize the cross-reacting materials detected in the livers of fat-fed (repressed) animals. Such studies should be able to resolve conflicts regarding the mechanisms of regulation of rat liver G6PD activity.

The present data, based on such a monoclonal antibody, indicate that the dietary regulatory step for G6PD is post-translational. More specifically, G6PD activity may be modulated downward by interaction of fat metabolites (rather than upward by carbohydrate metabolites) with the enzyme protein and not by repression of synthesis of the G6PD protein.

Besides reaction with inactive liver G6PD, the monoclonal antibody described here detects G6PD in extracts of human placenta and cultured human fibroblasts. The antibody does not differentiate between the A and B forms of human G6PD (unpublished data).

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