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# The Isolation, Detection, and Quantification of Glucose Transporters

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A Thesis Prospectus

Presented to

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of Pembroke State University

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by

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Advisor's Approval _____

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## Abstract

The purpose of this research was to select a method of glucose transporter study, which is used to isolate, detect, and quantify glut-4, the isoform of glucose transporters found in muscle. Our long term goal is to find the vital link between glucose transporters and diabetes. To do this, an antibody against glut-4 was supplied to us by East Carolina School of Medicine. Glut-4 was then isolated from homogenized rat skeletal muscle. Samples of brain, kidney, liver (none of which contain glut-4 glucose transporters) were taken as negative controls, while samples of adipose and cardiac muscle (which contain glut-4 glucose transporters) were taken as positive controls. The proteins of the each tissue-type were immobilized on nylon paper via dot blotting, which showed non-specific binding. Since the dot blot was over reactive, we employed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which would give better separation of the proteins. The proteins were then transferred from the gel to a nitrocellulose membrane. The primary glut-4 antibody, from East Carolina, was added to the membrane, which contained the immobilized proteins. The secondary glut-4 antibody, which was conjugated to alkaline phosphatase enzyme, was added to the protein-primary glut-4 antibody complex for binding. When the substrate for the alkaline phosphatase enzyme was added, a color change indicated where the glut-4 glucose transporter was present.

## Purpose

The objective of this experiment was to develop a procedure to isolate, detect, and quantify glucose transporters in an effort to find a link between glucose transporters and diabetes. Although most of the procedures used in studying glucose transporters are universal, many steps can be altered (according to the researcher's needs). For example, when our dot blot failed, we altered our experimental protocol by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, to give us improved results. Our main objective here was to see which of the many methods might we consider, if any, in our future study of glucose transporters.

This research was the first step in the eventual expansion, hopefully by a Chancellor's Scholars Program student, of this research to eventually take a more indepth look at the things listed above, as they pertain to diabetes.

## Introduction

### Characteristics of the Five Known Isoforms of Glucose Transporters

Glucose is the number one source of energy for animal cells; thus the cells of most animals use glucose transporter proteins which facilitate the diffusion of glucose (Bell, 18). There are five known types, or isoforms, of glucose transporters (all of which were discovered in the early 1980's). Each glucose transporter is tissue-specific and numbered from one to five, according their order of discovery. Besides being tissue-specific, each of the isoforms of glucose transporters has its own affinity for glucose. Each isoform is also made up of a characteristic number of amino acids and has a particular molecular weight (See table below) (Kasanicki and Pilch, 221).

Characteristics of facilitative glucose-transport proteins

| Isoform | Tissue | Size (# amino acids) | Affinity for Glucose Km (mM)* |
|---|---|---|---|
| Glut-1 | Ubiquitous, fetal placenta | 492 | 2-20 |
| Glut-2 | Liver, kidney, intestine | 524 | 20-40 |
| Glut-3 | Ubiquitous, brain | 496 | ? |
| Glut-4 | Cardiac/Skeletal muscle, fat | 509 | 2-10 |
| Glut-5 | Small intestine | 501 | ? |

* The lower the Km, the more attracted the glucose transporter and the glucose are for each other.

Glut-4 is the isoform we are interested in because it appears to be a factor in insulin resistance (Glucose, 59). Insulin resistance is when the body lacks the ability to respond normally to insulin; this condition is present in non-insulin dependent diabetes mellitus (diabetes type II). In diabetes type II, the patient is insulin resistant, although insulin levels are at or above its normal level (Glucose, 58). Insulin-stimulated glucose transport in muscle and adipose tissues is known to involve the translocation of [glut-4] glucose transporters from an extracellular site to the plasma membrane. In the resting cell, glut-4 resides primarily in intracellular vesicles. In response to insulin, these intracellular vesicles move to the surface and fuse with the plasma membrane, becoming functional. The mechanism by which insulin induces this recruitment of [glut-4] glucose transporters to the plasma membrane is not clearly known (Del Vecchio, 13278).

Studies have been done with two forms of low insulin. One form was a group of fasted rats and one form was a group with induced diabetes (via streptozotocin injection, which kills the beta-cells of the pancreas that produce insulin) propose that reduced levels of glut-4 is caused by the lack of efficiency of insulin in persons with diabetes type II. The study of glut-4 to find a treatment for diabetes type II seems to be the most promising means of prevention and treatment of this devastating disease which affects 10 million people in the United States alone (Glucose, 58). Diabetes type II can lead to blindness,

kidney failure, and cardiovascular disease (Rawn, 398).

Diabetes is associated with a decrease in glucose uptake in muscle; it has been hypothesized that this decreased uptake of glucose is due to one of the following three things:

1) the decreased number of glucose transporters in the muscle cells,
2) the glucose transporters are in the muscle cells, but do not come to the cells' surface to takeup glucose,
3) the glucose transporters are present and go to the surface to takeup glucose, but otherwise do not function properly.

## Steps in the Procedure for the Isolation and Detection of Glucose Transporters

The steps that we used for the study of glucose transporters included:

1) isolation of membrane proteins,
2) isolation of glucose transporters from other, unwanted membrane proteins,
3) the fixture of these transporters on a suitable surface so they can be detected,
4) detection of glucose transporters,
5) and the quantitation of these glucose transporters.

## Isolation of Membrane Proteins

As mentioned earlier, glut-4 is a membrane protein that can be found in muscle and fat. Since it is located in the membranes of muscle and fat cells, a sample of these tissues were taken as a positive control. Samples of brain, kidney, liver (none of which contain glut-4 glucose transporters) were taken as negative controls, while

samples of adipose and cardiac muscle (which contain glut-4 glucose transporters) were taken as positive controls. These tissues were then each freeze-fractured followed by homogenization. The resultant tissue homogenate was then stored in ice cold Pilch buffer prior to being homogenized. Upon centrifugation in an ultracentrifuge, we netted supernatant (liquid) waste and a pellet containing membranes, which contained their respective proteins. After the addition of 10% triton and another centrifugation, a supernatant that contains a mixture of the membrane proteins results. Since glut-4 transporters are membrane proteins, they too were present in the supernatant that was stored at -70 degrees Centigrade until needed (Maxwell).

Separation of Glucose Transporters from Other Membrane Proteins the Immobilization of Isolated Glucose Transporters

Following homogenation and centrifugation, the simplest method of detection without further separation of the proteins was a dot blot with horse radish peroxidase. The dot blot did not isolate the glucose transporters from the other membrane proteins to the degree we had hoped; therefore, we were forced to go to a more sophisticated technique. We employed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which is a technique in which molecules (such as proteins) are placed on a gel material. The gel is then exposed to an electrical field that exerts a force on the molecules.

This force causes the molecule to migrate in the gel. The speed and direction of this movement of the molecules depends on the strength of the electrical field and the charge of the molecules. Each molecule travels through the gel at a rate which depends on its molecular weight or size. In other words, smaller and/or lighter molecules travel farther through the gel, thus separating themselves from larger/heavier molecules which tend to lag behind.

After the proteins are subjected to SDS-PAGE, they are then transferred from the supporting gel to a nitrocellulose membrane (via Western Blotting) so that the glut-4 antibody can be used to identify the glut-4 glucose transporter.

Detection of Glucose Transporters

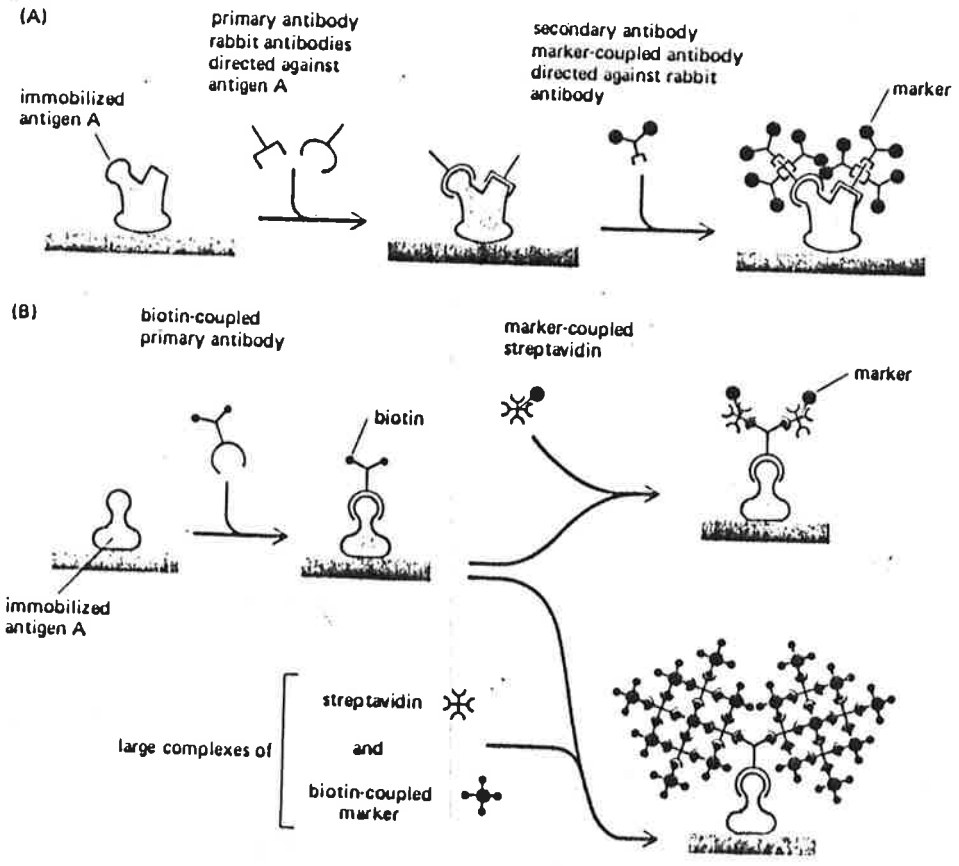
Upon the immobilization of the glucose transporters on the nitrocellulose membrane, the next step was to detect the proteins (which where not visible). To detect the glut-4 glucose transporter, we used a primary antibody that was made against the glut-4 glucose transporter and a secondary antibody that was made against the primary antibody. Antibodies are proteins that are produced by the immune system in response to the presence of a foreign antigen. There are two different types of antibodies: particulates (egs. red blood cells and viruses) and soluable antigenes (egs. proteins not normally in the body). Antibodies bind specifically to the antigen against

which they are created (Cooper, 13).

The primary antibody was anti-rat glut-4, which was made by injecting a rat with a 12 amino acid peptide specific for the caroxyl terminus of the insulin regulatable transporter. Serum is then removed from the rat, purified, and injected into a rabbit; serum is removed from the rabbit and the antibody is purified from this serum through the use of a Protein A column. This column contains Protein A, which binds the protein as the serum is pored through the column. The primary glut-4 antibody, from East Carolina, was added to the nitrocellulose membrane, which contained the immobilized proteins; this primary glut-4 antibody should bind to the glut-4 proteins. After an incubation with the primary antibody, the membrane was washed and the secondary glut-4 antibody, which was a goat anti-rabbit antibody, was then added. This secondary antibody was conjugated to alkaline phophatase (see figure on next page) When the color developer (the substrate for the alkaline phophatase) was added, a color change indicated where the glut-4 glucose transporter was present.

Quantification

Though (by this point) the glucose transporters are isolated, fixed on the membrane, and visible, it still is not clear just how much transporter is present; we could get a rough idea of how much glucose transporter is present via a visual inspection of the membranes: the darker the stain, the more transporter present. To better determine the amount (concentration) of glucose transporters, a densitometer can be used. This apparatus exposes light to the areas of the membrane where the transporters are; as the light passes through these areas, the densitometer measures how much light passes through: the more light that passes through the membrane, the lower the concentration of glucose transporter.



# PROCEDURE

## Steps in the Procedure for the Isolation and Detection of Glucose Transporters

The steps that we used for the study of glucose transporters included:

1) isolation of membrane proteins,
2) Isolation of glucose transporters from other, unwanted membrane proteins,
3) the fixture of these transporters onto some material suitable for detection,
3) detection of glucose transporters,
4) and the quantitation of these glucose transporters.

## Preparation of Tissues

Since our antibodies had been previously purified, we did not have to go through the purification process out-lined earlier. We were ready to make our Pilch's Homogenization Buffer (which was required for the homogenization of our tissues). This buffer was made as out-lined below:

1) To make 250 mL
   a) 1.49 g Hepes
   b) 0.37 g EDTA
   c) 0.98 g Benzamidine
   d) 10 uL leupeptin
   e) 17 uL pepstatin
   f) 25 uL aprotinin
   g) 0.087 g PMSF (Phenyl methyl-sufonyl fluoride) dissolved in 0.5 mL DMSO (dimethyl sulfoxide
2) Add th PMSF last.
3) Set pH to 7.4 and set on ice.

As mentioned earlier, glut-4 is a membrane protein that can be found in muscle and fat. Since it is located in the cellular membrane of muscle and fat tissues, a sample of these tissues were taken as a positive control. The skeletal muscle tissue was taken from the gastrocnemius and rectus femoris, while the cardiac muscle tissue was from the heart. Samples of brain, kidney, and liver (which contain glut-4 glucose transporters, and should not produce a color change on the nitrocellulose membrane) were taken as negative controls, while samples of adipose and cardiac muscle (which do not contain glut-4 glucose transporters) were taken as positive controls. Each of the samples taken from the rats were labeled by number and weighed as outlined below:

| Skeletal Muscle (mg) | Cardiac Muscle (mg) | Adipose (mg) | Brain (mg) | Liver (mg) | Kidney (mg) |
|---|---|---|---|---|---|
| 389 | 411 | 409 | 425 | 400 | 408 |
| 416 | 393 | 431 | 406 | 403 | 404 |
| 424 | 417 | 388 | 406 | 397 | 418 |
| 411 | 398 | 422 | 265 | 422 | 400 |

Separation of Proteins

Each tissue was then frozen in liquid nitrogen followed by freeze-fracture. The resultant powdered tissues were then each stored in 2 mL of ice cold Pilch buffer prior, during, and after being homogenized with the Polytron at speed 7, for 15 seconds. The homogenate was then centrifuged in the ultracentrifuge at 100,000g (40,000RPM) for 1 hour at 4 degrees Centigrade. This

centrifugation netted supernatant (liquid) and a pellet of particulate materials and packed cell membranes containing their respective proteins. Since we were interested in the glut-4 proteins, which were included in the membrane proteins, these pellets were saved and the supernatant was discarded. 720 uL of Pilch's buffer was then added to each centrifuge tube and the pellets were loosened from the sides of their respective tubes. Each tissue was once again homogenized with the Polytron at speed 6, for 10 seconds. 80 uL of 10% Triton (1mL 100% Triton and 10 mL of water) was then added; these contents were mixed with a vortex machine and allowed to set on ice for 1.5 hours, prior to another centrifugation at 100,000 g (40,000 RPM) for 1 hour at 4 degrees Centigrade. This centrifugation separated solid materials into a pellet and soluble proteins were suspended in the supernatant. The pellets were discarded. Since glut-4 transporters are membrane proteins, they too were present in the supernatant. The supernatant was placed in labeled microcentrifuge tubes, and stored at -70 degrees Centigrade until needed.

Now that we had our membrane proteins suspended in the supernatant, the next step was to do the dot blot assay using horse radish peroxidase. But first, solutions for this had to be made. Tris buffered saline (TBS) was made by dissolving 4.84g tris and 58.48g NaCl in 1.5L of water; the pH was then raised to 7.5 and the volume was raised to 2L by adding water. The next solution to be made was Tween tris buffered saline (TBS); it was made by adding

0.5mL Tween 20 detergent to 1L of the TBS. An antibody solution was needed; it consisted of 2g Bovine Serum Albumin (BSA) to 200mL TTBS. In addition, a blocking solution was needed; it was made by adding 1g BSA to 100mL TBS. But we still faced another major obstacle; we didn't know how much (what concentration) of the protein to use (as we had a crude sample of proteins of unknown concentration). Therefore, we did the procedure with several concentrations to determine which would give use the best results. This experiment was only conducted on skeletal muscle, brain, liver, and kidney tissues. The proteins were diluted as followed:

| Sample | Initial Conc. (ug/mL) | Amt. applied per well (ug/100uL) | uL of sample required | Amt. of TBS for final volume |
|---|---|---|---|---|
| Muscle1 | 1900 | 72/100 | 379 | 621 |
| Muscle2 | 1900 | 48/100 | 253 | 734.4 |
| Brain1 | 5000 | 72/100 | 144 | 832.5 |
| Brain2 | 5000 | 48/100 | 96 | 875.7 |
| Liver1 | 10000 | 72/100 | 72 | 897.3 |
| Liver2 | 10000 | 48/100 | 48 | 918.9 |
| Kidney1 | 8000 | 72/100 | 90 | 881.1 |
| Kidney2 | 8000 | 48/100 | 60 | 908.1 |

Each of the samples were then vortexed.

Isolation/Immobilization and Identification of Glut-4 Proteins

The samples were then loaded into the wells of the dot blot assay apparatus. The apparatus was made up of two trays, which had a membrane inserted between them. The wells were filtered by gravity initially, but as the concentration of the proteins increased, a vacuum system had to be used to pull the fluid through. Upon completion

of the filtration, the nylon membrane was soaked in the blocking solution, allowed to stay for 1 hour on a shaker, and then washed twice with TTBS. The proteins were then incubated overnight in blotto (a solution of 5% nonfat dry milk and water). The nylon paper was then washed with TBS followed by TTBS.

Now we were ready to bind the primary antibody. But we still faced yet another obstacle, I didn't know how much (what concentration) of the antibody to use (as we, again, had a crude sample of antibody with an unknown concentration). Since we did the experiment above three different times, we could try three different antibody concentrations. The antibody was exposed to the glut-4 proteins by adding 1mL, 2mL, and 3mL of the antibody to 10mL 1% blotto to the trays which contained the nylon membranes, where they remained for two hours. After this, the nylon membrane was wash twice with TTBS (as earlier). Now we were ready to bind the secondary antibody, goat anti-rabbit antibody (GAR), to the primary antibody. To do this, 32 uL of the GAR (only 32uL to minimize background) and 100uL 1% blotto was added to trays that contained the membranes. While they were incubating for an hour, the color developer was made by adding 900mL of water to the horse radish peroxidase (HRP); this was then added to 600uL of color reagent B (which was supplied in the kit from Bio Rad). Just before being needed, 20mL color reagent A was added.

After their 1 hour incubation, the membranes were

washed again and the color developer was added. We had color change, but it was over reactive. All samples tested positive for glut-4 transporters. Therefore, we needed to employ a more specific method of analysis to determine what was testing positive in all samples. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was selected as a more analytical test.

In order to do the SDS-PAGE procedure, we first had to make the stock solutions listed below:

1.) MONOMER SOLUTION
Acrylamide - 29.2g (note: acrylamide is neurotoxic and should be handled with gloves)
Bis - 0.8g
Water - to 100mL

2.) 4X RUNNING GEL BUFFER
Tris - 36.3g
Water - to 200mL
(adjust pH to 8.8 with HCl)

3.) 4X STACKING GEL BUFFER
Tris - 3.0g
Water - to 50mL
(adjust pH to 6.8 with HCl)

4.) 10% SDS
SDS - 0.10g
Water - 10mL

5.) INITIATOR
Ammonium persulfate - 0.5g
Water - to 5.0mL

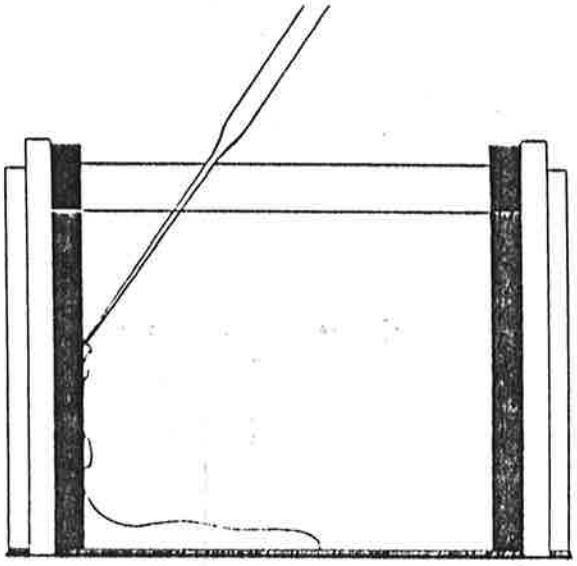
6.) 2X TREATMENT BUFFER
Solution (3) - 2.5mL
Solution (4) - 4.0mL
Glycerol - 2.0mL
2-mercaptoethanol - 1.0mL
Water - to 10mL

7.) TANK BUFFER
Tris - 12g
Glycine - 57.6g
Solution (4) - 40mL
Water - to 4.0L

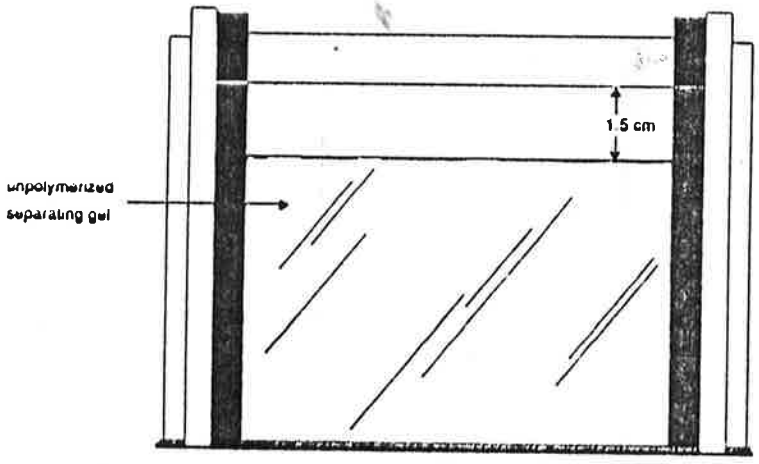
After our working solutions were ready, we set forth to assemble our gel, using the 1.5mm spacers. Then we were ready to make our separating gel and stacking gel, as shown on the next page.

| | SEPARATING GEL | STACKING GEL |
|---|---|---|
| Solution (1) | 20mL | 2.66mL |
| Solution (2) | 15mL | -- |
| Solution (3) | -- | 5.0mL |
| Solution (4) | 0.6mL | 0.2mL |
| Water | 24.1mL | 12.2mL |
| Solution (5) | 300uL | 100uL |
| Tetrametylene-ethylenediamine (TEMED) | 20uL | 10uL |

In a 125mL side arm vacuum flask with a magnetic stir bar, we mixed 60mL of separating gel solution, leaving out solution (5) and the TEMED. The flask was stopped and vacuumed for several minutes (as the gel will not polymerize if it is oxygenated too much). TEMED and Solution (5) were then added to make the solution begin to polymerize. Then I quickly pipetted the solution into the sandwich to a level a few centimeters from the top, as shown in the diagrams on the next page. I then added a thin layer of water to the top of the separating gel via a syringe fitted with a needle. When the gel polymerized, a very sharp water-gel interface became visible.



Introducing the separating gel solution into the gel sandwich.




Separating gel prior to polymerization.

After polymerization, we made the stacking gel in the same manner as the separating solution was made (in a vacuum adding the TEMED and solution (5) last). The water on the top of the polymerized separating gel was poured off and the sandwich was filled the rest of the way with stacking gel. The combs were then inserted into each sandwich and allowed to polymerize (see below).

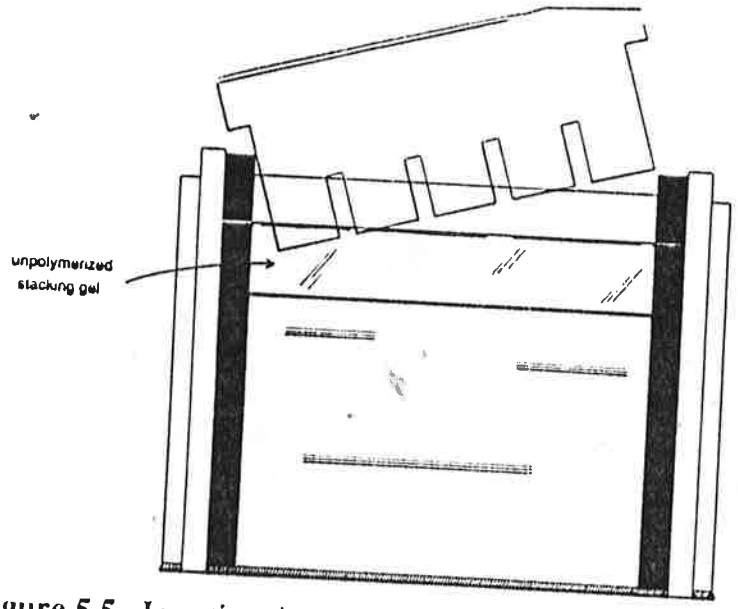



**Figure 5.5.** Inserting the sample-well comb into the stacking gel.

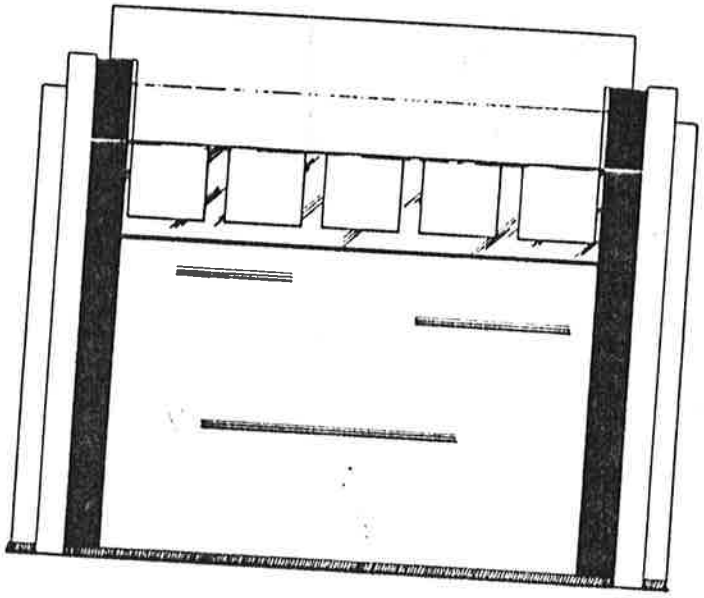
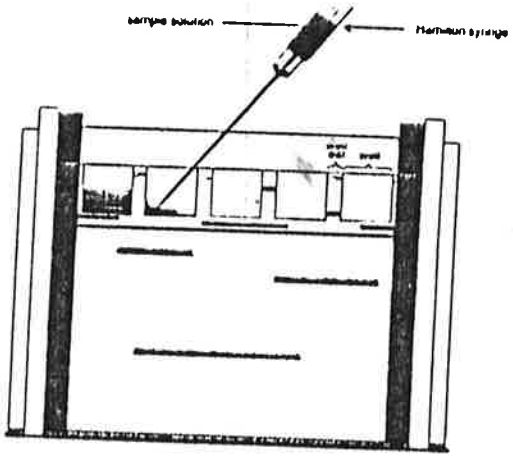


**Figure 5.6.** Stacking gel prior to polymerization.

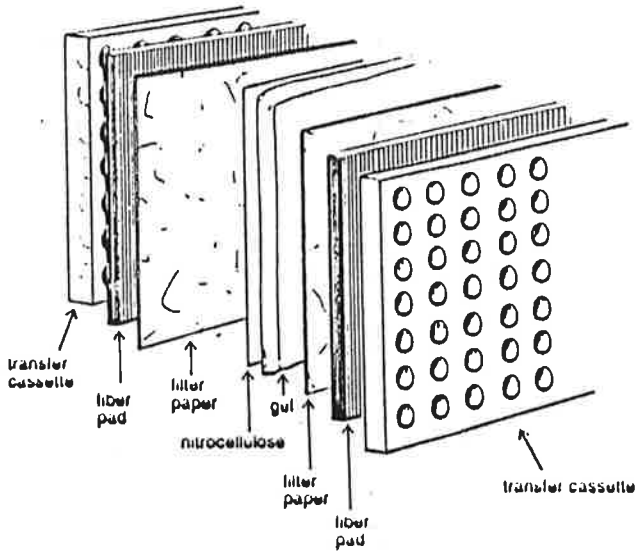
Our protein samples were treated for use in electrophoresis by mixing them with equal parts of 2X treatment buffer, put in a boiling bath for 90 seconds, and stored in the freezer until needed. When we were ready to load our protein samples, I removed the combs from the gel, placed a magnetic stirrer in the tank to maintain a uniform temperature, and filled the tanks and wells with tank buffer [solution (7)]. Then, using a micro pipetter, I loaded our samples into the wells (as shown below).



Introducing protein solution into sample well.

The lid was put on the unit and connections were made to its power supply, with the cathode connected to the upper buffer chamber. The power supply was then turned to a constant current and the electrophoretic run went to completion.

When the electrophoresis was finished, it was time to begin the test for the protein (glut-4). This test is known as a Western Blot. For this, we needed to make various reagents, transfer the protein from the gel to the nitrocellulose membrane, and then test for the proteins. We soaked the nitrocellulose sheet in some transfer buffer for 10-15 minutes. While this was soaking, I prepared the buffer chamber by filling it about half full of transfer buffer and added a magnetic stirrer. I then arranged the polyacrylamide gel-membrane sandwich as shown below. The cassette was then closed and slid into the transfer tank. The electrodes were connected to the power supply, and a constant current was applied to transfer the proteins from the gel to a membrane. The proteins, not yet visible, were immobilized on the nitrocellulose membrane and were ready for detection.

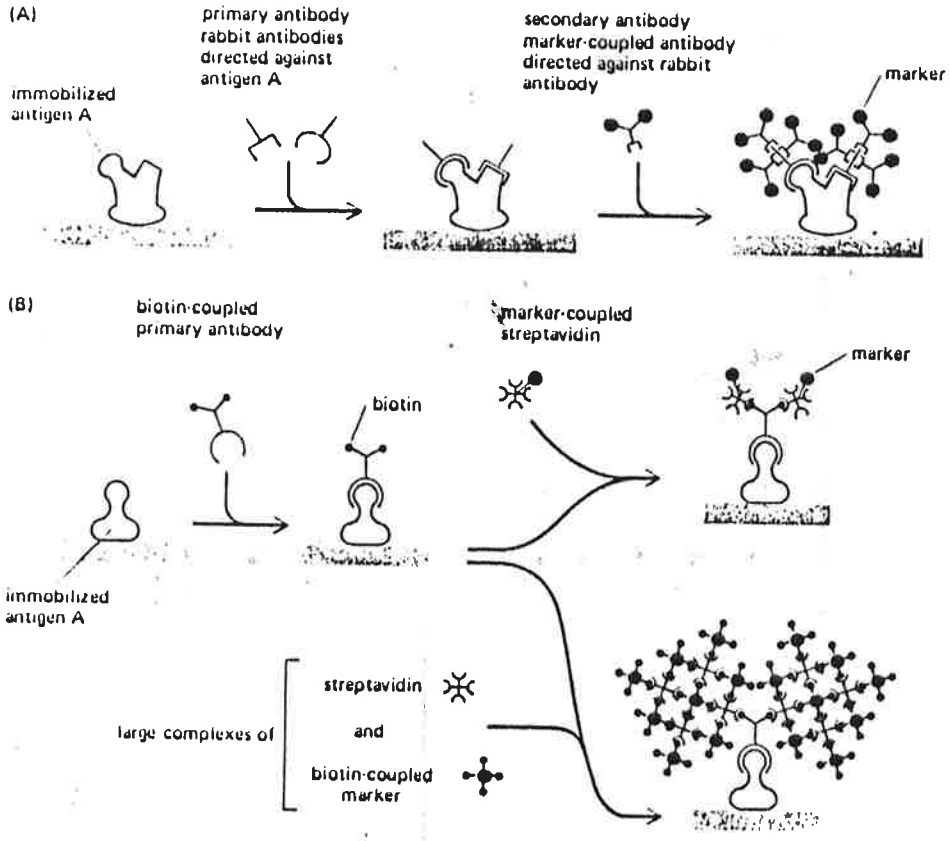



Sandwich involving polyacrylamide gel and nitrocellulose membrane for electroblotting.

The next step was to detect the proteins. To detect the glut-4 glucose transporter, we employed an immuno-assay. The immuno-assay used a primary antibody that was made against the glut-4 glucose transporter and a secondary antibody that is made against the primary antibody. Antibodies bind specifically to the antigen against which they are created (Cooper, 13). In this case, glut-4 is our antigen.

The primary antibody was anti-rat glut-4, which was made by injecting the rat glut-4 into a rabbit; serum is removed from the rabbit and the antibody is purified from this serum through the use of a Protein A column. This column contains Protein A, which binds the protein as the serum is pored through the column. We used primary glut-4 antibody, from East Carolina, which was added to the membrane that contained the immobilized proteins; as another control (in case the glut-4 antibodies from East Carolina were not purified well enough or were not specific enough), we ordered some purified glut-4 antibody from a biological control company. These primary glut-4 antibodies bound to the glut-4 proteins of the nitrocellulose membranes. After an incubation, the membranes were washed two times in TTBS for five minutes. The secondary glut-4 antibody, which was a goat anti-rabbit antibody, was then added (see figure on next page). Now we were almost ready for the color developer which was made by adding 1mL alkalyne phosphatase (AP) color reagent A to 1mL

of AP color reagent B and bringing this volume up to 100 mL by adding AP color development buffer (all supplied in the kit from Bio-rad). When the membranes were washed in the color developer (the substrate for the alkaline phophatase), a color change indicated where the glut-4 glucose transporters were present on the membrane.



# RESULTS

As stated earlier, when the color developer was added to the membrane after the dot blot assay, a definite color change occurred. Therefore, glut-4 was present; but the color change was too dark (due to non-specific binding of the antibodies to the glut-4 proteins on the membrane). As a result, we employed SDS-PAGE.

The SDS-PAGE, along with the Western Blot worked much better due to its ability to better isolate the glut-4 transporters from other proteins of similar size and/or structure. We thought this would remedy our problem of non-specific binding. SDS-PAGE did limit the amount of non-specific binding to some degree, but we still got a lot of non-specific binding after exposure to the antibodies. At first, we thought this non-specific binding was due to the concentration of the antibodies we used; but this proved not to be the case when we used various concentrations of antibodies (and, in one case, we even skipped the incubation with antibodies altogether), but still got the same results (non-specific binding). By this point, we had taken this work a step further than it had ever been taken in our laboratories. Since we did not know what our next step should be and the end of the semester was upon us, we concluded our experiment at this point.

## CONCLUSION

Since the antibodies, substrates, and enzymes all seemed to be working as they should, the only other things that could have caused the non-specific binding was our inability to properly isolate the glut-4 transporter or the antibodies' inability to distinguish between glut-4 and other similar proteins.

All photographs in this paper came from Alberts, Bruce et. al.

BIBLIOGRAPHY


Alberts, Bruce et. al. Molecular Biology of the Cell. New York: Garland Publishing, Inc., 1989

Bell, Graeme I. and Gould, Gwyn W. "Facilitative Glucose Transporters: An Expanding Family" Trends in Biochemical Sciences. Vol. 15, No. 1. January 1990.

Bollag, Daniel M. and Stuart J. Edelstein. Protein Methods. New York: Wiley-Liss, 1991

Cooper, Edwin L. General Immunology. Elmsford, New York: Pergamon Press, Incoporated, 1982.

Del Vecchio, Robert L. and Pilch, Paul F. "Phosphatidylinositol 4-Kinase is a Component of Glucose Transporter (GLUT 4)-containing Vesicles" The Journal of Biological Chemistry. Vol. 266, No. 20, Issue of July 15, 1991. 13278.

Kasanicki, Mary A. and Pilch, Paul F. "Regulation of Glucose Function" Diabetes Care. Vol. 13, No. 3, March 1990. 219-221.

Klip, Amira and Michel R. Paquet, "Glucose Transport and Glucose Transporters in Muscle and Their Metabolic Regulation," Diabetes Care, Vol. 13, No. 3, March 1990. 228-242.

Marieb, Elaine N., Human Anatomy and Physiology, New York: Benjamin/Cummings Publishing Company, 1989. 59.

Maxwell, Harold D. "Detection and Quantitation of Glucose Transporters." Pembroke State University, 1990.

Ramlal, Toolsie, et. al., "Decrease in Glucose Transporter Number in Skeletal Muscle of Mildly Diabetic (Streotozocin-Treated) Rats," Endocrinology, 1989, Vol. 125, No. 3. 890-897.

Rawn, J. David, Biochemistry. Burlington, NC: Neil Patterson Publishers. 1989. 1044.

Redhead, Stephen, "Glucose Transporter Defects Underlie Insulin Resistance," The Journal of NIH Research, Vol. 2, November 1990. 58-62.