A Protocol for the Harvesting of Monoclonal Antibodies

A Thesis

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by

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PURPOSES OF PROJECT

The purposes of this Chancellor's Scholars Project are: 1) to develop a protocol for the production of monoclonal antibodies using hollow fiber cells; 2) to give the reader a working knowledge of the antibody, and more specifically, the monoclonal antibody; 3) to examine the monoclonal antibody, and the different techniques employed for their production; 4) to highlight the purification and identification processes (chromatography and gel electrophoresis); and 5) to highlight their importance in biotechnological and chemical research and in clinical work.

THE IMMUNE RESPONSE

The ability of the human body to defend itself against foreign substances is referred to as immunity. These foreign substances are known as antigens, and the body has two immune responses to fight against them. They are the cell-mediated (cellular) immune (CMI) response and the antibody- mediated (humoral) immune (AMI) response. The CMI response involves the direct attack of antigens by "killer" T-cells, and is "directed against intracellular pathogens, such as viruses, some cancer cells, and tissue transplants (Tortora 687f), whereas in the AMI response, B Lymphocytes produce plasma cells, which in turn produce antibodies that are "directed against extracellular pathogens, such as bacteria" (Tortora 687f). Antibodies inactivate the antigen after binding to it at the antigenic determinant, or epitope-- "the specific portion of antigen molecules [that] trigger immune responses" (Tortora 688).

The body contains "millions of different B cells, each capable of responding to a specific

antigen" (Tortora 694). An antigen will bind itself to the B-cell--it will then be activated. After going through several processes, the activated B-cells "divide and differentiate into a clone of plasma cells" (Tortora 695). The plasma cells then secrete antibodies specific against the antigen bound to the B-cell.

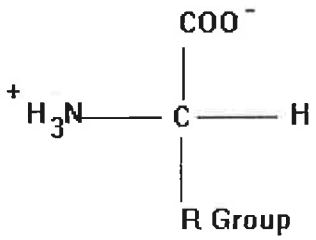
As will be covered in the following sections, there are five classes of antibodies, which are also referred to as immunoglobulins. Even though they are different, they all attack antigens in similar ways. These include: neutralizing antigens, the immobilization of bacteria, the agglutination and precipitation of antigens, the activation of complement, the enhancing of phagocytosis, and by providing fetal and newborn immunity.

BACKGROUND OF PROTEINS

Antibodies, also known as immunoglobulins, are proteins. Proteins are macromolecules that are composed of the 20 different kinds of amino acids. Proteins are extremely important in the operations of organisms because they "catalyze an extraordinary range of chemical reactions [enzymes], provide structural rigidity to the cell, control flow of material through membranes, regulate the concentrations of metabolites, act as sensors and switches, cause motion, and control gene function" (Lodish 52).

As stated before, proteins are composed of amino acids. There are only 20 common amino acids, and proteins are constructed from the various combinations of them. All amino acids have the same basic structure. There is an alpha carbon, which is adjacent to the carboxyl group (COOH). Also bonded to the carbon is an amino group (NH_2), a hydrogen (H) atom, and an R

group--which is "a variable group, called a side chain...[which varies] in size, shape, charge, hydrophobicity, and reactivity" (Lodish 52). Because one side of the protein has an amino group, and the other side a carboxyl group, when the amino acids link with each other, one end of the protein, or polypeptide, will also have an amino group at one end, and a carboxyl group at the other. The amino end is refferred to as the N-terminus, and the carboxyl end, the C-terminus. The size of the protein is measured in daltons.

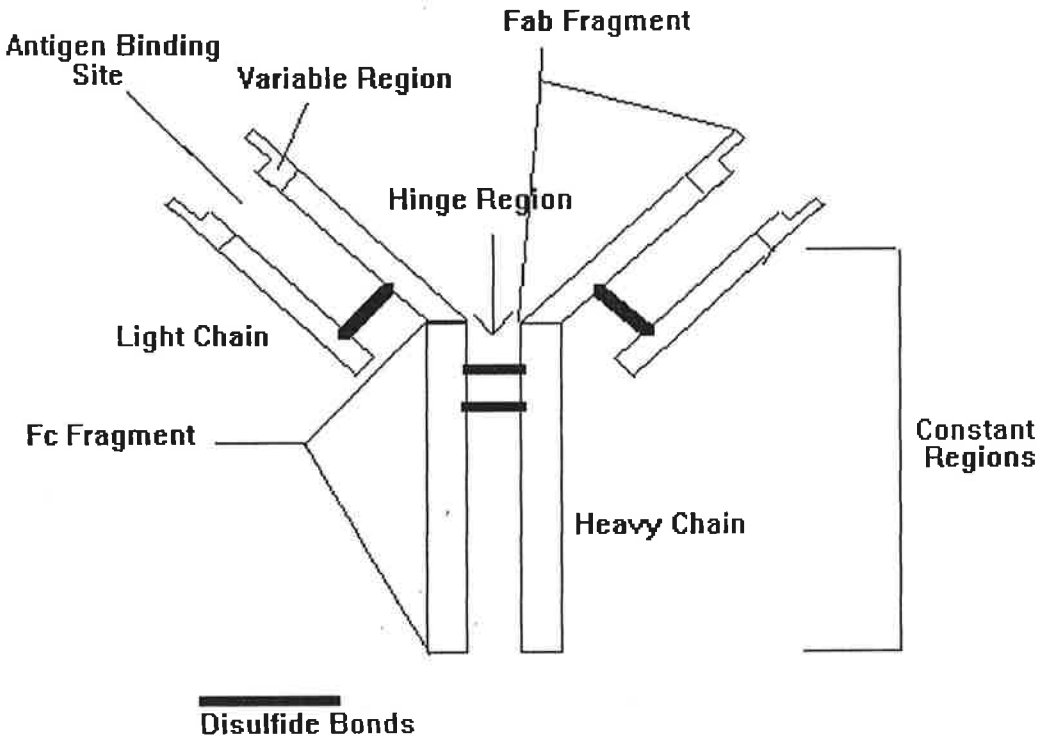


AMINO ACID

ANTIBODIES IN GENERAL

Antibodies are key in the protection of the human body against attack from foreign agents, or antigens. Also known as immunoglobulins, antibodies "are a group of glycoproteins present in the blood serum and tissue fluids of mammals" (Prescott 591). They are divided into five separate classes: IgD, IgA, IgM, IgG, IgE (DAMaGE). They "differ from each other in molecular size, structure, charge, amino acid composition, and carbohydrate content" (Prescott 591). Most antibodies, however, belong to the IgG class.

Each immunoglobulin has about the same structure "composed of four polypeptide chains connected to each other by disulfide bonds" (Prescott 592). Two of the chains are referred to as light chains, which are composed of 22 amino acids with a molecular weight of 25,000 Daltons, and heavy chains, composed of 440 amino acids, with a molcular weight between 50,000 and 77,000 Daltons. Each antibody also contains two constant regions, two variable fragments, two crystallizable fragments, two antigen-binding fragments, two antigen-binding sites, and a hinge region.



Each chain (light and heavy) have two different regions, constant and variable. The two contant regions (C_L and C_H) "have amino acid sequences that do not vary significantly between antibodies of the same subclass" (Prescott 592). Variable regions (V_L and V_H) "from different antibodies do have different sequences" (Prescott 592). The crystallizable fragment (F_C) is the

site where the antibody molecule can bind to the cell, and the antigen-binding fragments (Fab) bind the antibody with "compatible antigenic determinant sties" (Prescott 592). The F_C is only in constant regions, while Fabs are in both constant and variable regions [refer to above diagram].

Disulfide bonds (-S-S-) connect the light chains to the heavy chains, and the two heavy chains together near the hinge region. The bonds that link the chains together are referred to as interchain disulfide bonds, but intrachain disulfide bonds can cause loops comprised of about 25 amino acids in the chains. These areas are known as domains.

IgG: THE MOST ABUNDANT ANTIBODY

IgG, the most common and abundant immunoglobulin, can be further classified into four subclasses: IgG_1, IgG_2, IgG_3, IgG_4. The difference between each groups is a result of "the amino acid composition of the heavy chain" (Prescott 592). Of IgG serum, IgG_1 is the most abundant, composing about 65%, while IgG_2 is about 23%. These variations lead to further diversity of antibodies, because the different subclasses also have different functions. IgG_1 and IgG_3 both have anti-Rh factor antibodies, and "also best bind to monocytes and macrophages and activate component most effectively" (Prescott 594-95). IgG_2 antibodies are used to opsonize, and "develop in response to antitoxins...[and IgG_4] function[s] as skin-sensitizing immunoglobulins" (Prescott 594-95).

In human serum, about 70-75% of all immunoglobulins is IgG. It "acts against bacteria and viruses by opsonizing the invaders and neutralizing toxins" (Prescott 594). (Opsonization is the ability of an antibody to initiate phagocytosis, and neutralization is when "IgG antibodies can

inactivate viruses by blocking their attachment to host cells, and can neutralize bacterial toxins by blocking their active sites" (Tortora & Funke 452). Antibodies, however, do not kill the invaders, but instead, mark them for death. The neutrophils and macrophages are activated and destroy the antigen through phagocytosis--the engulfing and breakdown of a cell. This is an example of a non-specific immune repsonse. One aspect of IgG that makes it even more valuable is that "it is the only immunoglobulin molecule able to cross the placenta and provide naturally acquired passive immunity for the newborn" (Prescott 594).

TRADITIONAL PRODUCTION OF MONOCLONAL ANTIBODIES

Antibodies are synthesized by B-Lymphocytes--special kinds of white blood cells--which in animals, are "capable of producing a single type of antibody directed against a specific determinant, or epitope, on an antigen molecule" (Lodish 201). Each specific B-Lymphocyte forms "a clone of cells in spleen or lymph nodes, with each cell of the clone producing identical antibodies," (Lodish 201) which are referred to as monoclonal antibodies. However, because most antigens have multiple epitopes, many different types of antibodies are synthesized. This mixture of antibodies that reconginze different epitopes on the same antigen are described as polyclonal antibodies. Monoclonal antibodies are preferred to use in studies because of less variance, but it is difficult to separate monoclonal antibodies because of their low concentration in the body. They are also not produced in large quantities because "primary cultures of normal B lymphocytes do not grow indefinitely" (201). To avoid this, normal B lymphocytes, which cannot "survive more than a few hours in vitro" (Wilson 75) are fused with cancerous lymphocytes, or

myeloma cells, which are immortal.

There are two ways in which normal mammalian cells can synthesize DNA: "a de novo pathway in which nucleic acids are built up from purine and pyrimidine bases, deoxyribose and inorganic phosphate; and a 'salvage pathway' that converts similar nucleotides into the correct nucleic acid" (Wilson 75). The de novo process is inhibited by aminopterin, and cells containing HGPRT (hypoxanthine-guanine phophoribosyl transferase--an enzyme that is involved in nucleic acid synthesis) can undergo the "salvage pathway" and "survive in the presence of aminopterin" (Wilson 75). The HAT medium, which is used in the process of raising monoclonal antibodies, and contains hypoxyanthine, aminopterin, and thymidine, needs to have additional hypoxanthine and thymidine added to it, because of the additional demand placed on the "salvage pathway" by the cells with HGPRT. As a result, cells with HGPRT can grow in the HAT medium, while those that are $HGPRT^-$ are not able to. Since the myeloma cells lack HGPRT, they cannot grow in a HAT medium.

In 1975, Georges Kohler and Cesar Miltstein discovered how to fuse a myeloma cell with a noncancerous B-Lymphocyte from a mouse. First, a mouse is injected with a particular antigen, and after the animal has produced a large amount of antibodies, their spleens are removed. The spleen cells (the source of B Lymphocytes) are added with $HGPRT^-$ myeloma cells, along with 30% to 50% (w/v) polyethyleneglycol (PEG), which promotes cell membrane fusion. The fusion in PEG can only be done for a few minutes because "PEG is toxic to cells" (Wilson 75). The combination of the spleen cells and the myeloma cells results in the formation of hybridoma cells. The "fusion mixture is then transferred to a culture medium" (Prescott 602) containing HAT. Aminopterin, a previously mentioned, is "a poison that blocks a specific metabolic pathway in

cells" (Prescott 602). Because HGPRT is required for growth in the aminopterin, those with the enzyme surive, while the cells without it perish. As a result of this process, "lymphocytes and fused lymphocytes soon die, as they cannot be cultured in vitro[,and]...myeloma cells and fused myeloma cells are poinsoned by the aminopterin in the HAT medium, because they lack HGPRT" (Wilson 75). Only hybridomas survive because they are the "fusion products of B lymphocytes and HGPRT$^-$ myeloma cells" (Wilson 75), where the B lymphocytes have HGPRT and the myeloma cells can survive and grow in vitro. After 14 days, only the hybridomas will remain, and over the next 7 to 14 days, it is then necessary to reject and discard the hybridomas that are non-antibody-producing.

As stated before, the initial composition in the HAT medium contains spleen cells, of which the unfused ones die within a couple weeks, myeloma cells, which die because of the aminopterin, and the hybridomas, which produce monoclonal antibodies. This happens because "when a hybridoma is grown in a culture, its genetically identical cells continue to produce the type of antibody characteristic of the ancestral B cell" (Totora & Funke 436). Hybridomas, unlike normal antibody producing lymphocytes, can live indefinitely, and can produce large quantities of monoclonal antibodies.

RAISING AND HARVESTING MONOCLONAL ANTIBODIES

As previously described, the traditional manner in the production of monoclonal antibodies involves a wide variety of techniques and equipment, and even a live donor (e.g. rabbit) to facilitate the raising of the antbodies. For this project, a new way to culture these cells will be

utilized. It involves the use of the Cell-Pharm System 100, developed by Unisyn. The Cell-Pharm System 100, or (CP 100) "is a hollow fiber cell culture system for the production and continuous recovery of mamalian cell products." (Unisyn 2). It is a miniature laboratory with a sole purpose of culturing cells, and has the ability to "control temperature, medium recirculation, and CO_2 and air delivery" (Unisyn 2). The harvesting of monoclonal antibodies typically can be completed in a months time, and is capable of producing 200 mg to 400 mg of cellular product in this time span.

In the course of this project, the procedure follows that explained in the Operator's Manual for the CP 100. The following description is a brief overview of the procedure of the CP 100:

- **Peristaltic Pump**--circulates the medium through the system at a "fixed rate of 108 (50 MHz) or 130 mL/min (60 Hz)" (4). The pump will need to be properly adjusted to ensure proper usage.
- **Medium Formulation and Serum Usage**--syringe needles should be one inch (2.54 cm), but "syringe needles used for inoculation, cell feeding and harvesting should be 22 guage" (5).
- **Bioreactor Circuit Preparation**--the CP 100 should be place into a hood, and proper adjustments and connections should be made (e.g. power cord, Luer connections, tubing).
- **Flushing the Flowpath**--ensures a proper environment (e.g., temperature, circulation) in the system for cell culturing by allowing the system to be observed for problems, and to expell any waste from the system to protect against contamination. Two liters of sterile medium will be used for this technique After the flush is completed, the temperature is set, which is programmed for 37 degrees Celsius by the factory.

- **System Inoculation**--always performed in a hood. Before sample is introduced into system, its pH and initial glucose levels should be checked. To take a sample of the material to determine this, first take an empty 10 mL sterile syringe with a 22 guage needle and "depress the free plunger... [which] will remove any excess air and ensure smooth operation" (20). Then wipe tip of septum with 70% alcohol swab, insert syringe into septum, pull back on plunger, and allow it to fill with medium. Then wipe the septum, again, with the 70% alcohol swab. Then check the sample for pH and glucose levels.
- **Product Harvesting/Cell Feeding Protocol**--this technique will involved two syringes, "one empty syringe, to collect the harvest medium, and the second syringe containing fresh replacement ECS medium" (23). For the first two weeks, 5.0 mL are harvested, and then it will increase to 10 mL. This work should be done in a hood, and is similar to the inoculation procedure.
- **Recirculating Medium Replacement**--To ensure that the cells do not die, the medium, which supplies them with nutrients, will need to be periodically replaced. By monitoring glucose levels, an optimal level in the medium will be learned. Then the medium can be replaced and adjusted. About 500 mL to 2 liters of "circulating medium should be changed one or two times per week" (25). The medium used for this should also be pre-warmed to the system temperature, which is probably set at 37 degrees Celsius.
- **Recirculating Medium Sampling**-- "Samples of the circulating medium may be taken from a septum port attached to the 'Reservoir In' line" (27). These samples can be used to monitor levels of glucose and lactate, as well as pH and molecular components.

There are also several safety warnings to know while operating the CP 100. Precautions

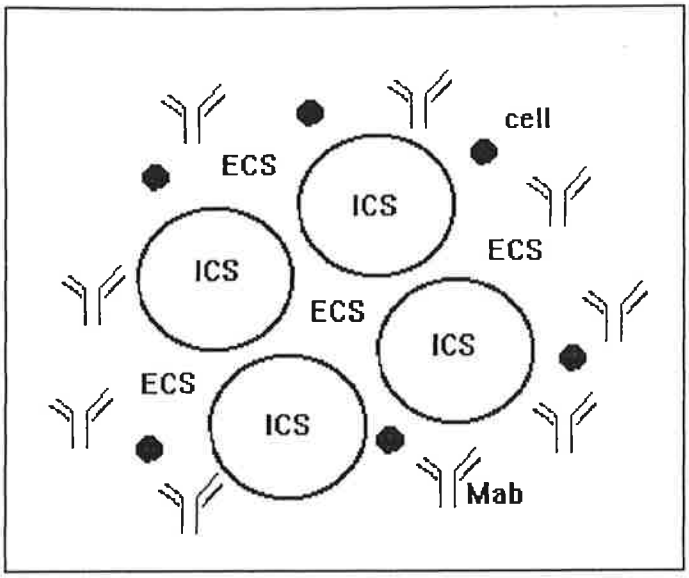
include:

- unplugging system before working on instruments and use correct fuses to prevent electric shock or fire,
- wearing eye protection while using bleach bottle (which aids in sterility), media solutions, and when handling pressured gases (e.g. air, CO_2),
- wearing gloves for contact with potentially hot surfaces,
- keeping body parts, hair, jewelry, and clothing away from the pumps because of use of rotors, and
- always perform lab work in properly ventillated hood.

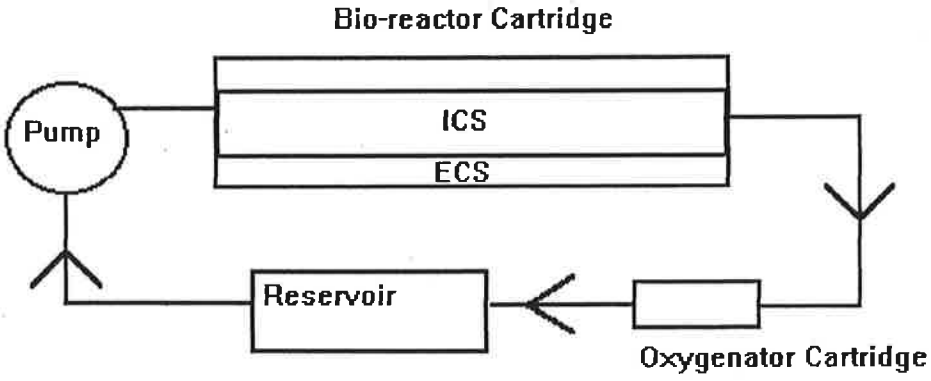
FUNCTIONS OF CELL PHARM 100

The Cell Pharm 100 houses a "hollow fiber" system, which is found in both the oxygenator and bio-reactor cartridges. The bio-reactor cartridge is where the cells are grown and cultured. The hollow fibers can be described as long tube-like structures in which media from the reservior flows through, and where nutrients are able to reach the cells, which are located outside of the hollow fibers. The media and nutrients nourish the cells by crossing the membrane of the hollow fibers into the Extra Cappillary Space, or ECS. The ECS is where the cells grow and produce antibodies. As the process of harvesting continues, the antibody concentration in the ECS increases. The inside of the hollow fiber, or lumen side, is referred to as the Inter Capillary Space, or ICS [see figure below]. There are between 2,000 and 3,000 hollow fibers in the Cell

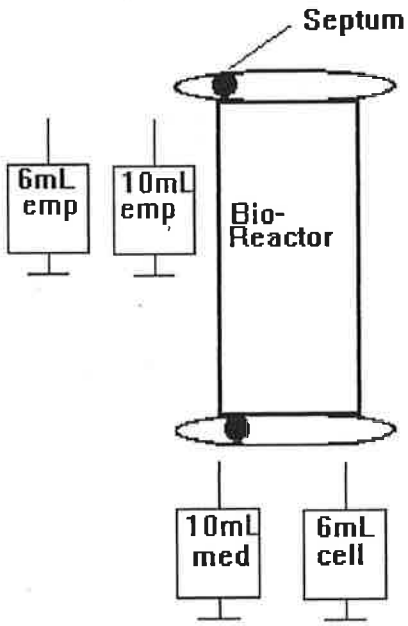
Pharm 100.



The media is circulated throughout the machine beginning in the reservoir, which should contain about 1 Liter of medium. It is then pumped to the rest of the apparatus by the pump, and then it runs through the bio-reactor, and then it goes through the oxygenator, and back to the reservoir. The machine also contains a heating block, to measure and maintain the temperature of the media (at 37°C, although the media should be pre-warmed).



Before the hybridomas, which will be harvested to produce monoclonal antibodies in the ECS, are introduced into the system, the Cell Pharm 100 must be flushed. This means that air, and any other contamenents, will be removed from the fibers. To inoculate, a 22-gauge needle is used, but the septa should only be penetrated by the needle for about three times. As 10 mL of the media is injected into the bio-reactor cartridge at one of the septa, an empty needle is withdrawing the displaced volume from the cartridge. After this is done, another syringe with 6 mL solution, with 3 mL of the conditioned media (which contains the cells being grown--hybridomas), and 3 mL of the normal media, which is what is in the reservoir [see diagram below].



The CO_2 gas is used to keep the pH level of the cell at the biological level of 7.4. The

carbonic acid produced, resulting from CO_2, helps to maintain it, but as the cells in the system begin to multiply and produce lactic acid, the lactic acid alone will maintain the pH, and the CO_2 will no longer be required.

WHY USE CELL-PHARM 100?

There are limitations with producing monoclonal antibodies in the classical manner. On disadvantage is that monoclonal antibodies "rely on the random fusion of a heterogeneous population of B lymphocytes with myeloma cells, resulting in much time being spent in growing and testing hybrids that secrete a vast variety of immunoglobulins" (Wilson 77). It is also very expensive and laborious to produce monoclonal antibodies.

USES OF MONOCLONAL ANTIBODIES

According to Tortora, Funke, and Case, monoclonal antibodies are useful for three reasons: "they are uniform, they are highly specific, and they can be readily produced in large quantities" (436). As a result, they are used as diagnostic and therapeutic tools. Monoclonal antibodies are extremely valuable in research. They can be used to label specific proteins, and then the protein can be isolated by affinity chromatography in which the monoclonal antibody is bound to the column. They can be used to treat diseases caused by pathogens in which they "bind to and inactivate toxic proteins (toxins) secreted by bacterial pathogens" (Lodish 202).

The monoclonal antibodies can also be used in the fight against cancer. Some are

"specific for cell-surface proteins expressed by certain types of tumor cells" (Lodish 202). These antibodies are being used to form complexes with toxic drugs that could be used for chemotherapy, which would destroy the tumor cells. As a result, the toxins would be delivered to an antigen win which the antibody is specific for--a tumor cell. The monoclonal antibody-toxin complex is referred to as an immunotoxin. It can be used to carry toxins "throughout the body by the cardiovascular system to specifically attach and kill cancer cells wherever they might be" (Tortora & Funke 437).

One specific example of using a monoclonal antibody to treat cancer is the B-cell-depleting monoclonal antibody IDEC-C2B8, or Rituximab. Designed by IDEC Pharmaceuticals, IDEC-C2B8 was unanimously recommended for approval "by a US Food and Drug Administration advisory committee for treatment of patients with low-grade or follicular B-cell non-Hodgkin lymphoma (NHL)" (Ault 416).

For diagnostic purposes, monoclonal antibodies are extremely beneficial and useful. They are used in identifying chlamydial and streptococcal bacteria, and are even used to test for pregnancies. The hormone Human chorionic gonadotropin (hCG) is present in the urine of a pregnant woman. The dipstick of a pregnancy test contains two types of monoclonal antibodies, free monoclonal antibodies and capture monoclonal antibodies. The free monoclonal antibodies are "specific for hCG and are color labeled" (Tortora & Funke 437). The capture monoclonal antibodies are specific for the hCG-free antibody complex. If hCG is in the urine, the free antibody will bind to it, and then the capture antibody will bind to them both--thus surrounding the human chorionic gonadotropin. As a result, "the color labeled antibodies then create a visible color change in the test window" meaning hCG is present in the urine and that there is a strong

possibility that pregnancy has occurred.

The reason, however, that more and more therapeutic uses with monoclonal antibodies are not being used is because they are produced by mouse cells, and "the immune systems of some people have reacted against the foreign mouse proteins" (Tortora & Funke 437).

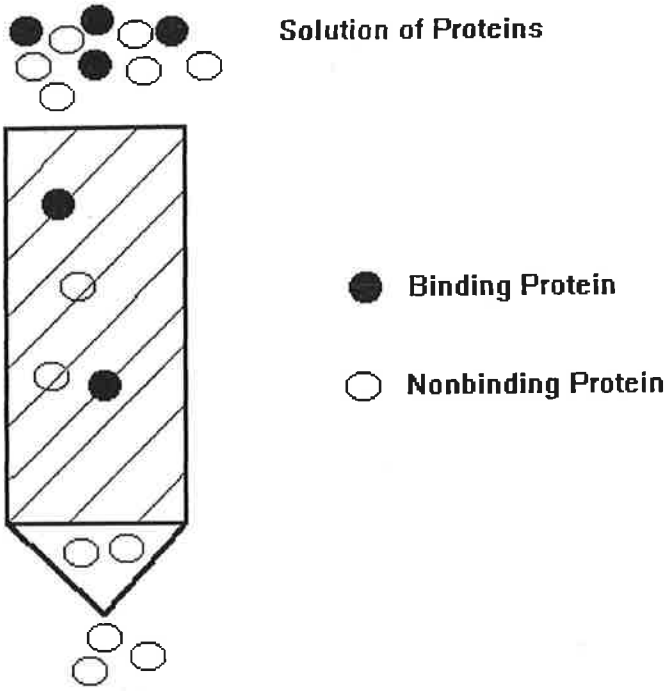
PURIFICATION TECHNIQUES OF MONOCLONAL ANTIBODIES

After the process of raising and culturing the monoclonal antibodies is completed, it is necessary to ensure that only monoclonal antibodies are present in the serum. To do so, it will need to be purified, which will result in a serum composed of only monoclonal antibodies. This can be done through many chromatographic techniques, but hydroxyapatite chromatography will be utilized for this project.

Chromatography is a method used to extract desired solutes from a solution containing different solutes. There are many different components of chromatography. The mobile phase is the "solvent moving through the column" (Harris 627), and the stationary phase "is the one that stays in place inside the column" (Harris 627). The fluid that is entering the column is called the eluent, and the fluid that emerges from the column at the bottom is referred to as eluate.

There are many different types and categories of chromatography, but the basic premise of them all is the same. For example, if a chemist wants to separate Solute A from a solution of both A and B solutes, he would pour the solution into a column with a stationary phase (or column packing) that attracts Solute A. Therefore, Solute A is "stuck" to the column side as Solute B passes straight through the column. To remove Solute A from the column, it is eluted by adding

an excess of the same substance of the column. Solute A would then attach to the new eluant and will flow from the column. The result is a solution of only Solute A.



Chromatography

Hydroxyapatite chromatography was first introduced in 1956, and "offers a unique assemblage of process characteristics that merit serious evaluation" (Gagnon 87). It has a formula of $Ca_{10}(PO_4)_6(OH)_2$, and is "both the ligand and the matrix" (Gagnon 87). The positively charged functional groups are the calcium ions (C-sites), while the negatively charged ones are the phosphate ions (P-sites).

When the antibodies (or proteins) are introduced into the column and matrix, the positive amino groups of the protein are attracted to the P-sites, but are repelled by the C-sites. However,

the carboxyl groups of the protein are attracted to the C-sites, but are repelled by the P-sites. The binding process of carboxyls to C-sites "involves [the] formation of much stronger coordination complexes between C-sites and clusters of protein carboxyls" (Gagnon 88).

GEL ELECTROPHORESIS

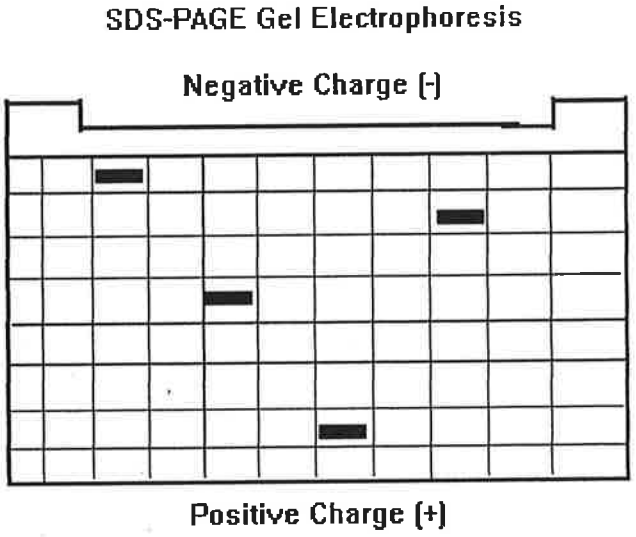
Gel electrophoresis is a technique used "for the separation of biological molecules based on their movement due to the influence of a direct electric current" (Holmes 1). They migrate down the gel at a "speed determined by their charge:mass ratio." (Lodish 92). The lighter molecules migrate further down the gel, while the heavier ones remain at the top. The technique can also be used to test the purity by either comparing the migration pattern of an unknown sample to that of a known, or by observing the number of bands that appear on the gel--if the desired number appear, than it is likely the sample is pure and contains no contaminants.

The gels used for the molecular migration are usually polyacrylamide gels. They "are cast between a pair of glass plates by polymerizing a solution of acrylamide monomers into polyacrylamide chains and simultaneously cross-linking chains into a semisolid matrix" (Lodish 93). The cross-linking of the acrylamide monomers for pores in which the molecules migrate through. These pores are quite small, which is why the smaller proteins migrate faster and further down the gel instead of the heavier molecules, which migrate slowly, and near the top, because the larger proteins cannot make it through the small pores.

For this project, SDS-polyacrylamide gel (PAGE) electrophoresis is used. The monoclonal antibodies cultured in this project will be exposed to SDS (sodium dodecylsulfate).

SDS, with a chemical formula of $CH_3(CH_2)_{11}SO_4^-Na^+$, is a detergent, which places negative charges on the molecules, causing them to repel each other. Thus the proteins will break up into their individual subunits. During their migration, the proteins "separate into bands according to their size...[and] are visualized by staining with a dye" (Lodish 92).

In the case of antibodies, because of SDS, the heavy chains will separate from the light chains--thus appearing as two bands. The heavy chains weigh about 45,000 Daltons, and the band will appear at the top because they are heavier, while the light chains, weighing about 35,000 Daltons, appear further down the gel.



PREPARATION OF SDS-PAGE BUFFER SYSTEM

On September 23, 1997, while taking a Chemistry Research course under the guidance of Dr. Mandjiny and Dr. Holmes, I began work to familiarize myself with gel electrophoresis; which will aid me in conveying a clear message to future students wishing to use SDS-PAGE to determine the purity, and identity, of their monoclonal antibody serum.

The first task in the preparation of this system, is the preparation of stock solutions. Acrylamide/bis is one of these. It was formed by using 87.6 grams of acrylamide, 2.4 grams of N´N´-bis-methylene-acrylamide, and diluting to 300 mL with distilled water. A 1.5 Molar Tris-HCL solution, with pH 8.8, was prepared next, with 27.23 grams of Tris base and about 80 mL of distilled water. The pH is adjusted with HCL. It was then made to 150 mL with distilled water. A 0.5 Molar Tris-HCL solution, this time with an acid pH of 6.8, was initially prepared using 6 grams of Tris base and about 60 mL of distilled water. The pH was then checked to ensure it was at 6.8 by using HCL. Afterwards, the solution was diluted to 100 mL with distilled water.

Instead of preparing our own gels, we used ready-made gels from Bio-Rad. The comb, which leaves indentions--or wells--in the gel, is removed. The gel is then placed into the gel cassettes, which will allow it to be positioned vertically. The protein samples are placed into the wells. In Well 1, 10 uL of Albumin Bovine was loaded with a molecular weight of about 66,000 Daltons. In Well 3, 50 uL of Albumin (Egg) was loaded, and has a molecular weight of about 45,000 Daltons. In Well 5, 25 uL G-3-P Dehydrogenase, and has a molecular weight of about 36,000 Daltons. A 25 uL sample of Trypsin Inhibitor was placed in Well 7, and has a molecular weight of about 20,100 Daltons. The gel cassettes were then submersed in a solution of buffer

solution, which aids in the transfer of the electric current--which is necessary to cause the migration of the proteins through the gel. The potential of the system was at 93 Volts, and the current was 40 milliamperes.

The resulting data of the gel electrophoresis supports the principle behind it. The Trypsin Inhibitor migrated the furthest down the gel, while the Albumin Bovine remained close to the top. In order for the bands of proteins to be visible for observation purposes, staining solutions are used. The most popular stain is Coomassie Blue.

HYDROXYAPATITE CHROMATOGRAPHY RESULTS

Bovine Serum Albumin was used for a test procedure using hydroxyapatite chromatography. It was collected from a blood sample, treated with 0.1M Citrate Buffer (pH 5), and then centrifuged to remove cells from the sample. The supernatant (the clear liquid remaining at the top after the solid material is forced to the bottom after centrifugation) was then filtered, and passed through the hydroxyapatite column. The IgG in the BSA was attracted to the ions of the column. To remove the IgG, a buffer with a higher solute concentration was used to pull the IgG from the column, thus coming out as a mostly pure IgG solution. Electrophoresis was then done on this sample, which showed Albumin at the top of the gel (heavy protein) which was an impurity. The heavy and light chains did appear on the gel--the heavy chain closer to the top.

The graph, "Absorbance vs. Elution Volume," shows the elution of IgG from the BSA. The first peak was non-IgG material, because the IgG was attracted to the column and did not come out with the buffer. The next two peaks show the removal of IgG using a higher solute

concentrated buffer. [See appendix for graph data.]

CONCLUSION

This project will hopefully be of some asset to future chemistry and biology students interested in producing, raising, and purifying monoclonal antibodies. The importance of these antibodies is unquestionable, but researchers need to continue to find alternative methods to produce them in ways that are not as time consuming or expensive. The uses of monoclonal antibodies may be endless, but they still must be produced, and possessing a desire and interest to do such that, is a start in the right direction.

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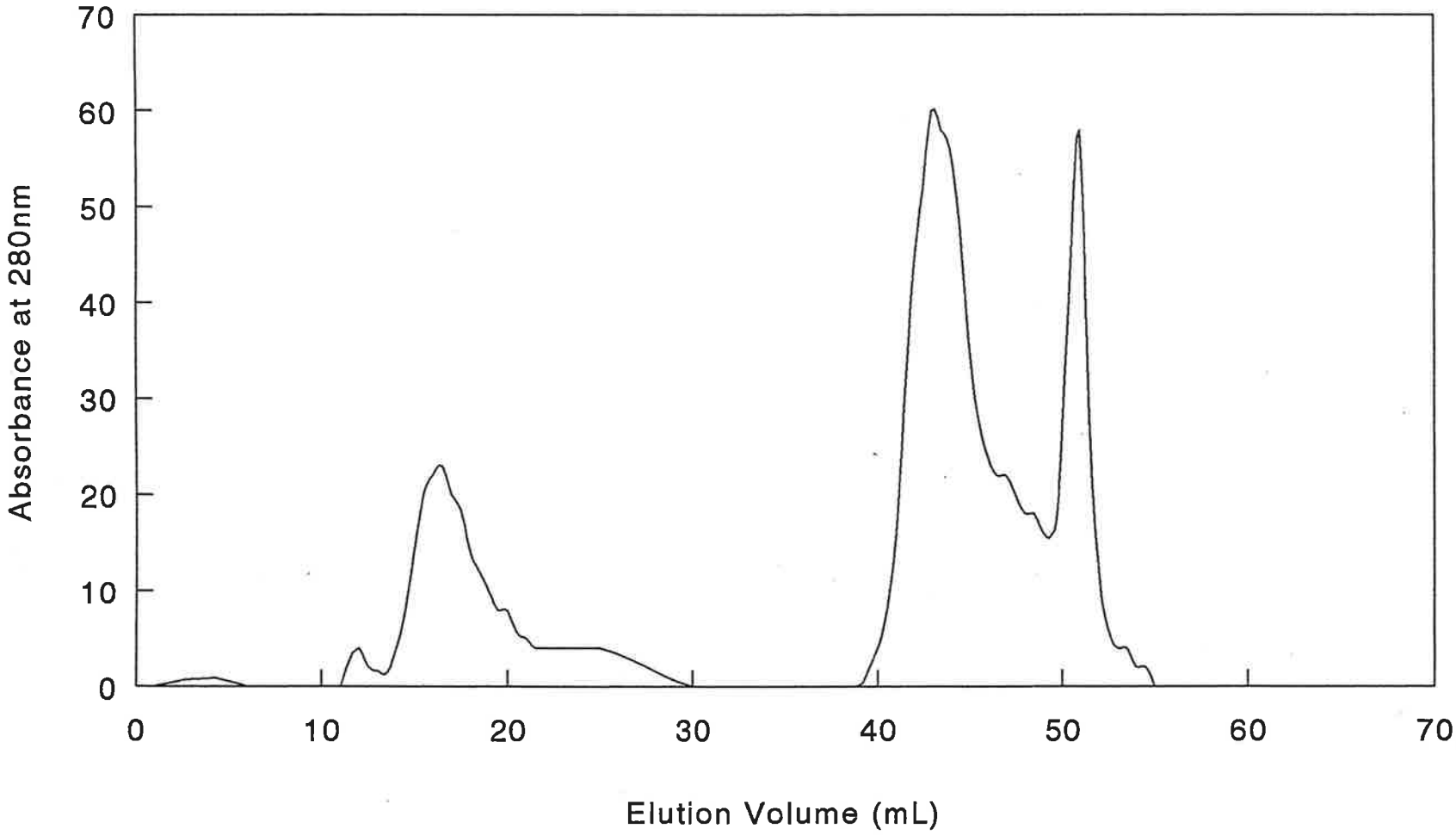
Absorbance vs. Elution Volume



DATA FOR ABSORBANCE VERSUS ELUTION VOLUME

Flow Rate: 1mL/min
Chart Speed: 12cm/hour
1cm=5mL

Point	Elution		Absorbance at 280nm
	Length (cm)	Volume (mL)	
1	0.2	1	0
2	1.2	6	0
3	2.2	11	0
4	2.4	12	4
5	2.5	12.5	2
6	2.6	13	1.6
7	2.7	13.5	1.4
8	2.8	14	4
9	2.9	14.5	8
10	3.0	15	14
11	3.1	15.5	20
12	3.2	16	22
13	3.3	16.5	23
14	3.4	17	20
15	3.5	17.5	18.4
16	3.6	18	14
17	3.7	18.5	12
18	3.8	19	10
19	3.9	19.5	8
20	4.0	20	8
21	4.1	20.5	5.6
22	4.2	21	5

23	4.3	21.5	4
24	4.5	22	4
25	5	25	4
26	5.5	27.5	2
27	6	30	0
28	6.5	32.5	0
29	7	35	0
30	7.5	37.5	0
31	8	40	4
32	8.1	40.5	8
33	8.2	41	16
34	8.3	41.5	30
35	8.4	42	44
36	8.5	42.5	52
37	8.6	43	60
38	8.7	43.5	58
39	8.8	44	56
40	8.9	44.5	48
41	9	45	36
42	9.1	45.5	28
43	9.2	46	24
44	9.3	46.5	22
45	9.4	47	22
46	9.5	47.5	20
47	9.6	48	18
48	9.7	48.5	18
49	9.8	49	16
50	9.9	49.5	16

51	9.95	49.75	18
51	10	50	26
53	10.1	50.5	44
54	10.2	51	58
55	10.3	51.5	28
56	10.4	52	12
57	10.5	52.5	6
58	10.6	53	4
59	10.7	53.5	4
60	10.8	54	2
61	10.9	54.5	2
62	11	55	0